

**SYSTEMS AND METHODS FOR PROCESSING CELLS****CROSS-REFERENCE**

**[0001]** This application claims of benefit of U.S. Provisional Patent Application No. 63/723,534, filed November 21, 2024, U.S. Provisional Patent Application No. 63/558,804, filed February 28, 2024, and U.S. Provisional Patent Application No. 63/623,024, filed January 19, 2024, each of which is entirely incorporated herein by reference.

**BACKGROUND**

**[0002]** Cell processing techniques have applications in a variety of fields such as life science research and industrial and therapeutic production. Bioreactor technologies and bioprocessing systems are useful tools for facilitating culturing, processing, and manufacturing of cells and valuable cell byproducts.

**SUMMARY**

**[0003]** The present disclosure provides systems and methods for processing cells. Cell processing systems may permit fully automated cell processing. Automated cell processing may include cellular expansion, gene delivery, development of cellular therapies, biologics manufacturing, or any combinations thereof. The systems and method described herein may include simpler fluidics as compared to other cell processing systems which may permit cell processing in smaller and lower cost instruments. The systems and methods described herein may permit cell processing with reduced cell handling and in an environment that replicates a cells natural environment which may maintain or produce healthier cells as compared to other cell processing systems and methods. Thus, the systems and methods described herein may provide for improved cell handling and processing such that produced cells or cell products have increased reproducibility, viability, and overall health as compared to cells produced using other systems and methods.

**[0004]** In an aspect, the present disclosure provides a system for manufacturing a cell product, comprising: a compartment configured to retain a plurality of cells; a fluid flow path in fluid communication with the compartment, wherein the fluid flow path is configured to (i) flow a fluid along a first direction having an angle with respect to a second direction of flow within the compartment, wherein the angle is greater than zero degrees, and (ii) provide material from the fluid to the compartment to thereby generate the cell product; and an analysis unit coupled to and within a same housing as the compartment and the fluid flow

path, wherein the analysis unit is configured to monitor the plurality of cells or the fluid as the materials are provided to the compartment during generation of the cell product.

**[0005]** In some embodiments, the material comprises reagents or nutrients. In some embodiments, the angle is greater than 45 degrees. In some embodiments, the angle is greater than 60 degrees. In some embodiments, the angle is greater than 80 degrees. In some embodiments, the cell product is a cell therapy product. In some embodiments, the system is configured to generate the cell therapy product at a scale of at least one human dose equivalent. In some embodiments, the compartment is configured to contain the plurality of cells. In some embodiments, the plurality of cells or a portion thereof is immobilized within the compartment. In some embodiments, the plurality of cells is encapsulated within the compartment.

**[0006]** In some embodiments, the analysis unit is configured to monitor the plurality of cells in the compartment. In some embodiments, the analysis unit is configured to monitor the plurality of cells in real time. In some embodiments, the analysis unit monitors or detects one or more properties selected from the group consisting of marker expression, phenotype, viability, concentration, potency, sterility, endotoxins, and safety. In some embodiments, the system further comprises a cell processing unit in fluid communication with the compartment. In some embodiments, the cell processing unit is configured to provide one or more processes selected from the group consisting of cell enrichment, cell selection, cell isolation, washing, concentration, buffer exchange, and de-clumping.

**[0007]** In some embodiments, the compartment is configured to provide to the plurality of cells one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection. In some embodiments, the selection is negative selection. In some embodiments, the housing has a volume of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ . In some embodiments, the volume is of at most 1  $\text{m}^3$ . In some embodiments, the volume is of at most 0.5  $\text{m}^3$ . In some embodiments, the system is an aseptically closed system.

**[0008]** In some embodiments, the analysis unit comprises one or more sensors configured to monitor an environment comprising the plurality of cells. In some embodiments, the one or more sensors are selected from the group consisting of metabolites sensors, dissolved gas sensors, pH sensors, temperature sensors, viscosity sensors, and pressure sensors. In some embodiments, the analysis unit comprises one or more sensors configured to analyze the cell product. In some embodiments, the one or more sensors are selected from the group consisting of Raman spectroscopy, infrared (IR) absorption spectroscopy, near-infrared (NIR)



spectroscopy, mid-infrared spectroscopy, Fourier Transform infrared (FTIR) spectroscopy, optical coherence tomography, confocal microscopy, multiphoton microscopy, microwave impedance microscopy, ultrasound imaging, photoacoustic microscopy, elastography, computed tomography, conductance sensor, radio-frequency impedance sensor, dielectric spectroscopy, permittivity sensor, fluorescence imaging, mass spectroscopy, optical density, and magnetic resonance angiography. In some embodiments, the system further comprises one or more temperature sensors and a temperature control unit. In some embodiments, the system further comprises a plurality of compartments comprising the compartment and a plurality of fluid flow paths comprising the fluid flow path.

**[0009]** In another aspect, the present disclosure provides a system for cell processing, comprising: a cell processing unit configured to retain and process a plurality of cells to generate a cell product; a preservation unit coupled to the cell processing unit, wherein the preservation unit is configured to preserve the cell product; and an analysis unit coupled to the cell processing unit or the preservation unit, wherein the analysis unit is configured to detect or monitor one or more properties of the plurality of cells or the cell product.

**[0010]** In some embodiments, the cell product is a cell therapy product. In some embodiments, the system further comprises a receiving unit configured to receive the plurality of cells. In some embodiments, the plurality of cells is a plurality of cryopreserved cells. In some embodiments, the receiving unit is configured to subject the plurality of cryopreserved cells to controlled thawing to generate a plurality of thawed cells. In some embodiments, the receiving unit is configured to automatically transfer the plurality of thawed cells to the cell processing unit. In some embodiments, the system is an aseptically closed system. In some embodiments, the preservation unit is configured to generate a cryopreserved cell product. In some embodiments, the cell processing unit is configured to provide one or more processes selected from the group consisting of cell enrichment, cell selection, cell isolation, washing, concentration, buffer exchange, and de-clumping. In some embodiments, the cell processing unit is configured to contain the plurality of cells. In some embodiments, the plurality of cells or a portion thereof is immobilized within the cell processing unit. In some embodiments, the plurality of cells is encapsulated within the cell processing unit.

**[0011]** In some embodiments, the system further comprises a bioreactor coupled to the cell processing unit, the preservation unit, or the analysis unit. In some embodiments, the bioreactor is a solid-state bioreactor. In some embodiments, the bioreactor is configured to

provide to the plurality of cells one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.

**[0012]** In some embodiments, the analysis unit is configured to provide in situ, real-time detection or monitoring of the one or more properties of the plurality of cells or the cell product. In some embodiments, the one or more properties of the plurality of cells or the cell product are selected from the group consisting of cell viability, density, concentration, cytometry, pH, dissolved oxygen, and metabolites. In some embodiments, the analysis unit is configured to provide release testing of the cell product. In some embodiments, the release testing comprises one or more testing methods selected from the group consisting of imaging cytometry, quantitative polymerase chain reaction (qPCR), digital polymerase chain reaction (dPCR), enzyme-linked immunosorbent assay (ELISA), cell-based assays, cell count, viable cell count, and imaging bead count.

**[0013]** In some embodiments, the cell processing unit, the analysis unit, and the preservation unit are integrated withing a housing. In some embodiments, the housing has a volume of about 0.01 cubic meters ( $\text{m}^3$ ) to about  $2 \text{ m}^3$ . In some embodiments, the volume is of at most  $1 \text{ m}^3$ . In some embodiments, the volume is of at most  $0.5 \text{ m}^3$ . In some embodiments, the analysis unit comprises one or more sensors configured to monitor an environment comprising the plurality of cells. In some embodiments, the one or more sensors are selected from the group consisting of metabolites sensors, dissolved gas sensors, pH sensors, temperature sensors, viscosity sensors, pressure sensors. In some embodiments, the analysis unit comprises one or more sensors configured to analyze the cell product. In some embodiments, the one or more sensors are selected from the group consisting of Raman spectroscopy, infrared (IR) absorption spectroscopy, near-infrared (NIR) spectroscopy, mid-infrared spectroscopy, Fourier Transform infrared (FTIR) spectroscopy, optical coherence tomography, confocal microscopy, multiphoton microscopy, microwave impedance microscopy, ultrasound imaging, photoacoustic microscopy, elastography, computed tomography, conductance sensor, radio-frequency impedance sensor, dielectric spectroscopy, permittivity sensor, fluorescence imaging, mass spectroscopy, optical density, and magnetic resonance angiography.

**[0014]** In another aspect, the present disclosure provides a method for manufacturing a cell product, comprising: providing a plurality of cells to a system comprising (i) a compartment that retains the plurality of cells, (ii) a fluid flow path in fluid communication with the compartment, and (iii) an analysis unit coupled to and within a same housing as the compartment and the fluid flow path; providing material from the a fluid in the fluid flow

path to the compartment to generate the cell product, wherein the fluid flow path flows the fluid along a first direction having an angle with respect to a second direction of flow within the compartment, wherein the angle is greater than zero degrees; and using the analysis unit to monitor the plurality of cells or the fluid as the materials are provided to the compartment during generation of the cell product.

**[0015]** In some embodiments, the material comprises reagents or nutrients. In some embodiments, the angle is greater than 45 degrees. In some embodiments, the angle is greater than 60 degrees. In some embodiments, the angle is greater than 80 degrees. In some embodiments, the cell product is a cell therapy product. In some embodiments, the cell therapy product is generated at a scale of at least one human dose equivalent. In some embodiments, the compartment is configured to contain the plurality of cells. In some embodiments, the plurality of cells or a portion thereof is immobilized within the compartment. In some embodiments, the plurality of cells is encapsulated within the compartment.

**[0016]** In some embodiments, the analysis unit monitors the plurality of cells in the compartment. In some embodiments, the analysis unit monitors the plurality of cells in real time. In some embodiments, the analysis unit monitors or detects one or more properties selected from the group consisting of marker expression, phenotype, viability, concentration, potency, sterility, endotoxins, and safety. In some embodiments, the method further comprises using a cell processing unit to generate the cell product. In some embodiments, the cell processing unit provides one or more processes selected from the group consisting of cell enrichment, cell selection, cell isolation, washing, concentration, buffer exchange, and de-clumping. In some embodiments, the method further comprises subjecting the plurality of cells to one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection. In some embodiments, the selection is negative selection. In some embodiments, the housing has a volume of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ .

**[0017]** In another aspect, the present disclosure provides a method for cell processing, comprising: providing a plurality of cells to a system comprising (i) a cell processing unit that retains the plurality of cells, (ii) a preservation unit coupled to the cell processing unit, and (iii) an analysis unit coupled to the cell processing unit or the preservation unit; processing the plurality of cells in the cell processing unit to generate a cell product; preserving the cell product in the preservation unit; and detecting or monitoring one or more properties of the plurality of cells or the cell product during the processing or the preserving.

**[0018]** In some embodiments, the cell product is a cell therapy product. In some embodiments, the method further comprises providing the plurality of cells to receiving unit coupled to the cell processing unit, the preservation unit, or the preservation unit. In some embodiments, the plurality of cells provided to the receiving unit is a plurality of cryopreserved cells. In some embodiments, the receiving unit subjects the plurality of cryopreserved cells to controlled thawing to generate a plurality of thawed cells. In some embodiments, the receiving unit automatically transfers the plurality of thawed cells to the cell processing unit. In some embodiments, the preservation unit generates a cryopreserved cell product.

**[0019]** In some embodiments, the method further comprises subjecting the plurality of cells to one or more processes selected from the group consisting of cell enrichment, cell selection, cell isolation, washing, concentration, buffer exchange, and de-clumping. In some embodiments, the method further comprises a bioreactor coupled to the cell processing unit, the preservation unit, or the analysis unit. In some embodiments, the bioreactor is a solid-state bioreactor. In some embodiments, the method further comprises using the bioreactor to subject the plurality of cells to one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.

**[0020]** In some embodiments, the analysis unit provides in situ, real-time detection or monitoring of the one or more properties of the plurality of cells or the cell product. In some embodiments, the one or more properties of the plurality of cells or the cell product are selected from the group consisting of cell viability, density, concentration, cytometry, pH, dissolved oxygen, and metabolites. In some embodiments, the method further comprises using the analysis unit for release testing of the cell product. In some embodiments, the release testing comprises one or more testing methods selected from the group consisting of imaging cytometry, qPCR, dPCR, ELISA, cell-based assays, cell count, viable cell count, and imaging bead count.

**[0021]** In another aspect, the present disclosure provides a system for cell processing, comprising: a plurality of three-dimensional (3D) matrices, wherein a 3D matrix of the plurality of 3D matrices is configured to retain a population of cells; and a plurality of fluid flow paths, wherein a fluid flow path of the plurality of fluid flow paths is independent from and adjacent to the 3D matrix, and wherein the fluid flow path is configured to deliver a fluidic material to the 3D matrix to process the population of cells into a cell product.

**[0022]** In some embodiments, the cell product is a cell therapy product. In some embodiments, the 3D matrix and the fluid flow path are separated by a membrane. In some

embodiments, the 3D matrix is configured as a slab comprising a first dimension and a second dimension that are larger than a third dimension. In some embodiments, the third dimension is less than or equal to about 10 millimeters (mm). In some embodiments, the third dimension is less than or equal to about 1 mm. In some embodiments, the third dimension is less than or equal to about 500 micrometers ( $\mu\text{m}$ ). In some embodiments, the third dimension is less than or equal to about 200  $\mu\text{m}$ . In some embodiments, the 3D matrix is configured as a fiber comprising a diameter that is smaller than the long dimension. In some embodiments, the diameter is less than or equal to about 10 mm. In some embodiments, the 3D matrix is configured as a sphere and wherein the tangential flow of the fluid flows along an outer circumference of the sphere. In some embodiments, the plurality of 3D matrices is fluidically coupled to one another. In some embodiments, the 3D matrix is configured to contain the plurality of cells. In some embodiments, the 3D matrix is configured to immobilize the plurality of cells. In some embodiments, the 3D matrix is configured to encapsulate the plurality of cells.

**[0023]** In some embodiments, the fluid flow path is disposed between a first 3D matrix and a second 3D matrix. In some embodiments, the fluid flow path is configured to deliver the fluidic material to the first 3D matrix and the second 3D matrix. In some embodiments, the 3D matrix comprises a pore size of less than or equal to about 400 micrometers ( $\mu\text{m}$ ). In some embodiments, the 3D matrix comprises a pore size from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the 3D matrix comprises a porosity of greater than or equal to about 80%. In some embodiments, an interfacial area between the fluid flow path and the 3D matrix is greater than or equal to about 5 square centimeters ( $\text{cm}^2$ ). In some embodiments, the 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within the 3D matrix. In some embodiments, the 3D matrix is a programmable 3D matrix. In some embodiments, the 3D matrix has a surface area to volume ratio of greater than or equal to about 0.1. In some embodiments, the 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m. In some embodiments, the 3D matrix is configured to provide activation of the plurality of cells via membrane signal transduction in the 3D matrix.

**[0024]** In another aspect, the present disclosure provides a method for cell processing, comprising: providing (i) a population of cells, (ii) a plurality of three-dimensional (3D) matrices, and (iii) a plurality of fluid flow paths, wherein the population of cells is retained within a 3D matrix of the plurality of 3D matrices, and wherein a fluid flow path of the plurality of fluid flow paths is independent from and adjacent to the 3D matrix; using the

fluid flow path to deliver fluidic material to the 3D matrix; and in a presence of the fluidic material delivered to the 3D matrix, processing the population of cells into a cell product.

**[0025]** In some embodiments, the cell product is a cell therapy product. In some embodiments, the cell therapy product is produced at a scale of at least one human dose equivalent. In some embodiments, the 3D matrix is configured to contain the plurality of cells. In some embodiments, the 3D matrix is configured to immobilize the plurality of cells. In some embodiments, the 3D matrix is configured to encapsulate the plurality of cells.

**[0026]** In some embodiments, the method further comprises functionalizing the 3D matrix to generate a functionalized 3D matrix. In some embodiments, the functionalizing comprises coupling one or more biomolecules to the 3D matrix. In some embodiments, the one or more biomolecules are selected from the group consisting of proteins, peptides, cytokines, antibodies, and nucleic acid molecules. In some embodiments, the one or more biomolecules are usable for membrane signal transduction. In some embodiments, the method further comprises polymerizing pre-functionalized polymer precursors to generate the 3D matrix. In some embodiments, the functionalizing occurs concurrently with providing (i) a population of cells, (ii) a plurality of three-dimensional (3D) matrices, and (iii) a plurality of fluid flow paths. In some embodiments, the functionalizing occurs subsequent to providing (i) a population of cells, (ii) a plurality of three-dimensional (3D) matrices, and (iii) a plurality of fluid flow paths.

**[0027]** In some embodiments, the method further comprises releasing the one or more biomolecules from the 3D matrix or replacing the one or more biomolecules with additional or different biomolecules. In some embodiments, the 3D matrix and the fluid flow path are separated by a membrane, and wherein the fluidic material is transported through the membrane. In some embodiments, the 3D matrix has a dimension less than or equal to about 10 millimeters (mm). In some embodiments, the dimension is less than or equal to about 1 mm. In some embodiments, the dimension is less than or equal to about 500 micrometers ( $\mu\text{m}$ ). In some embodiments, the dimension is less than or equal to about 200  $\mu\text{m}$ . In some embodiments, the plurality of 3D matrices is fluidically coupled to one another. In some embodiments, the fluid flow path is disposed between a first 3D matrix and a second 3D matrix of the plurality of 3D matrices. In some embodiments, the fluid flow path delivers the fluidic material to the first 3D matrix and the second 3D matrix.

**[0028]** In some embodiments, the 3D matrix comprises a pore size of less than or equal to about 400 micrometers ( $\mu\text{m}$ ). In some embodiments, the 3D matrix comprises a pore size from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the 3D matrix comprises a

porosity of greater than or equal to about 80%. In some embodiments, an interfacial area between the fluid flow path and the 3D matrix is greater than or equal to about 5 square centimeters (cm<sup>2</sup>). In some embodiments, the 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within the 3D matrix. In some embodiments, the 3D matrix has a surface area to volume ratio of greater than or equal to about 0.1. In some embodiments, the 3D matrix is a programmable 3D matrix.

**[0029]** In another aspect, the present disclosure provides a method for cell processing, comprising: providing a three-dimensional (3D) matrix containing a plurality of cells in a cell processing unit; in the cell processing unit, automatically: processing the plurality of cells within the 3D matrix to produce a plurality of processed cells; and separating the plurality of processed cells from the 3D matrix; and collecting the plurality of processed cells.

**[0030]** In some embodiments, the plurality of cells is encapsulated in the 3D matrix. In some embodiments, the 3D matrix is configured to retain the plurality of cells. In some embodiments, the 3D matrix is configured to immobilize the plurality of cells. In some embodiments, the plurality of processed cells is a cell therapy product. In some embodiments, the cell therapy product is produced at a scale of at least one human dose equivalent. In some embodiments, the method further comprises providing a mixture comprising a matrix precursor and the plurality of cells to the cell processing system.

**[0031]** In some embodiments, the method further comprises, in the cell processing unit, applying a first stimulus to the mixture comprising the matrix precursor and the plurality of cells to generate the 3D matrix encapsulating the plurality of cells. In some embodiments, the first stimulus is a thermal stimulus. In some embodiments, the first stimulus is a chemical stimulus. In some embodiments, the first stimulus is an enzymatic stimulus. In some embodiments, the method further comprises functionalizing the 3D matrix with one or more biomolecules concurrently with the applying the first stimulus to generate the 3D matrix. In some embodiments, (ii) comprises applying a second stimulus to the 3D matrix to release the cell product from the 3D matrix. In some embodiments, the second stimulus is a thermal stimulus. In some embodiments, the second stimulus is a chemical stimulus. In some embodiments, the second stimulus is an enzymatic stimulus.

**[0032]** In some embodiments, the method further comprises, prior to providing the 3D matrix, generating the 3D matrix in presence of the plurality of cells such that the 3D matrix encapsulates the plurality of cells. In some embodiments, providing the 3D matrix comprises loading the 3D matrix encapsulating the plurality of cells is loaded into a compartment in the

cell processing unit. In some embodiments, providing the 3D matrix comprises separately providing the plurality of cells and the 3D matrix to the cell processing unit. In some embodiments, the 3D matrix is generated within a compartment in the cell processing unit. In some embodiments, the 3D matrix is generated external to the cell processing system and subsequently provided to the cell processing unit.

**[0033]** In some embodiments, the method further comprises functionalizing the 3D matrix to generate a functionalized 3D matrix. In some embodiments, the functionalizing comprises coupling one or more biomolecules to the 3D matrix. In some embodiments, the one or more biomolecules are selected from the group consisting of proteins, peptides, cytokines, antibodies, and nucleic acid molecules. In some embodiments, the one or more biomolecules are usable for membrane signal transduction. In some embodiments, the method further comprises functionalizing the 3D matrix with the one or more biomolecules prior to (i). In some embodiments, the method further comprises functionalizing the 3D matrix with the one or more biomolecules concurrently with (i). In some embodiments, the method further comprises releasing the one or more biomolecules from the 3D matrix or replacing the one or more biomolecules with additional biomolecules. In some embodiments, (i) comprises activating the plurality of cells within the 3D matrix to obtain the cell product. In some embodiments, (i) comprises transducing the plurality of cells within the 3D matrix to obtain the cell product. In some embodiments, (i) comprises transfecting the plurality of cells within the 3D matrix to obtain the cell product. In some embodiments, the 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m. In some embodiments, the method further comprises activating the plurality of cells via membrane signal transduction in the 3D matrix.

**[0034]** In another aspect, the present disclosure provides a cell processing system, comprising: a cell processing unit configured to retain a three-dimensional (3D) matrix, wherein the 3D matrix is configured to encapsulate a plurality of cells; and one or more computer processors operatively coupled to the cell processing unit, wherein the one or more computer processors are individually or collectively programmed to automatically (i) process the plurality of cells within the 3D matrix to produce a plurality of processed cells, (ii) direct the cell processing unit to separate the plurality of processed cells from the 3D matrix, and (iii) direct the cell processing unit to collect the plurality of processed cells.

**[0035]** In some embodiments, the method further comprises a receiving unit configured to receive the plurality of cells from a user. In some embodiments, the one or more computer processors is operatively coupled to the receiving unit and individually or collectively



programmed to automatically direct the receiving unit to provide the plurality of cells to the cell processing unit. In some embodiments, the receiving unit is configured to receive the plurality of cells in a cryopreserved state, and wherein the receiving unit is configured to provide controlled thawing to the plurality of cells in the cryopreserved state. In some embodiments, the method further comprises an analysis unit configured to monitor the plurality of cells during processing of the plurality of cells. In some embodiments, the analysis unit is configured to monitor the plurality of cells in real time. In some embodiments, the analysis unit monitors or detects one or more properties selected from the group consisting of marker expression, phenotype, viability, concentration, potency, sterility, endotoxins, and safety. In some embodiments, the analysis unit comprises one or more sensors configured to monitor an environment comprising the plurality of cells. In some embodiments, the one or more sensors are selected from the group consisting of metabolites sensors, dissolved gas sensors, pH sensors, temperature sensors, viscosity sensors, pressure sensors. In some embodiments, the analysis unit comprises one or more sensors configured to analyze the cell product. In some embodiments, the one or more sensors are selected from the group consisting of Raman spectroscopy, infrared (IR) absorption spectroscopy, near-infrared (NIR) spectroscopy, mid-infrared spectroscopy, Fourier Transform infrared (FTIR) spectroscopy, optical coherence tomography, confocal microscopy, multiphoton microscopy, microwave impedance microscopy, ultrasound imaging, photoacoustic microscopy, elastography, computed tomography, conductance sensor, radio-frequency impedance sensor, dielectric spectroscopy, permittivity sensor, fluorescence imaging, mass spectroscopy, optical density, and magnetic resonance angiography. In some embodiments, the cell processing unit is further configured to provide one or more processes selected from the group consisting of cell enrichment, cell selection, cell isolation, washing, concentration, buffer exchange, and de-clumping. In some embodiments, the cell selection is negative selection. In some embodiments, the method further comprises a housing configured to retain the cell processing unit and the one or more computer processors. In some embodiments, the housing has a footprint of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ .

**[0036]** In some embodiments, the 3D matrix is configured to contain the plurality of cells. In some embodiments, the 3D matrix is configured to immobilize the plurality of cells. In some embodiments, the 3D matrix is configured to encapsulate the plurality of cells. In some embodiments, the cell processing unit is configured to generate the 3D matrix in presence of the plurality of cells. In some embodiments, the cell processing unit is configured to receive a pre-formed 3D matrix. In some embodiments, the cell processing unit is

configured to receive the pre-formed 3D matrix comprising the plurality of cells. In some embodiments, the cell processing unit is configured to encapsulate the plurality of cells in the pre-formed 3D matrix. In some embodiments, the 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m. In some embodiments, the 3D matrix is configured to provide activation of the plurality of cells via membrane signal transduction in the 3D matrix.

**[0037]** In another aspect, the present disclosure provides a method for cell processing, comprising: providing a plurality of cells ex vivo to a cell processing unit; in the cell processing unit, contacting the plurality of cells with a selection reagent, wherein, upon the contacting, the selection reagent (i) couples with cells of a sub-population of the plurality of cells and (ii) perturbs a state of one or more cells of the sub-population to product (A) a population of perturbed cells and (B) a population of non-perturbed cells; and processing either the population of perturbed cells or the population of non-perturbed cells into a cell therapy product.

**[0038]** In some embodiments, the cell therapy product is produced at a scale of at least one human dose equivalent. In some embodiments, the population of perturbed cells have an altered growth rate or viability as compared to the population of non-perturbed cells. In some embodiments, the method further comprises contacting the plurality of cells with the selection reagent and processing either the population of perturbed cells or the population of non-perturbed cells into a cell therapy product simultaneously. In some embodiments, the method further comprises processing either the population of perturbed cells or the population of non-perturbed cells subsequent to contacting the plurality of cells with a selection reagent.

**[0039]** In some embodiments, the cell processing unit comprises a three-dimensional (3D) matrix encapsulating the plurality of cells. In some embodiments, the method further comprises contacting the plurality of cells with the selection reagent within the 3D matrix. In some embodiments, the cell processing unit comprises a vessel comprising the plurality of cells. In some embodiments, the method further comprises contacting the plurality of cells with the selection reagent within the vessel.

**[0040]** In some embodiments, the selection reagent comprises a binding agent and a selection agent. In some embodiments, the selection agent is a cytotoxic agent. In some embodiments, the binding agent is coupled to the selection agent via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the binding agent is an antibody. In some embodiments, the binding agent is an aptamer. In some embodiments, the

population of perturbed cells comprises dead cells or cell degradation products. In some embodiments, the population of perturbed cells have an altered cell morphology as compared to the population of non-perturbed cells. In some embodiments, the processing comprises one or more processes selected from the group consisting of cell culture, transduction, transfection, transformation, expansion, in situ electroporation, and gene delivery.

**[0041]** In another aspect, the present disclosure provides a system for manufacturing a cell therapy product, comprising: a three-dimensional (3D) matrix configured to retain a population of cells at a density of greater than about 80 million cells per cubic centimeter ( $\text{cell}/\text{cm}^3$ ), wherein the system is capable of processing cells of the population of cells, with aid of the 3D matrix, into the cell therapy product.

**[0042]** In some embodiments, the density is at least 100 million cells/ $\text{cm}^3$ . In some embodiments, the 3D matrix is configured to contain the population of cells. In some embodiments, the 3D matrix is configured to immobilize the population of cells. In some embodiments, the 3D matrix is configured to encapsulate the population of cells. In some embodiments, the 3D matrix has a surface area to volume ratio greater than or equal to 0.1. In some embodiments, the 3D matrix has a porosity of at least about 80%. In some embodiments, the method further comprises a plurality of 3D matrices comprising the 3D matrix. In some embodiments, the method further comprises a fluid flow path in fluid communication with the 3D matrix, wherein the fluid flow path is adjacent to and independent from the 3D matrix. In some embodiments, the method further comprises a membrane disposed between the fluid flow path and the 3D matrix. In some embodiments, the 3D matrix is programmable.

**[0043]** In another aspect, the present disclosure provides a method for manufacturing a cell therapy product, comprising (i) providing a population of cells at a density of greater than about 80 million cells per centimeters ( $\text{cells}/\text{cm}^3$ ) retained within a three-dimensional (3D) matrix and (ii) with aid of the 3D matrix, processing cells of the population of cells into the cell therapy product.

**[0044]** In some embodiments, the processing of the cells comprises culturing the population of cells. In some embodiments, the processing of the cells comprises in situ electroporation of the cells. In some embodiments, the 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m. In some embodiments, the processing of the cells comprises activation of the cells in the 3D matrix.

**[0045]** In another aspect, the present disclosure provides a bioreactor for processing a population of cells comprising a three-dimensional (3D) matrix configured to retain the

population of cells, wherein the bioreactor has a volume of at most about 2 cubic meters ( $\text{m}^3$ ) and is configured to, with aid of the 3D matrix and at high reproducibility, processes the population of cells into a cell product.

**[0046]** In some embodiments, the cell product has a viability of greater than or equal to about 75%. In some embodiments, the volume is of at most  $1 \text{ m}^3$ . In some embodiments, the volume is of at most  $0.5 \text{ m}^3$ .

**[0047]** In another aspect, the present disclosure provides a method for processing a population of cells comprising (i) providing a population of cells retained within three-dimensional (3D) matrix of a bioreactor, wherein the bioreactor has a volume of at most 2 cubic meters ( $\text{m}^3$ ) and (ii) processing, with aid of the 3D matrix and at high reproducibility, the population of cells into a cell product.

**[0048]** In some embodiments, the cell product has a viability of greater than or equal to about 75%. In some embodiments, the method further comprises, prior to (i), generating the 3D matrix in presence of the population of cells. In some embodiments, the method further comprises subsequent to (ii), dissolving the 3D matrix and collecting the population of cells to generate the cell product.

**[0049]** In another aspect, the present disclosure provides a system for cell therapy manufacturing, comprising: a three-dimensional (3D) matrix configured to retain a population of cells at a density of at least about 5 million cells per cubic centimeters ( $\text{cells}/\text{cm}^3$ ), wherein the system is capable of processing cells of the population within the 3D matrix for a time period of at most about 48 hours to generate a cell product, and wherein the cell product is usable to generate a cell therapy.

**[0050]** In some embodiments, the density is at least 10 million cells/  $\text{cm}^3$ . In some embodiments, the density is at least 20 million cells/  $\text{cm}^3$ . In some embodiments, the 3D matrix has a surface area to volume ratio greater than or equal to 0.1. In some embodiments, the 3D matrix has a porosity of at least about 80%. In some embodiments, the 3D matrix has a dimension of less than about 200 micrometers ( $\mu\text{m}$ ). In some embodiments, the dimension is less than about 100  $\mu\text{m}$ . In some embodiments, the dimension is less than about 10  $\mu\text{m}$ . In some embodiments, the dimension is from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within the 3D matrix. In some embodiments, the system further comprises a plurality of 3D matrices comprising the 3D matrix. In some embodiments, the system further comprises a fluid flow path in fluid communication with the 3D matrix, wherein the fluid flow path is adjacent to

and independent from the 3D matrix. In some embodiments, the system further comprises a membrane disposed between the fluid flow path and the 3D matrix.

**[0051]** In another aspect, the present disclosure provides a method for manufacturing a cell therapy, comprising (i) providing a population of cells at a density of at least about 5 million cells per centimeters (cells/ cm<sup>3</sup>) retained within a three-dimensional (3D) matrix; (ii) processing the cells of the population of cells within the 3D matrix for a time period of at most about 48 hours to generate a cell product; and (iii) using the cell product to generate the cell therapy.

**[0052]** In some embodiments, the processing of the cells comprises culturing the population of cells. In some embodiments, the processing of the cells comprises in situ electroporation of the population of cells. In some embodiments, the 3D matrix has a conductivity from about 10<sup>-5</sup> Siemens per m (S/m) to about 10<sup>-1</sup> S/m. In some embodiments, the processing of the cells comprises activation via membrane signal transduction in the 3D matrix.

**[0053]** Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

#### **INCORPORATION BY REFERENCE**

**[0054]** All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede or take precedence over any such contradictory material.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0055]** The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative

embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “figure” and “FIG.” herein), of which:

[0056] **FIG. 1** shows example cell products that may be generated by the systems and methods described herein;

[0057] **FIG. 2** shows example chimeric antigen receptor (CAR)-T cell therapy manufacturing;

[0058] **FIG. 3** schematically illustrates an example integrated cell processing system and consumables;

[0059] **FIG. 4** schematically illustrates an example cell processing unit and solid-state bioreactor;

[0060] **FIGs. 5A and 5B** show example processes provided by an example integrated cell processing system; **FIG. 5A** schematically illustrates example processes provided by an example integrated cell processing system; and **FIG. 5B** schematically illustrates an example cell processing workflow;

[0061] **FIG. 6** schematically illustrates glucose levels as a function of time for example bioreactor configurations;

[0062] **FIG. 7** schematically illustrates an example workflow for producing a cell product from an example cell processing system;

[0063] **FIG. 8** schematically illustrates example units integrated into an example cell processing system;

[0064] **FIG. 9A** schematically illustrates fluid, nutrient, or reagent transfer in example bulk and microfluidic bioreactors; **FIG. 9B** schematically illustrates fluid, nutrient, or reagent transfer in example tangential or perfusion flow type bioreactors;

[0065] **FIGs. 10A and 10B** schematically illustrates example 3D matrix configurations and fluid interfaces;

[0066] **FIGs. 11A and 11B** schematically illustrate example 3D matrix functional physical properties;

[0067] **FIGs. 12A and 12B** schematically illustrate example 3D matrix functionalization;

[0068] **FIG. 13** schematically illustrates example interfaces between 3D matrices and fluid flow paths and 3D matrices and cellular substrates;

[0069] **FIG. 14** schematically illustrates example cell loading methods;

[0070] **FIG. 15** schematically illustrates example 3D matrix loading into a cell compartment;

- [0071] FIGs. 16A and 16B schematically illustrate example reagent delivery and removal from 3D matrix;
- [0072] FIG. 17 shows example delivery reagent sizes and corresponding size cutoffs;
- [0073] FIG. 18 show example cell processing unit comprising a 3D matrix and flow pathway;
- [0074] FIG. 19 schematically illustrates single and dual fluid flow path configurations;
- [0075] FIG. 20 schematically illustrates an example cell processing unit configured for tangential flow;
- [0076] FIGs. 21A-21C schematically illustrate example plate-like 3D matrix configurations and fluid flow pathways;
- [0077] FIGs. 22A and 22B schematically illustrates example fiber-like 3D matrix configurations and fluid flow pathways;
- [0078] FIG. 23 schematically illustrates example cell processing units including 3D matrix and fluid flow pathway;
- [0079] FIG. 24 shows an image of an example cell processing unit comprising a 3D matrix and fluid flow pathway;
- [0080] FIG. 25 shows an exploded view of an example cell processing unit;
- [0081] FIG. 26 shows an example cartridge comprising a plurality of cell processing units;
- [0082] FIG. 27 schematically illustrates an example cell processing consumable;
- [0083] FIG. 28 schematically illustrates an alternate vessel configuration;
- [0084] FIG. 29 schematically illustrates example 3D matrix configurations and bioreactor vessel types;
- [0085] FIG. 30 schematically illustrates example upstream modules for cell processing;
- [0086] FIG. 31 schematically illustrates additional example upstream modules for cell processing;
- [0087] FIG. 32 schematically illustrates example downstream modules for cell processing;
- [0088] FIG. 33 schematically illustrates additional example upstream and downstream modules for cell processing;
- [0089] FIG. 34 schematically illustrates an example aseptic fluidic interface;
- [0090] FIG. 35 schematically illustrates workflow for generating aseptic fluidic coupling;
- [0091] FIG. 36 schematically illustrates a comparison between a non-aseptic connector and aseptic fluidic connector;

- [0092] FIG. 37 schematically illustrates another workflow for generating aseptic fluidic coupling using in situ sterilized connectors;
- [0093] FIG. 38 schematically illustrates example hybrid interconnects;
- [0094] FIG. 39 schematically illustrates aseptic interconnection automation;
- [0095] FIG. 40 schematically illustrates negative selection and magnetic cell selection workflows;
- [0096] FIG. 41 shows example in situ stimulation of cells within a 3D matrix and in suspension;
- [0097] FIG. 42 shows example negative cell selection of CD19 cells from peripheral blood mononuclear cells (PBMCs);
- [0098] FIG. 43 shows example dose response curve for negative cell selection using an example antibody-drug conjugate;
- [0099] FIG. 44 shows example in situ processes for generating cell products;
- [0100] FIG. 45 shows example cell transduction as a function of cell viability for cell transduced in a suspension and 3D matrix;
- [0101] FIG. 46 shows example lentiviral transduction in suspended cells and cells encapsulated in a 3D matrix;
- [0102] FIG. 47 shows example electroporation within a 3D matrix;
- [0103] FIG. 48 shows example optical density measurements in a 3D matrix and suspension culture;
- [0104] FIG. 49 shows example optical density measurements taken in a 3D matrix and liquid media;
- [0105] FIG. 50 shows example monitoring of cell expansion within a 3D matrix and in a suspension culture;
- [0106] FIG. 51 shows example monitoring of cell viability within a 3D matrix and suspension;
- [0107] FIG. 52 shows example flow cytometry measurements for cultures expanded in a 3D matrix and suspension culture;
- [0108] FIG. 53 shows example release testing;
- [0109] FIG. 54 shows an example workflow for cell and gene therapy manufacturing;
- [0110] FIG. 55 schematically illustrates example processes performed during workflow processes;
- [0111] FIG. 56 shows an example workflow for CAR-T manufacturing;



- [00112] FIG. 57 schematically illustrates another example CAR-T cell manufacturing workflow;
- [00113] FIG. 58 shows a computer system that is programmed or otherwise configured to implement methods provided herein;
- [00114] FIGs. 59A and 59B schematically illustrate example configurations for cell processing scale up;
- [00115] FIGs. 60A-60C schematically illustrate example electroporation configurations;
- [00116] FIGs. 61A-61B schematically illustrate other example electroporation configurations;
- [00117] FIG. 62A and 62B schematically illustrates example optical density measurements;
- [00118] FIG. 63 schematically illustrates another example optical density measurement configuration;
- [00119] FIG. 64 schematically illustrates an example consumable;
- [00120] FIG. 65 schematically illustrates an internal portion of an example consumable;
- [00121] FIG. 66 schematically illustrates an example electronics and component diagram for an example cell processing system;
- [00122] FIG. 67 schematically illustrates an example perfusion module;
- [00123] FIG. 68 schematically illustrates another example perfusion module;
- [00124] FIG. 69 schematically illustrates another example perfusion module;
- [00125] FIG. 70 schematically illustrates an example perfusion module workflow;
- [00126] FIG. 71 schematically illustrates an example filtration module;
- [00127] FIG. 72 schematically illustrates another example filtration module;
- [00128] FIG. 73 schematically illustrates another example filtration module;
- [00129] FIG. 74 schematically illustrates another example filtration module;
- [00130] FIG. 75 shows example cell expansion for donor cells in 3D cell culture and suspension;
- [00131] FIG. 76 shows example cell expansion and viability after transduction;
- [00132] FIG. 77 shows an example image of a 3D matrix prior to cell harvest;
- [00133] FIG. 78 shows an example 3D bioreactor during cell expansion;
- [00134] FIG. 79 shows example sensors usable for 3D cell culture;
- [00135] FIG. 80 shows example phenotype quality of cells subjected to 3D culture versus suspension culture;

[00136] FIG. 81 shows example T cell activation and expansion using functionalized matrices;

[00137] FIG. 82 shows example antibody conjugation to a functionalized 3D matrix;

[00138] FIG. 83 shows example cell isolation using size selection;

[00139] FIG. 84 shows an example filtration module;

[00140] FIG. 85 shows another example filtration module;

[00141] FIG. 86 shows example cell filtration; and

[00142] FIG. 87 shows another example of cell filtration.

### DETAILED DESCRIPTION

[00143] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[00144] Whenever the term “at least,” “greater than,” or “greater than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “at least,” “greater than” or “greater than or equal to” applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

[00145] Whenever the term “no more than,” “less than,” or “less than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “no more than,” “less than,” or “less than or equal to” applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

[00146] The term “automatic” or “automatically,” as used herein, generally refers to a device, system, or process without or substantially without external direction or interference. For example, a system or device may be configured to run or may run a process from start to finish without or substantially without interference from a human operator. Alternatively, or in addition to, a system or device may be configured to run or may run portions of a process without or substantially without interference from a human operator.

[00147] The terms “cell processing system” or “bioprocessing system,” which may be used interchangeably, generally refer to a multicomponent system configured for processing cells. A cell processing system may comprise a bioreactor, cell processing unit, analysis

system, control system, preservation system, other components useful for processing cells, or any combinations thereof. In an example, a cell processing system comprises a bioreactor comprising a cell processing unit and additional cell processing unit(s) separate from the bioreactor. In another example, a cell processing system comprises a cell processing unit and does not comprise a bioreactor. In another example, a cell processing system may be a bioreactor comprising cell processing unit(s) integrated with one or more other components, such as analysis system, control system, or other system.

**[00148]** The term “cell processing unit” as used herein, generally refers to a device, module, or unit configured to process a cell. A cell processing unit may comprise a compartment configured to retain or which retains cells (e.g., cell compartment). A cell compartment, as described elsewhere herein, may include or comprise a three-dimensional substrate that provides cell retention. A cell processing unit may comprise one or more other components that facilitate or aid in processing cells. The one or more other components may include one or more of fluid flow path, interfaces, sensors, or other components useful for processing cells. A cell processing unit may be configured to process cells without expansion or culturing of the cells. Alternatively, or in addition to, a cell processing unit may be configured to expand or culture cells. A cell processing system or a bioreactor may comprise one or a plurality of cell processing units.

**[00149]** The term “bioreactor,” as used herein, generally refers to a device or system that supports a biologically active environment for growth or expansion of a cell population. A bioreactor may include a vessel, tank, compartment, or other environment in which whole cells are grown or cultured or in which cells are processed to generate a cell product or an expression product of a cell. A bioreactor may include a single vessel, tank, compartment, or other environment. Alternatively, or in addition to, a bioreactor may include a plurality of vessels, tanks, compartments, or other environments. A bioreactor may provide a controlled environment for biological reactions, chemical reactions, or both chemical and biological reactions. A bioreactor may include one or more cell processing units, as described elsewhere herein, that provide an environment for cellular expansion or culturing. A bioreactor may comprise one or a plurality of cell processing units.

**[00150]** The term “cell therapy product,” as used herein, generally refers to a therapy product including cells or tissues for implantation, transplantation, infusion, or transfer to a subject, such as a patient. A cell therapy product may be a cell product that is sufficient for treatment of a subject or patient. A cell therapy product may be a general product or may be

a personalized cell therapy product. A personalized cell therapy product may be manufactured from a subject's own cells or from a cell line or other cell source.

**[00151]** The term “automated system,” as used herein, generally refers to a system configured to process cells with little or no operator interventions. For example, an automated system may not have or permit the transfer of matter in or out of the system by an operator during operation. For example, an automated system may permit transfer of sample, cells, or consumables into the system by an operator when the system is not operating (e.g., before operation), but may not permit intervention during cell processing. In another example, the automated system may permit samples, cells, consumables, reagents, etc. to be input into the system before, during, or after operation, but may not permit intervention in the processing of the cells. The automated system may be coupled to one or more other modules or devices (e.g., a sequencer) such that matter (e.g., samples or cells) may be transferred to the coupled units, modules, or devices without or substantially without human intervention.

**[00152]** The term “closed system,” as used herein generally refers to a system configured to processes cells with minimal or without environmental contamination. A closed system may be an aseptically closed system. An aseptically closed system may be a system that prevents microorganisms from entering or leaving the system to protect a cell product, workers, or facilities from contamination.

**[00153]** The term “integrated system,” as used herein, generally refers to a system comprising a plurality of components, devices, apparatus, or combinations thereof that are coupled together such that the components, devices, apparatus, or combinations thereof function as a single system. Individual components of the system may be disposed in a single housing. Alternatively, or in addition to, individual components of the system may be disposed in separate houses and be electronically, fluidically, or pneumatically coupled to function as a single system. In an example, an integrated system may comprise robotic or mechanical transfer of objects (e.g., consumables, reagents, samples, cells, etc.) between separate houses. In an example, some components, devices, or apparatus may be disposed in a single housing while other components, devices, or apparatus may be disposed in another housing or in an individual housing. The various components, devices, or apparatus may comprise individual controllers. For example, an individual component, device, or apparatus may comprise a controller associated with that component, device, or apparatus. The system may further include a master controller that permits the individual components, devices, or apparatus to be controlled as one functional system. The components, devices, or apparatus may be coupled together via electronic, fluidic, pneumatic, or other connections. Fluidic or

pneumatic connections may provide aseptic coupling between the various components of the integrated system.

**[00154]** The terms “three-dimensional (3D) matrix” or “3D substrate,” as used interchangeably herein, generally refer to a matrix or substrate configured for 3D cell processing. The 3D matrix or substrate may be configured to permit cell proliferation, migration, differentiation, or any combination thereof. The 3D matrix, as described elsewhere herein, may comprise a polymer matrix, such as a hydrogel matrix, 3D scaffold, or combination thereof.

**[00155]** The term “microfluidic,” as used herein, generally refers to fluid handling components on the scale of micrometers ( $\mu\text{m}$ ). For example, a microfluidic fluid flow path (e.g., channel), chamber, or other fluid handling component may have one or more dimensions (e.g., diameter, depth, etc.) on the order of 1  $\mu\text{m}$ , 10  $\mu\text{m}$ , 100  $\mu\text{m}$ , or larger. Microfluidic devices may be configured to or otherwise used to process volumes of fluid on the order of milliliters (mL) to picoliters (pL). For example, a microfluidic device may process fluid volumes on the order of 1 pL, 10 pL, 100 pL, 1  $\mu\text{L}$ , 10  $\mu\text{L}$ , 100  $\mu\text{L}$ , 1 mL, 10 mL, 100 mL, or more.

**[00156]** The term “real time,” as used herein, generally refers to a response time of less than about 1 second, a tenth of a second, a hundredth of a second, a millisecond, or less. In an example, real time may refer to simultaneous or substantially simultaneous processing, detection, monitoring, analysis, or any combination thereof.

**[00157]** The term “subject,” as used here in, generally refers to a human or other mammal, avian, or other organism (e.g., plant). For example, the subject can be a vertebrate, a mammal, a rodent (e.g., a mouse), a primate, a simian or a human. Animals may include, but are not limited to, farm animals, sport animals, and pets. A subject can be a healthy or asymptomatic individual, an individual that has or is suspected of having a disease (e.g., cancer) or a pre-disposition to the disease, or an individual that is a candidate for a therapy (e.g., cell therapy). A subject may be a patient.

**[00158]** The term “sample,” as used herein, generally refers to a biological sample of a subject. A biological sample may comprise a population of cells, for example, a heterogenous or homogenous population of cells. The biological sample may provide a cell line or cell sample for cell processing. The sample may be a cell line or cell culture sample. The sample can include one or more cells. The sample can include one or more microbes. The biological sample may be derived from another sample. The sample may be a tissue sample, such as a biopsy, core biopsy, needle aspirate, or fine needle aspirate. The sample

may be a fluid sample, such as a blood sample, urine sample, saliva sample, apheresis sample, or leukapheresis sample. The sample may be a skin sample. The sample may be a cheek swab. The sample may be a plasma or serum sample.

#### **Cell processing applications**

**[00159]** Cells may be an important material resource for life science research and development across basic and applied scientific practices. Primary and secondary cells, including cell lines, are frequently propagated, expanded, and banked for use in research. Cells are also frequently modified, for example, via genome editing, for research use. Additionally, cell therapies, such as adoptive cell therapy and ex vivo cell-based gene therapies may represent an emerging therapeutic modality with the potential to treat many types of human diseases, for example, cancer, autoimmune diseases, neurological diseases, and age-related diseases.

**[00160]** The methods and systems described herein may be useful for production of cell therapies. Cell therapies including adoptive cell therapy (ACT) and ex vivo cell-based gene therapies may represent an emerging therapeutic modality with the potential to treat many types of human disease, including, for example, oncology, autoimmune, neurological, and aging-related diseases. In autologous therapies, cells may be collected from the patient, modified ex vivo, and reintroduced to the patient. In allogeneic therapies, a different cell source than the patient may be used, including primary donor sources (matched or unrelated), cells derived from primary cells, and secondary cell sources including stem cells and induced pluripotent stem cells (iPSC). Many emerging cell therapies may utilize genome editing technologies to engineer cells with therapeutic functions and mechanisms of action, which adds significant complexity to cell manufacturing (also referred to herein as cell processing, cell bioprocessing, or bioprocessing). Advanced cell therapies may be comprised of immune cells or cells of an immune lineage, such as T cells and hematopoietic stem and precursor cells (HSPCs). Some advanced cell therapies delivering other cell types may be approved or in various stages of development, including those which use various somatic cell types including fibroblasts, chondrocytes, keratinocytes, hepatocytes, pancreatic islet cells, cardiomyocytes, neurons, and other types of immune cells, such as dendritic cells (DCs), natural killer (NK) cells, and macrophages. Other types of cell products may include stem cells (e.g., both naturally occurring, such as in tissues, bone marrow, and cord blood, and induced, e.g., iPSC), and cell products derived from stem cells through directed differentiation or genetic programming to achieve a targeted therapeutic cell state. Despite the significant number of approved products and ongoing clinical trials, many challenges remain

in reproducible, cost-effective manufacturing procedures for cell and ex vivo cell-based gene therapy products and other expression products of a cell with therapeutic and industrial value.

**[00161]** The systems and methods described herein may be useful for development of a variety of cell products. For example, and as shown in **FIG. 1**, the systems and methods described herein may be useful for culturing or otherwise producing cell products from adherent cell types, stem and stem derived cells, rare cell types, or any combination thereof. The systems and methods described herein may be useful for personalized medicine, point of care treatment, or both.

**[00162]** Cell manufacturing may be a bottleneck that limits patient access, increases cost, and reduces commercial viability of cell therapy products. For example, cell therapy products may utilize  $10^6$ - $10^9$  therapeutic cells per clinical dose. Increasing the efficiency and rate of cell expansion while maintaining critical attributes may be challenging. Executing complex bioprocessing procedures at dose scale may also be challenging and may use multiple instrumentation platforms to perform various processes. The various processes that comprise complex or multi-process cell processing workflows may be performed in series, increasing manufacturing time. Manufacturing challenges may be reflected in the high cost of researching and developing novel cell therapies, as well as the high cost of approved cell therapy products. Reproducible manufacturing may also be a challenge due to functional heterogeneity of cells and variations that result in different quality attributes and clinical outcomes. Sources of product variability may include, but are not limited to: Patient-to-patient variability and intra-population heterogeneity in source materials, or variability introduced during manufacturing processes, including variations in cell isolation, expansion processes, or genome engineering processes.

**[00163]** Approximately ten advanced cell therapies are approved for human use in the US, and despite hundreds of thousands of patients being potentially eligible for cell therapy treatments, to date tens of thousands of patients have received cell therapy treatment. Currently hundreds of cell therapies are in development or being tested in clinical trials. Over the next decade, many tens of cell therapies are expected to be approved for human use. New advances in clinical cell manufacturing may be useful to reduce the research and development burden of novel cell therapies, as well as to deliver these potent therapies to more patients. In addition, cell therapies may be manufactured in centralized plants, adding complexity in the transport of fresh or cryopreserved cell products and manufacturing logistics. Advances in clinical cell therapy manufacturing that enable alternative manufacturing paradigms, such as distributed and point-of-care manufacturing may reduce

cost and otherwise improve patient access. Advances in cell manufacturing that reduce cost, labor, logistical complexity, biological variation, and turnaround time of cell therapy production may increase patient access to a powerful emerging therapeutic modality.

**[00164]** The systems and methods described herein may be useful for gene therapy, valuable cell byproduct, and biologic therapy production. Cellular processes, both natural and modified through genetic engineering, enable the production of various chemicals and biologic compounds that are essential in the pharmaceutical, chemical, food, energy, and agricultural industries. Bioproduction of clinically and commercially important biological products and chemicals from living cells may be complex and time-consuming, requiring complex infrastructure, manufacturing equipment, and workflows. Bioreactors are crucial tools for bioproduction, enabling the controlled and scalable production of valuable cellular byproducts. These specialized systems are designed to provide an optimal environment for the growth and metabolic activity of cells, allowing for the efficient production of biologics, such as enzymes, therapeutic proteins, vaccines, viral particles, and other biological agents. By precisely controlling parameters such as temperature, pH, oxygen levels, and nutrient supply, bioreactors ensure that cells are maintained in conditions that maximize their productivity.

**[00165]** One application of bioreactors is for production of biopharmaceuticals through the use of recombinant DNA technology. Cells (e.g., natural or genetically engineered cells) may be cultured in bioreactors to produce therapeutic proteins such as insulin, monoclonal antibodies, and hormones. These proteins may be useful for treating various diseases, including diabetes, cancer, and autoimmune disorders. For example, monoclonal antibodies produced in bioreactors may be used in targeted cancer therapies that may bind specifically to cancer cells and mediate their destruction without harming normal cells. Bioreactors may be useful for producing viral vectors that are capable of delivering therapeutic genes to patients in the form of in vivo gene therapies. For example, onasemnogene abeparvovec-xioi (Zolgensma) uses an adeno-associated virus vector (AAV9) to deliver a fully functional copy of the human survival motor neuron (SMN) gene directly into a patient's cells. The SMN gene is critical for the survival of motor neurons, and mutations in this gene may cause spinal muscular atrophy (SMA). By providing a new, working SMN gene, Zolgensma may address the genetic root cause of the disease, allowing motor neuron cells to produce proteins for improving muscle movement and function. This gene therapy may be useful to treat pediatric patients under the age of two with SMA, a rare and often fatal genetic disease that causes muscle weakness and progressive loss of movement. Zolgensma may be administered as a



one-time intravenous infusion, highlighting the practical advantages of in vivo gene therapies. However, the production of viral vectors such as gene therapies may be tightly controlled to ensure their safety and efficacy. Bioreactors may provide a controlled environment that can support the growth of virus-producing cells to high densities, facilitating the production of sufficient quantities of vectors for clinical applications. The use of bioreactors in bioproduction may offer reliable, efficient, and scalable methods for generating high-value biological products. As biotechnology continues to advance, the capabilities of bioreactors may expand, leading to greater innovations and applications in medicine and beyond.

**[00166]** Cells may be a material resource for life sciences research and development across basic and applied scientific practices. As such, cell culture and cell engineering workflows are integral to the practice of modern life sciences. Moreover, many research and development applications use significant quantities of cells or reproducible sources of cells over time. Examples of some research and development workflows and experimental paradigms which utilize cells include, but are not limited to, high-throughput screening, cell-based assays, and stem cell research. Primary and secondary cells including cell lines may be frequently propagated, expanded, and banked for use in research. Cells may also be frequently modified for research use, including by genome editing, through various bioprocessing workflows of considerable complexity. Across all these bioprocessing workflows, manual and semi-automated approaches require significant time, labor, and expense, which ultimately increases the cost and decreases the flexibility of cell-based research. New advances in cell manufacturing that reduce the turnaround time, labor, or cost of cell bioprocessing workflows may therefore accelerate cell-based life sciences research.

**[00167]** The systems and processes described herein may be useable for producing a variety of cell products. Cell products may include products comprising whole cells or products generated by whole cells. In an example, the systems and methods described herein may be usable to produce chimeric antigen receptor (CAR)-T cell therapy. As shown in **FIG. 2**, CAR-T cell therapy manufacturing may include rapid and non-rapid manufacturing methods. Manufacturing methods may include point of care, distributed, or centralized manufacturing. The systems and methods described herein may provide cell therapy manufacturing using smaller production volumes and higher cell densities than other cell therapy manufacturing systems and methods.

**[00168]** The systems described herein may be cell processing systems, integrated cell processing systems, automated cell processing systems, or any combination thereof. A cell

processing system may be an integrated cell processing system, as shown in **FIG. 3**. The integrated cell processing system **301** may be configured to permit flexible cell inputs **302**. The integrated cell processing system may include a graphical user interface (e.g., touch screen interface) that provides users with real time information related to cell processing. Cell inputs **302** may include whole blood, Leukopak, cryopreserved cells, or any combination thereof. The integrated cell processing system **301** may be configured to permit the use of flexible sample carriers **303**. The system may include radio frequency identification (RFID) readers for carrier identification and cloud-based electronic documentation systems. Sample carriers may include, but are not limited to cryo bags, tubes, vessels, cartridges, or any combination thereof. The integrated cell processing system may further include an integrated preservation unit **304**. The preservation unit **304** may be a cryopreservation unit. The preservation unit **304** may be configured to freeze or thaw cells, cell samples, or other biological products. The preservation unit **304** may be configured for rapid cryopreservation.

**[00169]** A cell processing system may comprise a processing unit and an analysis unit. The cell processing unit **401** and analysis unit **402** may be disposed separately. Alternatively, or in addition to, the cell processing unit and analysis unit may be integrated into a single cell processing and assay unit **403**, as shown in **FIG. 4**. The cell processing and assay unit **403** may be configured to provide various functions including, but not limited to, cell enrichment, cell isolation, washing, concentration, buffer exchange, de-clumping, or any combinations thereof. The cell processing and assay unit **403** may be configured to provide various product and release assays including, but not limited to, marker expression, phenotype, viability, concentration, potency, sterility, endotoxin, pyrogen, safety (e.g., vector copy number, replication competent virus, etc.), or any combinations thereof. The output (e.g., cells, media, etc.) from the cell processing and assay unit **403** may be provided to a bioreactor (e.g., solid state bioreactor) **404**. The bioreactor **404** may be configured to provide various cellular processing functions including, but not limited to, cell culture, membrane transduction, expansion, gene delivery, cell selection (e.g., negative or positive selection), or any combinations thereof.

**[00170]** A cell processing system may be a benchtop system configured to perform various cell processing processes, as shown in **FIG. 5A**. The cell processing system **501** may provide cell isolation **502**, activation **503**, gene transfer **504**, expansion **505**, formulation **506**, harvest **507**, or any combination hereof. The integrated cell processing system **501** may be useful for research, preclinical cell therapy development, clinical research, or any combination thereof. The cell processing system **501** may provide several, non-limiting benefits including reduce

costs and labor, increase process repeatability, improve and reduce processing time to accelerate breakthroughs, improve outcomes, or any combination thereof. The cell processing system **501** may provide fully or partially automated functions performed in any order for hands-off or reduced touch operation.

**[00171]** Cell processing systems described herein may be usable for running a variety of cell processing operations and workflows. An example cell processing workflow, as shown in **FIG. 5B**, may include cell thawing **510**, cell culture **511**, cell engineering **512**, assaying **513**, cell selection **514**, freezing **515**, or any combination thereof. The various processes may include automated processes and non-automated processes. Processes may be performed in any order and some processes may be performed more than once while processing a population of cells. Thawing **510** may comprise using a thermal unit to alter the state of provided cells. The thermal unit may be integrated with a preservation unit. Cell culture **511** may be conducted in a bioreactor (e.g., solid state bioreactor) and may include culture and expansion. Cell engineering **512** may include, but is not limited to, membrane transduction (e.g., activation), gene delivery (e.g., electroporation, lipid nanoparticles (LPN), viral), or any combination thereof. Assaying **513** may include, but is not limited to, cytometry, imaging, molecular assays, cell-based assays, or any combinations thereof. Cell selection **514** may include, but is not limited to, in situ selection (e.g., with or without beads), ex situ selection, upstream selection, downstream selection, or any combinations thereof. The cell or biological product produced may be subjected to freezing **515** via the thermal unit.

**[00172]** The systems and methods described herein may provide continuous nutrient delivery prior to, during, or after cell processing. **FIG. 6** schematically illustrates glucose levels, which may be an indicator of provided nutrients, as a function of time for example bioreactor configurations. In a static or batch configuration, glucose levels may begin high and decrease over time until depletion or near depletion occurs. In a batch fed configuration, glucose levels may begin high and exponentially decrease until a bolus of glucose is added returning the level to beginning or near beginning levels. This reduction and bolus cycle may repeat multiple times, for example, until the cell product is formed. In a continuous flow configuration, the glucose level may be maintained constant or otherwise closely controlled at a target level.

**[00173]** In an example, a cell processing system may be configured to provide fed batch workflows. **FIG. 7** schematically illustrates an example workflow for producing a cell product from an example cell processing system. A workflow may include providing isolating a population of cells **701**. The isolated cells may be mixed with a gel precursor

material **702**. The mixture comprising the cells and the gel precursor may be loaded into a compartment, for example, a compartment of a cassette and polymerized **703** to encapsulate the cells in a 3D polymer matrix. Activation reagents may be provided **704** to the cells encapsulated within the 3D matrix. The cells may be cultured or expanded **705** within the 3D matrix for at least 1, 2, 3, 4, 5, 6, 8, 10, or more days. In an example, the cells may be cultured or expanded **705** within the 3D matrix for two to three days. Genes may be delivered to the expanded cells **706**, for example, via providing a virus and media mixture to the cells. Cell may be cultured **707** during, or subsequent to, gene delivery to a target cell concentration or number of cells. Once the target concentration or number of cells is reached the 3D matrix may be liquified and recovered **708** from the compartment. The recovered cells may be harvested **709** as a cell product or for downstream processing.

**[00174]** A cell processing system, as described herein, may comprise multiple units and may be configured to provide multiple processes, as shown in **FIG. 8**. The cell processing system may be configured to receive liquid phase cellular material **801**, for example, via a sterile interconnection. The system may comprise a preservation unit comprising a thermal unit configured to provide a controlled-rate thaw. The preservation unit may be configured to receive frozen cellular material and to thaw the cellular material. The thawed or liquid phase cells may be provided to a cell processing module **802** for de-clumping, enrichment, concentration adjustments, mixing with liquid gel precursors, or any combination thereof. The cell and liquid gel precursor mixture may be provided to a compartment of a bioreactor **803** for polymerization. The bioreactor **803** may be configured for perfusion of media, in situ cell selection, cell stimulation, gene delivery, or any combination thereof. The cells processed in the bioreactor may be provided to a cell processing unit **804**. The cell processing unit **804** may be the same cell processing unit as **802** or a different cell processing unit. The cell processing unit **804** may be integrated with an analysis unit **805** and **806**. The cell processing unit **804** and analysis unit **806** may be configured for de-clumping, washing, concentration adjustment, material capture for release testing, release testing, buffer exchange for preservation, or any combinations thereof. The cells may be provided to a cryobag **807** or other vessel within a preservation unit for preservation (e.g., cryopreservation).

#### **Cell processing platforms**

**[00175]** In an aspect, the present disclosure provides cell processing systems, devices, and methods usable for cell processing. The described systems may include or provide for a variety of cell processing, cell culturing, or cell expansion applications. The described systems and methods may provide for two- or three-dimensional cell culture. In an example,

the described systems and methods provide for two-dimensional (2D) cell culture. In another example, the described systems and methods provide for three-dimensional (3D) cell culture.

*Two- and three-dimensional cell culture*

**[00176]** In cell culture, cells may be categorized based on cell growth characteristics and requirements, particularly in whether they attach or do not attach to surfaces. Adherent cells may be attached to a solid or semi-solid surface such as tissue culture plastic or microcarrier beads to grow and proliferate. These cells may be derived from organs where cells are naturally anchorage-dependent. Adherent cells may grow as a monolayer, attaching to the bottom of the culture dish, flask, or beads, or otherwise grow into three dimensional (3D) structures such as clusters or organoids. Adherent cells may be detached from the solid substrate using enzymatic or mechanical methods during or prior to subculturing, passaging, or harvesting. Suspension cells may not be attached to any surface for growth. Suspension cells may grow freely floating in the culture medium. Suspension cells may include cells derived from blood, such as lymphocytes or hematopoietic stem cells.

**[00177]** Both adherent and suspension cells may be cultured in two dimensional (2D) systems, where adherent cells survive and grow while adhered to a solid substrate (e.g., Petri dishes, flasks, or plates). For example, adherent cells may grow in a monolayer, while suspension cells may settle to the bottom surface due to gravity (e.g., in the absence of mixing) where they survive and grow. For adherent cell types, 2D culture approaches may lack scalability due to the linear relationship between maximum potential viable biomass and the available surface area for cells being cultured. In addition, the surface attachment may include more intricate handling during subculturing, passaging, or harvesting, and may require specific types of coated culture vessels for effective growth. Strategies to scale production by increasing the available surface area may utilize multi-layer plate or flask vessels which include multiple 2D surfaces. However, these types of 2D culture cell apparatus may have numerous challenges. For example, it may be challenging to uniformly distribute cells across or between the layers. To improve this process, procedures which change the orientation of the device (e.g., rotation) during cell seeding may be used to provide a more uniform distribution. It may be challenging to uniformly perfuse multi-layer vessels with media to support longer-term culture. It may also be challenging to provide uniform gas exchange (e.g., to provide a suitable supply of oxygen) by use of liquid-impermeable gas-permeable membranes, as each layer may include a gas exchange membrane to locally oxygenate cells. For suspension cell types, 2D culture approaches may also lack scalability and exhibit other biological challenges. Without a method of mixing or

keeping the cells in suspension, cells may settle to the bottom due to gravity. If the cell density is too high, the formation of a dense layer of settled cells can result in non-uniform delivery of nutrients and oxygen (whether nutrients or nutrients and gas are provided by excess media on top of the settled cells, or whether gas is provided by a gas-permeable liquid-impermeable membrane below the cell layer), resulting in increased variability or poor quality. **Table 1** shows example differences between 2D and 3D cultures. Solid-state cell processing may provide widespread use of various cell types for therapeutic applications. For example, the systems and methods described herein may permit cell product generation from low yielding cell sources such as umbilical cord blood sources with small quantity of hematopoietic stem and progenitor cells (HSPCs). Generating cell products from low yielding cell sources in 2D may be difficult due to low expansion ability, loss of stemness features, diminished migration and engraftment, reduced capacity for long-term repopulation, or any combination thereof. The solid-state cell processing systems and methods described herein may provide a 880-fold increase in HSPCs, 10- to 100-fold increase in stemness marker genes, 10-fold increase in homing genes, 73-fold increase in long-term HSC frequency and prolonged in vivo duration of hematopoietic reconstitution.

**Table 1.** Example differences between 2D and 3D cell culturing.

| Culture Aspect                            | 2D                                         | 3D                                       |
|-------------------------------------------|--------------------------------------------|------------------------------------------|
| Depth of cell culture                     | E.g., monolayer                            | Distributed in space                     |
| Cell Shape                                | Flattened or elongated                     | More natural and complex cell shapes     |
| Migration                                 | 2D                                         | 3D                                       |
| Exposure to Medium                        | May be accessed from a single dimension    | Can be accessed from multiple dimensions |
| Cell junctions and cell-cell interactions | Less common and less physiological         | More common and physiological            |
| Gene expression                           | Often vastly perturbed compared to in vivo | More closely resembles in vivo           |
| Effect of gravity and                     | Mostly absent                              | More closely resembles in                |

|                                                                                                            |                 |           |
|------------------------------------------------------------------------------------------------------------|-----------------|-----------|
| physical microforces                                                                                       |                 | vivo      |
| Control over microphysical environment (e.g., stiffness, elasticity, and presentation of factors to cells) | Minimal or none | Extensive |

**[00178]** Stirred or mixed suspension cultures, which may utilize horizontal-impeller, paddle, and rocking-wave mixing mechanisms, can generate unfavorable hydrodynamic environments and stresses (e.g., shear stress) that negatively impact cell growth and quality maintenance. In perfused suspension cultures, cells may be lost in spent media during recirculation, media removal, or both. Use of a cell retention device in a fluid perfusion system, which may separate cells from spent media or retains cells in the bioreactor, such as by the process of alternating tangential flow (ATF) filtration in which cells are retained in the filter of the ATF while the spent media is removed, may reduce or prevent the loss of cells during perfusion. Alternative cell retention techniques include other filtration approaches including, but not limited to, passive flow-based retention (e.g., approaches which use inertial or hydrodynamic forces, such as by using a spiral flow or deterministic displacement configuration), and use of active flow-based retention (e.g., approaches which use acoustics or magnetic forces, such as by using an acoustic transducer to separate cells from spent media via acoustic particle focusing). These cell retention methods for suspension culture perfusion may cause reliability issues or reduce cell quality. For example, membrane-based and filter-based cell retention strategies can suffer from membrane fouling, flow-based cell retention strategies can suffer from clogging (especially microfluidic embodiments), and active cell retention strategies (e.g., acoustic focusing) can subject cells to unphysiological and harsh forces. Moreover, neither 2D nor suspension culture approaches provide the benefits of a 3D cell culture environment described herein.

**[00179]** Suspension bioreactors may use carrier particles, for example, microcarriers. In cell culture applications, microcarriers can comprise non-porous bead or particle substrates with a 2D surface onto which cells are localized or adhered. Particle substrates may comprise glass, polystyrene, microporous materials (e.g., cell-impenetrable, polymer materials such as gelatin, collagen, chitin, cellulose, acrylamide, or combinations thereof), or combinations thereof. These types of carrier particles may not provide the benefits of 3D culture

environments described herein. Other carrier particle approaches may use porous or macroporous two and a half dimensional (2.5D)/3D particles, which may provide a 3D culture environment (e.g., forming a cell-laden matrix, such particles being composed of materials including but not limited to cellulose, gelatin, dextran, collagen, acrylamide, and PEG), suitable for culture of adherent cells in a suspension bioreactor. Such substrates may be unsuitable for many types of cells, including but not limited to migratory cells, cells capable of mixed suspension-adherent culture, and non-adherent / suspension cells, such as T cells, which may not remain localized to the porous carriers during culture and may relocate to the suspension media phase. Regardless of carrier particle type (porous or non-porous), carrier particle approaches may have significant limitations. Cell loading onto or into carrier particles may be inefficient, relying on an optimized seeding density and environment providing for collision between cells and carrier particles to facilitate the attachment of cells. In addition, as there may be little or no communication between individual carrier particles, a random or stochastic cell seeding procedure may result in carriers being seeded with a highly variable initial number of cells. Carrier particles seeded with a greater initial number of cells may reach confluence, while other carrier particles either not seeded or seeded with a lesser initial number of cells may remain empty or do not reach confluence. Frequent passaging may overcome these particle-localized space limitations and heterogeneity. In addition, empty carrier particles may not contribute to the overall available surface area or volume of the culture system, reducing the effective manufacturing scale or yield of such systems below the theoretical maximum.

**[00180]** Although suspension bioreactors (which may be static or mixed, such as stirred and impeller bioreactors) may be suitable for carrier particle culture approaches, they may be configured to create an excess suspension media environment, in which the carrier particles comprise a fraction of the total bioreactor volume, for example, 16 grams (g) of carrier per Liter (L) of culture. Moreover, mixed or stirred bioreactors may expose cells, carrier particles, or both to shear and other physical stresses that may damage or detach cells, or otherwise degrade the carrier particles. Bioreactors configured for carrier particle cell culture may use batch, fed-batch, or perfusion feeding. For fed-batch or perfusion feeding, a method of separating and retaining carrier particles from spent media is used. Such methods may be subject to challenges similar to the challenges of retaining suspension cells in such bioreactors. Carrier particle culture approaches are also subject to carrier particle clumping and aggregation, which may negatively affect the supply of nutrients, oxygen, and other supportive factors to cells within clumps or aggregates. Clumps and aggregates may further



clog, impede, or reduce the efficiency of retention, feeding, and harvest procedures. When non-dissolvable or non-biodegradable carrier particles are used, cells may be harvested and separated from the carrier particles. However, inefficient cellular detachment methods may reduce harvest efficiency or cell yield, while harsh cellular detachment methods may reduce the viability or quality of resulting cell products. For use in humans, carrier particle contamination is a significant risk and may rely on additional procedures and quality control to ensure efficient removal and minimal-to-no carry over of carrier particles.

**[00181]** Packed and fixed-bed bioreactors may be suitable for carrier particle culture approaches. These types of bioreactors may face numerous limitations and challenges (in addition to challenges of clumping and aggregation, harvesting cells from carriers, and others described above) which limit scale-up, increase product variability, or reduce product quality. For example, it may be challenging to achieve a uniform distribution within the bed, such that the cell distribution, liquid flow, or liquid communication across and between any subsets or cross sections of the bed may result in, for example, nutrient gradients in the bed or aeration limitations. It can also be challenging to maintain a uniform cell distribution or fluid circulation throughout a fixed or packed bed, over time within a given batch and from batch to batch. Variation in bioreactor packing and preparation can also create variability in both processes and products. Because both cell distribution and fluid circulation directly impact bioreactor performance (e.g., aeration, nutrient supply, shear stresses on cells), this can result in challenges with manufacturing consistency and performance over time. When scaling up, changes in the dimensions or volume of the packed bed may rely on extensive, time-consuming empirical optimization of other system attributes such as perfusion flow rate.

**[00182]** Three-dimensional (3D) culture systems may grow cells in a 3D matrix environment. Three-dimensional culture processes performed manually in the lab environment by static culture, including through the use of spheroids, droplets, and gels or other 3D substrates in multi-well plates and flasks, may be unsuited for scalable manufacturing and bioprocessing. Alternatively, automated 3D cell culture systems can provide significant benefits.

**[00183]** Compared to 2D cultures, cells cultured in a 3D microenvironment may exhibit substantially different and often superior properties. For example, 2D cultures may be limited in depth to a monolayer, while 3D cultures may be distributed in 3D space. Cells cultured in 2D may be flatter, more elongated, or both, while cells cultured in 3D may have more natural and complex cell shapes. Cells in 2D culture may migrate along two dimensions, and can be accessed from a single dimension, while cells in 3D culture may migrate in 3D and can be

accessed from multiple dimensions. Cell junctions and cell-cell interactions in 2D cultures may be less common and less physiological compared to 3D cultures. Gene expression may be perturbed in 2D cell cultures, but more closely resemble in vivo expression patterns in 3D cultures. In 2D cultures, the effects of gravity and physical microforces may be minimal or absent, while 3D cultures may expose cells to more natural, in vivo-like gravitational and physical microforces. Additionally, 3D cultures may enable increased control over the microphysical environment (e.g., stiffness, elasticity, and presentation of factors to cells), whereas 2D cultures may provide little or no control over these parameters. The additional control over cell microenvironment provided by 3D culture approaches may be beneficial to reducing variability in products, both resulting from variability in source materials and by reducing variation introduced during production. A 3D matrix which further provides for the retention of cells during bioprocessing may have process advantages, both biological and process/automation, including but not limited to, providing a low-shear environment, reducing or eliminating use of cell retention devices during feeding or media perfusion, reduced susceptibility to contamination, and instrument platforms with simplified designs or fewer components, increasing reliability and reducing cost. For equivalent production throughput, 3D cultures may use a smaller working volume and overall volume than 2D cultures, due to increased cell concentrations (e.g., cell volume per reactor volume, cell number per reactor volume, or both).

**[00184]** Three-dimensional cell culture processes may be challenging to implement with existing bioreactor designs, which includes static, hollow fiber, packed bed, and other bioreactor configurations. For example, it is challenging to culture cells in 3D while maintaining cellular localization to the 3D substrate and providing an adequate supply of culture components supporting cellular metabolism, such as media, nutrients, and dissolved oxygen. In a packed-bed reactor design, media may be substantially perfused through and around the cell-containing compartment (e.g., the packed bed). However, establishing a suitable rate of media flow through a 3D substrate to support cell growth may rely on unsuitably high fluid pressures or subject cells or the substrate to unsuitable forces negatively impacting the quality of cells or the quality and durability of the 3D substrate. Providing media flowing around a 3D substrate may not be a scalable delivery mechanism, as limited diffusion rates within a 3D substrate may limit the effective distance of perfusion, resulting in non-uniform perfusion or necrotic zones within the 3D substrate. The use of a 3D substrate that is porous enough to enable fluid perfusion at appropriate scale (e.g., woven fiber beds, aligned fiber beds, given the physical dimensions of the 3D substrate) may not provide a

suitable 3D microenvironment to capture the benefits of 3D cell culture (e.g. may be too microporous, such that cells are effectively in 2D culture attached to the scaffold, or in suspension culture in the interstitial spaces). In a hollow-fiber type perfusion bioreactor, a fraction of the intracapillary (IC) compartment, which comprises the space inside the hollow fibers as well as the head space on each end of the casing and the dead volume of the IC fluidic loop, is exposed to media perfusion by the extracapillary space of the casing. The rest of the volume may not be in fluidic communication with the extracapillary (EC) space. This may increase the challenges of cell seeding, gas exchange, and methods for providing nutrients to the cells. Because the 3D substrate substantially retains the cells, loading a cell-laden matrix in the IC may result in a substantial fraction of cells being retained in non-perfused IC spaces. Similarly, a fraction of the EC (which may comprise head spaces on each end of the casing and a dead volume of the EC fluidic loop) may be in fluidic communication with the IC. Loading a cell-laden matrix in the EC may similarly result in a substantial fraction of cells being retained in non-perfused EC spaces. Additionally, because hollow fibers may be distributed randomly within the casing, loading the EC with a cell-laden matrix may result in non-uniform perfusion by media flow in the IC.

#### *Cell processing systems*

**[00185]** The manufacture of cell therapies and other valuable chemical and biological cell products may use cell processing (e.g., bioprocessing) beyond simple bioreactor culture, such as cell isolation and gene delivery. Bioreactor systems without integrated cell processing capabilities may involve the transfer of cellular biomass between different workflows and instrumentation platforms. Biomass transfer and processing between workflows and instrumentation may increase human interaction, increasing the labor used for production and therefore increasing product cost. In “open” configurations, the cellular biomass may be exposed to the environment during processing, leading to contamination, manufacturing failures, batch-to-batch variability, difficulty meeting regulatory standards including for sample tracing, and using larger footprints in highly controlled environments (e.g., cleanroom) or more highly trained personnel. In “closed” configurations, the systems may be designed to avoid exposure of the cellular biomass to the environment (e.g., through use of sterile barriers and connectors, through the incorporation of single-use technologies, or both), which may reduce the risk of contamination and increases flexibility in terms of facility design (e.g., less controlled) and personnel staffing (e.g., less trained). Closed configurations may use manual or automated methods of forming the numerous sterile interconnections to aseptically transfer the biomass between process workflows, which may increase the amount

of human labor used or the complexity of automated systems. Non-integrated manufacturing systems may use multiple instrument systems and platforms, creating challenges in scheduling and instrument availability when many products are being manufactured concurrently, as well as challenges in documentation and accurate record keeping. Transferring cells between platforms and processes may be harsh on cells when there is a change in cell concentration or buffer, and when cells are held in non-optimal conditions between processes (e.g., in a transfer vessel). Transferring cells within a closed process via the establishment and termination of closed sterile interconnects (e.g., sterile welding inputs and outputs at each operation or on each separate instrument platform, during consumable manufacturing, system setup, or system operation) may use complex fluidic or automation architectures or labor-intensive, complex procedures. Automated transfer of cells between processes, such as by using one or more robotic arms or robotic systems to physically transport the cellular biomass between instruments (or alternatively, to transport instrumentation between cellular biomasses), may be challenging to scale. Such robotic systems may not scale well due to their size and complexity and may further exhibit fundamental limits in terms of scaling multiple parallel processes (e.g., the number of concurrent operations that can be supported by a single robot arm may be limited by time to perform each operation and physical reach of the robot arms or motion systems). For these reasons, closed integrated systems may improve consistency, purity, and safety while helping to lower overall manufacturing costs by reducing labor and facility requirements. Closed integrated systems which perform more, substantially all, or all processes involved in cell processing may be scalable by simply increasing the number of systems used in manufacturing.

**[00186]** Another challenge for cell processing platforms and technologies is the integration with cryopreserved input materials and the generation of cryopreserved output materials. When bioprocessing from a cryopreserved source material, the frozen sample may be submerged in a water bath at 37°C until a visual determination is made that the ice has melted (e.g., wet thawing). This method may be simple and low cost but may rely on specialized technicians to be executed successfully and with consistency. Variability in user behavior and laboratory settings can cause batch-to-batch variation. Untrained operators might choose slightly different, and often subjective, cryobag angles, agitation speeds, and end-of-thaw criteria, producing a spectrum of inconsistent results. While sufficient in a research setting, water baths present challenges in clinical and cleanroom environments. In addition to the risk of process and product variability, the presence of warm water may introduce contamination

risk that is unacceptable in controlled settings. The integration of an automated thawing module functionally connected to a bioprocessing system may solve these challenges.

**[00187]** When cell processing a cryopreserved product, the cell sample or other material may be suspended in a specialized buffer suitable for cryopreservation, and the temperature may be lowered to freezing using an optimized procedure to minimize damage to the cells or product. Cell cryopreservation buffers may be at least mildly cytotoxic, such that extended cell exposure to the liquid buffer may result in loss of post-thaw viability and other quality attributes. The integration of an automated cryopreservation module functionally connected to the cell processing system may minimize the time in which cells are exposed to cryopreservation buffer prior to freezing, while also reducing the timing and complexity of manufacturing, as the product may be stable once frozen and can be collected from the system at a convenient time.

**[00188]** As described herein, integrated bioprocessing systems (e.g., integrated cell processing systems) may provide various benefits over other bioprocessing or cell processing systems. For example, the described systems may provide simpler fluidics, such as fewer valves, tubing, and interconnects, which may provide for smaller and lower cost instrumentation, simpler consumables, and ease of sterilization. The described systems and methods may provide for a reduced routing of cells between processes as compared to other bioprocessing or cell processing systems. The systems and methods described herein may enhance cellular phenotypes and provide expanded applications as compared to other bioprocessing systems. For example, systems and methods described herein may provide for healthier cells due to reduced processing and handling. In some embodiments, three dimensional (3D) matrices may be used to replicate natural tissue extracellular matrix to provide superior phenotypes. The described systems and methods may be useful for processing adherent or suspension cells.

**[00189]** The cell processing systems described herein may be useful for immunoassays and target discovery, cell research and cell and gene therapy (CGT) development, clinical cell therapy, induced pluripotent stem cell (iPSC) and adherent cell bioprocessing, continuous biologics manufacturing, or any combination thereof. Immunoassays and target discovery applications may include, but is not limited to, massively multiplex ribonucleic acid (RNA)-based artificial antigen presenting cells (APC) for antigen screening and immunoreactivity validation or scalable receptor and target screening for autoimmune research and fully-human chimeric antigen receptor (CAR) development. Cell research and CGT development applications may include, but is not limited to, multiplex RNA cell engineering to understand

phenotypes and functional consequences of gene expression heterogeneity, development of phenotypic enhancements for cell and gene therapies, cell therapy process development, or any combination thereof. Clinical cell therapy applications may include, but is not limited to, multiplex RNA cell phenotype engineering and CAR delivery, safer profile due to transient kinetics and reduced or no risk of oncogenic integration, focus on oncology and autoimmune disease areas, long-term delivery of synthetic immune systems, or combinations thereof. iPSC and adherent cell bioprocessing applications may include, but are not limited to, bioreactor (e.g., solid-state) methods of scalable iPSC expansion and developmental programming, bioreactor (e.g., solid-state) methods of scalable production of adherent cell types such as islet cells and cardiomyocytes, research and clinical use, or combinations thereof. The systems and methods described herein may provide continuous biologics manufacturing for high density cell line culture in three-dimensional (3D) matrix with continuous media perfusion and collection of secreted proteins for purification.

#### **Three-dimensional matrices and methods of use**

**[00190]** In an aspect, the present disclosure provides systems for cell processing. A system for cell processing may include a plurality of three-dimensional (3D) matrices and a plurality of fluid flow paths. A 3D matrix of the plurality of 3D matrices may be configured to retain or may retain a population of cells. A fluid flow path of the plurality of fluid flow paths may be independent from and adjacent to the 3D matrix. The fluid flow path may be configured to deliver or may deliver a fluidic material to the 3D matrix to process the population of cells into a cell product.

**[00191]** In another aspect, the present disclosure provides methods for cell processing. A method for cell processing may include providing a population of cells, a plurality of 3D matrices, and a plurality of fluid flow paths. The population of cells may be retained within a 3D matrix of the plurality of 3D matrices. A fluid flow path of the plurality of fluid flow paths may be independent from and adjacent to the 3D matrix. The method may include using the fluid flow path to deliver fluidic material to the 3D matrix. The method may include processing the population of cells into a cell product in the presence of the fluidic material delivered to the 3D matrix.

**[00192]** In another aspect, the present disclosure provides methods for cell processing. A method for cell processing may include providing a 3D matrix retaining, containing, encapsulating, immobilizing, or any combination thereof a plurality of cells in a cell processing unit. Within the cell processing unit, the plurality of cells may be automatically processed within the 3D matrix to produce a plurality of processed cells. Within the cell

processing unit, the processed cells may be automatically separated from the 3D matrix. The method may further include collecting the plurality of processed cells.

**[00193]** In another aspect, the present disclosure provides systems for cell processing. A system for cell processing may include a cell processing unit and one or more computer processors. The cell processing unit may be configured to retain or may retain a 3D matrix. The 3D matrix may be configured to encapsulate or may encapsulate a plurality of cells. The one or more computer processors may be operatively coupled to the cell processing unit. The one or more computer processors may be individually or collectively programmed to automatically (i) process the plurality of cells within the 3D matrix to produce a plurality of processed cells, (ii) direct the cell processing unit to separate the plurality of processed cells from the 3D matrix, (iii) direct the cell processing unit to collect the plurality of processed cells, or any combination thereof.

**[00194]** In another aspect, the present disclosure provides systems for manufacturing a cell product. A system may include a 3D matrix configured to retain, contain, encapsulate, or immobilize or that retains, contains, encapsulates or immobilizes a population of cells at a density of greater than 80 million cells per cubic centimeter (cells/cm<sup>3</sup>). The system may be capable of processing cells of the population of cells, with aid of the 3D matrix, into a cell therapy product.

**[00195]** In another aspect, the present disclosure provides methods for manufacturing a cell therapy product. A method for manufacturing a cell therapy product may include providing a population of cells at a density of greater than 80 million cells/cm<sup>3</sup> retained, contained, encapsulated, or immobilized within a 3D matrix and, with the aid of the 3D matrix, processing cells of the population of cells into the cell therapy product.

**[00196]** In another aspect, the present disclosure provides bioreactors for processing a population of cells. A bioreactor for processing a population of cells may include a 3D matrix configured to retain, contain, encapsulate, or immobilize the population of cells. The bioreactor may have a volume of at most about two cubic meters (m<sup>3</sup>). The bioreactor may be configured to process or may process, with the aid of the 3D matrix and at high reproducibility, the population of cells into a cell product.

**[00197]** In another aspect, the present disclosure provides methods for processing a population of cells within a bioreactor. A method for processing a population of cells may include providing the population of cells retained, contained, encapsulated, or immobilized within a 3D matrix of a bioreactor. The bioreactor may have a volume of at most about 2 m<sup>3</sup>.

The method may include processing the population of cells into a cell product with aid of the 3D matrix and at high reproducibility.

**[00198]** In another aspect, the present disclosure provides systems for cell therapy manufacturing. A system for cell therapy manufacturing may include a 3D matrix configured to retain, contain, encapsulate, or immobilize or that retains, contains, encapsulates, or immobilizes a population of cells at a density of at least about 5 million cells/cm<sup>3</sup>. The system may be capable of processing cells or may process cells of the population within the 3D matrix for a time period of at most 48 hours to generate a cell product. The cell product may be usable to generate a cell therapy.

**[00199]** In another aspect, the present disclosure provides methods for manufacturing a cell therapy. A method for manufacturing a cell therapy may include providing a population of cells at a density of at least about 5 million cells/cm<sup>3</sup> retained, contained, encapsulated, or immobilized within a 3D matrix. The method may include processing the cells of the population of cells within the 3D matrix for a time period of at most about 48 hours to generate a cell product. The cell product may be used to generate a cell therapy.

**[00200]** 3D matrices may provide several benefits for cell processing over solution phase cell processing. Fluid, nutrient, or reagent transfer in bulk and microfluidic bioreactors may be challenging, as shown in **FIG. 9A**. Bulk and microfluidic bioreactors may transfer cells and fluids from one process to another thereby increasing cell handling and the likelihood of cell damage. Further, bulk and microfluidic bioreactors may use multiple wash and separation processes that may subject the cells to shear stresses that may damage the cells. In an alternative configuration, cells may be processed within a 3D matrix, as shown in **FIG. 9B**. Processing cells within a 3D matrix may reduce the shear stresses on the cells by permitting fluid, nutrient, or reagent transfer via tangential or perfusion flow of fluids. The 3D matrix and compartment retaining the 3D matrix may be configured such that all or substantially all the fluid flows along a surface of the 3D matrix such that nutrients and reagents within the fluid diffuse into the 3D matrix to contact the cells encapsulated therein. The cells may be subjected to multiple processes within the 3D matrix, thereby reducing cell handling. Tangential or perfusion flow of nutrients, reagents, or both may permit levels of those nutrients or reagents within the 3D matrix to be highly controlled to permit cells to be processed at high cell densities (e.g., ~10-100 million cells/cm<sup>3</sup>).

**[00201]** The 3D substrates utilized in cell-matrix formulations may comprise a porous, permeable solid, or mixtures thereof, suspended in an interstitial fluid predominantly consisting of water, and also comprising cells in suspension in the interstitial aqueous liquid,



adhered to the 3D substrate and in contact with the interstitial aqueous liquid, or both. The 3D substrate may comprise a three-dimensional structure with a defined or undefined structure described as, but not limited to, a matrix, mesh, network, or otherwise as a physically interconnected system of molecules such as polymers. The structure of the 3D substrate may support the structural integrity of the 3D cell culture environment, providing an interstitial space in which cells may reside, grow, proliferate, or any combination thereof, and facilitating the transmission of nutrients and other essential dissolved materials, as well as material reagents and constructs for genetic engineering. The configuration of the interstitial space, characterized by the organization and porosity of the 3D substrate, as well as the physical properties of the substrate such as hydrophilicity, cell adhesion properties, and biofouling properties, may allow for the diffusion of water-soluble substances, making it an ideal medium for cellular activities. The configuration of the interstitial space and structural properties of the 3D substrate may further allow for active exchange of the interstitial fluid, such as by use of a fluid flow which may partially, substantially, or totally displace a first interstitial fluid with a second interstitial fluid. In some embodiments, the 3D substrate additionally provides for the retention of cells within the 3D substrate during cell culture or passive or active exchange of interstitial fluid. Three-dimensional substrates may include, but are not limited to, hydrogel matrices, porous solid matrices, inverse opal matrices, and composite matrices (e.g., interpenetrating matrices).

**[00202]** Hydrogel matrices, for example, those used in aqueous environments for cell culture, may comprise hydrophilic polymers. The hydrogel matrices may comprise all or substantially all hydrophilic polymers. The hydrogel matrices may comprise greater than or equal to about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or more hydrophilic polymers. The hydrogel matrices may comprise from about 20% to 30%, 20% to 40%, 20% to 50%, 20% to 60%, 20% to 70%, 20% to 80%, 20% to 90%, 20% to 95%, 20% to 98%, 30% to 40%, 30% to 50%, 30% to 60%, 30% to 70%, 30% to 80%, 30% to 90%, 30% to 95%, 30% to 98%, 40% to 50%, 40% to 60%, 40% to 70%, 40% to 80%, 40% to 90%, 40% to 95%, 40% to 98%, 50% to 60%, 50% to 70%, 50% to 80%, 50% to 90%, 50% to 95%, 50% to 98%, 60% to 70%, 60% to 80%, 60% to 90%, 60% to 95%, 60% to 98%, 70% to 80%, 70% to 90%, 70% to 95%, 70% to 98%, 80% to 90%, 80% to 95%, 80% to 98%, 90% to 95%, 90% to 98%, or 95% to 98% hydrophilic polymers.

**[00203]** Hydrophilic polymers, while hydrophilic, or water-absorbent, may form a 3D matrix which itself is insoluble in water. The polymer matrix may swell in the presence of water without dissolving. This swelling may occur because the hydrophilic (water-attracting)

nature of the polymer chains allows them to absorb and retain a significant amount of water, which can be many times their dry weight. Despite their affinity for water, these polymers may be chemically or physically crosslinked, which prevents them from dissolving in the water. Crosslinking between and among polymers comprising the hydrogel may impart stability to the hydrogel matrix. Crosslinking may involve forming links between and among the individual polymer chains, thereby forming a three-dimensional network structure. This network can further comprise within its structure or volume other structural materials (such as in the cases of hybrid gel materials and matrices formed of interpenetrating networks), functional molecules (e.g., biomolecules), water molecules, and other particles, such as cells. Crosslinks can be formed through chemical bonds (e.g., covalent crosslinking) or through physical interactions. Physical interactions may include physical crosslinking such as ionic bonds, hydrogen bonds, hydrophobic interactions, polyelectric complexation, electrostatic interactions, condensation, or any combination thereof. The ratio of chemical and physical crosslinking may alter the mechanical strength, degradation rate, biological interactions, or any combination thereof of the hydrogel matrix.

**[00204]** Crosslinks may be stable or reversible under given conditions (e.g., stimulus), as described elsewhere herein. The insolubility and crosslinked nature of the hydrogel matrix in an aqueous environment may provide stable and supportive scaffold for cells. Such a 3D substrate may mimic the extracellular matrix in which cells naturally reside. A 3D substrate may further provide structural support and a medium or substrate through which nutrients and other soluble factors can diffuse or be presented to or otherwise be available for contact with cells. Additionally, a hydrogel's physical structure and properties, including porosity (e.g., the size and distribution of its pores), stiffness, elasticity, may be tailored or engineered as described herein, including for the purposes of regulating cell migration, proliferation, differentiation, phenotype, gene expression, or any combination thereof.

**[00205]** Hydrogels may be formed from natural polymers, modified polymers, synthetic polymers, or any combination thereof. Hydrogel materials may be selected for biocompatibility, biodegradability, mechanical strength, ease of modification, or any combination thereof. The material used for the 3D matrix may be modified or altered to be compatible with a given type of cell. Natural polymers may include, but are not limited to, fibrinogen, hyaluronic acid, collagen, Matrigel, gelatin, chitosan, alginate, cellulose, heparin, dextran, keratin, agarose, silk fibroin, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptide polymers, or any combination thereof. Synthetic polymers may include, but are not limited to, Poly(ethylene glycol) (PEG), Poly(vinyl alcohol) (PVA), Poly(acrylic acid)

(PAA), Poly(lactic-co-glycolic acid) (PLGA), Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), Poly(caprolactone) (PCL), Poly(vinyl acetate) (PVA), Poly(ethyleneimine) (PEI), Poly(vinylpyrrolidone) (PVP), Poly(N-isopropylacrylamide) (PNIPAM), Polysiloxanes, Polyurethanes, poly(ethylene glycol) diacrylate, poly(ethylene glycol) dimethacrylate, derivatives thereof, or any combination thereof. The 3D matrix may comprise a single polymer or may comprise a mixture of polymers configured for nutrient diffusion, mechanical support of cells, biocompatibility, or any combination thereof.

**[00206]** Polymers (e.g., hydrogel polymers) may or may not be chemically modified. In an example, polymers (e.g., hydrogel polymers) are chemically modified. For example, collagen may be provided as a chemically modified form of the natural protein collagen, such as, methacrylated collagen (often abbreviated as collagen-MA or methacryloyl collagen), in which collagen may be functionalized with methacrylate groups. The process of methacrylation may involve the introduction of methacryloyl groups into the amino acid residues of the collagen molecule, for example, using methacrylic anhydride. This chemical modification may occur mainly at the lysine residues and the N-terminal groups of the collagen. Using methacrylated collagen may be beneficial because of its ability to undergo rapid thermal gel-sol transition (e.g., exhibiting a reversible transition from a liquid to a gel with increasing temperature) and photocrosslinking when exposed to light (e.g., ultraviolet light) in the presence of a photoinitiator. The methacrylate groups may form covalent bonds with each other, creating a crosslinked network. Chemical modification may not alter the biocompatibility and biodegradability of natural collagen, making it an excellent scaffold material that can support cell adhesion, proliferation, and differentiation. Hydrogels formed from methacrylated collagen may have tunable mechanical properties, which may be adjusted to match the properties of a native tissue environment. The degree of substitution of methacrylate groups can be varied, altering the density of the crosslinking and the physical properties of the resulting hydrogels, such as their thermal responsiveness, stiffness, degradation rate, and pore size. In another example, a PEG polymer may be prepared as a block copolymer with Poly(N-isopropylacrylamide) (PNIPAM), also known as thermogelling PEG-PNIPAM block copolymers. PEG-PNIPAM block copolymers exhibit thermally responsive gel-sol transition (e.g., exhibiting a reversible transition from a liquid to a gel with increasing temperature). Each polymer segment may provide specific attributes that contribute to the overall behavior of the copolymer. PEG is a hydrophilic polymer that may be biocompatible and non-immunogenic polymer. PEG may be used in biomedical applications. In copolymers, PEG may serve as a segment that stabilizes and increases the

solubility of the copolymer in aqueous environments. PNIPAM may exhibit thermoresponsive behavior. Below its Lower Critical Solution Temperature (LCST), which may be around 32°C, PNIPAM is hydrophilic and soluble in water. Above this temperature, PNIPAM may become hydrophobic and insoluble, causing the polymer to precipitate or form a gel. In these copolymers, PEG and PNIPAM may be linked in a block copolymer architecture, where each polymer forms a distinct block within the copolymer chain. The ratio of each polymer component may be controlled, thereby affecting the properties of the copolymer. The copolymer may comprise PNIPAM and secondary polymer. The ratio of PNIPAM to secondary polymer (e.g., PNIPAM:PEG) may be from about 1000:1 to about 1:1000. The ratio of PNIPAM to secondary polymer may be selected based on target mechanical properties, chemical properties, responsivity, synthesis route, copolymer composition, or any combination thereof. The block copolymer structure may allow PEG-PNIPAM materials to leverage the water solubility and biocompatibility of PEG along with the temperature-sensitive gelation properties of PNIPAM. Other LCST polymers, which may be incorporated into stimulus-responsive copolymers, include but are not limited to poly((meth)acrylamide)s: poly(*N,N*-diethylacrylamide), poly(2-carboxyisopropylacrylamide), poly(*N*-(L)-(1-hydroxymethyl) propylmethacrylamide), poly(*N*-acryloyl-*N'*-propylpiperazine), poly(*N*-ethylacrylamide), poly(*N*-methyl-*N*-ethylacrylamide), poly(*N*-*n*-propylacrylamide), poly(*N*-ethylmethacrylamide), poly(*N*-methyl-*N*-isopropylacrylamide), poly(*N*-isopropylmethacrylamide), poly(*N*-*n*-propylmethacrylamide), poly(*N*-methyl-*N*-isopropylacrylamide), poly(*N*-cyclopropylacrylamide), poly(*N*-cyclopropylmethacrylamide), poly(*N,N*-bis(2-methoxyethyl) acrylamide), poly(*N*-(3-methoxypropyl) acrylamide), poly(ethoxypropylacrylamide), poly(aminomethoxypropylacrylamide), or combinations thereof. Alternatively, other polymers may be used or incorporated into the 3D matrix to provide stimuli (e.g., chemical, physical, thermal, or any combination thereof) responsive polymers. Other temperature-responsive polymers include, but are not limited to, Poly(*N*-vinylcaprolactam), poly(2-ethyl-2-oxazoline), poly(vinyl methyl ether), poly(2-isopropyl-2-oxazoline), poly((2-dimethylamino) ethyl methacrylate), poly(propylene oxide), and poly(*N*-acryloylpiperidine). Other pH responsive polymers include, but are not limited to, Poly(acrylic acid), poly(2-ethyl acrylic acid), poly(*N,N*-dimethyl aminoethyl methacrylate), poly(vinyl imidazole), Polyaspartate, polylysine, and polyhistidine. Other light responsive polymers include, but are not limited to, Poly(ethylene oxide-methacrylate) with pyrene pendant, nitrobenzyl and dithiodipropionic acid modified polyetherimide, poly-*N*-isopropylacrylamide-spiropyran,

poly[*N*-isopropylacrylamide-*b*-sodium 2-(acrylamido)-2-methylpropane sulfonate-spiropyran]. Other glucose-responsive polymers include, but are not limited to, Poly[(2-dimethylamino) ethyl methacrylate-*co*-3- acrylamidephenylboronic acid].

Stimulus responsive polymers, including LCST polymers such as PNIPAM and others listed above, may be synthesized as copolymers with other polymers, including but not limited to, poly(amino acids); polysaccharides such as alginate, cellulose, chitosan, guar gum and dextran; proteins; and synthetic polymers including poly(esters), poly(caprolactone), Polyethylenimine (PEI), poly(ethylene glycol). Poly( $\epsilon$ -caprolactone) (PCL), acrylic acid (AA) and acrylate monomers such as 2-hydroxyethyl methacrylate (HEMA) and poly( $\epsilon$ -caprolactone) dimethacrylate (PCLDMA), and propylacrylic acid (PAA). Examples of such copolymers include, but are not limited to, PEG-PNIPAM, alginate/*N*-isopropylacrylamide (NIPAM), PNIPAM/poly(sodium acrylate), and methacryloylchitosan/PNIPAM. Copolymers may comprise two or more components or block constituents, such as diblock copolymer, triblock copolymer, or blended copolymers. Polymers and copolymers may be designed and synthesized with a myriad of topologies/morphologies, such as linear polymers (homopolymers, di/ter/multi-block copolymers, and organic/inorganic hybrid polymers) and non-linear polymers (star polymers, (hyper)-branched polymers). Polymers and copolymers may be synthesized using various methods including but not limited to free radical polymerization (FRP), atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer (RAFT), and graft copolymerization.

**[00207]** Alternatively, or in addition to, 3D substrates for cell processing may include porous solids. These matrices can be made from a wide range of materials, including but not limited to plastics (e.g., polystyrene, polyethylene, polypropylene, polyethylene terephthalate, polyvinyl chloride, polycarbonate, acrylic, cyclic olefin copolymers, cyclic olefin polymers, or any combinations thereof), ceramics, glass, metals, natural fibers, or any combinations thereof. Unlike hydrogels, these materials may have defined, micro-scale porosity with precise control over pore size and distribution. This porosity may be created through methods like sintering, foaming, leaching, weaving, 3D printing, or a combination thereof. Solid porous matrices may be more rigid and stable than hydrogels and may not necessarily swell or change shape in the presence of water or biological fluids, which can be advantageous for certain applications requiring dimensional stability. Solid porous materials may also be modified to alter physical, chemical, or biological properties. Functionalization and modification of porous solid 3D scaffold materials may include all methods of modifying these materials for 2D cell culture applications, including but not limited to plasma surface

modification, self-assembled monolayer deposition, covalent grafting, surface coating or absorption, or any combination thereof. For example, plastic and other solid scaffold materials may be treated to modify the surface energy, surface tension, or wettability, such as by plasma treatment or oxidation reactions forming carbonyl ( $\text{-C=O-}$ ). Carboxyl ( $\text{HOOC-}$ ), hydroperoxide ( $\text{HOO-}$ ) and hydroxyl ( $\text{HO-}$ ) groups. As another example, materials may be treated to affect protein binding, protein deposition, biofouling, and cell adhesion, such as surface hydrolysis of polyester scaffolds to promote protein adsorption for improving cell attachment. Surface functionalization can be performed by exposing surfaces to RF plasma. Many gases can be excited and used to functionalize surfaces for a wide variety of applications. Surface functionalization techniques may include using air plasma, oxygen plasma, and ammonia plasma as well as other exotic gases. Each gas can have varying effects on a substrate. For example, ammonia plasma and n-heptylamine vapor treatments can be used to attach amine functional groups. Surfaces may be treated with thin polysaccharide films or coatings, including through covalent attachment, such as by using oxidized polysaccharides which form Schiff base linkages with amine-functionalized materials.

**[00208]** Alternatively, or in addition to, 3D substrates may comprise Inverse Opal (IOPAL) materials that mimic the ordered porous architecture of opals, but with an inverted morphology. To form IOPAL 3D substrates, a first sacrificial material (e.g., the opal template) may be deposited, packed, or otherwise formed into a 3D spatial configuration (e.g., by using a mold). For example, the opal template may be formed of monodisperse microspheres (e.g., made of polystyrene, silica, polymethyl methacrylate, etc.) that are packed into a dense structure, such as through sedimentation, vertical deposition, or centrifugation, which may allow the spheres to naturally order into a 3D configuration. The 3D structure may comprise a substantially regular structure (e.g., a face-centered cubic (FCC) lattice). A 3D substrate precursor material may be infiltrated into the interstices of the opal template to fill the voids without disturbing the 3D structure. The 3D substrate material may be subsequently formed into a 3D substrate, such as through sol-gel processes or polymerization. The sacrificial template may then be removed, such as by dissolution in a suitable solvent (e.g., toluene for polystyrene) or by another stimulus-responsive template chemistry (e.g., by using a temperature sensitive template material and subsequently heating or cooling to liquify the template facilitating removal from the IOPAL structure). The removal of the opal template may leave behind a porous structure with an ordered array of voids, which may mirror the original arrangement of the template. In some examples, the removal of the sacrificial porogen creates a network of interstitial spaces in the exact

geometric arrangement of the original template, resulting in interconnected voids in the inverse opal structure that may be uniform and highly ordered. Due to their ability to form 3D structures with highly controlled porosity and structural regularity, IOPAL materials can provide uniform diffusion and other desirable material properties. IOPAL substrates may comprise a variety of materials including, but not limited to, hydrogels, porous solids, or any combination thereof.

**[00209]** In an example, the 3D matrix may comprise a composite 3D substrate. Composite 3D substrates may be formed from mixtures of 3D substrate materials or interpenetrating 3D matrices. A composite 3D substrate may comprise any suitable mixture of hydrogels, polymers, non-hydrogel substrates (e.g., porous solids, 3D scaffolds, particles, or any combination thereof), or any combination thereof, which may support cell culture. To support cell culture, 3D substrates may provide a physical 3D space for cells, a supply of liquid media, dissolved gasses, or other reagents and compositions supporting cell culture. The various components, or a portion thereof, making up the composite 3D matrix may be physically crosslinked, may physically associate, or may not be physically associate. The 3D substrate may comprise one or more interpenetrating networks, such as a first mechanically strong, nonreversible network interpenetrated with a second network featuring reversible crosslinks. The use of composite 3D substrates or mixture of 3D substrate materials can provide advantages such as including separate materials to serve select functions. In another example, a 3D substrate may comprise a first material which provides for cell retention, and a second material which provides for compatible cellular growth microenvironments, such separate materials being configured separately and used together to form a 3D substrate usable for cell retention and growth. Other examples include, but are not limited to, interpenetrating hydrogel matrices, such as those formed by the combinations of thermally-responsive hydrogel materials (e.g., methacrylated collagen and PEG-PNIPAM) and separately, a porous solid matrix such as polystyrene, which features a large pore size (e.g., greater than 100 micrometers ( $\mu\text{m}$ )), filled with a separate hydrogel matrix such as collagen gel.

#### *3D substrate properties*

**[00210]** Physical properties of 3D substrates for cell culture may include, but are not limited to, stiffness, softness, rigidity, swelling, porosity, stability, optical properties, dimensional properties, or any combinations thereof. The physical characteristics of the 3D substrate may be modified or otherwise engineered by altering the composition of the matrix, the method of forming the matrix, the soluble environment of the matrix, or any combination

thereof. Methods for modifying or tuning the physical properties of a 3D substrate may include changing the crosslinking density (e.g., by altering the crosslinking potential of one or more underlying components), changing the proportions of components (e.g., by altering the percent of crosslinking components), changing the molecular weight of polymers or substrates, adding components (e.g., by adding nanomaterials, including degradable, removable, or sacrificial porogens which may not be a component of the formed matrix, but otherwise alter the distribution and network architecture of the matrix), changing the soluble environment (e.g., such as pH, ionic strength, etc.), or any combination thereof.

**[00211]** A 3D substrate may provide a high surface area to volume ratio to efficiently provide nutrients, gas exchange, and other reagents like viral particles to the 3D cell culture substrate. 3D matrices or substrates with higher surface area to volume ratio may have a high interfacial area as compared to the volume of the 3D substrate. 3D substrates with high surface area to volume ratios may provide more uniform cellular environments than 3D substrates with low surface area to volume ratio. For example, 3D matrices or substrates with higher surface area to volume ratios because fluidic materials such as nutrients and reagents may have shorter distances to diffuse or transport into the 3D matrix as compared to a similar matrix with a lower surface area to volume ration. Shorter diffusion or transport distance may provide smaller concentration gradients across the 3D substrate and more uniform cellular environment throughout the 3D matrix or substrate. A 3D matrix or substrate may have a surface area to volume ratio of greater than or equal to about 0.1, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, or more. A 3D matrix or substrate may have a surface area to volume ratio from 0.1 to 0.5, 0.1 to 1, 0.1 to 2, 0.1 to 3, 0.1 to 4, 0.1 to 5, 0.1 to 10, 0.1 to 15, 0.1 to 20, 0.5 to 1, 0.5 to 2, 0.5 to 3, 0.5 to 4, 0.5 to 5, 0.5 to 10, 0.5 to 15, 0.5 to 20, 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 10, 1 to 15, 1 to 20, 2 to 3, 2 to 4, 2 to 5, 2 to 10, 2 to 15, 2 to 20, 3 to 4, 3 to 5, 3 to 10, 3 to 15, 3 to 20, 4 to 5, 4 to 10, 4 to 15, 4 to 20, 5 to 10, 5 to 15, 5 to 20, 10 to 15, 10 to 20, or 15 to 20.

**[00212]** 3D matrices or substrates may comprise any shape. For example, a 3D matrix may be a slab, sphere, fiber, or any other shape as described elsewhere herein. In an example, a 3D matrix is a slab. In another example, a 3D matrix comprises a fiber shape. In another example, a 3D matrix comprises a spherical shape. A 3D matrix or substrate may have one dimension that is substantially smaller than any other dimension. For example, a 3D matrix may be a slab and the thickness of the slab may be substantially smaller than the length or width of the slab. In another example, the 3D matrix may be a sphere and the diameter may be substantially smaller than the circumference. In another example, the 3D



matrix may be a fiber and the diameter of the fiber may be substantially smaller than the length of the fiber. During cell processing, fluid may flow in a direction normal or substantially normal to the small dimension (e.g., the fluid flow may be tangential to a long dimension of the 3D matrix). For example, fluid flow may be parallel to a long dimension of the slab or along a surface of the slab disposed at approximately a 90-degree, 80-degree, 70-degree, or 60-degree angle from the short dimension (e.g., the fluid flows along the length or width of the slab). In an example, the 3D matrix may comprise a fiber and the fluid may flow along a length of the fiber. In another example, the 3D matrix may comprise a sphere, and the fluid may flow along an outer circumference of the sphere. In The 3D matrix may have a small dimension that is less than or equal to about 20 millimeters (mm), 10 mm, 8 mm, 6 mm, 5 mm, 4 mm, 3 mm, 2 mm, 1 mm, 900  $\mu\text{m}$ , 800  $\mu\text{m}$ , 700  $\mu\text{m}$ , 600  $\mu\text{m}$ , 500  $\mu\text{m}$ , 400  $\mu\text{m}$ , 300  $\mu\text{m}$ , 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , 50  $\mu\text{m}$ , 25  $\mu\text{m}$ , 10  $\mu\text{m}$ , or less. In an example, the 3D matrix may have a small dimension of less than or equal to about 10 mm. In an example, the 3D matrix may have a small dimension of less than or equal to about 1 mm. In an example, the 3D matrix may have a small dimension of less than or equal to about 500  $\mu\text{m}$ . In an example, the 3D matrix may have a small dimension of less than or equal to about 200  $\mu\text{m}$ . In an example, the 3D matrix may have a small dimension of less than or equal to about 100  $\mu\text{m}$ . In an example, the 3D matrix may have a small dimension of less than or equal to about 10  $\mu\text{m}$ . In an example, the 3D matrix may comprise a slab configuration with a small dimension of less than or equal to about 10 mm. In an example, the 3D matrix may comprise a slab configuration with a small dimension of less than or equal to about 1 mm. In an example, the 3D matrix may comprise a slab configuration with a small dimension of less than or equal to about 500  $\mu\text{m}$ . In an example, the 3D matrix may comprise a slab configuration with a small dimension of less than or equal to about 200  $\mu\text{m}$ . In an example, the 3D matrix comprises a fiber configuration and the fiber comprises a small dimension (e.g., diameter) that is less than or equal to about 10 mm. The small dimension may be from about 10  $\mu\text{m}$  to 25  $\mu\text{m}$ , 10  $\mu\text{m}$  to 50  $\mu\text{m}$ , 10  $\mu\text{m}$  to 100  $\mu\text{m}$ , 10  $\mu\text{m}$  to 200  $\mu\text{m}$ , 10  $\mu\text{m}$  to 200  $\mu\text{m}$ , 10  $\mu\text{m}$  to 300  $\mu\text{m}$ , 10  $\mu\text{m}$  to 400  $\mu\text{m}$ , 10  $\mu\text{m}$  to 500  $\mu\text{m}$ , 10  $\mu\text{m}$  to 600  $\mu\text{m}$ , 10  $\mu\text{m}$  to 700  $\mu\text{m}$ , 10  $\mu\text{m}$  to 800  $\mu\text{m}$ , 10  $\mu\text{m}$  to 900  $\mu\text{m}$ , 10  $\mu\text{m}$  to 1 mm, 10  $\mu\text{m}$  to 2 mm, 10  $\mu\text{m}$  to 3 mm, 10  $\mu\text{m}$  to 4 mm, 10  $\mu\text{m}$  to 5 mm, 10  $\mu\text{m}$  to 6 mm, 10  $\mu\text{m}$  to 8 mm, 10  $\mu\text{m}$  to 10 mm, 10  $\mu\text{m}$  to 20 mm, 25  $\mu\text{m}$  to 50  $\mu\text{m}$ , 25  $\mu\text{m}$  to 100  $\mu\text{m}$ , 25  $\mu\text{m}$  to 200  $\mu\text{m}$ , 25  $\mu\text{m}$  to 200  $\mu\text{m}$ , 25  $\mu\text{m}$  to 300  $\mu\text{m}$ , 25  $\mu\text{m}$  to 400  $\mu\text{m}$ , 25  $\mu\text{m}$  to 500  $\mu\text{m}$ , 25  $\mu\text{m}$  to 600  $\mu\text{m}$ , 25  $\mu\text{m}$  to 700  $\mu\text{m}$ , 25  $\mu\text{m}$  to 800  $\mu\text{m}$ , 25  $\mu\text{m}$  to 900  $\mu\text{m}$ , 25  $\mu\text{m}$  to 1 mm, 25  $\mu\text{m}$  to 2 mm, 25  $\mu\text{m}$  to 3 mm, 25  $\mu\text{m}$  to 4 mm, 25  $\mu\text{m}$  to 5 mm, 25  $\mu\text{m}$  to 6 mm, 25  $\mu\text{m}$  to 8 mm, 25  $\mu\text{m}$  to 10 mm, 25  $\mu\text{m}$  to 20 mm, 50  $\mu\text{m}$  to 100  $\mu\text{m}$ , 50  $\mu\text{m}$  to 200

μm, 50 μm to 200 μm, 50 μm to 300 μm, 50 μm to 400 μm, 50 μm to 500 μm, 50 μm to 600 μm, 50 μm to 700 μm, 50 μm to 800 μm, 50 μm to 900 μm, 50 μm to 1 mm, 50 μm to 2 mm, 50 μm to 3 mm, 50 μm to 4 mm, 50 μm to 5 mm, 50 μm to 6 mm, 50 μm to 8 mm, 50 μm to 10 mm, 50 μm to 20 mm, 100 μm to 200 μm, 100 μm to 300 μm, 100 μm to 400 μm, 100 μm to 500 μm, 100 μm to 600 μm, 100 μm to 700 μm, 100 μm to 800 μm, 100 μm to 900 μm, 100 μm to 1 mm, 100 μm to 2 mm, 100 μm to 3 mm, 100 μm to 4 mm, 100 μm to 5 mm, 100 μm to 6 mm, 100 μm to 8 mm, 100 μm to 10 mm, 100 μm to 20 mm, 200 μm to 300 μm, 200 μm to 400 μm, 200 μm to 500 μm, 200 μm to 600 μm, 200 μm to 700 μm, 200 μm to 800 μm, 200 μm to 900 μm, 200 μm to 1 mm, 200 μm to 2 mm, 200 μm to 3 mm, 200 μm to 4 mm, 200 μm to 5 mm, 200 μm to 6 mm, 200 μm to 8 mm, 200 μm to 10 mm, 200 μm to 20 mm, 300 μm to 400 μm, 300 μm to 500 μm, 300 μm to 600 μm, 300 μm to 700 μm, 300 μm to 800 μm, 300 μm to 900 μm, 300 μm to 1 mm, 300 μm to 2 mm, 300 μm to 3 mm, 300 μm to 4 mm, 300 μm to 5 mm, 300 μm to 6 mm, 300 μm to 8 mm, 300 μm to 10 mm, 300 μm to 20 mm, 400 μm to 500 μm, 400 μm to 600 μm, 400 μm to 700 μm, 400 μm to 800 μm, 400 μm to 900 μm, 400 μm to 1 mm, 400 μm to 2 mm, 400 μm to 3 mm, 400 μm to 4 mm, 400 μm to 5 mm, 400 μm to 6 mm, 400 μm to 8 mm, 400 μm to 10 mm, 400 μm to 20 mm, 500 μm to 600 μm, 500 μm to 700 μm, 500 μm to 800 μm, 500 μm to 900 μm, 500 μm to 1 mm, 500 μm to 2 mm, 500 μm to 3 mm, 500 μm to 4 mm, 500 μm to 5 mm, 500 μm to 6 mm, 500 μm to 8 mm, 500 μm to 10 mm, 500 μm to 20 mm, 600 μm to 700 μm, 600 μm to 800 μm, 600 μm to 900 μm, 600 μm to 1 mm, 600 μm to 2 mm, 600 μm to 3 mm, 600 μm to 4 mm, 600 μm to 5 mm, 600 μm to 6 mm, 600 μm to 8 mm, 600 μm to 10 mm, 600 μm to 20 mm, 700 μm to 800 μm, 700 μm to 900 μm, 700 μm to 1 mm, 700 μm to 2 mm, 700 μm to 3 mm, 700 μm to 4 mm, 700 μm to 5 mm, 700 μm to 6 mm, 700 μm to 8 mm, 700 μm to 10 mm, 700 μm to 20 mm, 800 μm to 900 μm, 800 μm to 1 mm, 800 μm to 2 mm, 800 μm to 3 mm, 800 μm to 4 mm, 800 μm to 5 mm, 800 μm to 6 mm, 800 μm to 8 mm, 800 μm to 10 mm, 800 μm to 20 mm, 900 μm to 1 mm, 900 μm to 2 mm, 900 μm to 3 mm, 900 μm to 4 mm, 900 μm to 5 mm, 900 μm to 6 mm, 900 μm to 8 mm, 900 μm to 10 mm, 900 μm to 20 mm, 1 mm to 2 mm, 1 mm to 3 mm, 1 mm to 4 mm, 1 mm to 5 mm, 1 mm to 6 mm, 1 mm to 8 mm, 1 mm to 10 mm, 1 mm to 20 mm, 2 mm to 3 mm, 2 mm to 4 mm, 2 mm to 5 mm, 2 mm to 6 mm, 2 mm to 8 mm, 2 mm to 10 mm, 2 mm to 20 mm, 3 mm to 4 mm, 3 mm to 5 mm, 3 mm to 6 mm, 3 mm to 8 mm, 3 mm to 10 mm, 3 mm to 20 mm, 4 mm to 5 mm, 4 mm to 6 mm, 4 mm to 8 mm, 4 mm to 10 mm, 4 mm to 20 mm, 5 mm to 6 mm, 5 mm to 8 mm, 5 mm to 10 mm, 5 mm to 20 mm, 6 mm to 8 mm, 6 mm to 10 mm, 6 mm to 20

mm, 8 mm to 10 mm, 8 mm to 20 mm, or 10 mm to 20 mm. In an example, the small dimension is from about 10  $\mu\text{m}$  to 100  $\mu\text{m}$ .

**[00213]** A 3D matrix may be configured such that fluid may flow along a surface of the 3D matrix. In an example, the 3D matrix may comprise a fiber-like or plate-like configuration, as shown in **FIG. 10A**. Alternatively, or in addition to, the 3D matrix may comprise a spherical-like configuration. The 3D matrix may comprise a surface area that is significantly larger than a volume of the 3D matrix to permit diffusion and transport of nutrients and reagents into the 3D matrix. In an example, the thickness or diameter of the 3D matrix may be substantially less than other dimensions of the 3D matrix. 3D matrices comprising large surface area to volume ratios may permit the use of small or large media volumes, as shown in **FIG. 10B**, depending upon cell product being produced. In an example, the system may be configured for low fluid volumes. Low fluid volumes may permit high flow rate and more frequent batch feeds. Lower fluid volume operations may be useful short process runs, such as zero to three or zero to five days. Low fluid volume configuration may minimize the amount of activation and genetic payload reagents used, saving costs and increasing reagent efficiency. Alternatively, the 3D matrix and system may be configured for large or larger media volumes. Larger media volumes may provide for lower fluid flow rates and less frequent batch feeds. Configurations with larger fluid volumes may be useful for longer process runs, for example, process runs of longer than five days.

**[00214]** The amount of cell product produced may depend on the volume available for cell processing. The volume available for cell processing may be the volume of a single 3D matrix or the combined volume of a plurality of 3D matrices. In an example, the volume available for cell processing is the volume of a single 3D matrix. In another example, the volume available for cell processing is the volume of a plurality 3D matrices. In another example, a cell processing system may have a plurality of 3D matrices and each 3D matrix may perform a separate cell process such that the volume available for generating the cell product from each process is the volume of a single 3D matrix. In another example, a cell processing system may have a plurality of 3D matrices each performing the same cell processing operations to generate a single cell product from the plurality of 3D matrices. In another example, a subset of the plurality of 3D matrices may perform a first operation or series of operations to generate a first cell product and another subset of the plurality of 3D matrices may perform a second operation or series of operations to generate another cell product. The volume of a 3D matrix may be at least about 1 milliliter (mL), 2 mL, 3 mL, 5

mL, 10 mL, 25 mL, 50 mL, 75 mL, 100 mL, 150 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL, 1000 mL, or more. The volume of a 3D matrix may be less than or equal to about 1000 mL, 900 mL, 800 mL, 700 mL, 600 mL, 500 mL, 400 mL, 300 mL, 200 mL, 150 mL, 100 mL, 75 mL, 50 mL, 25 mL, 10 mL, 5 mL, 3 mL, 2 mL, 1 mL, or less. The volume of a 3D matrix may be from about 1 mL to 2 mL, 1 mL to 3 mL, 1 mL to 5 mL, 1 mL to 10 mL, 1 mL to 25 mL, 1 mL to 50 mL, 1 mL to 75 mL, 1 mL to 100 mL, 1 mL to 150 mL, 1 mL to 200 mL, 1 mL to 300 mL, 1 mL to 400 mL, 1 mL to 500 mL, 1 mL to 600 mL, 1 mL to 700 mL, 1 mL to 800 mL, 1 mL to 900 mL, 1 mL to 1000 mL, 2 mL to 3 mL, 2 mL to 5 mL, 2 mL to 10 mL, 2 mL to 25 mL, 2 mL to 50 mL, 2 mL to 75 mL, 2 mL to 100 mL, 2 mL to 150 mL, 2 mL to 200 mL, 2 mL to 300 mL, 2 mL to 400 mL, 2 mL to 500 mL, 2 mL to 600 mL, 2 mL to 700 mL, 2 mL to 800 mL, 2 mL to 900 mL, 2 mL to 1000 mL, 3 mL to 5 mL, 3 mL to 10 mL, 3 mL to 25 mL, 3 mL to 50 mL, 3 mL to 75 mL, 3 mL to 100 mL, 3 mL to 150 mL, 3 mL to 200 mL, 3 mL to 300 mL, 3 mL to 400 mL, 3 mL to 500 mL, 3 mL to 600 mL, 3 mL to 700 mL, 3 mL to 800 mL, 3 mL to 900 mL, 3 mL to 1000 mL, 5 mL to 10 mL, 5 mL to 25 mL, 5 mL to 50 mL, 5 mL to 75 mL, 5 mL to 100 mL, 5 mL to 150 mL, 5 mL to 200 mL, 5 mL to 300 mL, 5 mL to 400 mL, 5 mL to 500 mL, 5 mL to 600 mL, 5 mL to 700 mL, 5 mL to 800 mL, 5 mL to 900 mL, 5 mL to 1000 mL, 10 mL to 25 mL, 10 mL to 50 mL, 10 mL to 75 mL, 10 mL to 100 mL, 10 mL to 150 mL, 10 mL to 200 mL, 10 mL to 300 mL, 10 mL to 400 mL, 10 mL to 500 mL, 10 mL to 600 mL, 10 mL to 700 mL, 10 mL to 800 mL, 10 mL to 900 mL, 10 mL to 1000 mL, 25 mL to 50 mL, 25 mL to 75 mL, 25 mL to 100 mL, 25 mL to 150 mL, 25 mL to 200 mL, 25 mL to 300 mL, 25 mL to 400 mL, 25 mL to 500 mL, 25 mL to 600 mL, 25 mL to 700 mL, 25 mL to 800 mL, 25 mL to 900 mL, 25 mL to 1000 mL, 50 mL to 75 mL, 50 mL to 100 mL, 50 mL to 150 mL, 50 mL to 200 mL, 50 mL to 300 mL, 50 mL to 400 mL, 50 mL to 500 mL, 50 mL to 600 mL, 50 mL to 700 mL, 50 mL to 800 mL, 50 mL to 900 mL, 50 mL to 1000 mL, 75 mL to 100 mL, 75 mL to 150 mL, 75 mL to 200 mL, 75 mL to 300 mL, 75 mL to 400 mL, 75 mL to 500 mL, 75 mL to 600 mL, 75 mL to 700 mL, 75 mL to 800 mL, 75 mL to 900 mL, 75 mL to 1000 mL, 100 mL to 150 mL, 100 mL to 200 mL, 100 mL to 300 mL, 100 mL to 400 mL, 100 mL to 500 mL, 100 mL to 600 mL, 100 mL to 700 mL, 100 mL to 800 mL, 100 mL to 900 mL, 100 mL to 1000 mL, 150 mL to 200 mL, 150 mL to 300 mL, 150 mL to 400 mL, 150 mL to 500 mL, 150 mL to 600 mL, 150 mL to 700 mL, 150 mL to 800 mL, 150 mL to 900 mL, 150 mL to 1000 mL, 200 mL to 300 mL, 200 mL to 400 mL, 200 mL to 500 mL, 200 mL to 600 mL, 200 mL to 700 mL, 200 mL to 800 mL, 200 mL to 900 mL, 200 mL to 1000 mL, 300 mL to 400 mL, 300 mL to 500 mL, 300 mL to 600 mL, 300 mL to 700 mL, 300 mL to 800 mL, 300 mL to

900 mL, 300 mL to 1000 mL, 400 mL to 500 mL, 400 mL to 600 mL, 400 mL to 700 mL, 400 mL to 800 mL, 400 mL to 900 mL, 400 mL to 1000 mL, 500 mL to 600 mL, 500 mL to 700 mL, 500 mL to 800 mL, 500 mL to 900 mL, 500 mL to 1000 mL, 600 mL to 700 mL, 600 mL to 800 mL, 600 mL to 900 mL, 600 mL to 1000 mL, 700 mL to 800 mL, 700 mL to 900 mL, 700 mL to 1000 mL, 800 mL to 900 mL, 800 mL to 1000 mL, or 900 mL to 1000 mL. In an example, a 3D matrix has a volume from about 5 mL to 100 mL.

**[00215]** The combined volume of a plurality of 3D matrix may be at least about 10 mL, 25 mL, 50 mL, 75 mL, 100 mL, 150 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL, 1 L, 2 L, 3 L, 4 L, 5 L, 6 L, 8 L, 10 L, or more. The combined volume of a 3D matrix may be less than or equal to about 10 L, 8 L, 6 L, 5 L, 4 L, 3 L, 2 L, 1 L, 900 mL, 800 mL, 700 mL, 600 mL, 500 mL, 400 mL, 300 mL, 200 mL, 150 mL, 100 mL, 75 mL, 50 mL, 25 mL, 10 mL, or less. The combined volume of a 3D matrix may be from about 10 mL to 25 mL, 10 mL to 50 mL, 10 mL to 75 mL, 10 mL to 100 mL, 10 mL to 150 mL, 10 mL to 200 mL, 10 mL to 300 mL, 10 mL to 400 mL, 10 mL to 500 mL, 10 mL to 600 mL, 10 mL to 700 mL, 10 mL to 800 mL, 10 mL to 900 mL, 10 mL to 1 L, 10 mL to 2 L, 10 mL to 3 L, 10 mL to 4 L, 10 mL to 5 L, 10 mL to 6 L, 10 mL to 8 L, 10 mL to 10 L, 25 mL to 50 mL, 25 mL to 75 mL, 25 mL to 100 mL, 25 mL to 150 mL, 25 mL to 200 mL, 25 mL to 300 mL, 25 mL to 400 mL, 25 mL to 500 mL, 25 mL to 600 mL, 25 mL to 700 mL, 25 mL to 800 mL, 25 mL to 900 mL, 25 mL to 1 L, 25 mL to 2 L, 25 mL to 3 L, 25 mL to 4 L, 25 mL to 5 L, 25 mL to 6 L, 25 mL to 8 L, 25 mL to 10 L, 50 mL to 75 mL, 50 mL to 100 mL, 50 mL to 150 mL, 50 mL to 200 mL, 50 mL to 300 mL, 50 mL to 400 mL, 50 mL to 500 mL, 50 mL to 600 mL, 50 mL to 700 mL, 50 mL to 800 mL, 50 mL to 900 mL, 50 mL to 1 L, 50 mL to 2 L, 50 mL to 3 L, 50 mL to 4 L, 50 mL to 5 L, 50 mL to 6 L, 50 mL to 8 L, 50 mL to 10 L, 75 mL to 100 mL, 75 mL to 150 mL, 75 mL to 200 mL, 75 mL to 300 mL, 75 mL to 400 mL, 75 mL to 500 mL, 75 mL to 600 mL, 75 mL to 700 mL, 75 mL to 800 mL, 75 mL to 900 mL, 75 mL to 1 L, 75 mL to 2 L, 75 mL to 3 L, 75 mL to 4 L, 75 mL to 5 L, 75 mL to 6 L, 75 mL to 8 L, 75 mL to 10 L, 100 mL to 150 mL, 100 mL to 200 mL, 100 mL to 300 mL, 100 mL to 400 mL, 100 mL to 500 mL, 100 mL to 600 mL, 100 mL to 700 mL, 100 mL to 800 mL, 100 mL to 900 mL, 100 mL to 1 L, 100 mL to 2 L, 100 mL to 3 L, 100 mL to 4 L, 100 mL to 5 L, 100 mL to 6 L, 100 mL to 8 L, 100 mL to 10 L, 150 mL to 200 mL, 150 mL to 300 mL, 150 mL to 400 mL, 150 mL to 500 mL, 150 mL to 600 mL, 150 mL to 700 mL, 150 mL to 800 mL, 150 mL to 900 mL, 150 mL to 1 L, 150 mL to 2 L, 150 mL to 3 L, 150 mL to 4 L, 150 mL to 5 L, 150 mL to 6 L, 150 mL to 8 L, 150 mL to 10 L, 200 mL to 300 mL, 200 mL to 400 mL, 200 mL to 500 mL, 200 mL to 600 mL, 200 mL to 700 mL, 200 mL to 800 mL, 200 mL to 900

mL, 200 mL to 1 L, 200 mL to 2 L, 200 mL to 3 L, 200 mL to 4 L, 200 mL to 5 L, 200 mL to 6 L, 200 mL to 8 L, 200 mL to 10 L, 300 mL to 400 mL, 300 mL to 500 mL, 300 mL to 600 mL, 300 mL to 700 mL, 300 mL to 800 mL, 300 mL to 900 mL, 300 mL to 1 L, 300 mL to 2 L, 300 mL to 3 L, 300 mL to 4 L, 300 mL to 5 L, 300 mL to 6 L, 300 mL to 8 L, 300 mL to 10 L, 400 mL to 500 mL, 400 mL to 600 mL, 400 mL to 700 mL, 400 mL to 800 mL, 400 mL to 900 mL, 400 mL to 1 L, 400 mL to 2 L, 400 mL to 3 L, 400 mL to 4 L, 400 mL to 5 L, 400 mL to 6 L, 400 mL to 8 L, 400 mL to 10 L, 500 mL to 600 mL, 500 mL to 700 mL, 500 mL to 800 mL, 500 mL to 900 mL, 500 mL to 1 L, 500 mL to 2 L, 500 mL to 3 L, 500 mL to 4 L, 500 mL to 5 L, 500 mL to 6 L, 500 mL to 8 L, 500 mL to 10 L, 600 mL to 700 mL, 600 mL to 800 mL, 600 mL to 900 mL, 600 mL to 1 L, 600 mL to 2 L, 600 mL to 3 L, 600 mL to 4 L, 600 mL to 5 L, 600 mL to 6 L, 600 mL to 8 L, 600 mL to 10 L, 700 mL to 800 mL, 700 mL to 900 mL, 700 mL to 1 mL, 700 mL to 2 mL, 700 mL to 3 mL, 700 mL to 4 mL, 700 mL to 5 mL, 700 mL to 6 mL, 700 mL to 8 mL, 700 mL to 10 mL, 800 mL to 900 mL, 800 mL to 1 L, 800 mL to 2 L, 800 mL to 3 L, 800 mL to 4 L, 800 mL to 5 L, 800 mL to 6 L, 800 mL to 8 L, 800 mL to 10 L, 900 mL to 1 L, 900 mL to 2 L, 900 mL to 3 L, 900 mL to 4 L, 900 mL to 5 L, 900 mL to 6 L, 900 mL to 8 L, 900 mL to 10 L, 1 L to 2 L, 1 L to 3 L, 1 L to 4 L, 1 L to 5 L, 1 L to 6 L, 1 L to 8 L, 1 L to 10 L, 2 L to 3 L, 2 L to 4 L, 2 L to 5 L, 2 L to 6 L, 2 L to 8 L, 2 L to 10 L, 3 L to 4 L, 3 L to 5 L, 3 L to 6 L, 3 L to 8 L, 3 L to 10 L, 4 L to 5 L, 4 L to 6 L, 4 L to 8 L, 4 L to 10 L, 5 L to 6 L, 5 L to 8 L, 5 L to 10 L, 6 L to 8 L, 6 L to 10 L, or 8 L to 10 L. In an example, a plurality of 3D matrix has a combined volume from about 100 mL to 10 L.

**[00216]** The efficiency of material transport and diffusion of nutrients and reagents from a fluid flowing over or adjacent to the 3D matrix may be dependent upon the interfacial area between the fluid or flowing fluid and the 3D matrix. The interfacial area between the fluid (e.g., fluid flow path or fluid compartment) and the 3D matrix may be greater than or equal to about 1 square centimeters ( $\text{cm}^2$ ), 2  $\text{cm}^2$ , 3  $\text{cm}^2$ , 4  $\text{cm}^2$ , 5  $\text{cm}^2$ , 6  $\text{cm}^2$ , 8  $\text{cm}^2$ , 10  $\text{cm}^2$ , 15  $\text{cm}^2$ , 20  $\text{cm}^2$ , 30  $\text{cm}^2$ , 50  $\text{cm}^2$ , or greater. The interfacial area between the fluid (e.g., fluid flow path or fluid compartment) and the 3D matrix may be from about 1  $\text{cm}^2$  to 2  $\text{cm}^2$ , 1  $\text{cm}^2$  to 3  $\text{cm}^2$ , 1  $\text{cm}^2$  to 4  $\text{cm}^2$ , 1  $\text{cm}^2$  to 5  $\text{cm}^2$ , 1  $\text{cm}^2$  to 6  $\text{cm}^2$ , 1  $\text{cm}^2$  to 8  $\text{cm}^2$ , 1  $\text{cm}^2$  to 10  $\text{cm}^2$ , 1  $\text{cm}^2$  to 15  $\text{cm}^2$ , 1  $\text{cm}^2$  to 20  $\text{cm}^2$ , 1  $\text{cm}^2$  to 30  $\text{cm}^2$ , 1  $\text{cm}^2$  to 50  $\text{cm}^2$ , 2  $\text{cm}^2$  to 3  $\text{cm}^2$ , 2  $\text{cm}^2$  to 4  $\text{cm}^2$ , 2  $\text{cm}^2$  to 5  $\text{cm}^2$ , 2  $\text{cm}^2$  to 6  $\text{cm}^2$ , 2  $\text{cm}^2$  to 8  $\text{cm}^2$ , 2  $\text{cm}^2$  to 10  $\text{cm}^2$ , 2  $\text{cm}^2$  to 15  $\text{cm}^2$ , 2  $\text{cm}^2$  to 20  $\text{cm}^2$ , 2  $\text{cm}^2$  to 30  $\text{cm}^2$ , 2  $\text{cm}^2$  to 50  $\text{cm}^2$ , 3  $\text{cm}^2$  to 4  $\text{cm}^2$ , 3  $\text{cm}^2$  to 5  $\text{cm}^2$ , 3  $\text{cm}^2$  to 6  $\text{cm}^2$ , 3  $\text{cm}^2$  to 8  $\text{cm}^2$ , 3  $\text{cm}^2$  to 10  $\text{cm}^2$ , 3  $\text{cm}^2$  to 15  $\text{cm}^2$ , 3  $\text{cm}^2$  to 20  $\text{cm}^2$ , 3  $\text{cm}^2$  to 30  $\text{cm}^2$ , 3  $\text{cm}^2$  to 50  $\text{cm}^2$ , 4  $\text{cm}^2$  to 5  $\text{cm}^2$ , 4  $\text{cm}^2$  to 6  $\text{cm}^2$ , 4  $\text{cm}^2$  to 8  $\text{cm}^2$ , 4  $\text{cm}^2$  to 10  $\text{cm}^2$ , 4  $\text{cm}^2$  to 15  $\text{cm}^2$ , 4  $\text{cm}^2$

to 20 cm<sup>2</sup>, 4 cm<sup>2</sup> to 30 cm<sup>2</sup>, 4 cm<sup>2</sup> to 50 cm<sup>2</sup>, 5 cm<sup>2</sup> to 6 cm<sup>2</sup>, 5 cm<sup>2</sup> to 8 cm<sup>2</sup>, 5 cm<sup>2</sup> to 10 cm<sup>2</sup>, 5 cm<sup>2</sup> to 15 cm<sup>2</sup>, 5 cm<sup>2</sup> to 20 cm<sup>2</sup>, 5 cm<sup>2</sup> to 30 cm<sup>2</sup>, 5 cm<sup>2</sup> to 50 cm<sup>2</sup>, 6 cm<sup>2</sup> to 8 cm<sup>2</sup>, 6 cm<sup>2</sup> to 10 cm<sup>2</sup>, 6 cm<sup>2</sup> to 15 cm<sup>2</sup>, 6 cm<sup>2</sup> to 20 cm<sup>2</sup>, 6 cm<sup>2</sup> to 30 cm<sup>2</sup>, 6 cm<sup>2</sup> to 50 cm<sup>2</sup>, 8 cm<sup>2</sup> to 10 cm<sup>2</sup>, 8 cm<sup>2</sup> to 15 cm<sup>2</sup>, 8 cm<sup>2</sup> to 20 cm<sup>2</sup>, 8 cm<sup>2</sup> to 30 cm<sup>2</sup>, 8 cm<sup>2</sup> to 50 cm<sup>2</sup>, 10 cm<sup>2</sup> to 15 cm<sup>2</sup>, 10 cm<sup>2</sup> to 20 cm<sup>2</sup>, 10 cm<sup>2</sup> to 30 cm<sup>2</sup>, 10 cm<sup>2</sup> to 50 cm<sup>2</sup>, 15 cm<sup>2</sup> to 20 cm<sup>2</sup>, 15 cm<sup>2</sup> to 30 cm<sup>2</sup>, 15 cm<sup>2</sup> to 50 cm<sup>2</sup>, 20 cm<sup>2</sup> to 30 cm<sup>2</sup>, 20 cm<sup>2</sup> to 50 cm<sup>2</sup>, or 30 cm<sup>2</sup> to 50 cm<sup>2</sup>. In an example, the interfacial area is greater than or equal to about 5 cm<sup>2</sup>.

**[00217]** Processing cells within a 3D matrix, as shown in **FIG. 11A**, may provide for improved processing or cell culture conditions that may improve the health of produced cells and reliability of cell manufacturing process. A 3D matrix may retain cells within a cell compartment, create a favorable biophysical microenvironment mimicking natural tissue extra cellular matrix (e.g., matrix stiffness), create high local concentrations of secreted factors, provide a programmable substrate for cell transduction and signaling, or any combinations thereof. The 3D matrix may provide for tunable physical characteristics, as shown in **FIG. 11B**. For example, 3D matrices that comprise a dense, soft matrix may deform as cells proliferate within the 3D matrix. 3D matrices that comprise porous, stiff matrices may permit cell proliferation within the matrix with small amounts of deformation. 3D matrices that comprise stiff, dense matrices may not permit cell proliferation.

**[00218]** The physical stiffness, softness, or rigidity of 3D substrates may alter the ability of cells to grow, proliferate, or otherwise be processed within the 3D matrices described herein. Physical stiffness, softness, or rigidity of 3D substrates may be described and measured in terms of mechanical parameters such as stiffness, elastic modulus, and viscoelasticity. The elastic modulus (e.g., Young's Modulus) is a measure of the stiffness of a material. It may be defined as the ratio of stress (force per unit area) to strain (deformation in response to stress). For 3D substrates such as hydrogels, the elastic modulus may be determined using compression or tensile tests, where a sample is deformed under a given load, and the resulting deformation is measured. The modulus may then be calculated from the slope of the stress-strain curve in the elastic region. The shear modulus may describe the material's response to shear stress (parallel to the surface) and is particularly relevant for materials like hydrogels that can undergo significant shear deformation. Shear modulus may be measured using rheometry, where a hydrogel sample is subjected to oscillatory shear, and the resulting shear stress and strain are used to calculate the modulus. The bulk modulus may describe a material's response to (uniform) hydrostatic pressure. For isotropic materials, shear modulus (G), elastic modulus (E), and bulk modulus (K) may be related to each other as a function of

a material's Poisson's ratio ( $\nu$ ) as shown by the equations:  $E = 2G(1+\nu) = 3K(1-2\nu)$ . In some embodiments, the 3D matrix may be configured to have an elastic modulus or other physical descriptor resembling or in the range of those exhibited by natural tissue extracellular matrix (ECM), e.g., of brain tissue ( $E \sim 1.4\text{--}1.9$  kPa), or another type of tissue. The 3D matrix may have an elastic modulus of greater than or equal to about 0.1 kilopascals (kPa), 0.25 kPa, 0.5 kPa, 0.75 kPa, 1 kPa, 1.25 kPa, 1.5 kPa, 1.75 kPa, 2 kPa, 2.5 kPa, 3 kPa, 4 kPa, 5 kPa, 6 kPa, 7 kPa, 8 kPa, 9 kPa, 10 kPa, or more. The 3D matrix may have an elastic modulus of less than or equal to about 10 kPa, 9 kPa, 8 kPa, 7 kPa, 6 kPa, 5 kPa, 4 kPa, 3 kPa, 2.5 kPa, 2 kPa, 1.75 kPa, 1.5 kPa, 1.25 kPa, 1 kPa, 0.75 kPa, 0.5 kPa, 0.25 kPa, 0.1 kPa, or less. The 3D matrix may have an elastic modulus from about 0.1 kPa to 0.25 kPa, 0.1 kPa to 0.5 kPa, 0.1 kPa to 0.75 kPa, 0.1 kPa to 1 kPa, 0.1 kPa to 1.25 kPa, 0.1 kPa to 1.5 kPa, 0.1 kPa to 1.75 kPa, 0.1 kPa to 2 kPa, 0.1 kPa to 2.5 kPa, 0.1 kPa to 3 kPa, 0.1 kPa to 4 kPa, 0.1 kPa to 5 kPa, 0.1 kPa to 6 kPa, 0.1 kPa to 7 kPa, 0.1 kPa to 8 kPa, 0.1 kPa to 9 kPa, 0.1 kPa to 10 kPa, 0.25 kPa to 0.5 kPa, 0.25 kPa to 0.75 kPa, 0.25 kPa to 1 kPa, 0.25 kPa to 1.25 kPa, 0.25 kPa to 1.5 kPa, 0.25 kPa to 1.75 kPa, 0.25 kPa to 2 kPa, 0.25 kPa to 2.5 kPa, 0.25 kPa to 3 kPa, 0.25 kPa to 4 kPa, 0.25 kPa to 5 kPa, 0.25 kPa to 6 kPa, 0.25 kPa to 7 kPa, 0.25 kPa to 8 kPa, 0.25 kPa to 9 kPa, 0.25 kPa to 10 kPa, 0.5 kPa to 0.75 kPa, 0.5 kPa to 1 kPa, 0.5 kPa to 1.25 kPa, 0.5 kPa to 1.5 kPa, 0.5 kPa to 1.75 kPa, 0.5 kPa to 2 kPa, 0.5 kPa to 2.5 kPa, 0.5 kPa to 3 kPa, 0.5 kPa to 4 kPa, 0.5 kPa to 5 kPa, 0.5 kPa to 6 kPa, 0.5 kPa to 7 kPa, 0.5 kPa to 8 kPa, 0.5 kPa to 9 kPa, 0.5 kPa to 10 kPa, 0.75 kPa to 1 kPa, 0.75 kPa to 1.25 kPa, 0.75 kPa to 1.5 kPa, 0.75 kPa to 1.75 kPa, 0.75 kPa to 2 kPa, 0.75 kPa to 2.5 kPa, 0.75 kPa to 3 kPa, 0.75 kPa to 4 kPa, 0.75 kPa to 5 kPa, 0.75 kPa to 6 kPa, 0.75 kPa to 7 kPa, 0.75 kPa to 8 kPa, 0.75 kPa to 9 kPa, 0.75 kPa to 10 kPa, 1 kPa to 1.25 kPa, 1 kPa to 1.5 kPa, 1 kPa to 1.75 kPa, 1 kPa to 2 kPa, 1 kPa to 2.5 kPa, 1 kPa to 3 kPa, 1 kPa to 4 kPa, 1 kPa to 5 kPa, 1 kPa to 6 kPa, 1 kPa to 7 kPa, 1 kPa to 8 kPa, 1 kPa to 9 kPa, 1 kPa to 10 kPa, 1.25 kPa to 1.5 kPa, 1.25 kPa to 1.75 kPa, 1.25 kPa to 2 kPa, 1.25 kPa to 2.5 kPa, 1.25 kPa to 3 kPa, 1.25 kPa to 4 kPa, 1.25 kPa to 5 kPa, 1.25 kPa to 6 kPa, 1.25 kPa to 7 kPa, 1.25 kPa to 8 kPa, 1.25 kPa to 9 kPa, 1.25 kPa to 10 kPa, 1.5 kPa to 1.75 kPa, 1.5 kPa to 2 kPa, 1.5 kPa to 2.5 kPa, 1.5 kPa to 3 kPa, 1.5 kPa to 4 kPa, 1.5 kPa to 5 kPa, 1.5 kPa to 6 kPa, 1.5 kPa to 7 kPa, 1.5 kPa to 8 kPa, 1.5 kPa to 9 kPa, 1.5 kPa to 10 kPa, 1.75 kPa to 2 kPa, 1.75 kPa to 2.5 kPa, 1.75 kPa to 3 kPa, 1.75 kPa to 4 kPa, 1.75 kPa to 5 kPa, 1.75 kPa to 6 kPa, 1.75 kPa to 7 kPa, 1.75 kPa to 8 kPa, 1.75 kPa to 9 kPa, 1.75 kPa to 10 kPa, 2.5 kPa to 3 kPa, 2.5 kPa to 4 kPa, 2.5 kPa to 5 kPa, 2.5 kPa to 6 kPa, 2.5 kPa to 7 kPa, 2.5 kPa to 8 kPa, 2.5 kPa to 9 kPa, 2.5 kPa to 10 kPa, 3 kPa to 4 kPa, 3 kPa to 5 kPa, 3 kPa to 6 kPa, 3 kPa to 7 kPa, 3 kPa to 8 kPa, 3 kPa to 9 kPa,



3 kPa to 10 kPa, 4 kPa to 5 kPa, 4 kPa to 6 kPa, 4 kPa to 7 kPa, 4 kPa to 8 kPa, 4 kPa to 9 kPa, 4 kPa to 10 kPa, 5 kPa to 6 kPa, 5 kPa to 7 kPa, 5 kPa to 8 kPa, 5 kPa to 9 kPa, 5 kPa to 10 kPa, 6 kPa to 7 kPa, 6 kPa to 8 kPa, 6 kPa to 9 kPa, 6 kPa to 10 kPa, 7 kPa to 8 kPa, 7 kPa to 9 kPa, 7 kPa to 10 kPa, 8 kPa to 9 kPa, 8 kPa to 10 kPa, or 9 kPa to 10 kPa.

**[00219]** The viscoelasticity of the 3D matrix may be tuned or altered for select cell processing applications. Viscoelasticity may describe materials that exhibit both viscous and elastic characteristics when deformed. Hydrogels may display significant viscoelastic behavior such that the hydrogel may dissipate energy (e.g., like a viscous fluid) and recover the original shape (e.g., like an elastic solid). Dynamic mechanical analysis (DMA) may be used to measure viscoelasticity, where a 3D matrix such as a hydrogel may be subjected to a sinusoidal stress and the resulting strain may be measured. The phase difference between stress and strain may provide insights into the material's viscoelastic properties.

Viscoelasticity and relaxation time, independent of stiffness, may impact cellular behaviors. For example, in hydrogels that exhibit stress relaxation, each force or strain a cell applies to the matrix over time may initially be resisted with a certain stiffness, defined by the initial elastic modulus, followed by a decrease in resistance over time. Stress relaxation, for example, may be measured by a stress relaxation test ( $\tau_{1/2}$ ). Stress relaxation timescales may be less than or equal to about 1 week, 1 day, 1 hour, 1 minute, 1 second, or less. Stress relaxation timescales may be greater than or equal to about 1 second, 1 minute, 1 hour, 1 day, 1 week, or more.

**[00220]** The swelling properties of 3D matrices may be tuned or otherwise modified for select cell processing applications. Swelling properties may be described and measured in terms of swelling ratio, e.g., the amount of water or buffer a 3D substrate (e.g., a hydrogel or other polymer matrix) can absorb and retain. Swelling may be an indicator of the network hydrophilicity as well as of the relative crosslinking density. 3D matrices with stiffer networks may exhibit lower swelling and matrices with more flexible networks may exhibit greater swelling. Swelling ratio may indirectly influence mechanical softness and flexibility. The swelling ratio may be a measure of how much a matrix swells beyond an initial state, or the ratio of its swollen volume to its dry volume. For example, to determine a swelling ratio, dry material may be weighed or its volume measured, submerged in water or other suitable aqueous buffer until equilibrium swelling is reached, then reweighed or its volume re-measured to determine the swelling ratio (e.g.,  $Q_{1a} = V_s/V_d$ ;  $Q_{1a}$  volume swelling ratio from dry,  $V_s$  volume saturated,  $V_d$  volume dry;  $Q_{1b} = (V_s - V_d)/V_d$ ;  $Q_{1c} = M_s/M_d$ ;  $Q_{1c}$  mass swelling ratio from dry,  $M_s$  mass saturated,  $M_d$  mass dry;  $Q_{1d} = (M_s - M_d)/M_d$ ).

Alternatively, the swelling ratio may be determined by weighing or determining the volume of a solvated or hydrated matrix in an initial state (e.g., an initial hydrogel of 2% collagen w/v hydrogel), submerging the matrix in water or other suitable aqueous buffer until equilibrium swelling is reached, then reweighing or re-measuring the volume to determine the swelling ratio (e.g.,  $Q_{2a} = V_s / V_i$ ;  $Q_2$  volume swelling from initial state;  $V_s$  volume saturated;  $V_i$  initial volume;  $Q_{2b} = (V_s - V_i) / V_i$ ;  $Q_{2c} = M_s / M_i$ ;  $Q_{1c}$  mass swelling ratio from initial,  $M_s$  mass saturated,  $M_i$  initial mass;  $Q_{2d} = (M_s - M_i) / M_i$ ). In some embodiments, the 3D matrix does not swell substantially (e.g., swelling ratio  $Q_{2d}$  of less than about 50%). The swelling ratio of the 3D matrix may be greater than or equal to about 25%, 50%, 75%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 400%, 500%, or more. The swelling ratio of the 3D matrix may be less than or equal to about 500%, 400%, 300%, 250%, 200%, 175%, 150%, 125%, 100%, 75%, 50%, 25%, or less. The swelling ratio of the 3D matrix may be from about 25% to 50%, 25% to 75%, 25% to 100%, 25% to 125%, 25% to 150%, 25% to 175%, 25% to 200%, 25% to 250%, 25% to 300%, 25% to 400%, 25% to 500%, 50% to 75%, 50% to 100%, 50% to 125%, 50% to 150%, 50% to 175%, 50% to 200%, 50% to 250%, 50% to 300%, 50% to 400%, 50% to 500%, 75% to 100%, 75% to 125%, 75% to 150%, 75% to 175%, 75% to 200%, 75% to 250%, 75% to 300%, 75% to 400%, 75% to 500%, 100% to 125%, 100% to 150%, 100% to 175%, 100% to 200%, 100% to 250%, 100% to 300%, 100% to 400%, 100% to 500%, 125% to 150%, 125% to 175%, 125% to 200%, 125% to 250%, 125% to 300%, 125% to 400%, 125% to 500%, 150% to 175%, 150% to 200%, 150% to 250%, 150% to 300%, 150% to 400%, 150% to 500%, 175% to 200%, 175% to 250%, 175% to 300%, 175% to 400%, 175% to 500%, 200% to 250%, 200% to 300%, 200% to 400%, 200% to 500%, 250% to 300%, 250% to 400%, 250% to 500%, 300% to 400%, 300% to 500%, or 400% to 500%. In an example, the swelling ratio of the 3D matrix is less than or equal to about 100%.

**[00221]** In some embodiments, the 3D volume of the matrix may be constrained by the configuration of the vessel, such as a flow cell or compartment thereof, such that the 3D matrix is prevented from absorbing additional water and swelling beyond the defined volume of the container. In some embodiments, the 3D volume of the matrix may be variable, exhibiting swelling over time or in response to the addition of solvents or other reagents. For example, the 3D matrix may be a programmably swellable matrix which undergoes a change in swelling properties in response to an external stimulus. The external stimulus may be a chemical stimulus, thermal stimulus, electromagnetic stimulus, physical stimulus, or any combination thereof. In some embodiments, the vessel containing the 3D matrix may not physically constrain or otherwise limit swelling of the 3D volume of physical dimensions of

the 3D matrix. For example, the container retaining the 3D matrix may comprise a deformable vessel. The deformable vessel may be internally configured such that 3D matrix swelling occupies internal spaces otherwise occupied by fluid or gas when the 3D matrix is in a non-swollen state, and which become displaced by the 3D matrix upon swelling.

**[00222]** The porosity of 3D substrates may be tuned or otherwise modified for a given cell processing application. The porosity of 3D substrates may be determined by a number of methodologies, including through microscopy (e.g., by stimulated emission depletion (STED) microscopy, atomic force microscopy (AFM), or cryogenic scanning electron microscopy (Cryo-SEM)) to visualize and measure the morphology and size distribution of pores.

Porosity may also be quantified by measuring the pore volume fraction, e.g., porosity  $e = V_p / V_t \times 100$ ;  $V_p$  = pore volume =  $(W_w - W_d)/p$  ( $W_w$  = weight wet,  $W_d$  = weight dry,  $p$  = fluid density);  $V_t$  = total volume). 3D matrix porosity can influence the swelling behavior and other mechanical properties of the 3D matrix. For example, lower swelling and higher modulus may be correlated with a smaller porosity. The mesh size, or molecular porosity, of the matrix may be on the nanometer to micrometer scale and can influence nutrient flux throughout the matrix and cell retention, proliferation, growth, and other aspects of cellular state or phenotype. In some embodiments, the matrix porosity is on average smaller than a cell, but large enough to allow the diffusion of macromolecules for maintenance of cellular viability. In some embodiments, the matrix porosity is on average larger than a cell, such as to facilitate cellular proliferation and migration within the 3D matrix, while still retaining cells. The average pore diameter may be constant throughout the 3D matrix or may vary throughout the 3D matrix. The 3D matrix may have a porosity of greater than or equal to about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more. In an example, the 3D matrix has a porosity of greater than or equal to about 80%. The 3D matrix may have a porosity of less than or equal to about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or less. The 3D matrix may have a porosity from about 20% to 30%, 20% to 40%, 20% to 50%, 20% to 60%, 20% to 70%, 20% to 80%, 20% to 90%, 30% to 40%, 30% to 50%, 30% to 60%, 30% to 70%, 30% to 80%, 30% to 90%, 40% to 50%, 40% to 60%, 40% to 70%, 40% to 80%, 40% to 90%, 50% to 60%, 50% to 70%, 50% to 80%, 50% to 90%, 60% to 70%, 60% to 80%, 60% to 90%, 70% to 80%, 70% to 90%, or 80% to 90%.

**[00223]** The 3D matrix or substrate may have an average pore size that permits nutrients, reagents, and other materials to diffuse or otherwise move through the 3D matrix while retaining or substantially retaining cells within the 3D matrix. In some examples, the 3D matrix comprises a porous, solid matrix having interconnected pores. The average pore size

of the interconnected pores may be sufficiently large to permit growth of cells (e.g., lymphocytes or other target cells) within the 3D matrix. The 3D matrix or substrate may have an average pore size of less than or equal to about 1000  $\mu\text{m}$ , 900  $\mu\text{m}$ , 800  $\mu\text{m}$ , 700  $\mu\text{m}$ , 600  $\mu\text{m}$ , 500  $\mu\text{m}$ , 400  $\mu\text{m}$ , 300  $\mu\text{m}$ , 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , 75  $\mu\text{m}$ , 50  $\mu\text{m}$ , 25  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , 1  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , 0.1  $\mu\text{m}$ , or less. In an example, the 3D matrix or substrate has an average pore size of less than or equal to about 400  $\mu\text{m}$ . The 3D matrix or substrate may have an average pore size of greater than or equal to about 0.1  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , 1  $\mu\text{m}$ , 5  $\mu\text{m}$ , 10  $\mu\text{m}$ , 25  $\mu\text{m}$ , 50  $\mu\text{m}$ , 75  $\mu\text{m}$ , 100  $\mu\text{m}$ , 200  $\mu\text{m}$ , 300  $\mu\text{m}$ , 400  $\mu\text{m}$ , 500  $\mu\text{m}$ , 600  $\mu\text{m}$ , 700  $\mu\text{m}$ , 800  $\mu\text{m}$ , 900  $\mu\text{m}$ , 1000  $\mu\text{m}$ , or greater. The 3D matrix or substrate may have an average pore size from about 0.1  $\mu\text{m}$  to 0.5  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 1  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 5  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 10  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 25  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 50  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 75  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 100  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 200  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 300  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 400  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 500  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 600  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 700  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 800  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 900  $\mu\text{m}$ , 0.1  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 1  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 5  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 10  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 25  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 50  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 75  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 100  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 200  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 300  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 400  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 500  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 600  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 700  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 800  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 900  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 1  $\mu\text{m}$  to 5  $\mu\text{m}$ , 1  $\mu\text{m}$  to 10  $\mu\text{m}$ , 1  $\mu\text{m}$  to 25  $\mu\text{m}$ , 1  $\mu\text{m}$  to 50  $\mu\text{m}$ , 1  $\mu\text{m}$  to 75  $\mu\text{m}$ , 1  $\mu\text{m}$  to 100  $\mu\text{m}$ , 1  $\mu\text{m}$  to 200  $\mu\text{m}$ , 1  $\mu\text{m}$  to 300  $\mu\text{m}$ , 1  $\mu\text{m}$  to 400  $\mu\text{m}$ , 1  $\mu\text{m}$  to 500  $\mu\text{m}$ , 1  $\mu\text{m}$  to 600  $\mu\text{m}$ , 1  $\mu\text{m}$  to 700  $\mu\text{m}$ , 1  $\mu\text{m}$  to 800  $\mu\text{m}$ , 1  $\mu\text{m}$  to 900  $\mu\text{m}$ , 1  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 5  $\mu\text{m}$  to 10  $\mu\text{m}$ , 5  $\mu\text{m}$  to 25  $\mu\text{m}$ , 5  $\mu\text{m}$  to 50  $\mu\text{m}$ , 5  $\mu\text{m}$  to 75  $\mu\text{m}$ , 5  $\mu\text{m}$  to 100  $\mu\text{m}$ , 5  $\mu\text{m}$  to 200  $\mu\text{m}$ , 5  $\mu\text{m}$  to 300  $\mu\text{m}$ , 5  $\mu\text{m}$  to 400  $\mu\text{m}$ , 5  $\mu\text{m}$  to 500  $\mu\text{m}$ , 5  $\mu\text{m}$  to 600  $\mu\text{m}$ , 5  $\mu\text{m}$  to 700  $\mu\text{m}$ , 5  $\mu\text{m}$  to 800  $\mu\text{m}$ , 5  $\mu\text{m}$  to 900  $\mu\text{m}$ , 5  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 10  $\mu\text{m}$  to 25  $\mu\text{m}$ , 10  $\mu\text{m}$  to 50  $\mu\text{m}$ , 10  $\mu\text{m}$  to 75  $\mu\text{m}$ , 10  $\mu\text{m}$  to 100  $\mu\text{m}$ , 10  $\mu\text{m}$  to 200  $\mu\text{m}$ , 10  $\mu\text{m}$  to 300  $\mu\text{m}$ , 10  $\mu\text{m}$  to 400  $\mu\text{m}$ , 10  $\mu\text{m}$  to 500  $\mu\text{m}$ , 10  $\mu\text{m}$  to 600  $\mu\text{m}$ , 10  $\mu\text{m}$  to 700  $\mu\text{m}$ , 10  $\mu\text{m}$  to 800  $\mu\text{m}$ , 10  $\mu\text{m}$  to 900  $\mu\text{m}$ , 10  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 25  $\mu\text{m}$  to 50  $\mu\text{m}$ , 25  $\mu\text{m}$  to 75  $\mu\text{m}$ , 25  $\mu\text{m}$  to 100  $\mu\text{m}$ , 25  $\mu\text{m}$  to 200  $\mu\text{m}$ , 25  $\mu\text{m}$  to 300  $\mu\text{m}$ , 25  $\mu\text{m}$  to 400  $\mu\text{m}$ , 25  $\mu\text{m}$  to 500  $\mu\text{m}$ , 25  $\mu\text{m}$  to 600  $\mu\text{m}$ , 25  $\mu\text{m}$  to 700  $\mu\text{m}$ , 25  $\mu\text{m}$  to 800  $\mu\text{m}$ , 25  $\mu\text{m}$  to 900  $\mu\text{m}$ , 25  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 50  $\mu\text{m}$  to 75  $\mu\text{m}$ , 50  $\mu\text{m}$  to 100  $\mu\text{m}$ , 50  $\mu\text{m}$  to 200  $\mu\text{m}$ , 50  $\mu\text{m}$  to 300  $\mu\text{m}$ , 50  $\mu\text{m}$  to 400  $\mu\text{m}$ , 50  $\mu\text{m}$  to 500  $\mu\text{m}$ , 50  $\mu\text{m}$  to 600  $\mu\text{m}$ , 50  $\mu\text{m}$  to 700  $\mu\text{m}$ , 50  $\mu\text{m}$  to 800  $\mu\text{m}$ , 50  $\mu\text{m}$  to 900  $\mu\text{m}$ , 50  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 75  $\mu\text{m}$  to 100  $\mu\text{m}$ , 75  $\mu\text{m}$  to 200  $\mu\text{m}$ , 75  $\mu\text{m}$  to 300  $\mu\text{m}$ , 75  $\mu\text{m}$  to 400  $\mu\text{m}$ , 75  $\mu\text{m}$  to 500  $\mu\text{m}$ , 75  $\mu\text{m}$  to 600  $\mu\text{m}$ , 75  $\mu\text{m}$  to 700  $\mu\text{m}$ , 75  $\mu\text{m}$  to 800  $\mu\text{m}$ , 75  $\mu\text{m}$  to 900  $\mu\text{m}$ , 75  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 100  $\mu\text{m}$  to 200  $\mu\text{m}$ , 100  $\mu\text{m}$  to 300  $\mu\text{m}$ , 100  $\mu\text{m}$  to 400  $\mu\text{m}$ , 100  $\mu\text{m}$  to 500  $\mu\text{m}$ , 100  $\mu\text{m}$  to 600  $\mu\text{m}$ , 100  $\mu\text{m}$  to 700  $\mu\text{m}$ , 100  $\mu\text{m}$  to 800  $\mu\text{m}$ , 100  $\mu\text{m}$  to 900  $\mu\text{m}$ , 100  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 200  $\mu\text{m}$  to 300  $\mu\text{m}$ , 200  $\mu\text{m}$  to 400  $\mu\text{m}$ , 200  $\mu\text{m}$  to 500  $\mu\text{m}$ , 200  $\mu\text{m}$  to 600  $\mu\text{m}$ , 200  $\mu\text{m}$  to 700  $\mu\text{m}$ , 200  $\mu\text{m}$  to 800  $\mu\text{m}$ , 200  $\mu\text{m}$  to 900  $\mu\text{m}$ , 200  $\mu\text{m}$  to 1000  $\mu\text{m}$ ,

300  $\mu\text{m}$  to 400  $\mu\text{m}$ , 300  $\mu\text{m}$  to 500  $\mu\text{m}$ , 300  $\mu\text{m}$  to 600  $\mu\text{m}$ , 300  $\mu\text{m}$  to 700  $\mu\text{m}$ , 300  $\mu\text{m}$  to 800  $\mu\text{m}$ , 300  $\mu\text{m}$  to 900  $\mu\text{m}$ , 300  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 400  $\mu\text{m}$  to 500  $\mu\text{m}$ , 400  $\mu\text{m}$  to 600  $\mu\text{m}$ , 400  $\mu\text{m}$  to 700  $\mu\text{m}$ , 400  $\mu\text{m}$  to 800  $\mu\text{m}$ , 400  $\mu\text{m}$  to 900  $\mu\text{m}$ , 400  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 500  $\mu\text{m}$  to 600  $\mu\text{m}$ , 500  $\mu\text{m}$  to 700  $\mu\text{m}$ , 500  $\mu\text{m}$  to 800  $\mu\text{m}$ , 500  $\mu\text{m}$  to 900  $\mu\text{m}$ , 500  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 600  $\mu\text{m}$  to 700  $\mu\text{m}$ , 600  $\mu\text{m}$  to 800  $\mu\text{m}$ , 600  $\mu\text{m}$  to 900  $\mu\text{m}$ , 600  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 700  $\mu\text{m}$  to 800  $\mu\text{m}$ , 700  $\mu\text{m}$  to 900  $\mu\text{m}$ , 700  $\mu\text{m}$  to 1000  $\mu\text{m}$ , 800  $\mu\text{m}$  to 900  $\mu\text{m}$ , 800  $\mu\text{m}$  to 1000  $\mu\text{m}$ , or 900  $\mu\text{m}$  to 1000  $\mu\text{m}$ . In an example, the 3D matrix or substrate comprises a pore size from about 0.5  $\mu\text{m}$  to 400  $\mu\text{m}$ .

**[00224]** The stability of 3D substrates may be tuned or otherwise modified for select cell processing applications. Certain 3D substrate materials may be substantially chemically or biologically stable. For example, porous solid matrices formed of materials such as glass, ceramics, or plastics can be considered stable over indefinite timescales because they are not subject to substantial chemical or biological material degradation under cell processing use cases (e.g., cell culture conditions). In some embodiments, the 3D substrate is substantially stable at macroscale and over relevant timescales (e.g., hours, days, or weeks). For example, 3D matrices comprising natural polymeric hydrogels, such as collagen and fibrin, may degrade in the presence of cells by the activity of cell-mediated proteases including matrix metalloproteinases (MMPs). 3D hydrogel matrices comprising synthetic polymers can also be engineered with peptide crosslinkers, which may be designed to degrade in response to cell-mediated protease activity. 3D matrices comprising degradable moieties may be stable at macroscale despite these biological processes. For example, such degradation processes may remodel the 3D matrix locally around cells, thereby permitting cellular proliferation, cellular migration, or both within the local 3D environment. During local remodeling the macroscale matrix may remain substantially structurally intact (e.g., gelled). In some embodiments, the 3D matrix may be substantially degraded during cell culture processes. For example, after a given time the 3D matrix may no longer be substantially structurally intact. In some embodiments, matrix materials may provide “self-healing” properties or other dynamic reorganization properties over time. The “self-healing” properties may permit the 3D matrix to autonomously recover an original state after damage or degradation. 3D matrices configured for dynamic reorganization may permit polymer disentanglement, disruption of weak physical interactions, rearrangement of reversible interactions, degradation processes, or any combination thereof.

**[00225]** The optical properties of 3D matrices may be tuned or otherwise modified for select cell processing applications. For example, the optical properties of 3D matrices used in

3D cell culture may provide in-process monitoring and analysis. In an example, the optical properties of a 3D substrate may determine which imaging and spectroscopic techniques may be effectively applied to observe and measure the biological and physical processes occurring within the 3D substrate during cell culture. In some embodiments, 3D matrices (e.g., hydrogel matrices) with high transparency may be used to enable optical techniques such as measurement of optical density and backscatter. To measure optical density, light with one or more specific wavelength(s) may be emitted into a 3D substrate and the intensity of light transmitted through the 3D substrate may be measured by a detector. A 3D substrate containing relatively more cells or denser cells may transmit less light. Using the Lambert Beer law, the transmitted light signal may be used to calculate the optical density. To measure backscatter, light with one or more specific wavelength(s) may be emitted into a 3D substrate, and a sensor close to the light source may detect the amount or intensity of light scattered back by cells and other particles present in the 3D substrate. A 3D substrate containing relatively more cells or denser cells may backscatter more light. Measuring optical density (OD) and backscatter can be a useful in situ process analytical tool in 3D cell culture, for example, when assessing cell growth, proliferation, or metabolic activity within the 3D matrix. Such measurements may be used to estimate the concentration of cells or other substances in a solution by measuring the absorbance of light at specific wavelengths. In some embodiments, 3D substrates which do not absorb significantly at the measurement wavelength or scatter light, which can interfere with accurate readings, are used to enable optical density and backscatter measurements. In some embodiments, such as those utilizing thicker or more opaque hydrogels, calibration or correction factors can be used to account for reduced light penetration in optical density measurements. In some embodiments, 3D matrices with high transparency may be used to permit the use of microscopy techniques, including high-resolution methods like confocal microscopy. Optical transparency may provide minimal light scattering and absorption, allowing for clear imaging of cells and structures within a 3D matrix. In some embodiments, a 3D matrix such as a hydrogel may exhibit optical scattering properties, particularly with thicker or more densely crosslinked hydrogels. Adjusting the refractive index of the hydrogel to match that of the immersion medium or the cells may help reduce scattering. Matching the refractive index of the 3D substrate with that of the surrounding medium (usually buffer or culture medium) may reduce refraction at interfaces within the imaging field, enhancing image clarity and depth penetration. A matched refractive index may provide for deep imaging, allowing for more effective use of techniques such as multi-photon microscopy. Certain 3D substrates, such as

porous solids and hydrogels derived from natural polymers, may exhibit autofluorescence, which can interfere with fluorescence microscopy. In some embodiments, 3D matrices with low autofluorescence may be used to permit fluorescence imaging techniques, or configuration of an imaging modality using specific wavelengths of excitation and emission (e.g., wavelengths where autofluorescence is reduced or minimal for a certain material) can minimize the negative impact of autofluorescence on optical sensing.

**[00226]** The 3D matrix may be tuned or otherwise modified for optical homogeneity to permit accurate spectroscopic measurements. Inhomogeneities may lead to variable light pathlengths and affect the accuracy of spectroscopic data, such as with Near-Infrared (NIR) and Raman spectroscopy. In some embodiments, a 3D substrate is selected for compatibility with specific spectroscopic techniques. In an example, a 3D matrix is configured to permit Near-Infrared (NIR) Spectroscopy, which can penetrate deeper into 3D matrices such as hydrogels (compared to visible light) making it useful for monitoring cellular processes, water content, solvent interactions, and other bulk properties of a cell-laden 3D substrate. In another example, a 3D matrix is configured to permit Raman spectroscopy, a measurement technique sensitive to molecular vibrations that can provide detailed information about the chemical environment within a 3D substrate. The optical properties of the 3D matrix may be selected or designed to enhance the quality of the Raman signals. The 3D matrix may incorporate or otherwise couple with optical sensors that respond to changes in pH, temperature, specific biomolecules, or any combinations thereof. These sensors may provide real-time, in situ monitoring of the microenvironment which may provide monitoring of dynamic cellular processes. In some embodiments, 3D matrix compositions may support the propagation of light with minimal distortion for technologies like optical coherence tomography (OCT) or photonic crystal-based sensing. Another optical consideration may be the stability of the 3D substrate under light exposure. Some 3D substrates, such as hydrogels, may be susceptible to photodegradation, especially under intense or prolonged light exposure used in certain imaging techniques. This may affect both the structural integrity of the hydrogel and the viability of encapsulated cells. In some embodiments, a 3D substrate which is not susceptible to photodegradation under relevant exposure conditions may be used. As described herein, in some embodiments, the 3D matrix may be optically clear, substantially optically clear, light scattering, opaque, and have other photonic properties such as refractive index and light scattering. In some embodiments, 3D matrices are optically transparent, substantially optically transparent, or otherwise optically suitable for imaging, such as to permit in situ cell imaging, including by microscopy methods such as widefield, brightfield,

light sheet, confocal microscopy, or any combination thereof. In some embodiments, 3D matrices are optically suitable for spectroscopy, such as to permit in situ process analytics using NIR spectroscopy or Raman spectroscopy.

*Electrically conductive 3D substrates*

**[00227]** The 3D matrix may be configured for or may provide electrical conductance. Electrical conductance may be useful for processing cell types that exhibit phenotypically relevant electrochemistry or electrophysiology. In some embodiments, electrical conductance of 3D substrates may permit in situ electroporabilization within a 3D matrix, such as for the purpose of electroporation cell transfection and genetic engineering. In some embodiments, an electrically conductive matrix can be used to incorporate electrical signals as biorelevant physical cues. Electrical biorelevant cues may be useful for the culture and study of electrochemically active cell types such as neurons and cardiomyocytes. These cells may be highly responsive to electrical stimuli, which play a role in their function, development, and communication. Incorporating electrical cues into cell culture environments may enhance physiological relevance and thereby support the formation and maintenance of relevant cellular phenotypes. Direct electrical stimulation may be applied by delivering electrical pulses directly to the 3D substrate, culture medium, or through electrodes embedded in, around, or throughout the cell culture substrate. This method may mimic the natural electrical activity that cells, such as neurons and cardiomyocytes, experience in vivo. The stimulation may be configured in various ways (e.g., amplitude, frequency, pulse duration, or combinations thereof) to match physiological conditions as closely as possible. For neurons, this can promote axon growth and synapse formation, while for cardiomyocytes, it can enhance the maturation, alignment, contraction strength, or any combination thereof.

**[00228]** In some embodiments, the material(s) comprising the 3D substrate, and the 3D substrate itself, may be a weak electrical conductor(s). For example, collagen hydrogel matrices may have electrical conductivity that is generally very low, for example, close to that of an insulator. In another example, PEG is a non-ionic polymer and does not conduct electricity. A 3D substrate formed from PEG hydrogels may be non-conductive unless modified. In some embodiments, the 3D substrate may be considered a volume conductor, or material that allows electric current to pass through its entire volume (e.g., in contrast to surface conductors, where current flows primarily along the surface). Volume conductors may permit current flow through their three-dimensional mass. A 3D substrate may have an electrical conductivity of greater than or equal to about  $10^{-10}$  Siemens per meter (S/m),  $10^{-9}$  S/m,  $10^{-8}$  S/m,  $10^{-7}$  S/m,  $10^{-6}$  S/m,  $10^{-5}$  S/m,  $10^{-4}$  S/m,  $10^{-3}$  S/m,  $10^{-2}$  S/m,  $10^{-1}$  S/m,  $10^0$  S/m,



$10^1$  S/m, or more. A 3D substrate may have an electrical conductivity of less than or equal to about  $10^1$  S/m,  $10^0$  S/m,  $10^{-1}$  S/m,  $10^{-2}$  S/m,  $10^{-3}$  S/m,  $10^{-4}$  S/m,  $10^{-5}$  S/m,  $10^{-6}$  S/m,  $10^{-7}$  S/m,  $10^{-8}$  S/m,  $10^{-9}$  S/m,  $10^{-10}$  S/m, or less. A 3D substrate, depending on composition, may have an electrical conductivity from about  $10^{-10}$  to  $10^{-9}$  S/m,  $10^{-10}$  to  $10^{-8}$  S/m,  $10^{-10}$  to  $10^{-7}$  S/m,  $10^{-10}$  to  $10^{-6}$  S/m,  $10^{-10}$  to  $10^{-5}$  S/m,  $10^{-10}$  to  $10^{-4}$  S/m,  $10^{-10}$  to  $10^{-3}$  S/m,  $10^{-10}$  to  $10^{-2}$  S/m,  $10^{-10}$  to  $10^{-1}$  S/m,  $10^{-10}$  to  $10^0$  S/m,  $10^{-10}$  to  $10^1$  S/m,  $10^{-9}$  to  $10^{-8}$  S/m,  $10^{-9}$  to  $10^{-7}$  S/m,  $10^{-9}$  to  $10^{-6}$  S/m,  $10^{-9}$  to  $10^{-5}$  S/m,  $10^{-9}$  to  $10^{-4}$  S/m,  $10^{-9}$  to  $10^{-3}$  S/m,  $10^{-9}$  to  $10^{-2}$  S/m,  $10^{-9}$  to  $10^{-1}$  S/m,  $10^{-9}$  to  $10^0$  S/m,  $10^{-9}$  to  $10^1$  S/m,  $10^{-8}$  to  $10^{-7}$  S/m,  $10^{-8}$  to  $10^{-6}$  S/m,  $10^{-8}$  to  $10^{-5}$  S/m,  $10^{-8}$  to  $10^{-4}$  S/m,  $10^{-8}$  to  $10^{-3}$  S/m,  $10^{-8}$  to  $10^{-2}$  S/m,  $10^{-8}$  to  $10^{-1}$  S/m,  $10^{-8}$  to  $10^0$  S/m,  $10^{-8}$  to  $10^1$  S/m,  $10^{-7}$  to  $10^{-6}$  S/m,  $10^{-7}$  to  $10^{-5}$  S/m,  $10^{-7}$  to  $10^{-4}$  S/m,  $10^{-7}$  to  $10^{-3}$  S/m,  $10^{-7}$  to  $10^{-2}$  S/m,  $10^{-7}$  to  $10^{-1}$  S/m,  $10^{-7}$  to  $10^0$  S/m,  $10^{-7}$  to  $10^1$  S/m,  $10^{-6}$  to  $10^{-5}$  S/m,  $10^{-6}$  to  $10^{-4}$  S/m,  $10^{-6}$  to  $10^{-3}$  S/m,  $10^{-6}$  to  $10^{-2}$  S/m,  $10^{-6}$  to  $10^{-1}$  S/m,  $10^{-6}$  to  $10^0$  S/m,  $10^{-6}$  to  $10^1$  S/m,  $10^{-5}$  to  $10^{-4}$  S/m,  $10^{-5}$  to  $10^{-3}$  S/m,  $10^{-5}$  to  $10^{-2}$  S/m,  $10^{-5}$  to  $10^{-1}$  S/m,  $10^{-5}$  to  $10^0$  S/m,  $10^{-5}$  to  $10^1$  S/m,  $10^{-4}$  to  $10^{-3}$  S/m,  $10^{-4}$  to  $10^{-2}$  S/m,  $10^{-4}$  to  $10^{-1}$  S/m,  $10^{-4}$  to  $10^0$  S/m,  $10^{-4}$  to  $10^1$  S/m,  $10^{-3}$  to  $10^{-2}$  S/m,  $10^{-3}$  to  $10^{-1}$  S/m,  $10^{-3}$  to  $10^0$  S/m,  $10^{-3}$  to  $10^1$  S/m,  $10^{-2}$  to  $10^{-1}$  S/m,  $10^{-2}$  to  $10^0$  S/m,  $10^{-2}$  to  $10^1$  S/m,  $10^{-1}$  to  $10^0$  S/m,  $10^{-1}$  to  $10^1$  S/m, or  $10^0$  to  $10^1$  S/m. In an example, the 3D matrix or substrate has a conductivity from about  $10^{-5}$  to  $10^{-1}$  S/m.

**[00229]** Incorporating ionic solutions or 3D matrix components with ionic or conductive components may increase matrix conductivity. In an example, the 3D matrix comprises ionic or conductive components that increase matrix conductivity. The cell-laden 3D substrate environment may comprise a conductive material or biomaterial, featuring ionic or electrical conductivity, such as by the presence of mobile ion species as dissolved electrolytes, which may be present in cell culture medias and buffers. For example, cell culture media and buffers may have an electrical conductivity around  $\sim 1.5$  to  $2.0$  S/m, similar to extracellular fluid. The conductivity of such media may be dependent upon the concentration of dissolved ions, such as from added salts (e.g., NaCl, KCl,  $\text{CaCl}_2$ ), nutrients, and buffering agents. The conductivity of cell culture media may aid in maintaining osmotic balance and pH and also permit various cell functions that depend on ion transport and electrical gradients. The conductivity parameter may provide that an applied electrical field effectively interacts with the cells without causing excessive heat or electrolytic effects. In some embodiments, the 3D substrate may provide electrical and electrochemical properties via integration of conductive materials into the 3D substrate, or usage of a 3D substrate comprising electrically conductive materials, including, but not limited to, gold (e.g.,  $\sim 4.1 \times 10^7$  S/m), carbon nanotubes (e.g.,  $\sim 10^5$  S/m), graphene (e.g.,  $\sim 10^6$  S/m), conductive polymers (e.g., polypyrrole,  $\sim 10$ - $100$  S/cm,

PEDOT:PSS, ~0.1-1 kS/cm), or any combination thereof. These materials can conduct electrical signals, provide localized electrical stimulation, facilitate in situ electroporation, or any combination thereof.

**[00230]** In some embodiments, the 3D substrate is filled with an ionic solution acting as an electroporation or membrane electropermeabilization, buffer. The electrical conductivity of electroporation buffers can vary and may be tailored to the specific application and cell type. Lower conductivity buffers (around 0.1 to 1.0 S/m) may be used to reduce joule heating during the electroporation process. Some electroporation processes may use lower or higher conductivities, depending on the field strength and pulse duration used. Electroporation buffers may include phosphate buffers or solutes such as sucrose to maintain osmolarity with minimal ionic strength. The composition and electrical conductivity may be modified to maximize pore formation in the cell membrane while minimizing cell death. Electroporation efficiency may depend on the conductivity and permittivity of the media, cell membrane, cell cytosol, and by cell size and shape. In some embodiments, the electrical conductivity of the 3D substrate and interstitial fluids may be tuned to a specific system configuration and biological application.

**[00231]** As described elsewhere herein, the apparatus and systems for cell processing may further incorporate electrical elements which may couple to or are otherwise in electrical communication with the 3D substrate, ionic solutions, or both to provide electrical interfaces (e.g., stimulation, sensing) with processed cells or to provide membrane electropermeabilization.

#### *Programmable 3D substrates*

**[00232]** A 3D matrix may be functionalized, as shown in **FIG. 12A**. Functionalization of a 3D matrix may provide for a structural microenvironment that enhances or otherwise improves signal transduction. For example, a 3D matrix may be functionalized with cytokines, antibodies, or other components useful for cell processing. Functionalization may be static such that the functionalization does not change during cell processing. Alternatively, the functionalization may change during cell processing such that the 3D matrix may be programmable. A programmable matrix, as shown in **FIG. 12B**, may comprise chemical tethering moieties. The chemical tethering moieties may be configured to permit tethering of biomolecular factors, such as cytokines and antibodies, to the 3D matrix. Functional biomolecules may be removably coupled to the 3D matrix such that, during cell processing, the functional biomolecules may be released from the 3D matrix and removed via washing.

**[00233]** In some embodiments, the 3D matrix is a programmable 3D matrix. Programmable 3D matrices may physically interact with cells by virtue of comprising a 3D local environment in which living cells reside (e.g., with physical properties such as stiffness and porosity). The 3D substrate may further comprise cell-interacting aspects with designed biological properties. Such cell-interacting aspects may include, but are not limited to, factors related to cell adhesion and membrane signal transduction.

**[00234]** In some embodiments, the 3D substrate comprises features which determine cellular adhesivity or attachment. 3D matrix materials may provide complex and mechanistically diverse adhesivity interactions with cells. Cellular surface proteins and the cell membrane may interact with a 3D substrate via steric, electrostatic, hydrophobic, hydrogen bonding, or other interactions. Transmembrane proteins and cell surface receptors, such as integrins and other proteoglycans, may bind to ligands (e.g., individually or synergistically) on or in 3D substrate (e.g., when cognate adhesion moieties are provided by a certain 3D substrate composition), thereby providing cellular adhesion, mechanical interactions with cells, cell signaling, or a combination thereof. The density of various cell adhesion moieties may depend on the composition of a matrix and may be tuned to promote a cellular phenotype, morphology, motility, or other cellular characteristic. In certain embodiments, substrates without intrinsic cell-interaction moieties may still permit cell adhesion through the cellular production of extracellular matrix and other proteins secreted by cells within the matrix, which may form a pericellular matrix with which the cells can interact and onto which the cells can adhere over time. In some embodiments, the matrix is substantially non-adhesive to cells. In some embodiments, the matrix is substantially adhesive to cells.

**[00235]** The 3D matrix or 3D substrate may be functionalized to generate a functionalized 3D matrix or substrate. Functionalizing the 3D matrix may permit the 3D matrix to be stimuli-responsive. Alternatively, or in addition to, functionalizing the 3D matrix may permit the 3D matrix to interact with the plurality of cells, for example, to activate or otherwise stimulate cells of the plurality of cells. Alternatively, or in addition to, functionalizing the 3D matrix may permit the matrix to be a substrate for the plurality of cells or to otherwise provide a given cellular environment to the plurality of cells. The 3D matrix or substrate may be functionalized using pre-functionalized polymer precursors to generate the 3D matrix. For example, pre-functionalized polymer precursors may be provided to a cell chamber in tandem with or in replacement of non-functionalized polymer precursors. The pre-functionalized polymer precursors may be incorporated into the 3D substrate upon polymerization of the

polymer precursors. In an example, cells and pre-functionalized polymer precursors may be provided simultaneously such that formation of the functionalized 3D matrix occurs concurrently entrapment or encapsulation of the plurality of cells. Alternatively, or in addition to, the 3D matrix or substrate may be formed, and functionalization may occur subsequent to polymerization of the 3D matrix or substrate. In an example, functionalizing the 3D matrix comprises coupling or immobilizing one or more biomolecules to the 3D matrix. The one or more biomolecules may be proteins, peptides, cytokines, antibodies, nucleic acid molecules, or any combinations thereof. In an example, the one or more biomolecules may be usable for membrane cell transduction. The one or more biomolecules may remain conjugated or cognate to the 3D matrix during cell processing. Alternatively, or in addition to, the functionalized biomolecules may be released from the 3D matrix or substrate and, in some examples, replaced with other biomolecules.

**[00236]** In some embodiments, the 3D substrate comprises cell-interacting factors. Such factors may provide one or more functions, including for example, without limiting other possible functions, mimicking a tissue microenvironment, simulating or providing an extracellular matrix composition, providing membrane signal transduction, modulating cellular properties including cell adhesion, proliferation, migration, differentiation (e.g., including immune cell stimulation), survival, providing stimulatory, co-stimulatory, pro-survival, or pro-phenotype cellular cues, or any combinations thereof. Cell-interacting factors may include, but are not limited to, matrix proteins, glycoproteins, glycosaminoglycans, proteoglycans, ECM-sequestered growth factors, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), as well as other secreted proteins, or any combinations thereof. For example, peptides may be incorporated into a 3D substrate to mimic the specific molecules, sequences, or moieties found in a natural extracellular matrix that interact with cell receptors, influencing cell adhesion, migration, and fate. RGD or peptides with related motifs may be used to permit cell attachment via the adhesive sequence arginine–glycine–aspartic acid naturally found in the extracellular matrix protein fibronectin, which may enhance cell adhesion by interacting with integrin receptors. IKVAV Peptides (Ile-Lys-Val-Ala-Val) may promote neuronal cell adhesion and neurite outgrowth. YIGSR Peptides (Tyr-Ile-Gly-Ser-Arg) may be used to influence cell attachment and proliferation. As another class of examples, growth factors may be incorporated within the 3D substrate to promote cell proliferation, differentiation, tissue regeneration, or any combination thereof. Fibroblast Growth Factors (FGFs) may be used in neural stem cell cultures to promote proliferation. Vascular Endothelial Growth Factor (VEGF) may be used

to promote angiogenesis. Bone Morphogenetic Proteins (BMPs) may be incorporated for bone tissue engineering to stimulate osteogenic differentiation. In some embodiments, the 3D substrate may comprise signal transduction factors that include, but are not limited to, antibodies, such as those which bind CD3 and CD28 for T cell stimulation, or co-stimulatory factors, such as cytokines and interleukins, including, but not limited to, IL-2 and IL-10. Such factors may be used to modulate cellular immune responses or promote specific pathways of immune lineage cell differentiation, regeneration, and function. In some embodiments, hormones can be used within the 3D substrate to influence hormonal pathways and support the growth and function of hormone-responsive cell types.

**[00237]** Cell-interacting factors may be coupled to a 3D matrix. Alternatively, or in addition to, cell-interacting factors may be non-covalently associated with the 3D matrix via electrostatic interactions, hydrogen bonding, van der Waals interactions, hydrophobic interactions, or any combination thereof. Cell-interacting factors may be covalently coupled to the 3D matrix via crosslinking, immobilization, binding, or any combination thereof. Cell-interacting factors may be encapsulated or otherwise physically retained within the 3D matrix. Cell-interacting factors may be irreversibly coupled to the 3D matrix. Alternatively, or in addition to, the 3D matrix may be configured for controlled release of cell-interacting factors.

**[00238]** In some embodiments, one or more cell-interacting factors are provided via covalent binding or crosslinking to a 3D substrate, which may stabilize the molecules within the 3D matrix or provide a uniform or homogenous and controlled signal (e.g., to present such molecules to cells at a particular average concentration within the 3D substrate, such as an equivalent milligram per milliliter (mg/mL) of a solution-phase formulation). 3D matrix functionalization may be tuned considering the selection of 3D substrate material, crosslinking density, functionalization strategy, or any combination thereof to provide a select bioactive composition for cellular contact and interaction. Alternatively, the 3D matrix may be an inactivated composition, for example, comprising an antibody which may be crosslinked to the 3D substrate in such a manner as to prevent binding or the otherwise targeted biological interaction. Functionalization chemistry may include, but are not limited to, covalent bonding chemistries, photocrosslinking, enzymatic crosslinking, Michael Addition, or any combination thereof. Carbodiimide chemistry may be used for crosslinking proteins to various substrates and involves the use of carbodiimides, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). EDC may activate carboxyl groups to react with amine groups, forming stable amide bonds. This chemistry may be used in conjunction with

N-hydroxysuccinimide (NHS) to improve efficiency. Glutaraldehyde crosslinking may be effective for crosslinking proteins through their amine groups, forming Schiff bases. This chemistry has the benefit of its simplicity and the robustness of the resulting crosslinks. Click chemistry, including copper-catalyzed azide-alkyne cycloaddition (CuAAC) and strain-promoted azide-alkyne cycloaddition (SPAAC), may be highly specific, efficient, and work under mild or biocompatible conditions, making them useful for coupling sensitive biomolecules. Incorporating photocrosslinkable moieties such as benzophenone, aryl azide, or cinnamoyl groups into a biomolecule or polymer may permit crosslinking upon exposure to ultraviolet (UV) or visible light. Photocrosslinking may provide spatial and temporal control over the crosslinking process. A thiol-ene reaction, a UV-induced reaction between thiol groups and alkenes, may generate thioether bonds. Enzymatic crosslinking may include examples such as transglutaminase, an enzyme which catalyzes the formation of covalent bonds between glutamine and lysine residues in proteins, and laccase and peroxidase enzymes, which may catalyze the oxidation of phenolic compounds to form crosslinks, such as those used in the modification of lignin or other natural polymers. Thiol-Michael Addition reactions between a thiol group and an electron-deficient double bond, such as acrylates or maleimides, may have the benefit of cytocompatibility and reactivity under physiological conditions. Certain conjugation chemistries may involve the chemical modification or conjugation of carbohydrates (sugars) to proteins or on carbohydrate-modified proteins. Chemoenzymatic bioconjugation may use enzymes, for example, glycosyltransferases, to attach sugars with specific functional groups to proteins. Utilizing glycosyltransferases to transfer a sugar molecule with a pre-attached functional group (e.g., biotin, or other conjugation group) to targeted sites on a protein to provide highly specific bioconjugation that permits retention of protein functionality for sensitive proteins such as antibodies. These sugars or functionalized sugars may serve as linkers for subsequent conjugation operations. Sugars on proteins may be modified to introduce azides or alkynes, and then used in copper-catalyzed azide-alkyne cycloaddition (CuAAC) or strain-promoted azide-alkyne cycloaddition (SPAAC) to attach various functional groups. Glycosylation sites on proteins may be used to attach polyethylene glycol (PEG) molecules that have been functionalized to react with sugars. This may involve using a linker that can react with the hydroxyl groups of the sugars to form stable bonds. Carbohydrates on the protein surface may be chemically modified to introduce aldehyde or ketone groups, which may be used to form oxime or hydrazone linkages with aminoxy- or hydrazine-functionalized molecules. Other methods of 3D matrix bioconjugation may be used to prepare a biofunctional 3D substrate.

**[00239]** In some embodiments, protein-protein interactions are used to immobilize bioactive proteins, leveraging specific and high-affinity binding pairs to create functional 3D substrates. For example, the biotin-streptavidin interaction, one of the strongest non-covalent interactions in nature, has a remarkably high affinity ( $K_d \approx 10^{-15}$  M). Biotinylated proteins can be immobilized in hydrogels that contain streptavidin or vice versa. In another example, collagen-binding peptides (CBP) can be used to link peptides via specific affinity for collagen molecules. This targeted protein interaction can be leveraged for protein immobilization by incorporating functionalized CBPs into a 3D matrix comprising collagen. The His-Tag and Ni-NTA (Nitrilotriacetic Acid) interaction may use short sequences of histidine residues added to proteins, which can bind to nickel ions on Ni-NTA groups integrated within a 3D substrate. This interaction may further be used for reversible immobilization as described elsewhere herein. Incorporating Protein A or G into a 3D substrate can facilitate the immobilization of antibodies. Protein A and Protein G have high affinity for the Fc region of IgG-type antibodies. In some embodiments, multivalent linkers may be employed to increase functionalization efficiency and control the density of cell-interacting factors within a 3D substrate. For example, four-arm, six-arm, eight-arm, ten-arm, twelve-arm, and other multi-armed PEG molecules, which may be provided in particular molecular weights or distributions thereof, further comprising chemical conjugation groups (e.g., 4-arm PEG Succinimidyl Carboxymethyl Ester), can be used to create stable linkages between 3D substrates and cell-interacting factors.

**[00240]** In some embodiments, one or more cell-interacting factors are provided via non-covalent binding to a 3D substrate. Transient binding, such as between positively and negatively charged polymers, proteins, or other biomolecules, can retain factors via electrostatic interactions. Hydrophobic interactions, such as those driven through self-assembly of amphiphilic molecules, which aggregate in aqueous environments to minimize exposure of their hydrophobic segments, can retain factors within a 3D substrate. In some embodiments, transient binding of one or more cell-interacting factors to a 3D substrate may be stabilized by additional crosslinking, such as by using the chemistries described elsewhere herein.

**[00241]** In some embodiments, the 3D matrix may be configured with a controlled release mechanism that provides cell-interacting factors within the 3D matrix. The controlled release mechanism may include encapsulation, cleavable linkers, ligand-binding interactions, or any combination thereof. In some embodiments, the cell-interacting factor(s) are one or more proteins. In some embodiments, the cell-interacting factor(s) are viral vectors. In some

embodiments, the cell-interacting factors comprise both proteins and viral vectors. Protein encapsulation may provide controlled release of bioactive factors into a culture environment or within a biological system such as a 3D matrix. Protein encapsulation may provide for the localized and sustained release of proteins and other factors that can, for example, direct cell behavior or modulate immune responses. Controlled release mechanisms may involve entrapping proteins or other factors within a carrier material, such as a hydrogel, nanoparticle, microsphere, liposome, or combination thereof. The carrier material may protect the molecule from degradation and permit controlled release through various mechanisms. In a diffusion-controlled release system, the protein or other factor may diffuse out of an encapsulating material over time. Diffusion may be driven by a concentration gradient. The rate of diffusion can be adjusted by modifying the porosity and crosslinking density of the encapsulation material. In a degradation-controlled release system, the encapsulating material may be biodegradable and, as it breaks down, the encapsulated proteins or other cell-interacting factors may be gradually released. The degradation rate can be tuned by the selection of materials or crosslinking agents in encapsulation materials.

**[00242]** In a stimulus responsive release system, the encapsulating material may be designed to respond to specific stimuli (e.g., pH, temperature, enzyme activity, or any combination thereof) present in the environment. The stimulus may trigger the release of protein(s) or cell-interacting factor(s). For example, proteins can be encapsulated within alginate beads using ionic crosslinking with calcium ions. The release rate may be controlled by the ionic strength and pH of the medium, which alter the stability of the alginate matrix. In another example, a temperature-sensitive hydrogel, such as poly(N-isopropylacrylamide) (PNIPAM) may undergo a phase transition at a given temperatures. The phase transition may release encapsulated proteins in response to a temperature changes. In another example, PLGA microspheres formed from Poly(lactic-co-glycolic acid) (PLGA), a biodegradable polymer which degrades over time, may release encapsulated proteins upon hydrolytic degradation and other processes which degrade the polymer matrix. Polymer composition and molecular weight may alter or modify the degradation rate of such encapsulation materials, and thus the release kinetics. In another example, dendrimers or other branched synthetic polymers can encapsulate proteins or other cell-interacting factors within their structure, such that the release can be controlled by the design of the polymer, including its size and surface functionality.

**[00243]** In an example, proteins or other cell-interacting factors may be provided to the 3D matrix in liposomes. Liposomes may be spherical vesicles with a lipid bilayer that can



encapsulate hydrophilic proteins or cell-interacting factors within their aqueous core. The liposomes may be introduced within the 3D substrate environment and release a payload over time via liposomal destabilization and leeching. Modifications to the liposome structure, such as the inclusion of pH-sensitive or thermosensitive lipids, can further allow for the release of proteins from liposomal formulations in response to environmental changes. In another example, hydroxyapatite (HA) particles can be used for the sustained release of cell-interacting factors such as viral particles within a 3D substrate. Hydroxyapatite can adsorb proteins and other biological molecules onto its surface due to its high surface area and porous structure, or by encapsulation of these molecules within its matrix, depending on formulation. Viral particles can be bound to HA particles, allowing for their gradual release as the HA degrades or as the particles desorb from the surface. The release rate can be adjusted by modifying the particle size, porosity, and surface characteristics of HA. For example, smaller particles or higher porosity particles may provide faster release rates than larger particles or particles with lower porosity. Viral particles and other cell-interacting factors can be encapsulated within biodegradable fibers, with release rates tailored by the fiber composition and structure. Viral particles can be encapsulated within fibers produced by electrospinning and other methods. These fibers may provide a high surface area and can be engineered to degrade over targeted timeframes, releasing cell-interacting factors such as viral particles. Combining different polymers or incorporating nanoparticles into the fibers can further refine the release profiles and enhance the stability of the factors such as viral particles. Other types of nanoparticles, such as silica nanoparticles, and protein-based matrices, such as those formed from gelatin, collagen, and silk, can adsorb factors such as viral particles onto their surface or encapsulate them within their porous structure, providing protection and a controlled release mechanism. Each controlled-release mechanism or encapsulation method may offer unique advantages and can be selected based on the specific requirements of the application, such as the targeted release profile, the stability of the factor(s), and the environmental conditions of the cell processing system. These strategies may permit precise control over the spatial and temporal availability of bioactive factors, enhancing the effectiveness of cell processing systems.

**[00244]** In some embodiments, elastin-like polypeptides (ELPs) may be used for the immobilization, controlled release, or both of cell-interacting factors within a 3D substrate. ELPs may comprise repeating pentapeptide sequences (Val-Pro-Gly-Xaa-Gly, where Xaa can be any amino acid except proline) that exhibit Lower Critical Solution Temperature (LCST) behavior. Below the LCST, ELPs may be soluble in water. Above the LCST ELPs may

undergo a transition to become insoluble and aggregated in water. This property can be harnessed to control the solubility and localization of ELP-tagged proteins within 3D matrices. By adjusting the environmental temperature to just above the LCST, ELPs can be induced to form aggregates, effectively capturing and immobilizing the linked proteins within the matrix. ELPs can be chemically modified to include reactive groups (such as acrylates). Through copolymerization or crosslinking reactions, ELPs can be integrated into a 3D matrix structure. This integration provides that when the ELP undergoes a phase transition, the linked protein(s) may be retained within the matrix, allowing for controlled spatial distribution. Proteins of interest can be genetically fused to ELP sequences. This fusion may permit proteins to inherit the phase transition properties of the ELP, providing responsive and reversible immobilization within 3D matrices. ELPs can be used to encapsulate proteins within microspheres or nanoparticles that are then embedded in a 3D substrate. Upon temperature change, these particles undergo a transition that can control the release rate of the encapsulated proteins. ELPs can also contribute to or increase the mechanical reinforcement of hydrogels. By behaving somewhat like natural elastin, they can impart elasticity and durability to a 3D matrix, which may be beneficial for creating scaffolds that mimic a natural extracellular matrix.

**[00245]** The linkages between or spatial colocalization of the cell-interacting factor(s) and a 3D matrix may be formed external to a cell processing system or on demand within the cell processing system. In some embodiments, a pre-functionalized 3D substrate is provided, such as a functionalized porous solid support material contained within a suitable consumable apparatus. In some embodiments, a 3D matrix precursor solution, which comprises polymers functionalized with cell-interacting factor(s), is provided, optionally mixed with a population of cells and allowed to gel, forming a functionalized cell-interacting 3D substrate. In some embodiments, a stock 3D matrix precursor solution comprising polymer(s) bearing attachment moieties may be provided such that upon mixing with one or more additional reagent(s) (e.g., reagents including cell-interacting factors), a functionalized cell-interacting 3D matrix or functionalized cell-interacting 3D matrix precursor solution (e.g., which upon gelation forms a functionalized cell-interacting 3D matrix) is formed. In some embodiments, a 3D substrate bearing attachment moieties or a 3D matrix precursor solution comprising attachment moieties may be provided and subsequently an aqueous reagent comprising at least one cell-interacting factor is provided and brought into contact with the 3D substrate, thereby forming linkages between the 3D substrate and the cell-interacting factor(s).

[00246] In some embodiments, a 3D matrix precursor solution comprising a controlled release formulation of cell-interacting factor(s) is provided. The 3D matrix precursor solution may be mixed with cells and permitted to gel, thereby forming a functionalized 3D substrate with controlled-release of cell-interacting factors. In some embodiments, a stock 3D matrix precursor solution is provided, and separately, a controlled release formulation of cell-interacting factor(s) is provided, such that upon mixing and gelation, a 3D substrate comprising controlled-release of cell-interacting factors is generated. In some embodiments, a 3D substrate, or a 3D matrix precursor solution is provided and subsequently an aqueous reagent comprising at least one controlled-release formulation of cell-interacting factor(s) is provided and brought into contact with the 3D substrate, thereby forming a 3D substrate comprising controlled-release cell-interacting factor(s).

[00247] In some embodiments, a functional aspect of a 3D substrate, such as a linkage between the 3D substrate and a cell-interacting factor, may be reversed, removed, cleaved, or any combination thereof. Linkages may be generated from any reversible conjugation or 3D deposition method, or reversible controlled-release formulation. These reversible linkages may respond to specific stimuli (e.g., chemical, thermal, physical, enzymatic, etc.) or incorporate mechanisms for competitive displacement. Cleavable linkers may be stable until exposed to certain internal or external stimuli, after which they cleave and release the conjugated molecule. Stimuli responsive linkers may include, but are not limited to, pH-sensitive linkers (e.g., hydrazone), enzyme-responsive linkers (e.g., enzyme cleavable peptide linkers), redox-responsive linkers (e.g., linkers comprising disulfide bonds configured to cleave in a reducing environment), and photo-cleavable linkers (e.g., O-Nitrobenzyl derivatives which cleave upon UV or near-UV light exposure). In an example, the linker is hydrazone which may be stable at physiological pH and cleaves in acidic environments. In another example, the linker is a peptide linker configured to be cleaved by matrix metalloproteinases (MMPs). Alternatively, a competitive displacement mechanism may be used in which binding of a bioconjugated molecule may be reversed by introducing a competitive molecule that has a higher affinity for the binding partner to displace the first molecule. For example, a cell-laden 3D matrix functionalized with one or more cell-interacting factors may be provided. Contacting the cell-laden 3D matrix with a reagent or other stimulus which reverses a linkage between the cell-interacting factors and the matrix may release the factor(s) from the 3D matrix to remove the factors from the 3D microenvironment. Released factors may be removed from the 3D microenvironment by washing, diffusion, fluid flow, or any combination thereof.

**[00248]** In some embodiments, a combination of conjugation and cleavage chemistries are used to temporally program signaling cascades using a 3D matrix to deliver cellular cues with specified timing, such as for a specified duration. These capabilities can be used to precisely control complex stimulatory, co-stimulatory, pro-survival, and pro-phenotype cellular cues to provide biological controls over cellular processes and products. For example, a cell-laden 3D matrix substrate comprising one or more functionalization moieties is provided. An aqueous reagent comprising one or more component(s) may be introduced into and brought into contact with the 3D matrix such that the functional component is incorporated into or onto the matrix via the functionalization moieties (e.g., by binding, chemical bonding, ionic bonding, electrostatic interaction, etc.). The 3D substrate may be contacted with a second reagent or other stimulus which reverses the linkage between the first component(s) and the matrix functionalization moiety. Reversing the linkage may release the first component(s) such that it may be removed from the 3D microenvironment via washing, diffusion, fluid flow, or any combination thereof. A third reagent comprising one or more functional component(s) may be introduced into and brought into contact with the 3D substrate such that the functional component is incorporated into or onto the 3D matrix via the first or a second functionalization moiety. Combination or combinatorial functionalization may provide dynamic or iterative temporal control and programming of the of 3D microenvironment. Dynamic or iterative temporal control may permit the use of multiplexed or concurrent cellular signaling workflows. The use of two or more orthogonal functionalization moieties or chemistries further permits temporally dynamic and controllable programs for cellular membrane signal transduction.

**[00249]** In some embodiments, the 3D substrate may be sterilized prior to use in cellular processing. Sterilizing the 3D substrate may reduce or prevent contamination during cell processing and may permit clinical-regulatory suitability of using 3D matrices in cell processing workflows. 3D substrates may be sterilized before cell processing (e.g., cell culturing) using temperature (e.g., autoclave), gamma or germicidal UV irradiation, ethylene oxide exposure, ethanol treatment, dense carbon dioxide gas sterilization, other methods, or combinations thereof. For workflows including a gel-sol transition, the precursor solution(s) may be sterilized before matrix formation. Sterilization of the precursor may include, but is not limited to, filtering (e.g., of lower viscosity solutions), gamma or germicidal UV irradiation of the solution or dry polymer, temperature (e.g., autoclave), or any combination thereof. The sterilization method used may be selected based on compatibility and cell processing application. For example, sterilization methods that do not degrade, denature, or

otherwise alter the physical and biological properties of the 3D substrate material(s) may be used. 3D substrate materials used in the manufacture of clinical products, such as for human use, may be subject to rigorous safety and performance standards. For example, a 3D substrate material may be selected for biocompatibility properties or capacity for facile or substantially complete removal (e.g., within an acceptance criteria) from a formulated product. A 3D substrate material which may carry over into a formulated product may be selected from a group of materials which does not evoke harmful immune reactions, cause toxicity, or lead to infection. Alternatively, a 3D substrate material may be selected or designed based on its capacity for facile or substantially complete removal from a formulated product. A 3D substrate material may be selected from a group of materials which do not or substantially do not carry over to product formulation. For example, the 3D substrate material may be removed during cell washing. A 3D substrate material may be selected for nonspecific interactions with cellular product. For example, materials may be selected that have little or no nonspecific interactions with the cellular product to reduce carry over of the material into the product. A 3D substrate material may be selected for the ability to remove the material from the formulated product. For example, the material may be easily washed away, degraded, or both to reach an acceptable level within the cell product. A 3D substrate may be selected or manufactured to minimize the presence of endotoxins, which can cause severe inflammatory responses, and may be subject to regulated limits in formulated clinical products. A 3D substrate material may be selected from a group of materials which can be manufactured consistently to provide uniformity in produce attributed or clinical outcomes. Uniformity may be subject to regulatory approval. The specific for these attributes can vary based on the intended application and regulatory guidelines.

*Reversible gel-sol 3D substrates*

**[00250]** In some embodiments, a 3D substrate is formed using a matrix composition which undergoes stimulus-responsive or reversible gel-sol transition. For example, a stimuli-responsive hydrogel may be used which responds to physical or biochemical stimuli. Physical or biochemical stimuli may include, but are not limited to, temperature, light, electromagnetic fields, acoustic waves, pH, ions, crosslinkers, other reagents, or any combination thereof.

**[00251]** Thermoresponsive 3D matrices may exhibit changes in their physical state or properties in response to temperature changes. This behavior may be governed by critical temperatures that define transitions in a 3D matrix's solubility and structure. The 3D matrix may exhibit a first phase at temperatures below the Lower Critical Solution Temperature

(LCST) and a second phase at temperatures above the Upper Critical Solution Temperature (UCST). LCST may be the temperature below which a polymer is soluble in a given solvent and above which it becomes insoluble. When the temperature is raised above the LCST, hydrophilic-hydrophobic balance may shift towards hydrophobicity due to changes in polymer-solvent interactions. The shift towards hydrophobicity may cause the polymer to aggregate and precipitate out of solution or form a gel. This behavior often results from an increase in hydrophobic interactions within the polymer chains that overcomes the polymer-solvent interactions as temperature rises. For polymers like Poly(N-isopropylacrylamide) (PNIPAM), the hydrogen bonding with water that maintains solubility at lower temperatures is disrupted at higher temperatures, leading to phase separation. Such materials may exhibit reversible phase transitions to permit re-solubilization of the matrix substrate upon reduction in temperature. This temperature-controlled gel-sol transition may permit facile harvesting of cell products. Polymers which may undergo thermally responsive gel-sol transition include, but are not limited to, gelatin, collagen, cellulose, chitosan, starch, carrageenan, hyaluronic acid, xanthan, xyloglucan, elastin, dextran, or combinations thereof. Additional chemical modifications may alter the thermal responsiveness of polymeric hydrogel materials. For example, methacrylated collagen may be capable of reversible thermal gel-sol transitions where the thermal gelation of natural collagen may be thermally irreversible.

**[00252]** Other classes of stimulus-responsive 3D substrate materials which may be used include materials that are pH-responsive, chemical-responsive, enzyme-responsive, electro-responsive, photo-responsive, or combinations thereof. pH responsive polymer materials may comprise polymers with pendant acidic or basic groups which either accept or donate protons in response to changes in pH, resulting in gel-sol transition. Chemically responsive polymer materials may use reversible chemical bonds to mediate gel-sol transition. Examples of materials and mechanisms with reversible chemical bonds include but are not limited to: those which feature disulfide bonds, such as in the PEGSSDA crosslinker; ionic condensation of alginate, which is crosslinked in the presence of cations such as  $\text{Ca}^{2+}$  and reversed by chelators such as EDTA; and reversible chemical bonds that may be broken by introduction of a competitive molecular species, e.g. FLAG-tag-based interactions which may be broken by introduction of competing FLAG peptides. Enzyme-responsive materials, such as those composed of naturally derived materials such as collagen (collagenase), fibrin (nattokinase), and HA (hyaluronidase) may permit gel-sol transition and cell liberation by degradation of the hydrogel matrix in the presence of, or upon addition of, or under the activity of, enzymes in a suitable buffer and reaction environment. When using enzyme-responsive materials, care

may be taken not to disrupt cell surface receptors through extended enzyme treatment. Use of a specific enzyme, such as collagenase, may provide benefits over non-specific proteases. Electro-sensitive hydrogels may be responsive to electric fields, which affect changes in their internal polymeric binding, structure, or both. For example, polyelectrolyte polymer materials may contain ionizable groups in the polymer backbone or side chains. Examples include synthetic polymers such as polyvinyl alcohol, polypyrrole, polyaniline, polythiophene, acrylic acid/vinyl sulfonic acid, and sulfonated polystyrene. Light-sensitive polymer materials may respond to particular wavelengths of light. For example, a gel matrix comprising deoxycholic acid-modified  $\beta$ -cyclodextrin derivative and an azobenzene-branched poly(-acrylic acid) copolymer may convert from gel to solution phase upon exposure to a first light (e.g., 355 nanometer (nm) wavelength light) and transition from solution phase to gel upon exposure to a second light (e.g., 450 nm light) irradiation. As described herein, other embodiments of 3D matrix substrates do not undergo gel-sol transition, such as in the case of a static or pre-formed matrix, such as a porous solid matrix.

**[00253]** In some embodiments, a liquid 3D matrix precursor solution is provided, mixed with cells, and subjected to gelation (e.g., via application of a stimulus) to form a 3D matrix. For example, a precursor solution, such as of a PEG-PNIPAM polymer, or of a collagen or collagen-based polymer, may be provided, which is in a liquid phase at 4°C, but undergoes a gel-sol transition when raised to 37°C, a suitable temperature for cell processing. In another example, a precursor solution, such as of an alginate or alginate-based polymer, may be provided, which is in a liquid phase until mixed with a suitable cation source (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Br}^{2+}$ , or  $\text{Sr}^{2+}$  salt solution). The precursor solution may be mixed with cells and loaded into a cell processing compartment (e.g., compartment of a bioreactor). A salt solution may be added to the compartment resulting in gelation of the 3D matrix. Subsequently, a stimulus may be provided which reverses the gel-sol transition. For example, the compartment temperature may be lowered to 4°C, resulting in gel-sol transition of thermally-responsive hydrogel matrices, or a ion chelator such as EDTA may be introduced to an alginate-based chemically-responsive hydrogel matrix, resulting in gel-sol transition. The liquid comprising the solubilized gel matrix material and cells may be harvested, such as by removing the liquid mixture from the compartment and washing or otherwise isolating the cells for downstream use as a liquid suspension. In some embodiments, a 3D substrate comprising a stimulus-responsive gel-sol transition property is provided, and cells may be seeded onto or into the 3D matrix. Stimulus may be provided which reverses the gel-sol transition facilitating the harvest of cells from the solubilized gel matrix material.

*Alternate 3D substrate configurations*

**[00254]** In some embodiments, a 3D substrate or cell-matrix may not comprise a macroscale monolithic matrix composition. For example, the 3D substrate may comprise other configurations, including liquid-like solids, colloidal solutions, packed bed, and dense suspensions (e.g., colloidal mixture configurations), which may also provide a matrix- or 3D-like environment and function at least in part in retaining cells. In some embodiments, the 3D substrate resembles a colloidal mixture, in which the matrix substrate comprises suspended, dispersed insoluble particles, including porous particles such as beads, gel beads, or gels, and ribbon-like and fibrous particles. Such configurations, despite not forming a monolithic hydrogel or monolithic 3D matrix, may exhibit hydrogel or matrix-like properties when densely suspended in a fluid medium such as cell culture medium. In some embodiments, a colloidal formulation of 3D substrates may be suspended within a porous matrix, such as a hydrogel, effectively forming an interpenetrating network of 3D matrix substrates supporting cell processing. The compositions of matter, chemistries, and methods described herein relating to 3D matrices, programmable substrates, membrane signal transduction, biological properties, reversible gel-sol, and other features described elsewhere herein may be utilized in the described non-monolithic matrix configurations.

**[00255]** In some embodiments, a solid-state bioreactor or cell processing system may be configured with a cell compartment which does not comprise a monolithic 3D substrate, such as a monolithic porous solid scaffold or a monolithic 3D matrix such as a hydrogel. In some embodiments, a solid-state bioreactor or cell processing system may be configured to permit maintenance of a cell compartment and optionally a perfusion compartment. For example, a 3D substrate may comprise a dense colloidal solution of particles, ribbons, or other substrates that may support cell culture and provide a cell processing environment similar to the systems and methods described elsewhere herein. For example, a system comprising a non-monolithic substrate may further comprise integrated sensing, perfusion flow, in situ genetic engineering, or any combination thereof. Such a configuration may make use of a perfusion flow configuration which does not substantially disturb or suspend the 3D substrate environment, such as a low flow rate or a flow configuration which applies minimal disruptive fluidic forces (e.g., shear) to the 3D substrate. A solid-state bioreactor or cell processing system may be configured with a semi-permeable membrane interface between the cell and perfusion or fluid flow compartments, which may retain or substantially retain both the cells and 3D substrate in the cell compartment (e.g., a pore or filtration size that, for example, neither microgels or fibers forming a 3D substrate nor the cells can pass through).



In such a configuration, nutrient supply and other reagents may be primarily delivered via diffusion from the perfusion compartment, by secondary flows directed through a cell compartment, or both. In such a configuration, nutrient supply and other reagents may be provided via other solid-state bioreactor configurations providing described herein to provide nutrients and other materials to the 3D substrate, such as those using vessels including flasks and bags.

**[00256]** A 3D matrix may be in contact with fluid within the fluid flow path or may be separated from the fluid flow path, as shown in **FIG. 13**. The 3D matrix may be separated from the fluid flow pathway by a membrane. The membrane may be porous such that reagents and waste may diffuse through the membrane. Alternatively, the interface between the 3D matrix and the fluid flow path may be a liquid interface such that the fluid flowing in the fluid flow path directly contacts the 3D matrix. In another example, the interface between the fluid flow path and the 3D matrix may be a thin gel layer. The thin gel layer may be a hydrogel. The thin gel interface may be configured such that reagents and waste may diffuse through the thin gel to or from the fluid. As described herein, the 3D matrix may be a stimulus-responsive 3D matrix. The 3D matrix may be configured to retain a variety of cellular substrates. For example, cells may be directly embedded within the 3D matrix. In another example, cells may be disposed in or on two dimensional (2D) carriers (e.g., ribbons), 3D carriers (e.g., gel beads), 2D beads (e.g., magnetic beads), microporous scaffolds (e.g., polystyrene mesh), or any combination thereof. Carriers or scaffolds comprising cells may then be encapsulated or embedded within the 3D matrix. In some examples, carriers or scaffolds comprising cells may be disposed in a solution and not encapsulated or embedded within a 3D matrix. In such examples, the carriers or scaffolds may be retained within the cell compartment by a membrane disposed between the cell compartment and the fluid flow pathway.

#### *Cell loading*

**[00257]** Various systems and methods may be used to provide components into a cell compartment or 3D matrix within a cell compartment. Systems and methods may provide that cells are localized or substantially localized in the 3D substrate, cell compartment, or both. Alternatively, cells may be localized or substantially localized within a region or area of a bioreactor or cell processing system in fluid communication with cell culture medium or perfusion flow. In some embodiments, one or more liquid 3D substrate precursor(s) are provided, mixed with one or more cell source(s), introduced to the solid-state bioreactor or a compartment thereof, and permitted to or directed to form a 3D substrate containing,

embedding, or encapsulating cells. For example, a solid-state bioreactor may be configured with, or configured to interface with a cell processing system with, a mixing vessel (which may function to mix cells with a liquid 3D matrix precursor solution and, in some examples, other reagents continuously or in batches). In another example, a solid-state bioreactor may be configured with, or configured to interface with a cell processing system with, a mixing module in a flow configuration such that a 3D matrix solution(s), cells, and, in some examples, other reagents are brought together and mixed in a continuous or semi-continuous manner as they are transferred within or into a bioreactor, cell processing unit, or compartment thereof. Methods may provide that the cell-laden 3D matrix is localized or substantially localized to a region or compartment of a bioreactor, such as a region or compartment configured to be in fluid communication with a cell culture media or perfusion flow. In an example, a sacrificial material, such as 3D matrix material not containing cells, another liquid, or a gas, may be introduced into the fluidic system and used to push the cell-laden matrix volume into a targeted position within the bioreactor. In another example, a negative pressure may be applied to pull the cell-laden matrix volume into a targeted position within the bioreactor (e.g., as shown in **FIG. 15**). As another example, an extrusion, deposition, or printing method may be used to place a cell-laden matrix volume in a targeted position within the bioreactor. These and other methods may be used in any combination. The solid-state bioreactor may be configured with fluidic paths and systems which minimize dead volumes of liquid cell-matrix mixtures.

**[00258]** Cells may be loaded into or onto a 3D substrate using any retention, encapsulation, or adhesion methods. In an example, a plurality of cells is encapsulated or entrapped within the 3D matrix. In an example, the 3D matrix or substrate is formed in presence of the plurality of cells to generate a 3D matrix encapsulating the plurality of cells. The 3D matrix encapsulating the plurality of cells may be generated internal to or otherwise within a compartment (e.g., cell compartment) as described elsewhere herein. Alternatively, the 3D matrix encapsulating or entrapping the plurality of cells may be generated external to the compartment and, subsequently, loaded into the cell compartment. In an example, the cell processing unit may be configured to receive a pre-formed 3D matrix with or within the plurality of cells. In an example, the plurality of cells may be mixed with matrix precursors in a solution and the solution may be provided to a compartment of the cell processing system or bioreactor and polymerized in place. Polymerizing the 3D matrix in place may comprise applying a stimulus to the matrix or polymer precursors. In an example, the stimulus may be applied to the matrix or polymer precursor in a compartment of the cell processing system or

bioreactor to generate the 3D matrix encapsulating the plurality of cells in situ. As described elsewhere herein, the stimulus may be a thermal stimulus, chemical stimulus, enzymatic stimulus, or any combination thereof. In another example, the 3D matrix may be generated in absence of a plurality of cells, either internal or external to a compartment, and the plurality of cells may be added to the pre-formed 3D matrix such that the cells adhere to the 3D matrix, migrate into the 3D substrate, or both.

**[00259]** In some embodiments, cells may be loaded or otherwise deposited into a preformed 3D cell culture substrate. For example, a cell source in suspension may be introduced to the bioreactor comprising a 3D substrate such that cells are passively deposited, or permitted to or directed to, actively penetrate the 3D substrate to become substantially retained in the 3D matrix. In another example, a solid-state bioreactor may be configured with a 3D substrate cell compartment and perfusion compartment oriented such that cells introduced to the perfusion compartment, as through a fluid flow, settle onto and penetrate into the 3D substrate by gravity. In some embodiments, the 3D substrate component of the solid-state bioreactor or cell processing system may be configured to entrap cells within the 3D matrix environment. For example, a 3D substrate may be provided in the solid-state bioreactor apparatus which is configured with a matrix environment promoting cellular retention or cellular migration into the 3D substrate. In some embodiments, the 3D substrate is functionalized (e.g., statically or programmatically) with factors that promote cellular interactions and such factors may promote the specific retention of cells in the matrix, or the specific migration of cells into the matrix where they are substantially retained. In some embodiments, substrate functionalization may be configured to promote retention or migration of cells of a specific type or nature. For example, a 3D substrate of a solid-state bioreactor may be configured with a 3D microenvironment that attracts or retains cells of a specific type due to the nature of the 3D microenvironment. In another example, a 3D substrate of a solid-state bioreactor may be configured with a 3D microenvironment that supports the viable culture of a specific type of cell, such that other types of cells are unable to maintain a viable cell state or proliferate. In some embodiments, a solid-state bioreactor may be configured with a stimulus-responsive 3D substrate capable of attracting or retaining cells upon a stimulus which modulates one or more properties of the 3D substrate. For example, the solid-state bioreactor may be configured with a stimulus-responsive 3D matrix which modulates the porosity of the substrate. Subsequent to bringing a cell suspension in contact with the 3D substrate in a highly porous configuration, such as by the addition or seeding of cells, a stimulus (e.g., a crosslinker) may be applied which causes a reduction in

porosity of the 3D substrate, thereby causing the cells to become entrapped or substantially retained within the substrate.

**[00260]** Cells may be loaded into a pre-formed matrix or, alternatively, a matrix may be generated in presence of a plurality of cells, as shown in **FIG. 14**. Cells may be loaded into a pre-formed matrix by flowing cells into a compartment comprising a pre-formed 3D matrix. One side of the compartment may comprise a membrane or other interface configured to retain the 3D matrix. The cells may adhere to the 3D matrix and migrate into and proliferate within the 3D matrix. The cells may settle into the 3D matrix due to gravity. In another example, the cells may be mixed with polymer or gel precursors. The polymer or gel precursors may be stimuli responsive such that the 3D matrix is formed or degraded by the application of a stimulus (e.g., thermal, chemical, or enzyme stimulus). The 3D matrix may be a thermoresponsive matrix that is a liquid or semi-liquid at low temperatures and solid at higher temperatures. The cells may be embedded or encapsulated in the matrix upon formation of the matrix.

**[00261]** The 3D matrix may be generated in a cell compartment. Alternatively, as shown in **FIG. 15**, the 3D matrix may be pre-formed and loaded into a cell compartment. A preformed 3D matrix may be loaded via a pressure differential or application of a mechanical force. In some examples, the cell compartment includes a sacrificial 3D matrix that is pushed out via the non-sacrificial 3D matrix or pulled out via application of negative pressure.

**[00262]** In some embodiments, a solid-state bioreactor comprises a plurality of hollow fibers within a casing and cell-laden 3D substrate is substantially localized to the region of the intracapillary space (IC) in fluid communication with the extracapillary space (EC) of the casing for perfusion. In some embodiments, a solid-state bioreactor may be configured with a hollow fiber structure with significantly reduced head space and fluidic system dead volume compared to existing hollow fiber bioreactors. In some embodiments, a solid-state bioreactor configured with a hollow fiber design may be configured with features that permit localizing the cell-matrix substantially into the fibers (and not in the head space and dead volume), such as those described above. Alternatively, a hollow fiber configuration that provides a substantially spatially uniform, defined, or adequate distribution of hollow fibers within a casing may permit loading the EC with a cell-laden 3D substrate that is in substantially uniform fluid communication with perfused media in the IC. Such a configuration may also utilize modified head space, dead volume, or loading procedure to maximally localize the cell-matrix to the perfused compartment of the EC.

*Cell culture and expansion*

[00263] In some embodiments, a solid-state bioreactor is configured to culture cells to produce cellular biomass, cellular products, or both. In an example, the cell product is a cell therapy product. Cellular biomass growth can be divided into several distinct phases. Biomass growth can comprise an initial lag phase, characterized by a low growth rate while cells are adapting to a new environment inside the bioreactor. During an exponential growth (or log) phase, cell division may continue at a constant or near constant rate. As nutrients and free space are depleted and by-products accumulate, the rate of cellular biomass growth may slow, and the culture may enter a stationary phase or cellular biomass may decline. The duration of each phase may be dependent on a number of factors including the initial seeding density, expansion or proliferation rate, culture media, cell type, or other bioreactor and cellular factors. A solid-state bioreactor may be usable for various cell culture methods. In some embodiments, a perfusion method that minimizes environmental variation throughout biomass growth and provides a targeted cellular environment may be used. For example, in a continuous perfusion configuration, fresh media is provided and spent medium is removed continuously, at specific or variable rate to maintain a fixed total volume in the perfusion fluidic system. Alternatively, the total volume of the fluidic system may be variable, for example, by using a fluid reservoir within the fluidic system that can accommodate variable volumes. In some embodiments, a solid-state bioreactor is configured to perform a static batch culture process such that the bioreactor is loaded with a fixed working volume of culture media used for the duration of the culture period, and media is not replaced during the period of cell incubation. While such a configuration may provide cell culture for a specified duration or may produce lower cell densities and production yields compared to batch-fed and perfusion processes, such a configuration may be suitable for certain applications including short-term culture during which other cell bioprocessing operations are used. For example, a solid-state bioreactor may be provided in a batch static configuration for a rapid cell therapy production process (e.g., T-Charge, FasT-CAR), in which cells are not substantially expanded, or are not permitted to substantially proliferate during manufacturing, but instead are maintained in a viable state during cell selection, activation, gene engineering, and other bioprocess operations. In such a configuration, a perfusion compartment may not exhibit flow (e.g., is configured with a static media volume). In such a configuration, the bioreactor may be further configured with a gas-exchange capability (e.g., via using a gas-permeable membrane as described elsewhere herein), such as to continuously supply oxygen to the static media. In an example, a solid-state bioreactor may be configured with a perfusion compartment that provides fluid flow, such as for the purpose of recirculation or gas

exchange without providing fresh media to the volume of cell media in the perfusion fluidic compartment or the fluidic system of the perfusion compartment. In some embodiments, a solid-state bioreactor is configured to perform a batch-fed or fed-batch culture process such that fresh media is provided during bioreactor operation to replace a volume of spent media or to dilute the spent media with fresh media in a non-continuous manner. For example, in a batch-fed configuration, an equal volume of spent media may be removed from the perfusion fluidic system to each volume of fresh media introduced to the perfusion fluidic system, such that the perfusion fluidic system maintains a constant total volume up to the working volume of the perfusion system (e.g., substantially all the media may be replaced during each feeding). In a fed-batch configuration, fresh media may be added non-continuously or continuously over time, but spent media may not be removed, increasing the total volume of the perfusion fluidic system over time. Such a configuration may be permitted by using a reservoir in line with the perfusion fluidic system, including fixed-volume and deformable reservoir vessel types, which may be configured to hold a variable amount of culture media or other fluid. For example, a solid-state bioreactor configured with a fixed volume fluid reservoir may hold a variable amount of liquid over time and increases or decreases in fluid volume may be accommodated by the displacement of gas (e.g., air). In another example, such a configuration may be permitted by the use of a deformable vessel within the solid-state bioreactor, such as a perfusion compartment capable of expanding upon addition of fluid (e.g., in an accordion configuration).

*Washing and buffer exchange*

**[00264]** In some embodiments, a solid-state bioreactor or cell processing system is configured to perform fluid exchange operations, such as to substantially replace a first fluid within the bioreactor cell processing unit or compartment thereof with a second fluid. For example, a fluid exchange operation may be used to substantially replace a first interstitial fluid within a 3D substrate with a second fluid. Such an operation may provide buffer exchange or to wash cells retained within the bioreactor. During cell processing media exchange may be frequently performed to change or substantially change a media condition (e.g., to replace the media with an electroporation buffer suitable for in situ electroporation), or to substantially remove a factor, such as an activation reagent or viral particles. Fluid exchange may purge or replace the perfusion media or fluid present in the 3D substrate and cell compartment. Fluid exchange may be performed iteratively to substantially exchange a first liquid from a cell compartment as it is diluted by diffusive mixing with the second liquid provided in a perfusion compartment. In configurations with two or more perfusion

compartments in fluid communication with a cell compartment, differences in pressure or flow rate between the two or more compartments can be used to accelerate wash-out and reagent exchange of the cell compartment and 3D substrate. For example, flow rate or fluid pressure in one fluid flow path may be less than or significantly less than flow rate or fluid pressure in another fluid flow path such that fluid from the higher flow rate or fluid pressure is driven through the cell compartment or 3D matrix to provide fluid exchange.

**[00265]** In some embodiments, a solid-state bioreactor is configured to provide one or more methods for retaining cells within the bioreactor to permit efficient cell washing and buffer exchange in the presence and absence of the 3D substrate. For example, the 3D matrix may provide cell retention. Alternatively, or in addition to, the solid-state bioreactor may comprise one or more membranes, cell trapping features, or other cell capture mechanisms to retain the cells in absence of the 3D substrate (e.g., before formation or after dissolution or disintegration). For example, a solid-state bioreactor or cell processing system may be configured with a 3D substrate which provides for cell retention during operation, and one or more semi-permeable membrane interface(s) between a 3D substrate (cell compartment) and one or more media or perfusion compartment(s). During solid-state cell culture, the 3D substrate may function to retain cells and provide a 3D environment for cell culture and growth, while the semi-permeable membrane interface functions to retain the 3D substrate, and, in some cases, to provide for additional cell retention, such as by use of a membrane with a filter size cutoff unsuitable for cells to pass through. In some embodiments, a 3D substrate may be configured to provide a reversible gel-sol transition in response to a stimulus such that cells may be washed in situ within a solid-state bioreactor in such a configuration. For example, upon a gel-sol stimulus, such as a change in temperature, addition of a chemical factor or other reagent, or other stimulus of gel-sol transition, the 3D substrate may transition from a substantially solid or semi-solid 3D substrate into a substantially liquid or solubilized state; upon such a 3D substrate gel-sol transition, cells contained within the 3D substrate may become suspension-state surrounded and suspend in the liquified or solubilized matrix. For hybrid 3D substrates, such as those which comprise a porous solid scaffold interpenetrated with a hydrogel scaffold onto which cells attach or with which the cells primarily interact and grow, a stimulus-responsive gel-sol transition may affect a subset of the 3D substrate while providing a cell culture transition, for example, from 3D culture to effectively suspension culture within the porous solid. Subsequently, buffers or media such as wash buffers or cryopreservation buffers may be pumped into and throughout the bioreactor or cell processing system, as described herein, while the semi-permeable

membrane acts to retain the cells in the same volume, space, or compartment such that cells may be in a suspension in the liquified 3D matrix or otherwise not attached to residual solid 3D substrate after such gel-sol transition. A solid-state bioreactor in such a configuration may permit in situ washing or buffer exchange. Washed or buffer exchanged cells may subsequently be collected from the cell compartment, such as through a dedicated port connected to the cell compartment, or by subsequently moving, removing, or degrading the membrane or interface between the cell and other compartment(s). In some embodiments, a solid-state bioreactor or cell processing system may be configured to perform an in situ clinical formulation operation using one or more in situ washing or buffer exchange procedures to suspend the cells in a suitable formulation buffer for harvest and downstream clinical use.

#### *Cell harvesting*

**[00266]** In some embodiments, a solid-state bioreactor is configured to perform a cell harvest operation. In some embodiments, cells are harvested from the solid-state bioreactor at a fixed time, on demand, or once they reach a target number, concentration, phenotype, or other relevant metric, such as provided by one or more sensor(s) and feedback systems within the bioreactor or bioprocessing system as described elsewhere herein. The 3D matrix may be dissolved, and the plurality of cells, or population thereof, may be collected to generate the cell product. In some embodiments, a solid-state bioreactor is configured with a stimulus-responsive gel-sol 3D substrate which facilitates cell harvest. The stimulus may be a thermal stimulus, chemical stimulus, enzymatic stimulus, or any combination thereof. In an example, application of a single stimulus may permit the plurality of cells to be released from the 3D matrix. In another example, application of at least two stimuli permits the plurality of cells to be released from the 3D matrix. Application of a single stimulus may release a select amount of cells and application of a second, different stimulus may release another amount of cells from the 3D matrix. By inducing a phase transition of the 3D substrate from solid-phase or semi-solid phase to liquid-phase (in whole or in part), such as through the application of a stimulus, cells may be harvested by collecting suspension-phase cells or cells suspended in the solubilized matrix. For example, a solid-state bioreactor may be configured to culture cells in a thermally-responsive reversible gel-sol material, such as methacrylated collagen or a polyethylene glycol (PEG)-Poly(N-isopropylacrylamide) (PNIPAAm) block copolymer. Upon lowering the temperature of the bioreactor or cell compartment, the 3D substrate may undergo a phase transition to a solubilized or liquid state and cells previously attached to the 3D substrate may be transitioned to suspension-phase. The liquified substrate containing the



cells may be harvested from the bioreactor, such as by using fluid flow. In some embodiments, an permeable or semi-permeable interface may be disposed adjacent to the cell compartment or perfusion compartment and the washing and buffer exchange methods described elsewhere herein may be used to partially or substantially remove the liquid matrix components from the cells while the cells are retained in the compartment, e.g., to facilitate cell harvest by reducing viscosity, or to facilitate the exchange of cells from the matrix reagent into a buffer suitable for downstream processing, including but not limited to cryopreservation buffers and formulation buffers.

**[00267]** In some embodiments, the solid-state bioreactor or cell processing system is configured to enable the harvest of cells directly from a 3D substrate. For example, fluid flow in or through the cell compartment may be configured to remove cells from the 3D substrate, such as by applying fluid force(s) capable of disrupting cell interactions with the 3D substrate to wash cells out of and from the 3D substrate. In some embodiments, the solid-state bioreactor or cell processing system may be configured with a programmable or stimulus-responsive 3D substrate which facilitates cell harvest. For example, by using a 3D substrate with the property of stimulus-responsive modulation of mass transfer properties, such as porosity or swelling, the harvest of cells being cultured within and retained by a 3D substrate may be facilitated by a stimulus which causes the 3D substrate to swell or increase porosity, such that cells are no longer substantially retained by the 3D substrate or can be removed by fluid flow. In an example, by using a stimuli-responsive 3D substrate configured to modulate cell attachment or migration properties of the 3D matrix, the harvest of cells being cultured within and retained by a 3D substrate may be facilitated by a stimulus which causes the 3D substrate to interact differently with the cells, such that cells may no longer be retained or substantially retained by the 3D substrate or can be removed by fluid flow. In an example, a 3D matrix may comprise programmable cell attachment factors or cell migration factors, which are cleaved, blocked, competitively displaced, or otherwise neutralized by a stimulus, thereby causing a reduction in cell retention properties and facilitating the harvest of cells, such as by fluid flow. In another example, a 3D substrate may be programmably functionalized with factors that promote cellular egress from the matrix environment to facilitate harvest of cells, such as by cell migration or by application of fluid flow. In some embodiments using a stimulus-responsive 3D substrate to facilitate cell harvest, the chemical or biological mechanisms facilitating cell harvest may be directed at a subset of a plurality of cells. For example, in an embodiment utilizing the modulation of 3D substrate cell attachment factors to facilitate cell harvest, one or more of a plurality of cell attachment

factors may be specifically modulated, such that the cell adhesion properties of the 3D substrate are modulated for a specific population of a plurality of cells to permit the harvest of a subset of a plurality of cells. A solid-state bioreactor may be configured with a 3D substrate comprising two or more cell attachment factor(s) directed at two or more populations of cells, such as feeder cell type, which may support the growth of one or more distinct cell type(s) in culture by contributing an undefined and complex mixture of soluble and extracellular matrix (ECM) components. Upon application of a stimulus which modulates the attachment of one of these classes of cells (e.g., the feeder cells or the other cell type(s)), a specific subset of cells may be harvested from the 3D substrate while the other subset(s) remain substantially retained within the 3D substrate. Such a configuration may provide benefits of streamlining cell harvest and formulation workflows or increasing the purity of harvested cells in co-culture applications.

**[00268]** In some embodiments, cells may be harvested by collecting the cell-laden 3D substrate. In some embodiments, cells may be subsequently isolated from the 3D substrate, outside of the solid-state bioreactor. In other embodiments, one or more types of cells may be cultured in a 3D substrate in a manner and composition suitable for downstream use. In an example, one or more types of cells present in a natural tissue may be cultured in a solid-state bioreactor using a 3D substrate which resembles a natural tissue extracellular matrix, and which is otherwise biocompatible. Upon the harvest of the cultured tissue (e.g., the resulting cell-laden 3D substrate), such a composition may be used for a purpose of tissue replacement or regeneration. Examples of such applications include the growth of tissue grafts, such as skin, muscle, cartilage, brain tissue, lymph nodes, or other types of tissues and cell-matrix biological structures. In another example, animal muscle cells may be cultured in a 3D substrate suitable for human consumption using a solid-state bioreactor. Upon the harvest of the cultured tissue, such a composition may be used as a source of food.

**[00269]** In an example, a solid-state bioreactor may be configured to harvest cells during operation for the purpose of in-process monitoring outside of or separate from the solid-state bioreactor. In some embodiments, a solid-state bioreactor comprises one or more specific cell compartment(s) or cell processing units separate from and may not be in fluidic communication with one or more other compartments or cell processing units of the bioreactor, from which cells may be harvested without harvesting cells from the other compartment(s) or cell processing units, thereby enabling in-process monitoring without disrupting the manufacturing process. Cells harvested in-process through the use of a solid-

state bioreactor in such a configuration may be further analyzed within a bioprocessing system or collected and analyzed separately from the bioreactor and bioprocessing system.

**[00270]** In some embodiments, cells harvested from a 3D substrate may be subjected to one or more additional cell culture operations (e.g., cell passaging) within the solid-state bioreactor. Passaging may comprise harvesting cells from a culture and transferring the cells or a subset thereof to one or more culture vessel(s) under a different condition, such as at a new (e.g., lower or higher) cell concentration or density, or in fresh growth medium, thereby establishing new culture(s). A solid-state bioreactor apparatus and cell processing system may be configured to harvest cells from a solid-state bioreactor, and subsequently re-seed a new 3D cell culture within the solid-state bioreactor. In some embodiments, a solid-state bioreactor may be configured with a number of cell processing units, some of which are used in a first passage, and others which are used in a second or subsequent passage. In certain embodiments, a solid-state bioreactor may be configured with a cell processing unit that is reused in one or more passages. In some embodiments, a solid-state bioreactor may be configured to re-seed passaged cells in the first 3D substrate, or in a separate 3D substrate, such as by mixing the cells with a fresh liquid formulation of soluble 3D matrix material and subsequently forming a de novo 3D substrate. In some embodiments, the solid-state bioreactor is configured for a re-seeding or passaging procedure designed to extend the duration of 3D cell culture, increase the total capacity for cell expansion and proliferation, or perform multiple cell engineering or other bioprocessing processes in series. In some embodiments, a solid-state bioreactor may be configured to perform a Multiplex Automated Genome Engineering (MAGE) procedure, in which cells are cultured, engineered, harvested, and optionally selected or subsets of cells isolated, over two or more passages, such as for the purpose of executing a series of cell engineering procedures or iterative or multiplex genome engineering. For example, over two or more passages of 3D cell cultures using a solid-state bioreactor, cells may be exposed to multiple rounds of genome engineering, such as by using CRISPR-Cas9, for the purpose of developing or manufacturing highly engineered cell products. In an example, the product harvested from the cell processing system is a cell therapy product. In another example, the product harvested from the cell processing system is usable to generate a cell therapy product. The cell therapy product may be generated at a scale of one human dose.

#### **Cell processing systems and methods**

**[00271]** In an aspect, the present disclosure provides systems for manufacturing a cell product. A system may include a compartment, or a plurality of compartments, configured to

retain or that retains a plurality of cells. The system may include a fluid flow path in fluid communication with the compartment. The fluid flow path may be configured to flow or may flow a fluid along a first direction having an angle with respect to a second direction of flow within the compartment. The angle may be greater than zero degrees. The fluid flow path may be further configured to provide or may provide material from a fluid to the compartment. The material from the fluid may be usable to generate a cell product. The system may include an analysis unit coupled to the compartment and the fluid flow path. The analysis unit may be disposed within the same housing as the compartment, the fluid flow path, or both the compartment and the fluid flow path. Alternatively, the analysis unit may be disposed in a separate housing than the compartment, fluid flow path, or both the compartment and fluid flow path. The analysis unit may be configured to monitor or may monitor the plurality of cells or the fluid as the materials are provided to the compartment during generation of the cell product.

**[00272]** In another aspect, the present disclosure provides systems for cell processing. A system may include a cell processing unit, a preservation unit, an analysis unit, or combinations thereof. The cell processing unit may be configured to retain and process or may retain and process a plurality of cells to generate a cell product. The preservation unit may be coupled to the cell processing unit. The preservation unit may be configured to preserve or may preserve the cell product. The analysis unit may be coupled to the cell processing unit, the preservation unit, or both. The analysis unit may be configured to detect or monitor or may detect or monitor one or more properties of the plurality of cells of the cell product.

**[00273]** In another aspect, the present disclosure provides methods of manufacturing a cell product. A method may include providing a plurality of cells to a system. The system may include a compartment or a plurality of compartments that retain the plurality of cells. The system may include a fluid flow path in fluid communication with the compartment. The system may include an analysis unit coupled to the compartment and the fluid flow path. The analysis unit may be disposed in the same housing as the compartment, the fluid flow path, or both the compartment and the fluid flow path. Alternatively, the analysis unit may be disposed in a different housing than the compartment, the fluid flow path, or both. The method may include providing material from a fluid in the fluid flow path to the compartment to generate the cell product. The fluid flow path may flow the fluid along a direction having an angle with respect to a second direction of flow within the compartment. The angle may be greater than zero degrees. The method may include using the analysis unit to monitor the

plurality of cells of the fluid as the materials are provided to the compartment during generation of the cell product.

**[00274]** In another aspect, the present disclosure provides methods for cell processing. A method may include providing a plurality of cells to a system. The system may include a cell processing unit, a preservation unit, an analysis unit, or combinations thereof. The cell processing unit may retain the plurality of cells. The preservation unit may be coupled to the cell processing unit. The analysis unit may be coupled to the cell processing unit, the preservation unit, or both. The method may include processing the cells in the cell processing unit to generate a cell product. The method may include preserving the cell product in the preservation unit. The method may include detecting or monitoring one or more properties of the plurality of cells of the cell product during processing of the cells, preserving the cell product, or both.

**[00275]** The systems and methods described herein (e.g., integrated cell processing systems) may permit the use of the 3D matrix and compositions of matter described elsewhere herein for the purpose of processing cells. Processing cells may include automated cell processing, cell culture, cell manipulation, or any combination thereof for production of cellular products. Cellular products may include cell therapies, gene therapies, biologics, biobanked cell stocks, or combinations thereof. The systems and methods described herein may be usable for manufacturing or research applications (e.g., genetic and environmental manipulation of calls and cell line creation). In some embodiments, the systems and methods may include a solid-state bioreactor. The solid-state bioreactor may permit culture or cellular manipulation of adherent, microcarrier, or suspension cell culture. The systems and methods described herein may be usable for reproducing cell products with high cell health and viability. A cell product produced using the systems and methods described herein may have a viability of greater than or equal to about 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or higher. In an example, a cell product produced using the systems and methods described herein may have a cell viability of greater than or equal to about 75%. In another example, the cell viability may be greater than or equal to about 90%. In another example, the cell viability may be greater than or equal to about 95%.

**[00276]** Cell processing systems as described herein may include one or more receiving units, bioreactors, cell processing units, preservation units, analysis units, control units, thermal control units, fluidic units, or any combination thereof. In an example, a cell processing system comprises a bioreactor coupled to a receiving unit, cell processing unit, preservation unit, or analysis unit. In another example, a cell processing system comprises a

bioreactor coupled to a cell processing unit, preservation unit, and analysis unit. In an example, the bioreactor is a solid-state bioreactor. The bioreactor may be configured to subject a plurality of cells to cell culture, membrane transduction, expansion, gene delivery, cell selection, or any combination thereof.

**[00277]** In an example, the cell processing system comprises a receiving unit. The receiving unit may be coupled to the bioreactor, a cell processing unit, preservation unit, an analysis unit, or any combination thereof. The receiving unit may be configured to receive a plurality of cells from an environment external to the cell processing system. The receiving unit may be configured to receive a plurality of cryopreserved (e.g., frozen) cells. The receiving unit may comprise a thermal unit, as described elsewhere herein, and may subject cryopreserved cells to controlled thawing. The receiving unit may be configured to automatically transfer the plurality of cell to the processing unit or to a bioreactor subsequent to thawing. Alternatively, or in addition to, the receiving unit may be configured to automatically transfer the cells to a thermal unit for thawing or a cell processing unit upon receipt of the plurality of cells.

**[00278]** Cell processing systems may comprise one or more control systems. Control systems may include computer systems configured to control or operate the cell processing system. The cell processing system may comprise one or more computer processors operatively coupled to one or more units of the cell processing system. The one or more computer processors may be operatively coupled to a cell processing unit, analysis unit, bioreactor, thermal control unit, receiving unit, preservation unit, fluid flow unit, or any combination thereof. In an example, the one or more computer processors are operatively coupled to the cell processing unit and are configured to direct the cell processing unit to automatically process the plurality of cells within the cell processing unit (e.g., within the 3D matrix), direct the cell processing unit to separate the plurality of processed cells from the 3D matrix, and direct the cell processing unit to collect the plurality of processed cells. In another example, the one or more computer processors are operatively coupled to the receiving unit and may be programmed to automatically direct the receiving unit to provide the plurality or cells to the cell processing unit.

**[00279]** Cell processing system or a bioreactor may include one or more cell processing units. In an example, a cell processing system comprises one or more bioreactors comprising one or more cell processing units. In an example, a cell processing system comprises one or more bioreactors comprising one or more cell processing units and additional cell processing

units separate from the bioreactor(s). In another example, the cell processing system may comprise one or more cell processing units and may not include a bioreactor.

**[00280]** A cell processing unit may comprise any components or feature configured to or usable to process cells. A cell processing unit may be configured or otherwise used to generate a cell product. A cell process unit may comprise a compartment configured to retain a plurality of cells (e.g., cell compartment), fluid flow path configured to provide fluid to the cell compartment, one or more interfaces disposed between fluid flow paths, cell compartments, or both, or any combination thereof. In an example, a cell processing unit comprises at least one cell compartment, at least one fluid flow path, and at least one interface. In another example, a cell processing unit comprises at least one cell compartment, at least one fluid flow path, and does not include an interface. In another example, a cell processing unit comprises at least one compartment, at least one interface, and no fluid flow path. In another example, a cell processing unit comprises at least one compartment and no fluid flow path or interface.

**[00281]** A cell processing unit may comprise at least 1, 2, 3, 4, 5, 6, 8, 10 or more cell compartments. A cell processing unit may comprise at least 1, 2, 3, 4, 5, 6, 8, 10, or more fluid flow paths. In an example, the fluid flow path(s) are perfusion compartments. A cell processing unit may comprise at least 1, 2, 3, 4, 5, 6, 8, 10, or more interfaces. The interfaces may be disposed between one cell compartment and another cell compartment or fluid flow path. Interfaces may be disposed between one fluid flow path and another fluid flow path. The number of interfaces may be greater than, equal to, or less than the number of cell compartments or fluid flow paths. The number of fluid flow paths may be less than, equal to, or greater than the number of cell compartments.

**[00282]** A bioreactor may comprise one or more cell processing units. A bioreactor may comprise at least 1, 2, 3, 4, 5, 6, 8, 10, or more cell processing units. A cell processing system may comprise a bioreactor and one or more additional cell processing units. A cell processing system may include at least 1, 2, 3, 4, 5, 6, 8, 10, or more cell processing units separate from a bioreactor (e.g., the cell processing system does not include a bioreactor, or the cell processing units are not a part of the bioreactor).

**[00283]** A cell processing unit may be configured to process or may process a plurality of cells. Processing cells may include, but is not limited to, cell enrichment, cell selection, cell isolation, cell expansion or culturing, washing, concentration, buffer exchange, de-clumping, or any combination thereof. A cell processing unit may comprise a compartment (e.g., cell compartment) configured to provide one or more processes to the cells. For example, the cell

compartment may be configured to subject the plurality of cells to cell culture, membrane transduction, expansion, gene delivery, cell selection, or any combination thereof.

#### *Cell retention*

**[00284]** The systems and methods described herein may provide integrated cell retention. Cell processing may include providing media or other liquids to cells, removal of media or other liquids from cells, or replacement of media or other liquids in the system. Cells may be retained within the system to provide effective separation of cells from spent media and other fluids, molecules, reagents, and cellular byproducts to be removed or harvested from the culture environment. The volume of the cell processing system containing cells may be a subset of the total working volume of the cell processing system. In the cell processing system at least some other volume (e.g., not containing cells) may contain other fluid components, such as growth media and buffers, or gasses. The former cell-containing volume is referred to herein as a cell compartment(s). The cell compartment may be disposed in a cell processing unit, a solid-state bioreactor, or any combination thereof. A solid-state bioreactor may be a cell processing unit. A cell processing unit may be any device, unit, or module configured to process or that processes cells.

**[00285]** Cell compartment(s) may be provided in a single contiguous configuration, or multiple discrete configurations. Cell compartment(s) provided in a discrete configuration may comprise a plurality of cell compartments physically separated from one another, for example, by a cell-free volume. The cell compartment, regardless of configuration, may provide for the substantial retention of cells by virtue of its design or its configuration utilizing a 3D substrate. The cell compartment may be in fluidic communication with a connected cell-free or substantially cell-free volume. The cell-free or substantially cell-free volume may be a fluid flow path (e.g., perfusion compartment), gas exchange compartment, or both. The cell compartment may be a vessel or other compartment configured to retain a 3D matrix. Alternatively, the 3D matrix may be the cell compartment. The cell compartment may represent a physical 3D volume within the cell processing unit (e.g., solid-state bioreactor or other cell processing unit) and comprises a physical form defined by the dimensions and shape of the 3D matrix or the bounds of cell occupancy within the cell processing unit.

**[00286]** In some embodiments, a cell compartment comprises a 3D matrix for cell processing (e.g., cell culture, gene delivery, transfection, etc.), as described elsewhere herein. In such a configuration, the 3D substrate, such as a 3D matrix, may provide for the substantial retention of cells. For example, a 3D substrate may retain cells, thereby



comprising the cell compartment, by virtue of cellular adhesion to the substrate, by encapsulation, by the porosity of the 3D substrate being unsuitable for cell egress, by a flow configuration or the fluid dynamics within the 3D substrate during perfusion or fluidic flow being unable to substantially displace cells from the 3D substrate, or by other physical and biological interactions among the 3D substrate, cells, and fluids, or combinations of these, as well as the effect of gravity, depending on configuration. In some embodiments, the interface(s) between the cell compartment(s) and the other volume(s) (e.g., fluid flow path) of the cell processing unit (e.g., solid-state bioreactor or other cell processing unit) may be defined by the boundary of the 3D substrate in which the cells reside. Such an interface and configuration may be considered a phase separation, e.g., the cell compartment(s) being defined as a solid or semi-solid phase comprising the 3D substrate, and the other compartment(s) being defined as liquid or gas phases, comprising media, atmosphere, or another aspect of the cell processing environment. In some embodiments, a pre-formed 3D substrate for cell culture is provided within the cell processing unit. Cells may be loaded into the cell processing unit and allowed to settle by gravity, attach by cell adhesion, migrate into, or otherwise become localized to or within the 3D substrate. In some embodiments, cell-signaling or cell-binding factors are provided to promote such cellular localization. In some embodiments, a liquid matrix formulation (e.g., polymer precursor mixture) is loaded into a cell processing unit and allowed to form a 3D substrate, such as by gel-sol transition, in situ polymerization, in situ crosslinking of the matrix, or combinations thereof. In some embodiments, the 3D substrate is configured such as to not occupy the entire volume of cell processing unit, e.g., by loading a suitable volume of liquid matrix such as to form a “head space” which is not occupied by 3D substrate upon matrix formation. The liquid matrix formulation (e.g., polymer precursor mixture) may contain cells to permit formation of a cell-laden 3D substrate upon matrix formation. Alternatively, cells may be added to the 3D substrate subsequent to matrix formation. In some embodiments, cells may be added prior to matrix formation and the generated 3D matrix may subsequently be seeded with additional cells. In some embodiments, the volume and specific spatial configuration of the cell compartment(s) may be further defined by a phase separation, such as gas (e.g., “head space”) or by use of a phase-separated liquid. For example, liquid matrix (e.g., polymer precursors) may be loaded into the cell processing unit with a separate immiscible liquid such as oil (concurrently, prior to, or subsequent to), such that the immiscible liquid phase excludes the matrix from a specific volume of the bioreactor during formation of the 3D substrate. Such phase-separated non-matrix liquid may be removed (e.g., washed out) from

the cell processing unit such as to form a perfusion or gas exchange volume and interface(s) with the 3D substrate within the cell processing unit. In some embodiments, multiphasic liquids are utilized. Multiphasic liquid may comprise at least a liquid matrix (e.g., polymer precursors) and a separate immiscible liquid which form two or more discrete phases or volumes separated by one or more interfaces upon formation of the 3D matrix.

**[00287]** A cell processing unit may comprise at least one fluid flow path. The fluid flow path may be configured to provide or may provide reagents to the 3D matrix and to remove waste (e.g., waste metabolites) from the 3D matrix, as shown in **FIGs. 16A** and **16B**. In an example, during cell culture, the fluid flow path may provide reagents to the 3D matrix to provide conditions sufficient for cell culture as shown in **FIG. 16A**. The reagents may include, but are not limited to, glucose, oxygen, buffering agents, proteins, amino acids, lipids, inorganic salts, trace elements, vitamins, reducing agents, or any combinations thereof. The fluid flow path may be configured to remove or may remove waste from the 3D matrix to maintain conditions sufficient for cell culture. Removed waste may include, but are not limited to, lactate, carbon dioxide, ammonia, other metabolites, or any combination thereof. In another example, during cell activation, the fluid flow path may be configured to provide or may provide growth factors, signaling factors, or both to the cells encapsulated within the 3D matrix. For example, the fluid flow path may provide interleukins (e.g., IL-2), antibodies, complexes (e.g., CD3/28), or any combination thereof to the 3D matrix. In another example, the fluid flow path may be configured to provide viruses, DNA, RNA, reagents, or any combination thereof for gene delivery. Gene delivery reagents may include, but are not limited to, viruses, DNA, RNA, lipid-complexes (LNP), electroporation buffer, adjuvants, or any combination thereof. The fluid flow path may be configured to wash the 3D matrix before, during, or subsequent to activation or gene delivery. The wash-out may include providing media or other wash solution to the 3D matrix to remove growth factors, signaling factors, gene delivery reagents, or any combination thereof. In another example, a cell processing unit may comprise a fluid flow path configured to deliver liquid nutrients and reagents and another fluid flow path configured to deliver gaseous nutrients and reagents, as shown in **FIG. 16B**. The 3D matrix may be disposed between a fluid flow pathway (e.g., first fluid flow pathway) configured to provide fluidic nutrients (e.g., nutrients disposed in a liquid phase, such as media) to the 3D matrix and another fluid flow pathway (e.g., second fluid flow pathway) configured to provide gaseous nutrients and reagents to the 3D matrix. Either the first or the second fluid flow pathway, or both, may be configured to flow a gas-exchange liquid (e.g., an oxygenated media) to provide gaseous nutrients and reagents to the

3D matrix. In an example, the second fluid flow pathway may be configured to flow a gas-phase fluid such that gas from the gas-phase fluid can diffuse into the 3D matrix or media compartments via a gas-permeable and liquid-impermeable membrane (e.g., polydimethylsiloxane (PDMS), silicones, etc.). In an example, a liquid delivering and gas delivering fluid flow path may be disposed on the same side of a 3D matrix.

**[00288]** A cell processing unit may comprise a membrane or interface. The membrane or interface may be disposed between a compartment, such as a cell compartment, and a fluid flow path. The membrane may be disposed between a first cell compartment and another cell compartment. The membrane may be disposed between a first fluid flow path and another fluid flow path. The membrane may be disposed between a cell compartment and another compartment configured to flow fluid (e.g., liquid or gas). The membrane may be configured to permit transport of material through the membrane. For example, the membrane may permit transport of nutrients, reagents, or other components in a liquid or gas phase through the membrane. In some embodiments, the cell processing unit does not comprise a membrane or semi permeable membrane between the compartment and the fluid flow path or other volume. In some embodiments, a cell compartment further comprises, or is coupled to, one or more membranes or semi-permeable substrate(s), such as a semi-permeable membrane. A membrane may have a thickness that permits transport of fluids or fluids comprising nutrients, reagents, or other components from a fluid flow path to a cell compartment (e.g., 3D matrix or 3D matrix in a cell compartment) or vice versa. A membrane may have a thickness of greater than or equal to about 5 micrometers ( $\mu\text{m}$ ), 10  $\mu\text{m}$ , 15  $\mu\text{m}$ , 20  $\mu\text{m}$ , 30  $\mu\text{m}$ , 40  $\mu\text{m}$ , 50  $\mu\text{m}$ , 75  $\mu\text{m}$ , 100  $\mu\text{m}$ , 200  $\mu\text{m}$ , 300  $\mu\text{m}$ , 400  $\mu\text{m}$ , 500  $\mu\text{m}$ , 600  $\mu\text{m}$ , 700  $\mu\text{m}$ , 800  $\mu\text{m}$ , 900  $\mu\text{m}$ , 1 millimeter (mm), 1.5 mm, 2 mm, 3 mm, 4 mm, 5mm, or thicker. A membrane may have a thickness of less than or equal to about 5 mm, 4 mm, 3 mm, 2 mm, 1.5 mm, 1 mm, 900  $\mu\text{m}$ , 800  $\mu\text{m}$ , 700  $\mu\text{m}$ , 600  $\mu\text{m}$ , 500  $\mu\text{m}$ , 400  $\mu\text{m}$ , 300  $\mu\text{m}$ , 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , 75  $\mu\text{m}$ , 50  $\mu\text{m}$ , 40  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 15  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , or less. A membrane may have a thickness from about 5  $\mu\text{m}$  to 10  $\mu\text{m}$ , 5  $\mu\text{m}$  to 15  $\mu\text{m}$ , 5  $\mu\text{m}$  to 20  $\mu\text{m}$ , 5  $\mu\text{m}$  to 30  $\mu\text{m}$ , 5  $\mu\text{m}$  to 40  $\mu\text{m}$ , 5  $\mu\text{m}$  to 50  $\mu\text{m}$ , 5  $\mu\text{m}$  to 75  $\mu\text{m}$ , 5  $\mu\text{m}$  to 100  $\mu\text{m}$ , 5  $\mu\text{m}$  to 200  $\mu\text{m}$ , 5  $\mu\text{m}$  to 300  $\mu\text{m}$ , 5  $\mu\text{m}$  to 400  $\mu\text{m}$ , 5  $\mu\text{m}$  to 500  $\mu\text{m}$ , 5  $\mu\text{m}$  to 600  $\mu\text{m}$ , 5  $\mu\text{m}$  to 700  $\mu\text{m}$ , 5  $\mu\text{m}$  to 800  $\mu\text{m}$ , 5  $\mu\text{m}$  to 900  $\mu\text{m}$ , 5  $\mu\text{m}$  to 1 mm, 5  $\mu\text{m}$  to 1.5 mm, 5  $\mu\text{m}$  to 2 mm, 5  $\mu\text{m}$  to 3 mm, 5  $\mu\text{m}$  to 4 mm, 5  $\mu\text{m}$  to 5 mm, 10  $\mu\text{m}$  to 15  $\mu\text{m}$ , 10  $\mu\text{m}$  to 20  $\mu\text{m}$ , 10  $\mu\text{m}$  to 30  $\mu\text{m}$ , 10  $\mu\text{m}$  to 40  $\mu\text{m}$ , 10  $\mu\text{m}$  to 50  $\mu\text{m}$ , 10  $\mu\text{m}$  to 75  $\mu\text{m}$ , 10  $\mu\text{m}$  to 100  $\mu\text{m}$ , 10  $\mu\text{m}$  to 200  $\mu\text{m}$ , 10  $\mu\text{m}$  to 300  $\mu\text{m}$ , 10  $\mu\text{m}$  to 400  $\mu\text{m}$ , 10  $\mu\text{m}$  to 500  $\mu\text{m}$ , 10  $\mu\text{m}$  to 600  $\mu\text{m}$ , 10  $\mu\text{m}$  to 700  $\mu\text{m}$ , 10  $\mu\text{m}$  to 800  $\mu\text{m}$ , 10  $\mu\text{m}$  to 900  $\mu\text{m}$ , 10  $\mu\text{m}$  to 1 mm, 10  $\mu\text{m}$  to 1.5 mm, 10  $\mu\text{m}$  to 2 mm, 10  $\mu\text{m}$  to 3 mm, 10  $\mu\text{m}$  to

4 mm, 10 μm to 5 mm, 15 μm to 20 μm, 15 μm to 30 μm, 15 μm to 40 μm, 15 μm to 50 μm, 15 μm to 75 μm, 15 μm to 100 μm, 15 μm to 200 μm, 15 μm to 300 μm, 15 μm to 400 μm, 15 μm to 500 μm, 15 μm to 600 μm, 15 μm to 700 μm, 15 μm to 800 μm, 15 μm to 900 μm, 15 μm to 1 mm, 15 μm to 1.5 mm, 15 μm to 2 mm, 15 μm to 3 mm, 15 μm to 4 mm, 15 μm to 5 mm, 20 μm to 30 μm, 20 μm to 40 μm, 20 μm to 50 μm, 20 μm to 75 μm, 20 μm to 100 μm, 20 μm to 200 μm, 20 μm to 300 μm, 20 μm to 400 μm, 20 μm to 500 μm, 20 μm to 600 μm, 20 μm to 700 μm, 20 μm to 800 μm, 20 μm to 900 μm, 20 μm to 1 mm, 20 μm to 1.5 mm, 20 μm to 2 mm, 20 μm to 3 mm, 20 μm to 4 mm, 20 μm to 5 mm, 30 μm to 40 μm, 30 μm to 50 μm, 30 μm to 75 μm, 30 μm to 100 μm, 30 μm to 200 μm, 30 μm to 300 μm, 30 μm to 400 μm, 30 μm to 500 μm, 30 μm to 600 μm, 30 μm to 700 μm, 30 μm to 800 μm, 30 μm to 900 μm, 30 μm to 1 mm, 30 μm to 1.5 mm, 30 μm to 2 mm, 30 μm to 3 mm, 30 μm to 4 mm, 30 μm to 5 mm, 40 μm to 50 μm, 40 μm to 75 μm, 40 μm to 100 μm, 40 μm to 200 μm, 40 μm to 300 μm, 40 μm to 400 μm, 40 μm to 500 μm, 40 μm to 600 μm, 40 μm to 700 μm, 40 μm to 800 μm, 40 μm to 900 μm, 40 μm to 1 mm, 40 μm to 1.5 mm, 40 μm to 2 mm, 40 μm to 3 mm, 40 μm to 4 mm, 40 μm to 5 mm, 50 μm to 75 μm, 50 μm to 100 μm, 50 μm to 200 μm, 50 μm to 300 μm, 50 μm to 400 μm, 50 μm to 500 μm, 50 μm to 600 μm, 50 μm to 700 μm, 50 μm to 800 μm, 50 μm to 900 μm, 50 μm to 1 mm, 50 μm to 1.5 mm, 50 μm to 2 mm, 50 μm to 3 mm, 50 μm to 4 mm, 50 μm to 5 mm, 75 μm to 100 μm, 75 μm to 200 μm, 75 μm to 300 μm, 75 μm to 400 μm, 75 μm to 500 μm, 75 μm to 600 μm, 75 μm to 700 μm, 75 μm to 800 μm, 75 μm to 900 μm, 75 μm to 1 mm, 75 μm to 1.5 mm, 75 μm to 2 mm, 75 μm to 3 mm, 75 μm to 4 mm, 75 μm to 5 mm, 100 μm to 200 μm, 100 μm to 300 μm, 100 μm to 400 μm, 100 μm to 500 μm, 100 μm to 600 μm, 100 μm to 700 μm, 100 μm to 800 μm, 100 μm to 900 μm, 100 μm to 1 mm, 100 μm to 1.5 mm, 100 μm to 2 mm, 100 μm to 3 mm, 100 μm to 4 mm, 100 μm to 5 mm, 200 μm to 300 μm, 200 μm to 400 μm, 200 μm to 500 μm, 200 μm to 600 μm, 200 μm to 700 μm, 200 μm to 800 μm, 200 μm to 900 μm, 200 μm to 1 mm, 200 μm to 1.5 mm, 200 μm to 2 mm, 200 μm to 3 mm, 200 μm to 4 mm, 200 μm to 5 mm, 300 μm to 400 μm, 300 μm to 500 μm, 300 μm to 600 μm, 300 μm to 700 μm, 300 μm to 800 μm, 300 μm to 900 μm, 300 μm to 1 mm, 300 μm to 1.5 mm, 300 μm to 2 mm, 300 μm to 3 mm, 300 μm to 4 mm, 300 μm to 5 mm, 400 μm to 500 μm, 400 μm to 600 μm, 400 μm to 700 μm, 400 μm to 800 μm, 400 μm to 900 μm, 400 μm to 1 mm, 400 μm to 1.5 mm, 400 μm to 2 mm, 400 μm to 3 mm, 400 μm to 4 mm, 400 μm to 5 mm, 500 μm to 600 μm, 500 μm to 700 μm, 500 μm to 800 μm, 500 μm to 900 μm, 500 μm to 1 mm, 500 μm to 1.5 mm, 500 μm to 2 mm, 500 μm to 3 mm, 500 μm to 4 mm, 500 μm to 5 mm, 600 μm to 700 μm, 600 μm to 800 μm, 600 μm to 900 μm, 600 μm to 1 mm, 600 μm to 1.5 mm,

600  $\mu\text{m}$  to 2 mm, 600  $\mu\text{m}$  to 3 mm, 600  $\mu\text{m}$  to 4 mm, 600  $\mu\text{m}$  to 5 mm, 700  $\mu\text{m}$  to 800  $\mu\text{m}$ , 700  $\mu\text{m}$  to 900  $\mu\text{m}$ , 700  $\mu\text{m}$  to 1 mm, 700  $\mu\text{m}$  to 1.5 mm, 700  $\mu\text{m}$  to 2 mm, 700  $\mu\text{m}$  to 3 mm, 700  $\mu\text{m}$  to 4 mm, 700  $\mu\text{m}$  to 5 mm, 800  $\mu\text{m}$  to 900  $\mu\text{m}$ , 800  $\mu\text{m}$  to 1 mm, 800  $\mu\text{m}$  to 1.5 mm, 800  $\mu\text{m}$  to 2 mm, 800  $\mu\text{m}$  to 3 mm, 800  $\mu\text{m}$  to 4 mm, 800  $\mu\text{m}$  to 5 mm, 900  $\mu\text{m}$  to 1 mm, 900  $\mu\text{m}$  to 1.5 mm, 900  $\mu\text{m}$  to 2 mm, 900  $\mu\text{m}$  to 3 mm, 900  $\mu\text{m}$  to 4 mm, 900  $\mu\text{m}$  to 5 mm, 1 mm to 1.5 mm, 1 mm to 2 mm, 1 mm to 3 mm, 1 mm to 4 mm, 1 mm to 5 mm, 1.5 mm to 2 mm, 1.5 mm to 3 mm, 1.5 mm to 4 mm, 1.5 mm to 5 mm, 2 mm to 3 mm, 2 mm to 4 mm, 2 mm to 5 mm, 3 mm to 4 mm, 3 mm to 5 mm, or 4 mm to 5 mm. In an example, a semipermeable membrane may have a thickness from about 18 to 25  $\mu\text{m}$  or from about 110 to 150  $\mu\text{m}$ .

**[00289]** Semipermeable membrane materials may include Polysulfone (PS/PSF), Polyethersulfone (PES), Polyvinylidene fluoride (PVDF), Polytetrafluoroethylene (PTFE), Nylon (Polyamide), Polycarbonate (PC, PCTE), Teflon (Polytetrafluoroethylene), Cellulose Nitrate, Cellulose Acetate, Mixed Cellulose (MCE), Glass Fiber, Polypropylene (PP), Polyacrylonitrile (PAN), Polyester (PETE), Polyethylene (PE), Polyether ether ketone (PEEK), thermoplastic, metal (such as silver, aluminum), ceramic, or any combinations thereof. Membranes may be surface treated, such as with a wetting agent. For example, a wetting agent may comprise a few molecular thicknesses of polyvinylpyrrolidone (PVP) deposited on the membrane surfaces of materials such as PCTE. As another example, a wetting agent may comprise a deposition of a polymer such as a protein, including but not limited to collagen, albumin, fibronectin, and laminin. For example, treatment, such as adsorption or coating, of collagen may increase the contact angle and hydrophilicity of a plastic material including membranes and other aqueous-fluid contacting surfaces. Porous or semipermeable membranes may be manufactured by track etching or as a woven mesh, nanofiber mesh, non-woven matrix, or combination thereof. Membranes may be selected based on properties including porosity (percent of total area occupied by pores, for example from less than about 1% to 16% for track-etched PC and PE, from about 40% to 80% for other materials and construction methods), pore size (e.g., pore diameter), hydrophilicity and hydrophobicity, bubble point (minimum amount of pressure required to push air bubbles through the largest pore of a wet membrane), maximum operating temperature, sealing compatibility (e.g., ultrasonic, heat, radiofrequency, insert molding), burst strength, water flow rate (e.g., mL/min/cm<sup>2</sup> at a given pressure), air flow rate (e.g., L/min/cm<sup>2</sup> at a given pressure), optical properties (e.g., clarity), solvent resistance, chemical compatibility, sloughing or particle shedding, biocompatibility, extractables and adsorption characteristics,

biofouling and protein binding (e.g., certain hydrophilic materials, such as nylon and cellulose nitrate, exhibit high protein binding capacity, while regenerated cellulose and cellulose acetate bind virtually no protein).

**[00290]** In an example, a membrane may be disposed between the 3D matrix or cell compartment and the media compartment. The membrane may be a track etched PC or PETE membrane with a thickness from about 18 to 25  $\mu\text{m}$ . The membrane may have a pore size from about 1 to 12  $\mu\text{m}$  pore size and a porosity from about 1 to 16%. In an example, the membrane has a pore size of less than about 1  $\mu\text{m}$ . In another example, the membrane has a pore size of less than about 2  $\mu\text{m}$ . In another example, the membrane has a pore size of less than about 3  $\mu\text{m}$ .

**[00291]** In another example, the system may comprise a gas-permeable membrane. The gas-permeable membrane may be dense (e.g., nonporous and devoid of interconnected pore networks such that gas molecules transport through diffusion) or porous (e.g., assisted by hydrophobicity, porous membranes may not permit the pressure-driven permeation of liquid into the gaseous pores of the membrane up to a certain critical pressure). Gas-permeable membranes may be made from materials including PDMS, silicone, Teflon (e.g., Teflon AF2400), poly[1-trimethylsilyl-1-propyne] (PTMSP), polyimide, polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), polycarbonate (PC), or any combination thereof. Other membrane materials used may include polymers of intrinsic microporosity (PIMs), thermally rearranged (TR) polymers, and heterogeneous membranes, including thin-film composite (TFC) membranes (e.g., an ultrathin dense polymer layer formed or deposited onto a rigid membrane support, permitting the simultaneous integration of the selectivity/permittivity of the dense membrane and the mechanical strength of the support layer) and mixed-matrix membranes (MMMs, consisting of organic polymers blended with inorganic additives, such as zeolites, metal-organic frameworks (MOFs), nanocarbons, and other nanoparticles, in which inorganic material improves gas selectivity, while polymers provide mechanical stability). Membranes or membrane materials may be treated, such as by ionization, to further enhance membrane properties. In an example, a gas-permeable liquid-impermeable membranes may comprise PDMS and may have a thickness of at least about 20  $\mu\text{m}$ , 100  $\mu\text{m}$ , 0.25 mm, 0.5 mm, or more.

**[00292]** The interface or membrane may be configured to permit reagents of select sizes to be delivered to or removed from the 3D matrix. Example reagent sizes are shown in **FIG. 17**. The properties of the interface or membrane may be modified for size selection. A semi-permeable membrane may provide cell retention or 3D substrate retention or enhance cell

retention (e.g., which contributes to cell retention when used in combination with a 3D substrate comprising cell retention properties, as described elsewhere herein). In some embodiments, a semi-permeable membrane provides an interface(s) between a cell compartment and one or more other compartments or volumes within the cell processing unit. The semi-permeable membrane may be configured to permit the transfer of substances between a cell compartment(s) and another compartment(s) (e.g., fluid flow paths). For example, a semi-permeable membrane interface may be configured to permit or may permit the transfer of water molecules and soluble or suspended components provided in a aqueous medium, such as cell culture media or other buffer, into the cell compartment, or reciprocally to permit the transfer of soluble or suspended components in the interstitial fluid of the 3D substrate or fluid component of the cell compartment(s) into the other compartment(s) (e.g., fluid flow path). In such a configuration, the transfer of fluidic, soluble, or suspended components between compartments may be driven by diffusion, such as by a concentration differential, by electrochemical gradients, such as under an applied electric field (e.g., electrophoresis), or by fluid flow, such as by hydrophilic absorption of the 3D substrate, a pressure gradient, or applied fluidic pressure or flow. Regardless of the mechanism of transfer (e.g., diffusion, flow-based, etc.), the nature of the semi-permeable membrane influences the rate of mass transfer. In such a configuration, the choice of semi-permeable membrane may be determined by a mass transfer property and other attributes such as biocompatibility, fouling, stability, compatibility, clinical safety, and so on. For example, for bioprocessing workflows in which predominantly metabolites and culture factors are transferred through the interface, an approximate 100 kDa size cutoff membrane may be used, whereas for bioprocessing workflows in which a virus is directed to pass through the interface, a size cutoff of less than or equal to about 1  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , or less size cutoff membrane may be used. Metabolites for cellular metabolism, such as glucose (180 Da) and Lactate (89 Da), may be within a range of approximately 10-100's of Daltons in mass, and may transfer through a semi-permeable membrane with a filter size of less than or equal to about 10 kDa, 8 kDa, 6 kDa, 5 kDa, 4 kDa, 3 kDa, 2 kDa, 1 kDa, 0.5 kDa, or less. The membrane may have a filter size of greater than or equal to about 0.5 kDa, 1 kDa, 2 kDa, 3 kDa, 4 kDa, 5 kDa, 6 kDa, 8 kDa, 10 kDa, or greater. In an example, the membrane may have a filter size from about 1 to 10 kDa. Culture factors, such as cytokines (e.g., IL-2 ~16 kDa, IL-7 ~25 kDa, IL-15 ~15 kDa) and antibodies or fragments thereof (e.g., anti-CD3 ~61 kDa or greater, anti-CD28 ~88 kDa or greater), may be within a range of approximately 10-100's of kDa in mass, and may transfer through a semi-permeable membrane with a filter size

of greater than or equal to about 100 kDa, 1 mDa, 0.1  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 1  $\mu\text{m}$ , or greater. Components related to the genetic engineering of cells, such as viral particles (e.g.,  $\gamma$ -retroviral, Lentiviral vectors,  $\sim 50$ -150 nm), nucleic acids including plasmid DNA, DNA, and RNA (e.g.,  $\sim 1000$ 's kDa), and ribonucleoproteins such as Cas9 (e.g.,  $\sim 200$  kDa,  $\sim 10$  nm), may be within a range of approximately 1000's of kDa in mass or 10's to 100's of nanometers in size, and may transfer through a semi-permeable membrane with a filter size of greater than or equal to about 1 mDa, 10 mDa, 0.1  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 1  $\mu\text{m}$ , or greater. Beads, polymeric complexes, and polypeptide complexes, such as immunostimulatory antibody tetramers ( $\sim 500$  kDa) and antibody-laden polymeric complexes, beads, and gel beads ( $\sim 100$  nm or greater), may be within a range of approximately 100's to 1000's of kDa in mass or 10's to 100's of nanometers or greater in size, and may transfer through a semi-permeable membrane with a filter size of greater than or equal to about 1 mDa, 10 mDa, 0.1  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 1  $\mu\text{m}$ , 4  $\mu\text{m}$ , 10  $\mu\text{m}$ , or greater. Cells, such as CD3+ T cells ( $\sim 6$ -14  $\mu\text{m}$ ), may be within a range of approximately 1-100  $\mu\text{m}$ , and may not substantially transfer through a semi-permeable membrane with a filter size of less than or equal to about 100  $\mu\text{m}$ , 75  $\mu\text{m}$ , 50  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 12  $\mu\text{m}$ , 10  $\mu\text{m}$ , 8  $\mu\text{m}$ , 5  $\mu\text{m}$ , 4  $\mu\text{m}$ , 1  $\mu\text{m}$ , or less. In some embodiments, a semi-permeable membrane is configured to retain or substantially retain the 3D cell culture substrate. A membrane configured to retain or substantially retain the 3D cell culture may comprise a pore size greater than or equal to about 0.1 mm, 0.2 mm, 0.5 mm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, or greater. In some embodiments, a semi-permeable membrane is configured to retain or substantially retain a liquid matrix formulation prior to formation of the 3D substrate, such as by its viscosity, by fluid tension, by hydrophobic interactions, and by other physical interactions with the membrane or physical properties of the membrane such as wetting. In some embodiments, a semi-permeable membrane is configured to substantially retain cells in a solution phase within the cell compartment. For example, a semi-permeable membrane may be configured to retain or substantially retain cells in a fluid suspension, such as during a harvest procedure in which a 3D substrate is solubilized, such as by a gel-sol transition of a suitable 3D substrate material. For example, the semi-permeable membrane may be configured to retain or substantially retain cells during a washing procedure, in which solubilized matrix or 3D substrate is displaced from the cell compartment and replaced with a different fluid, such as a wash buffer or cryopreservation buffer, through washing, diffusion, or fluidic flow.

**[00293]** In some embodiments, the semi-permeable membrane interface(s) between a cell compartment and one or more other compartments or volumes within a solid-state bioreactor



or cell processing unit is configured to permit or permits the transfer of substances between cell compartment(s) and other compartment(s), such substances being atoms or molecules of gas or dissolved gas. For example, one or more volumes within a solid-state bioreactor comprise a gas compartment(s) in molecular communication a cell compartment(s). The solid-state bioreactor may be configured with a gas-permeable liquid-impermeable membrane forming an interface between a gas compartment and a cell compartment, thereby permitting gasses such as O<sub>2</sub> to be absorbed by a liquid in the cell compartment. In some embodiments, gasses (which may be sterilized, such as by filtration or another sterilization method), including environmental air, O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>, or mixtures thereof, may be provided. Oxygen is useful for aerobic respiration in cells, serving as an electron acceptor in the mitochondrial electron transport chain, for ATP production. The concentration of oxygen may be carefully controlled to meet cellular demands without causing oxidative stress or hyperoxia, which can be damaging to cells. Carbon dioxide may be used primarily to control the pH of the culture medium through its equilibrium with bicarbonate in a buffer system. The concentration of CO<sub>2</sub> in the bioreactor may be maintained at a level that matches physiological conditions, for example, around 5% for mammalian cell cultures, to keep the pH within a range suitable for cell growth and function. Nitrogen may be used as a balance gas to make up the remainder of the bioreactor atmosphere. It can also be used to purge oxygen from the system to create a hypoxic or anoxic environment. Nitrogen can be used to maintain the targeted oxygen levels and prevent oxidative damage or excessive cell growth if lower oxygen levels are required (e.g., hypoxia). Air may provide a natural balance of gasses, including about 21% oxygen and 78% nitrogen, which can be suitable for many cell culture applications. When using air, it may be supplemented with additional CO<sub>2</sub> to achieve a given carbon dioxide concentration for pH control, O<sub>2</sub> to achieve a given oxygen concentration for cellular respiration, or other gasses. In some applications, helium may be used because of its physical properties such as high thermal conductivity and low solubility, which can be advantageous in select bioreactor designs. Helium is non-reactive and can be used to create controlled gas mixtures, especially when manipulating the physical properties of the gas phase is useful. All gasses introduced into a bioreactor may be sterilized or sterile to avoid contaminating the culture. Gasses may be sterilized using sterile filters. Gasses may be humidified before entering the bioreactor to prevent evaporation of the culture medium, which can alter the concentration of solutes and disrupt cellular activities. The pressure and flow rate of gasses may be controlled to ensure that the cells are not subjected to stress and that gas exchange is adequate without disrupting the cells or medium.

[00294] In some embodiments, one or more interface(s) between a cell compartment and one or more other compartment(s) may be formed in situ within the solid-state bioreactor. For example, a polymer layer may be formed in situ on or cognate to one or more dimensions of the cell compartment, thereby forming an interface which further defines the cell compartment(s), provides for retention of the 3D substrate, or provides for retention of cells, while permitting the transfer of aqueous reagents or gasses between the cell compartment and other compartment(s) of the bioreactor. A polymer layer, such as a thin film, may be formed using methods described herein, or by other methods and compositions for forming interface(s). In an example, a phase separation approach may be used to form a polymer interface, such as by saturating one compartment with a secondary matrix material and providing a cross-linker or catalyst in a separate phase, such as an oil phase, such that the secondary polymer matrix forms at the interface between the two compartments or phases. As another example, an interface may be formed by the configuration and method of forming a 3D matrix, such as by the use of an alginate hydrogel crosslinked by exogenous supply of an ion source such as  $\text{Ca}^{2+}$ , wherein a concentration or exposure timing gradient in the ion crosslinker results in a more highly crosslinked region of the 3D substrate (e.g., the interface), and a more porous region of the 3D substrate in which cells may proliferate and be retained (e.g., in the cell compartment). In some embodiments, an additional layer or volume of a 3D matrix composition not containing cells, which may be the same composition as that in which the cells are embedded or a separate composition, may be used as an interface between compartments. For example, a collagen hydrogel matrix may be used to embed, surround, or layer on top of a 3D cell culture substrate, which may also comprise a collagen hydrogel. In such examples, the additional collagen matrix, which may be substantially free of cells, may act as an interface between the cell compartment and another compartment, retaining the cells within the cell compartment. In other examples, an additional matrix, which is cognate to the cell-laden 3D matrix but substantially free of cells or free of cells at an initial timepoint, may provide a space for cell growth, proliferation, and migration. Such dynamic configurations may provide a variable cell compartment and interface, e.g., an additional matrix, which is cognate to a cell compartment and at least one other compartment(s) but substantially free of cells or free of cells at an initial timepoint, forming an interface with the cell compartment, may become cell-laden at a later timepoint, and become, in part or in whole, an additional volume of the cell compartment. Other methods of forming an in situ interface specific to particular 3D substrate compositions and methods, which similarly provide for retention of the 3D substrate or retention of cells while enabling the transfer of aqueous reagents or gasses

between the cell compartment(s) and other compartments of the bioreactor, follow from these examples.

**[00295]** The shape and volume of a cell compartment may be fixed or variable. In an example, the shape and volume of a cell compartment are fixed. In another example, the shape and volume of the cell compartment is variable. In another example, the shape of the cell compartment is fixed and the volume is variable. In another example, the volume of the cell compartment is fixed and the shape is variable. In some embodiments, a cell compartment comprises a fixed spatial configuration, predetermined by the configuration of the solid-state bioreactor apparatus or by the procedure of establishing the cell compartment, as in the following example cases. In an example, a cell compartment may comprise a compartment whose shape and volume are defined by the presence of a preformed 3D cell culture substrate. In another example, a cell compartment may comprise a compartment whose shape and volume are defined by a combination of impermeable surfaces (e.g., walls) and semi-permeable membrane(s), into which a liquid matrix formulation is loaded and directed to form or permitted to form a 3D substrate. In another example, a cell compartment may comprise a compartment whose shape and volume is defined by the volume and method of forming the 3D substrate component of the cell-matrix, for example, by a casting or extrusion procedure. The 3D substrate may comprise the cells at the time of forming. Alternatively, the cells may be added to the 3D substrate subsequent to formation of the 3D substrate. In some embodiments, a cell compartment comprises a variable spatial configuration, as in the following example cases. In an example, a cell compartment may comprise a compartment whose shape and volume are defined by the volume and method of forming a 3D substrate component of the cell-matrix, for example, by a casting or extrusion procedure, which may vary from time to time, or may vary in each given usage. The cells may be included in the 3D substrate during formation, or the cells may be added to the 3D substrate subsequent to formation of the 3D substrate. In another example, a cell compartment may comprise a compartment whose shape and volume varies over time due to swelling or other changes in the shape or volume of the 3D substrate itself. In another example, a cell compartment may comprise a compartment whose shape and volume are defined by at least one deformable surface (e.g., being permeable or impermeable), such that the shape and volume of the compartment may vary between separate processes, or change over time, such as to accommodate different volumes of 3D substrate or deformations of a 3D substrate (e.g., swelling, compression). In another example, a cell compartment may comprise a compartment whose shape and volume is defined by at least one movable surface

(e.g., being permeable or impermeable), such that the shape and volume of the compartment may vary between separate processes, or change over time, such as to accommodate different volumes of 3D substrate or to accommodate or to cause deformations of the 3D substrate (e.g., swelling, compression) by movement of the movable surface, such as driven by an applied pressure, a motor, or other motion system within or cognate to the solid-state bioreactor.

**[00296]** In some embodiments, a cell compartment is substantially homogeneous at least one time point during use. For example, a mixture of liquid matrix precursor solution and cells, which is a substantially homogeneous suspension, may be loaded into a solid-state bioreactor to form a 3D cell culture substrate containing cells in a substantially homogeneous initial distribution. In some embodiments, cells may be loaded into or onto a 3D substrate in a spatially heterogeneous manner (e.g., such as onto the surface of a preformed 3D substrate), or an inhomogeneous liquid cell-matrix precursor solution may be used to form an initially inhomogeneous distribution of cells within a 3D substrate. The homogeneity of the cell compartment may decrease over time due to cell migration, differential proliferation, or concentration gradients within the cell compartment. Cells in such a configuration may grow to a substantially homogeneous spatial distribution within the matrix, such as by migrating into and proliferating in regions of the 3D substrate which are initially devoid of cells or bearing cells at a lower initial concentration. Alternatively, cells may grow in such a way to decrease the homogeneity of the cell compartment. In some embodiments, the cell compartment is substantially inhomogeneous at one or more time points during use. For example, different regions of a cell compartment may support higher cellular proliferation or migration rates due to any differences in the spatial distribution of factors or metabolites.

**[00297]** A cell compartment can be configured to be in closed fluidic communication with a bioprocessing system, such as by configuration of inlets and outlets for the purpose of transferring cells, cell-laden matrix precursor solution, or other reagents into the cell compartment of the solid-state bioreactor, or for the purpose of retrieval of the cellular biomass from the cell compartment, including for retrieval of the cellular biomass from a 3D substrate without substantially solubilizing the substrate.

**[00298]** A cell process unit may comprise one or more components configured for cell processing, as shown in **FIG. 18**. As shown in **FIG. 18**, a cell processing unit may comprise a 3D matrix retained by a semi-permeable membrane. The cell processing unit may comprise a fluid flow path or perfusion compartment. The perfusion compartment may include an inlet and an outlet. The inlet may be configured to flow a fresh media or reagent supply to the

fluid flow path and, subsequently, to the 3D matrix. One side of the flow cell may comprise a gas permeable membrane configured to provide gas to the fluid in the fluid flow path and gaseous nutrients to the 3D matrix.

*Fluid flow compartments*

**[00299]** The cell processing unit may comprise one or more fluid flow paths. A fluid flow path may be a compartment or other configuration. In an example, the fluid flow path is a perfusion compartment configured to provide perfusion of material into the cell compartment. In an example, materials may include reagents or nutrients. The nutrients, reagents, and other components may be provided to the compartment via perfusion flow. The perfusion flow may be tangential flow perfusion. For example, during solid-state bioreactor operations using extended cell culture, a tangential flow perfusion may be used to provide for the metabolic needs of cultured cells retained within the cell compartment, such as by the 3D substrate. In some embodiments, the solid-state bioreactor is configured with one or more perfusion compartments, which are separate from, but in fluid communication with, one or more cell compartments. Fluid communication between the cell and perfusion compartment(s) may permit bioprocessing procedures, including genetic engineering, by providing the addition and removal of reagents and factors. In some embodiments, the perfusion compartment(s) are configured for tangential flow perfusion. Further according to this embodiment, the perfusion compartment(s) are configured with a fluid flow path, separate from a 3D substrate, such as in a tangential orientation to a cell compartment, so as to provide a source of nutrients or other reagents from a perfused fluid to a cell compartment, thereby providing for the metabolic needs of cells and enabling the generation of cell product(s). In some embodiments, nutrients, dissolved gasses, and other materials and reagents may be transported by diffusion between the perfusion compartment(s) and cell compartment(s) by passing through an interface, such an interface being defined as the boundary of the 3D cell culture substrate, which substantially contains the cells, regardless of whether such an interface is configured with a semi-permeable membrane, film, or other cell or 3D substrate retention mechanism. In some embodiments, the transport of aqueous reagents between the perfusion compartment(s) and cell compartment(s) may be driven by a difference in fluid pressures among or within the compartments of the solid-state bioreactor.

**[00300]** The 3D matrix and fluid flow paths may be configured in a single flow path or dual flow path configuration, as shown in **FIG. 19**. A single flow configuration may comprise a single flow path disposed adjacent to a long dimension of the 3D matrix to provide tangential flow of across a surface or adjacent to a surface of the 3D matrix. The

fluid flow path may provide unidirectional flow (e.g., tangential flow in a single direction). Alternatively, or in addition to, the fluid flow path may be configured to provide or may provide fluid flow in alternate directions (e.g., the direction of fluid flow may be dynamic and/or switch directions). For example, the fluid flow path may provide fluid flow in a first direction during a first time period and a second direction during a second time period. The fluid flow path may be configured to provide an aerated media. Alternatively, one side of the 3D matrix may be disposed adjacent to a fluid flow path configured to flow a gas. The fluid flow path (e.g., that flows liquid or gas) may be separated from the 3D matrix by a permeable membrane. The permeable membrane may be gas permeable, liquid permeable, or both liquid and gas permeable. Alternatively, the fluid flow path may not be separated from the 3D matrix by a membrane. For example, the fluid in the fluid flow path may contact the 3D matrix during flow (e.g., during tangential flow). In another example, the fluid flow path may be configured to provide or may provide dual flow of media. A dual flow configuration may comprise a fluid flow path disposed adjacent to a first surface of the 3D matrix and a second fluid flow path disposed adjacent to a second surface of the 3D matrix. The first and second fluid flow paths may be disposed directly across, and on opposite sides, of the 3D matrix. The two fluid flow paths may flow fluid in the same direction (e.g., unidirectional flow) or different directions (e.g., bidirectional or counter directional flow). The direction of fluid flow may be consistent during processing or may vary during processing. For example, the fluid flow in the dual fluid flow paths may initial be unidirectional and may change to bidirectional, or vice versa, during cell processing. The dual fluid flow paths may be configured to provide or may provide consistent volumetric flow or flow rate in each fluid flow path. Alternatively, the fluid flow paths may provide differential flow such that one of the fluid flow paths may provide greater volumetric flow or flow rate than the other fluid flow path. The differential flow may be maintained constant during cell processing or may vary during processing.

**[00301]** In some embodiments, a perfusion compartment is configured with one or more fluid flow path(s) tangential to or substantially tangential to a cell compartment, such as by a fluidic flow along an axis of the cell compartment. For example, in a configuration in which the cell compartment occupies a shape and volume which has a long axis, the fluid flow may be configured to flow along the long axis. In an embodiment for which the fluidic flow of the perfusion compartment is substantially tangential to the cell compartment, suspended and dissolved solutes may be transported between the cell and perfusion compartment by diffusion, electrochemical gradients, or electrical fields. For example, the metabolic activity

of cells within the cell compartment may drive a concentration gradient between a supply of fresh cell culture media provided in the perfusion compartment and cell culture media present within the 3D substrate to provide diffusive transport of materials between compartments. In another example, a buffer or fluidic reagent separate from that present within the 3D substrate may be introduced to the perfusion compartment by fluidic flow, providing concentration gradients which may drive diffusive transport of materials between compartments (e.g., buffer exchange, cell washing). In another example, a fluid within the perfusion compartment or cell compartment may contain charged molecules or ions, which can be transported between or within compartments by the application of an electric field (e.g., electrophoresis).

**[00302]** A cell processing unit may be configured for tangential flow or perfusion flow. As shown in **FIG. 20**, a cell processing unit may comprise a uniform 3D matrix comprising a polymer or gel (e.g., hydrogel). Tangential fluid flow may provide fresh media to the 3D matrix while also removing spent media. Tangential fluid flow may provide media in absence of bubble formation. Fluid flow(s) within a cell processing unit may be configured with low Reynolds numbers (e.g., dominated by laminar or sheet-like flow), high Reynolds numbers (e.g., turbulent), or both. Fluid flow(s) within a cell processing unit may be configured for higher or lower flow rates. The use of higher flow rates, turbulent flow, or both, may aid in removing particulate or caking from a membrane separating two compartments. High flow rates may decrease boundary layer thickness observed in laminar flow regimes which may increase local concentration of permeates at the separating membrane, leading to increased local concentration gradients leading to faster diffusion. Fluid flow, continuous fluid flow, or changes in fluid flow in perfusion flows may maintain stability in terms of concentration of nutrients and waste in the 3D matrix. Higher flow rates may create a more stable environment, uniform environment, or both, across the volume of a compartment, such as and between an inlet and outlet of a fluid compartment. Higher flow rates, more turbulent flow, or both, may create increased Taylor dispersion mixing. Lower flow rates, less turbulent flow, or both, may subject cells and other system components, such as membranes and the 3D substrate or matrix, to reduced forces, such as shear forces.

**[00303]** In some embodiments, a perfusion compartment is configured with a fluid flow path primarily tangential to a cell compartment that also causes one or more secondary flow path(s) between the perfusion compartment and cell compartment, such as by application of a pressure gradient between or within the perfusion and cell compartments. For example, application of a pressurized fluid flowing tangential to a long dimension of a cell compartment may generate fluid flow into the cell compartment (e.g., non-tangential fluid

flow). In some embodiments, a perfusion compartment is configured with multiple fluid flow paths. In some embodiments, a perfusion compartment is configured with one or more fluid flow paths to or from other compartments. In some embodiments, a fluid flow path may be configured to flow or may flow a fluid in a first direction and another fluid flow path may be configured to flow or may flow a fluid in a second direction different than the first direction. An angle between the first and second flow directions may be greater than or equal to about zero degrees, 15 degrees, 30 degrees, 45 degrees, 60 degrees, 80 degrees or more. An angle between the first and second flow directions may be less than or equal to about 80 degrees, 60 degrees, 45 degrees, 30 degrees, 15 degrees, 0 degrees, or less. An angle between the first and second flow directions may be from about 0 to 15 degrees, 0 to 30 degrees, 0 to 45 degrees, 0 to 60 degrees, 0 to 80 degrees, 15 to 30 degrees, 15 to 45 degrees, 15 to 60 degrees, 15 to 80 degrees, 30 to 45 degrees, 30 to 60 degrees, 30 to 80 degrees, 45 to 60 degrees, 45 to 80 degrees, or 60 to 80 degrees. In an example, the angle between the first and second flow directions is greater than or equal to about zero degrees. In an example, the angle between the first and second flow directions is greater than or equal to about 45 degrees. In an example, the angle between the first and second flow directions is greater than or equal to about 60 degrees. In an example, the angle between the first and second flow directions is greater than or equal to about 80 degrees. In some embodiments, fluid flow between and within compartments may be utilized to transport suspended or dissolved solutes between compartments.

**[00304]** In some embodiments, diffusion, fluidic flow, or electrical fields are used in combination to transport aqueous reagents to cells within the compartment(s). In some embodiments, a solid-state bioreactor is configured to support alternating between primarily diffusive, flow-based, or electrochemical transport mechanisms, such as over time during a process. For example, a solid-state bioreactor may be configured to operate using a flow-based mechanism for transporting fluids between compartments for a specified interval of time, and then be dynamically reconfigured to operate using diffusion as the primary mechanism of transporting dissolved or suspended solutes between compartments (e.g., as driven by a concentration gradient), and then be dynamically reconfigured to operate using electrochemical gradients under an electric field as the primary mechanism of transporting dissolved or suspended solutes between compartments. In some embodiments, one or more of these material transport mechanisms are used simultaneously. For example, a solid-state bioreactor may be configured to provide a primary fluidic flow tangential to a cell compartment and a secondary fluidic flow through a cell compartment, while diffusive



transport provides for additional transport, mixing, or equilibration of materials between and within compartments.

**[00305]** Multiple perfusion compartments or fluid flow paths may be in fluid communication with a cell compartment. At least 1, 2, 3, 4, 5, 6, 7, 8, 10, or more perfusion compartments or fluid flow paths may be in fluid communication with a cell compartment. In some embodiments, two or more perfusion compartments or fluid flow paths are in fluid communication with a cell compartment. For example, two perfusion compartments may be configured such that fluid flow is directed along two separate axes or surfaces of a cell compartment. In certain embodiments, a perfusion compartment or fluid flow path is in fluid communication with two or more cell compartments. For example, two cell compartments may be in a sandwich configuration, such that the single fluid flow path of a perfusion compartment is directed along the two axes or surfaces of the separate cell compartments.

**[00306]** In an example, the cell processing system (e.g., bioprocessing system) may comprise at least one perfusion compartments. A perfusion compartment may be configured to be in closed fluidic communication with a bioprocessing system. The fluid flow(s) of one or more perfusion compartment(s) may be driven by one or more fluidic system(s) of the bioprocessing system. For example, a single fluidic system may be used to drive fluid flow within one or more perfusion compartments. Using a single fluidic system to drive fluid flow across two or more perfusion compartments may simplify the design and manufacturing of an integrated cell processing system, reducing overall costs. In another example, two or more fluidic systems may be used to drive fluid flows within two or more perfusion compartments. Using two or more fluidic systems to independently regulate the two or more fluid flows among two or more perfusion compartments may have a benefit of providing additional fluidic modes or functions of a cell processing system. The fluidic system(s) of a cell processing system may be integrated into a solid-state bioreactor apparatus, partially integrated into a solid-state bioreactor apparatus and partially provided by a cell processing system, or substantially provided by a cell processing system. For example, the various components of a fluidic system, such as pumps, valves, tubing, connectors, sensors, mixing elements, gas exchange elements, thermal regulation elements, pressure regulators, and other common components of fluidic systems, may be shared among and between one or more perfusion compartments to facilitate fluid flow, in part or in totality, or such components may be configured to operate with one perfusion compartment to facilitate fluid flow. In some embodiments, a fluidic system functionally connected to a perfusion compartment may be configured to operate in a variety of modes and functions. For example, a fluidic system may

operate to generate pressure differentials among or within the compartments, such that the flow rate or pressure of two or more perfusion compartments in fluid communication with a cell compartment may be different, thereby driving additional indirect flow, or secondary flow, through a cell compartment. Such a mode of operation may be used, for example, to enhance the transport rate of solution-phase components to a cell compartment, or to enable washing and buffer exchange procedures within a cell compartment. In certain embodiments, a perfusion compartment, and functionally coupled fluidic system, may be connected to a number of reagent sources, such as by the use of switches or valves. In some embodiments, a perfusion compartment, and functionally coupled fluidic system, may be configured to operate as a fluidic recirculation system. For example, a fluidic system functionally coupled to a perfusion compartment may be configured to recirculate a fluid through a perfusion compartment and one or more additional fluidic components within the fluidic system, such as a reservoir, gas exchange module, or thermal control module. Alternatively, or in addition to, a fluidic system functionally coupled to a perfusion compartment may be configured to supply a fluid from a fluid source to the perfusion compartment, and subsequently to a waste vessel, such as to replace the fluid in the perfusion compartment with a new fluid, e.g., a fluid replacement mode. In some embodiments, a fluidic system functionally coupled to a perfusion compartment may be configured to alternate between modes of operation, such as a recirculation mode, a fluid replacement mode, or a hybrid fluid replacement and recirculation mode, in which the fluid within a fluidic system is replaced in part. For example, a fluidic system functionally coupled to a perfusion compartment may operate by recirculating fluid within a fluid loop which comprises in part the perfusion compartment while, continuously or from time to time, adding a volume of fluid from an input source and bleeding a volume of the recirculating fluid to waste. In such embodiments, the flow rates, pressures, timing, and other aspects of fluid replacement vs. recirculation may be variable or configurable by a cell processing system.

**[00307]** In some embodiments, one or more semi-permeable interface(s) between a perfusion compartment or another aspect of a fluidic system functionally coupled to the perfusion compartment and one or more other functional elements, such as a compartment, module, or other volume, within the bioreactor or fluidic system are configured to permit the transfer of substances between the fluid flow of the perfusion compartment or functionally connected fluidic system and the other functional elements, such substances being atoms or molecules of gas or dissolved gas. For example, one or more volumes within a fluidic system functionally connected to a perfusion compartment, or cognate to the perfusion compartment,

comprises a gas compartment in molecular communication with the fluid, such as by using a gas-permeable membrane to permit gasses such as O<sub>2</sub> to be absorbed by the fluid. Gasses (which may be sterile, such as by filtration or another sterilization method), including environmental air, O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>, or mixtures thereof, may be provided. A gas-permeable membrane may be any membrane (e.g., porous or non-porous) at least partially permeable to gas molecules. For example, a gas permeable membrane may provide sufficient permeability under operating conditions (e.g., gas flow rates, liquid flow rates, pressures, temperatures, etc.) adjacent to the membrane to provide sufficient oxygenation to cells within the 3D matrix. A liquid impermeable membrane may be any membrane (e.g., porous or non-porous) that is impermeable or substantially impermeable to a liquid (e.g., media, wash solution, etc.) under operating conditions (e.g., gas flow rates, liquid flow rates, pressures, temperatures, etc.) adjacent to the membrane. For example, a gas exchange mechanism, which may be in a membrane or hollow-fiber configuration, may be provided as a component of a fluidic system functionally connected to a perfusion compartment, such that fluids flowing into or recirculating through a perfusion compartment are gas exchanged. In another example, a gas-exchange membrane, such as a PDMS membrane, which allows the transfer of gasses but not liquids, may form an interface of a perfusion compartment, thereby gas-exchanging fluids present or flowing within the perfusion compartment.

**[00308]** A perfusion compartment may be configured to be in closed fluidic communication with a bioprocessing system, such as by the configuration of inlets and outlets for the purpose of transferring cells, culture media, or other reagents to and from a perfusion compartment or cell compartment of the solid-state bioreactor, or for the purpose of retrieval of the cellular biomass from the cell compartment, including for retrieval of the cellular biomass from a 3D substrate without substantially solubilizing the substrate. In an example, a perfusion compartment may be configured to provide or may provide polymeric precursors, cells to the cell compartment, or both to the cell compartment. In another example, a perfusion compartment may be configured to provide a variety of nutrients, reagents, stimuli, or any combination thereof to permit processing of cells within the cell compartment. In another example, a perfusion compartment may be configured to receive or may receive cells from the cell compartment.

**[00309]** The cell processing system may comprise a 3D matrix. The 3D matrix may be configured in a plate-like or slab configuration, as shown in **FIGs. 21A-21C**. The 3D matrix may be retained within a compartment. The 3D matrix may be disposed adjacent to a fluid flow path (e.g., perfusion compartment), as shown in **FIG. 21A**. The thickness of the 3D

matrix may be configured to provide efficient mass transfer of nutrients and reagents. The fluid in the fluid flow path may directly contact the 3D matrix. Alternatively, the fluid in the fluid flow path may be separated from the 3D matrix by a permeable or semi-permeable membrane disposed between the fluid flow path and the 3D matrix. The fluid flow path may be configured to provide fluid, nutrients, reagents, or any combination thereof to the 3D matrix and remove waste metabolites, unused reagents, unused nutrients, or any combination thereof from the 3D matrix via diffusion or perfusion mass transfer. The cell processing unit may comprise a plurality of stacked 3D matrices and fluid flow paths. A fluid flow path may provide fluid, nutrients, reagents, or any combination thereof to a single 3D matrix. A 3D matrix may have fluid, nutrients, reagents, or any combination thereof provided by a single fluid flow path. Alternatively, and as shown in **FIG. 21B**, a 3D matrix may be sandwiched between two fluid flow compartments and both fluid flow compartments may be configured to provide fluid, nutrients, reagents, or any combination thereof to the 3D matrix. In a stacked configuration, an individual fluid flow path may provide fluid, nutrients, reagents, or any combination thereof to two or more 3D matrices. In such a configuration, mass transport of materials (e.g., nutrients or reagents) into the 3D matrix and waste metabolites from the 3D matrix may be bi-directional such that mass is transported to and from the fluid flow paths on each side of the 3D matrix. Alternatively, and as shown in **FIG. 21C**, mass transport of materials (e.g., nutrients or reagents) may be unidirectional. In such a configuration, one of the fluid flow paths may be configured to provide nutrients or reagents to the 3D matrix. The other fluid flow path in the sandwich may comprise fluids without nutrients or reagents, generating a driving force for mass transport from the first fluid flow path, through the 3D matrix, to the second fluid flow path.

**[00310]** In an alternative configuration, a cell processing system may include 3D matrices in a fiber-like configuration, as shown in **FIGs. 22A** and **22B**. The cell processing unit may comprise a fluid flow path (e.g., media perfusion compartment). The fluid flow path may be tube, cylinder, or other compartment configured to flow a fluid. The fluid flow path may comprise a plurality of 3D matrix fibers disposed down a length of the fluid flow path, as shown in **FIG. 22A**. Alternatively, the fluid flow path may comprise a plurality of fiber-like pathways surrounded by a 3D matrix, as shown in **FIG. 22B**. The fluid flow path may provide fluid, nutrients, reagents, or any combination thereof to a plurality of fiber-like 3D matrices such that nutrients or reagents diffuse radially into the 3D matrix and waste metabolites diffuse or otherwise transport radially out of the 3D matrix.

[00311] As shown in **FIG. 23**, a cell processing unit may comprise a 3D matrix. The 3D matrix may be in a cell compartment. The cell compartment may be disposed adjacent to a fluid flow pathway. The fluid flow pathway may provide tangential flow in which fluid flows along or adjacent to a surface of the 3D matrix. Alternatively, or in addition to, the fluid flow pathway may comprise a perfusion manifold. A perfusion manifold may comprise a plurality of fluid inlets and a plurality of fluid outlets. The plurality of fluid inlets and outlets may be disposed on a single side of a 3D matrix. The fluid flow manifold may include a perfusion manifold on a top and bottom side (e.g., or any side across a 3D matrix from one another) of the 3D matrix. A cell processing system may comprise a plurality of cell processing units, each comprising a cell compartment and one or more fluid flow paths. The plurality of cell processing units may be arranged in a parallel configuration. Alternatively, or in addition to, the multiple configurations may be disposed in series. To scale up production, additional cell processing units may be added to a cell processing system to increase the total available volume for cell processing.

[00312] An individual cell processing unit, as shown in **FIG. 24**, may be configured with an inlet on one side of the cell processing unit and an outlet on another side of the cell processing unit. The inlet and outlet may be configured to provide tangential fluid flow to a cell compartment in the cell processing unit. Alternatively, the inlet and outlet may be configured to provide perfusion flow via a plurality of inlets and a plurality of outlets disposed adjacent to a side of the cell 3D matrix comprising a long dimension.

*Alternate flow configurations*

[00313] In some embodiments, a solid-state bioreactor may comprise a configuration which does not utilize tangential flow systems and methods described herein. In some embodiments, a solid-state bioreactor may comprise a vessel which is not configured for fluidic flow. In some embodiments, a solid-state bioreactor may comprise a vessel with a cell compartment as described and defined herein, and an additional space or volume configured to hold cell culture media or other liquids in fluidic communication with the cell compartment. For example, a 3D cell culture substrate, which is configured to substantially retain cells, including by the use of interfaces or membranes as described herein, may be configured within a bag, flask, or other vessel. Cell culture media may be added to the vessel, thereby providing a source of components which provide for the metabolic needs of cells residing within the 3D substrate. For example, a 3D substrate, such as a hydrogel layer, may be deposited in a flask, such as a tissue culture flask or G-Rex flask, along with cell culture media. Over time, additional volumes of such cell culture media or other fluid may be added

to the vessel, or volumes of the culture media or other fluid may be removed from the vessel, to provide for additional components supporting cell metabolic needs and bioprocessing, or to dilute or remove reagents or metabolic and other cellular byproducts which are present in the culture system or which accumulate as a result of cellular and metabolic activities. Alternatively, or in addition to, the cell culture media or other fluid may be replaced, continuously or from time to time, in part or in full, to provide an adequate environment for cell culture. In some embodiments, the fluid flow may not be tangential to a cell compartment. For example, a fluid may be substantially static throughout bioreactor operation.

**[00314]** In some embodiments, the cell compartment may be configured with a 3D substrate, including those which resemble a packed bed, which is structured to provide a plurality of perfusion flow paths through the cell compartment. For example, a cell compartment may be configured with a plurality of channels distributed to provide uniform local perfusion. The channels may be formed by any number of methods, including through the use of a sacrificial material during formation of the 3D substrate and by solid-state bioreactor apparatus which contain pre-fabricated fluid paths in the cell compartment.

#### *Fluidic control systems*

**[00315]** A solid-state bioreactor may comprise one or more fluidic systems, including fluidic control systems. Alternatively, a solid-state bioreactor may not comprise a fluidic system or fluidic control system. In an example, a solid-state bioreactor may comprise a cartridge-like configuration that is coupled to fluidic systems or fluidic control systems of a cell processing system. Fluidic control systems may be entirely integrated into a bioreactor apparatus, entirely integrated into a bioprocessing system separate from but configured to interface with a bioreactor apparatus, or in any combination. In some embodiments, fluidic control systems include permanent ex situ components, which are wholly incorporated into a cell processing system, but configured to interface with a solid-state bioreactor apparatus or other cell processing unit and be reused over time in various operations. For example, one or more peristaltic pumps in a bioreactor apparatus may be configured to interface with tubing in a solid-state bioreactor apparatus to facilitate movement of liquids within fluidic paths and systems of the bioreactor apparatus. In some embodiments, fluidic control systems include single-use fluidic control components which are wholly or partially incorporated into the bioreactor apparatus or other cell processing unit, but configured to be in communication with control, electronic, and computer systems of a bioprocessing system. For example, any components of a fluidic control system which are in contact with biomaterials, and which

may otherwise be sterilized between manufacturing batches between patients, may be configured as single-use components within a solid-state bioreactor apparatus. Alternatively, components in contact with biomaterials may be configured to be sterilized between manufacturing batches.

**[00316]** The fluidic systems may comprise pressure and flow regulation system(s), including positive and negative pressure embodiments, or combinations thereof, among the various fluidic systems. Fluidic system components may include pumps, including but not limited to peristaltic pumps, syringe pumps, diaphragm pumps, impeller pumps, or any combinations thereof. Pump configurations may application dependent and may be modified to provide a given flow rate, pressure, fluid characteristics, or any combination thereof. Fluidic system components may include valves which control the direction, flow, and pressure of fluids within the system, including but not limited to solenoid valves, check valves to prevent backflow, pinch valves (e.g., used with peristaltic pumps to control flow without contamination), and proportional valves for precise flow rate control. Fluidic system components may include tubing, connectors, and other conduits through which fluids are transported within the system. Fluid conduits may be comprised of materials which include, but are not limited to, silicone, Tygon, PTFE, PVC, or any combination thereof, which may be selected based on chemical and biological compatibility, flexibility, and sterility. Fluidic system components may include sensors to monitor various parameters including, but not limited to pressure, flow rate, pH, temperature, oxygen levels, or any combination thereof within the fluidic system. Sensors may include, but are not limited to, pressure sensors, flow sensors, temperature sensors, chemical sensors (e.g., pH and dissolved oxygen sensors), or any combination thereof. Fluidic system components may include mixers to ensure that fluids within the system are homogeneously combined, including but not limited to static mixers, which may use a series of geometric mixing elements or turbulent flows, and dynamic mixers, which use mechanical agitation (e.g., impellers, paddles). For example, the fluidic system of a solid-state bioreactor may comprise a mixer which is used to mix cells with a liquid 3D substrate precursor solution, which subsequently forms the cell compartment. In another example, the fluidic system may comprise a mixer which is used for on-demand mixing of fluid reagents, such as the mixing of cell culture additives into a base media (e.g., to introduce a reagent such as a cytokine, like IL-2, into a cell culture media). Such mixing elements can increase the flexibility of the solid-state bioreactor by permitting multiple formulations of cell culture media to be created on demand within a bioreactor from separate input sources or a reagent system provided by the cell processing system. Solid-state

bioreactor configurations with mixing elements may increase the stability, and therefore the working life, of system reagents. For example, a reagent which has increased stability at 4°C may be stored at its optimal temperature, while a separate reagent which has adequate stability at 22°C may be stored at ambient or room temperature, and the two mixed on demand within the bioreactor, thereby reducing the volume of 4°C reagent storage (as compared to storing both reagents at the lower optimal temperature) or increasing the shelf life of the temperature-sensitive reagent (as compared to storing both reagents at ambient room temperature). In some embodiments, mixing components are capable of mixing at least 2, 3, 4, 5, 6, 8, 10, or more fluid reagents. Fluidic system components may include one or more gas exchange modules, for example, to oxygenate liquid media. Fluidic system components may include pressure regulators, for example, to maintain a consistent pressure within the fluidic system which may prevent damage to system components and provide accurate operation. Such pressure regulators may include but are not limited to, back-pressure regulators, which control upstream pressure, pressure-reducing regulators, which control downstream pressure, or both. Fluidic system components may include flow cells, or chambers where fluids can be analyzed or processed. In bioreactors, flow cells can be used for inline monitoring of cell cultures or reactions without disturbing the system. Flow cells may be compatible with sensors and to allow easy integration with the flow path. Fluidic system components may include filters, including membranes and other filtration mechanisms, such as tangential flow filtration units and alternating flow filtration units, which can remove particulates and contaminants from fluids. Fluidic system components may include in-line sterilization units, such as UV sterilization systems, which may provide fluids free from viable microbes. The fluidic system of the bioreactor or cell processing system may be configured with one or more input(s) and output(s), such as for reagents, in-process sampling, and waste. The fluidic system of the bioreactor or cell processing system may be configured with compartments for internal fluid storage, such as reservoir(s) or vessel(s). The fluidic system may comprise a control system, which integrates sensors and actuators to regulate the fluidic system automatically. Such systems may utilize microcontrollers, programmable logic controllers (PLCs), or personal computer (PC)-based control systems equipped with software for real-time data processing and control. The fluidic systems of the bioreactor may be configured to be in fluid or electronic communication with a cell processing system. Components of the fluidic system may function together to precisely control the environments and conditions required for solid-state bioreactor applications.



Integration of the fluidic system with the bioreactor or cell processing system may increase overall system reliability, performance, or effectiveness.

*Thermal control systems*

**[00317]** The cell processing system may include a thermal control system configured to maintain, control, or otherwise provide thermal regulation to one or more components of the bioreactor (e.g., perfusion compartment, reagents, cell compartment, etc.) or cell processing unit (e.g., reagents, fluidic system, fluids, analytical systems, cell processing components, etc.). In some embodiments, the temperature of the bioreactor or of the cell compartment may be entirely regulated by a system of conditioning the temperature of the perfused liquid, with such a thermal conditioning system being integrated into the bioreactor or provided by the cell processing system. For example, the solid-state bioreactor may be configured with a thermal element, such as a Peltier cooler, thermal sheath (e.g. liquid sheath not in fluid communication with the perfusion compartment), heater, thermoelectric heat pump, which interfaces with a perfusion fluidic system, or any combination thereof such that media flowing through the perfusion compartment or in fluid communication with the cell compartment is thermally conditioned to provide thermal conditioning (e.g., temperature regulation) to the cell compartment. In some embodiments, the cell processing system may be configured with one or more thermal zones, which are configured to heat, cool, or maintain a temperature of one or more cell processing units, or components thereof, when disposed therein. In some embodiments, the bioreactor may be configured with a one or more thermal interfaces between the bioreactor and a conductive element or thermal element, such as a Peltier cooler, heater, thermal sheath, thermoelectric heat pump, or any combination thereof to directly regulating the temperature of the bioreactor. In some embodiments, the bioreactor may be configured with one or more integrated thermal element such as a Peltier cooler, heater, thermal sheath, thermoelectric heat pump, or any combination thereof which is configured to be in communication with the bioreactor system, for example, in electrical communication with a power supply or in electrical communication with a control system provided by the bioreactor system. In an example, the cell processing system or bioreactor comprises one or more temperature sensors and a temperature control unit. Methods of solid-state bioreactor or cell processing thermal control may include, but are not limited to, internal coils, heat exchangers, incubators, baths, Peltier elements, thermal jackets or sheaths, or any combination thereof, which can be electrically heated or cooled or circulate fluids such as coolants. The solid-state bioreactor or cell processing system may be configured with, or configured to be in communication with (e.g., via the bioreactor system or cell processing

system), advanced process control systems that monitor and adjust the temperature continuously. This integration may include the use of proportional, integral, derivative (PID) controllers, which can adjust the heat input based on the difference between the actual and set-point temperatures, minimizing deviations and enhancing process stability.

*Sensors and feedback systems*

**[00318]** In some embodiments, the solid-state bioreactor or cell processing system comprises an integrated sensor system. An integrated sensor system may be used to monitor processes, either continuously or non-continuously, in real time or not in real time. An integrated sensor system may record process data and permit feedback-based biomanufacturing processes via use of a control system that incorporates data from one or more sensor(s). Incorporation of these advanced manufacturing strategies can reduce process-related and patient-specific variability of cellular products, such as by monitoring and controlling bioreactor environmental conditions and cellular attributes such as cell health, number, phenotype, or any combination thereof to provide consistent product quality. The configuration and choice of specific sensors and feedback paradigms may be determined by the critical materials attributes (CMAs) and critical process parameters (CPPs) that provide consistent, safe, and effective product manufacturing. A control strategy can be used to monitor and control set points in the process to ensure Critical Quality Attributes (CQAs) are met. In some embodiments, sensors and feedback systems are entirely integrated into the bioreactor apparatus, entirely integrated into the cell processing system separate from but configured to interface with the bioreactor apparatus, or in any combination thereof. In some embodiments, a solid-state bioreactor is configured to interface with ex situ sensors, which are partially or wholly incorporated into a bioprocessing system and are re-used over time in various operations. For example, a solid-state bioreactor may contain a feature such as an optical window which is configured to functionally interface with an optical system provided by a cell processing system, such as for the acquisition of optical measurements. Such a configuration may permit the reuse of expensive or sensitive optical systems over time. In some embodiments, the solid-state bioreactor is configured with one or more single-use in situ sensor(s), or components thereof, and may be configured to be in communication with control, electronic, or computer systems of a cell processing system. For example, a sensor or component of a sensor may be integrated into a solid-state bioreactor apparatus, and configured to interface with one or more other components of a sensor system, such as an electrical or optical component provided by a cell processing system. Such configurations may permit production of sterile, single-use consumables containing sensor elements which

may be in fluid or physical communication with the internal bioreactor environment (e.g., a cell compartment or perfusion compartment), while expensive or sensitive components of the sensor system, such as electrical or optical components, may be provided by the bioprocessing systems and reused.

**[00319]** In some embodiments the bioreactor comprises sensors and sensor systems suitable for monitoring, identifying, or controlling relevant product CMAs and CPPs including, but not limited to, 3D substrate properties, cell properties, proteins, chemicals, metabolites, gasses, volatile organic compounds (VOCs), temperature, pH, or other physical attributes of the system, such as mass, physical dimensions, physical configurations, or any combinations thereof.

**[00320]** Measurable attributes of the system, including the solid-state bioreactor, may depend on physical properties and configurations of various apparatus and components, including but not limited to, the state of various interfaces and interconnections, relative or absolute spatial configurations, volume, and mass. For example, a solid-state bioreactor may be configured with sensors suitable for monitoring connections or interfaces, either internal or with a bioprocessing system, to determine if connections or interfaces are intact. In another example, a solid-state bioreactor may be configured with sensors suitable for monitoring the volume or mass of an aspect of the bioreactor, such as a compartment (e.g., perfusion compartment or cell compartment), 3D matrix, or combination thereof. Measurable attributes of a 3D substrate and cell compartment may include those usable to characterize structure and function, including but not limited to elastic modulus, viscosity, contractility, conductivity, matrix organization, or combinations thereof. Measurable attributes of cells may include those usable to characterize cellular properties, including but not limited to viability, motility, confluence, cell number, cell health, or any combinations thereof. Other measurable attributes of cells may include cellular identity or phenotype properties, including but not limited to, cellular differentiation state, morphology, gene expression, genome state, including 3D conformation, DNA modifications, genome-protein interactions, genome stability, or any combinations thereof. Measurable attributes of cellular metabolism and cellular activity may include properties related to expression or production of cellular components, including but not limited to extracellular matrix production, protein expression, protein secretion, production of viral particles, lipid production, nutrient, metabolite, waste analysis, cell signaling, cellular bi-products, respiratory activity, oxygen and carbon dioxide transfer rates, or any combinations thereof. Measurable attributes of the cell culture environment, in addition to measurable attributes of the system and 3D cell culture substrate,

may include pH, osmolality, temperature, media composition, chemical composition, sterility, or any combination thereof.

**[00321]** A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, a wide variety of sensing methods, technologies, and platforms described elsewhere herein. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more pH sensors, including but not limited to porous glass electrode-based electrolyte-filled sensors, MOSFET-based ISFET pH sensors, optical pH sensors, potentiometric sensors, those utilizing other electrochemical-sensing technologies, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more temperature sensor(s), including but not limited to thermocouples, resistance temperature detectors (RTD), optical temperature sensors, thermistors, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more gas sensor(s), including but not limited to electrochemical sensors, sensors that utilize electron paramagnetic resonance, piezoelectric absorption sensors, spectroscopic and optical sensors (e.g., IR absorption or Raman), or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more sensors related to the fluidic system, as described above. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more dissolved oxygen sensors, such as a Clark sensor. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more spectroscopic sensors, including but not limited to Raman, Fourier transform infrared spectroscopy (FTIR), near-infrared (NIR) spectroscopy, mid-infrared (MIR) spectroscopy, or any combination thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more optical sensors, including but not limited to sensors which measure optical density, absorption, reflectance, scattering, fluorescence, transmittance, colorimetry, refractive index, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more microscope(s). For example, a solid-state bioreactor may be configured with an optical interface which couples with an optical system provided by the cell processing system, such as to acquire microscopic images. Such microscopes may utilize a variety of modalities including but not limited to light microscopy, brightfield, phase contrast, darkfield, fluorescence, widefield, confocal, electron microscopy, Raman, super-resolution microscopy (e.g., stimulated emission depletion (STED) microscopy,

photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), or any combinations thereof), light sheet, structured illumination, digital holography, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more biosensors, including but not limited to electrochemical, optical, thermal, piezo-electric devices, or combinations thereof for sensing biocatalysts such as enzymes or biomolecules such as antibodies or other binding agents or ligands. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more sensors capable of sensing within a 3D substrate, such as optical coherence tomography (OCT), FTIR, confocal modalities, multiphoton modalities, structured illumination modalities, micro-impedance sensors, or any combinations thereof which can measure the resistance and reactance encountered by an electrical current as it passes through biological materials at the microscale. For example, micro-impedance techniques can be used to monitor cell health, growth, proliferation, or any combinations thereof non-invasively. Changes in cellular impedance can indicate alterations in cell morphology, adhesion, and confluence during growth. As another example, FTIR measures the infrared spectrum of absorption or emission of a solid, liquid, or gas, which can be used to identify chemical compounds through their vibrational spectra. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more acoustic sensors, including but not limited to ultrasound, such as for ultrasound imaging or ultrasound elastography, photoacoustic microscopy (PAM) modalities, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more advanced imaging and sensing technologies, including but not limited to X-ray, Magnetic Resonance Angiography (MRA), Computed Tomography (CT), or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more mechanical sensors, including but not limited to sensors for mechanical deformation, viscosity, elastic modulus, contractility, conductivity, other sensors of 3D substrate organization, or any combinations thereof. A solid-state bioreactor or cell processing system may be configured with, or configured to interface with, one or more liquid sensors, such as to detect the presence or absence of liquids within the bioreactor or compartments thereof.

**[00322]** In some embodiments, the solid-state bioreactor or cell processing system comprises, or is configured to interface with, sensors capable of, and in a configuration in which, sensor data can be acquired relating to the state of or activities of cells. For example, a

solid-state bioreactor or cell processing system may be configured with, or configured to interface with, viability sensors, confluence sensors, sensors which can measure a cell density or cell number, sensors which can detect other cellular attributes, or any combinations thereof. For example, bioreactors or cell processing systems may comprise capacitance sensors, impedance sensors, or both for monitoring or otherwise detecting viable biomass in the bioreactor or cell processing system. These monitor or detect electrical properties of a medium—for example capacitance and impedance—change in response to variations in the composition and properties of the medium, such as the presence and concentration of living cells. In some embodiments, a solid-state bioreactor or cell processing system is configured with, or is configured to interface with, sensors capable of measuring capacitance, impedance, or both within a bioreactor compartment, such as the cell compartment or 3D cell culture substrate. For example, capacitance sensors can measure the capacitance between two electrodes that are integrated into or coupled with the bioreactor or cell processing system. The capacitance may be altered by the permittivity of the medium between the electrodes, which may change with the concentration of cells. Living cells may have a high permittivity compared to the culture medium due to their cytoplasmic content. In another example, impedance sensors, which may combine resistance and capacitance measurements, can detect an impedance of bioreactor or cell processing system contents, which may be altered by the quantity and properties of cells within the bioreactor or cell processing system, such as due to cell membrane characteristics, which may affect how the electrical current passes through or around cells. In an example, a solid-state bioreactor or cell processing system may be configured with, or configured to interface with, dielectric spectroscopy sensors. Dielectric spectroscopy may involve measuring the dielectric properties (e.g., permittivity and conductivity) of a material across a range of frequencies. This method may provide insight into the electrical characteristics of the cell suspensions and how these characteristics vary with frequency. Dielectric spectroscopy may assess the total biomass and provide detailed information about the state of cells (e.g., cell viability, phase of growth, etc.). The measurement of permittivity and conductivity across a spectrum of frequencies can distinguish between the contributions of different cellular components (e.g., membranes, organelles, etc.) and the extracellular medium. For example, at low frequencies, current may not pass through the cell membrane easily, resulting in high impedance, while at high frequencies, current can pass through both the cells and the medium, lowering the impedance. Dielectric spectroscopy of cell cultures may provide information related to beta dispersion, caused by the polarization of cell membranes. The characteristic frequency of a dispersion

may provide information about cell size, viability, and membrane integrity. By integrating dielectric spectroscopy, capacitance sensors, impedance sensors, or any combinations thereof into the solid-state bioreactor or cell processing system, or configuring the solid-state bioreactor or cell processing system to interface with dielectric spectroscopy, capacitance sensors, impedance sensors, or any combination thereof multiple cell characteristics may be continuously monitored, such as, for example, viable cell density, growth phase, health status, or any combinations thereof of the culture without invasive sampling. This real-time monitoring can also be used for improving culture conditions across various applications and for scaling up bioprocesses.

**[00323]** In some embodiments, a solid-state bioreactor or cell processing system comprises, or is configured to interface with, cytometry systems, such as in a configuration in which cytometry data can be acquired relating to the state of or activities of cells within the bioreactor or cell processing system. Cytometry may include a variety of technologies designed to measure the physical and chemical characteristics of cells, including but not limited to flow cytometry, imaging cytometry, mass cytometry, acoustic cytometry, impedance cytometry, spectral cytometry, or any combinations thereof. For example, a solid-state bioreactor or cell processing system may be configured with, or configured to interface with, a flow cytometer, which utilizes fluid flow to pass cells through a laser beam while a detector measures light scatter and emitted fluorescence. In another example, a solid-state bioreactor or cell processing system may be configured with, or configured to interface with, an imaging cytometer, which uses a high-resolution camera to acquire image data of cells. In some embodiments, immunostaining and other cell staining or analyte labeling workflows may be integrated into, or functionally coupled with, a solid-state bioreactor or cell processing system to provide cytometric measurements of specific analytes such as cell-surface proteins, intracellular proteins, cellular states (e.g., viability), or any combinations thereof. Immunostaining and other cell staining or analyte labeling workflows may be used in the context of a 3D cell culture substrate, although incubation and washing may be lengthened or include agitation or liquid mixing to provide transfer of reagents into the 3D substrate such that they come into contact with cells, or are washed away to reduce background signals. In some embodiments, a solid-state bioreactor or cell processing system may be configured with an analysis compartment, cell compartment, perfusion compartment, fluid flow pathways, etc., which may be separate from and may not be in fluidic communication with one or more other analysis compartment, cell compartment, perfusion compartment, fluid flow pathways, etc., of the bioreactor or cell processing system. For

example, a solid-state bioreactor or cell processing system may be configured with one or more compartments or analysis systems directed at manufacturing a cell product or cell byproduct, and one or more compartments or analysis systems directed at analysis, such as a compartment configured with, or to interface with, an imaging cytometer. In such a configuration, cells or cell byproducts harvested for the purpose of product manufacturing may not be contaminated by, or otherwise comixed with, the separate plurality of cells subjected to cytometry analysis and, when optionally including analyte labeling, exposed to reagents used for such a purpose, which may not be compatible with a certain manufactured product specification. In such a configuration, providing a separate plurality of cells for analysis may permit flexible analysis of cell characteristics without concern for contamination of manufactured product(s) with analysis reagents or subjecting manufactured product(s) to analysis workflows.

**[00324]** In some embodiments, a solid-state bioreactor or cell processing systems comprises, or is configured to interface with, sensors with suitable depth capacity for sensing within a cell compartment and in a configuration in which sensor data can be acquired relating to the state of or activities within the cell compartment. For example, a solid-state bioreactor or cell processing systems may be configured with, or is configured to interface with, sensors and systems for Raman spectroscopy configured to measure the composition of a 3D substrate containing cells. The Raman spectroscopy analysis system may be configured to detect the presence of or quantify the concentration of a variety of analytes within the 3D culture environment. A Raman spectroscopy analysis system may be configured to detect or infer the presence of, or quantify the concentration of, cellular identities or states, including but not limited to differentiation state (e.g., stem vs non-stem) or immune lineage states (e.g., CD3+, CD4+, CD8+ T cells). To permit such in situ measurements, a solid-state bioreactor or cell processing systems may be configured with, or configured to interface with, optical or spectrophotometric sensors or systems capable of acquiring measurements under the physical constraints imposed by a 3D substrate. These devices may be capable of accurately measuring attributes such as light absorption through potentially uneven, semi-solid materials. In some embodiments, instrument and sensor calibration against a standard or control sample may be used to ensure that measurements, such as in situ optical density, microscopy, spectroscopy, or dielectric spectroscopy, accurately reflect the conditions within a 3D substrate. This may include systems and methods for measuring, calibrating for background of, or correcting for, the 3D substrate itself or for any changes in optical or other



measurement properties, including but not limited to changes in path length due to swelling or degradation of a 3D substrate over time.

**[00325]** In some embodiments, a solid-state bioreactor or cell processing system comprises electrode arrays or microelectrode arrays (MEAs) integrated into the 3D culture apparatus or 3D substrate, which can function to record the electrical activity of cells and provide localized stimulation without direct contact. MEAs may be useful for long-term studies of electrophysiological activity in neurons and cardiomyocytes, permitting real-time monitoring and control of electrical stimuli. In some embodiments, electrodes placed above or below the cell culture substrate can be used to create electric field(s) that indirectly stimulate or otherwise perturb the cells, including for the purpose of membrane electroporation. This technique may be less invasive than direct stimulation and may reduce the risk of electrode degradation and toxicity. Such a configuration for field stimulation may be effective for uniformly exposing a large number of cells within a 3D substrate to an electric field. In some embodiments, the cell processing system, consumable, or bioreactor may be configured to provide or may provide one or more of mechanical cues, electrical cues, flow cues, or any combinations thereof. In some embodiments, simultaneous mechanical cues, electrical cues, flow cues, or any combination thereof can be delivered, such as by using a bioreactor or system configuration which integrates both mechanical, electrical, and flow-based stimulation to mimic the dynamic environment of certain tissues more accurately, where electrical impulses and mechanical forces are intimately linked. In some embodiments, mechanical cues, electrical cues, flow cues, or any combinations thereof may be provided sequentially. In some embodiments, one or more of mechanical cues, electrical cues, flow cues, or any combinations thereof may be provided to cells or the 3D matrix separately over time during a process. For example, the cell processing system may be configured provide structural cues to the 3D matrix via stretching or compressing the 3D matrix. In another example, the cell processing system may be configured to provide fluid flow configured to mimic dynamic fluid environments of certain tissues, for example, by providing fluids at a given pressure or providing pulsed flow to mimic pulsatile flow within a body. In another example, the cell processing system may be configured to provide electrical signals to the 3D matrix or cells within the 3D matrix to mimic electrical environments of certain tissues. In some embodiments, the solid-state bioreactor or cell processing system may be configured to perform cell membrane electroporation, or electroporation. Parameters which may affect cell membrane electroporation include cell attributes, the conductivity and composition of an electroporation buffer, voltage, pulse duration, electrode configuration,

which can be configured to achieve effective permeabilization with minimal cytotoxicity, or any combinations thereof. In some embodiments, continuous monitoring is used by the solid-state bioreactor or cell processing system to permit precise control of electrical parameters during electroporation to provide consistent results, as variability in these parameters, such as conductivity, may affect the efficiency of molecule uptake and cell viability. Compared to suspension electroporation methods, in situ (solid-state) electroporation may permit precise control over electroporation parameters including strength and homogeneity of applied electrical fields.

**[00326]** In some embodiments, sensors and methods described herein may be used for identifying materials, quality control, purity analysis, mixture analysis, or any combinations thereof within a solid-state bioreactor or cell processing unit. In some embodiments, sensors and methods described herein are used to determine the internal state of a bioreactor or cell processing system or to otherwise provide feedback on bioreactor or cell processing system operation. In some embodiments, the solid-state bioreactor or cell processing system is configured with, or configured to interface with, one or more sensor array(s), which may collect a variety of data types from different aspects, areas, or compartments of a bioreactor or cell processing system, or from a plurality of aspects, areas, or compartments of a bioreactor or cell processing system. Maintaining and controlling bioreactor conditions may provide control over target yields and cell characteristics. In some embodiments, a solid-state bioreactor or bioprocessing system uses data from one or more sensor(s) to control a bioreactor or cell processing procedure. For example, various sensors including but not limited to pH sensors, metabolite sensors, dissolved oxygen sensors, or any combinations thereof may be used to control the timing of fresh media addition in a cell perfusion configuration. In such embodiments, a solid-state bioreactor may utilize or be configured to interface with a bioprocessing system with control systems including but not limited to those of the following descriptions: Classical control strategies, such as PID; Digital control strategies, such as adaptation, gain scheduling, and self-tuning concepts, which can be integrated into PID and other control schemes to control complex bioprocessing procedures subject to highly nonlinear dynamics; Distributed control strategies (DCS) utilizing a computer system to receive data from one or more sensors or local control systems which communicate and interact with controllers, inputs, and outputs; or systems and methods for autocalibration, autoranging, signal conditioning, characterization, and instrument or component self-diagnosis.

*Integrated consumable*

[00327] In some embodiments, the cell processing system comprises a consumable for processing cells, as shown in **FIG. 64**. The consumable may be a consumable that integrates with the cell processing system. Cells may be completely, or partially, processed within the consumable. Alternatively, the consumable may provide cells to the cell processing system for processing. In an example, the consumable is configured to completely process or partially process cells. The consumable may be configured to receive cells. The cells may be received in a frozen or solution phase (e.g., thawed) state. The cells may be directly input into the consumable by a user. Alternatively, a cell source (e.g., bag, reservoir, etc. of cells) may be fluidically connected with the consumable to provide cells to the consumable. The consumable may be configured to provide process cells to a preservation unit (e.g., cryopreservation unit) for preserving. Alternatively, the consumable may be configured to provide a preserved cell product. The consumable may comprise a sensor interface configured to permit the cell processing system sensors and sensor systems to interface with the consumable. The consumable may comprise a thermal control system configured to heat, cool, or heat and cool cells, reagents, or other materials within the consumable. The consumable may comprise a locking mechanism configured to couple or that couples the consumable to the cell processing system. The consumable may comprise a mechanical interface for pumps and valves configured to fluidically connect the consumable to the fluidic or pneumatic systems of the cell processing unit. The consumable may comprise an electrical interface, data interface, or both configured to electrically couple the consumable to the cell processing system. The consumable may be configured to provide negative or positive cell selection, as described elsewhere herein. For example, the consumable may comprise bead isolation reagents for cell selection.

[00328] The consumable may comprise the solid-state bioreactor (e.g., 3-D matrix for cell processing), as shown in **FIG. 65**. The consumable may comprise a single 3-D matrix or a plurality of 3-D matrices as described elsewhere herein. The consumable may comprise a warm zone, a cold zone, or both a warm zone and a cold zone. The warm zone may be configured to incubate or heat cells, reagents, media, or any combination thereof. The cold zone may be configured to cool or preserve cells, reagents, media, or any combination thereof. The consumable may comprise a media reservoir. The media reservoir may be configured to store media, prepare media via mixing reagents or media components together, provide media to the 3-D matrix or matrices, remove media from the 3-D matrix or matrices, or any combination thereof. The consumable may further comprise reagent storage. Reagent storage may be configured to store reagents, for example, in a temperature controlled

environment. The consumable may be provided to the cell processing system with cell or cell may be provided to the consumable after the consumable has been integrated with the cell processing system. A consumable may be one time use or may be reusable. For example, a consumable may be reusable at least 2, 3, 4, 5, 6, 8, 10, 12, or more times. In an example, the consumable may be configured as a single use consumable. The consumable may or may not be configured to be deconstructed. In an example, the consumable is configured to be deconstructed. Deconstruction of the consumable may comprise reducing the consumable to a plurality of components (e.g., plastics, electronics, sensors, etc.). Select components of the consumable may be configured to be sterilized and discarded via an appropriate waste stream (e.g., recycle, biohazard, etc.). Select components of the consumable (e.g., electronics, sensors, harvest elements, etc.) may be cleaned, sterilized (e.g., by autoclaving, peroxide vapor, etc.), and reused in subsequent consumables. Reusable components may include structural framing (e.g., metal or rigid components), sensor components (e.g., electrical components), computer components, or any other components that are not contacted by biological materials during cell processing. The consumable may be configured for facile deconstruction, for example, through incorporation of modular construction within internal component attachment mechanisms (e.g., clips, magnets, screws, etc.). The various reusable components of the consumable may be reused at least 2, 3, 4, 5, 6, 8, 10, 12, or more times. The consumable may comprise reagents, single-use sensors, single-use filtration and fluidic subsystem components. The cell processing system or consumable may comprise a plurality of integrated components, as shown in **FIG. 66**. For example, the consumable may integrate electronics components sensing components, fluidic components, or any combination thereof for processing cells.

**[00329]** In some embodiments, the cell processing system may provide perfusion to one or more 3D matrixes using a perfusion unit or module. The perfusion unit may be integrated into the consumable or may be integrated into the cell processing system. In an example, the perfusion unit is integrated into the consumable. In another example, the perfusion unit is integrated into the cell processing unit and interfaced with the consumable. **FIGs. 67-70** schematically illustrate example perfusion units or modules integrated into a cell processing system. **FIG. 67** schematically illustrates an example perfusion module with a single perfusion loop. A perfusion module may be fully within a thermal zone, partially within a thermal zone, or outside of a thermal zone. The thermal zone may maintain a solid-state bioreactor (e.g., 3D matrices, media compartment, etc.) at a user defined temperature. A perfusion module may comprise one or more pumps that circulate media through the media

compartment of the bioreactor for perfusion. A perfusion module may comprise one or more inline bubble traps configured to remove bubbles from the fluid stream before it enters the bioreactor. The fluid stream may pass through a gas exchanger unit connected to a gas supply to control the dissolved gasses in perfused media. A perfusion module may comprise one or more inline active media reservoirs configured to contain or that contain perfusion media. The active media reservoir(s) may be bypassed using manual or automated valves permitting operation of the perfusion loop in a low volume configuration that excludes active media reservoir. Alternatively, the perfusion loop may be operated in a higher volume configuration that includes the active media reservoir(s). A perfusion module may comprise automated valves that permit the perfusion stream to be directed from the loop to a waste reservoir, to remove spent media from the perfusion module. Automated valves may permit the pump(s) to pull media from a media reservoir (e.g., shown as a chiller) to maintain bulk media at a temperature to extend reagent shelf-life. Reagents (e.g., bulk) in the chiller may be volumetrically dispensed, such as by the action of a syringe pump or other pump, into the perfusion module or the bioreactor. As shown in **FIG. 67**, a perfusion module may comprise a plurality of reagent or active media reservoirs. Multiple reagent reservoirs may permit volumetric dispense of reagents directly into the bioreactor without dilution by the active media reservoir. For example, expensive reagents such as viral particles may be volumetrically dispensed into the fluidic path and transferred by the pump to the bioreactor, at which time the pump may stop circulation allowing the viral particles to diffuse into the gel compartment to transduce cells. Alternatively, reagents may be volumetrically dispensed into fluid path of the active media reservoir, for mixing and dilution. For example, IL-2, which has limited stability at higher temperatures and may be replenished periodically by dispensing it into the fluidic path after the bioreactor, and the pump may circulate the fluid to mix the IL-2 into the media volume in the active media reservoir.

**[00330]** A perfusion unit may comprise or be coupled to a control unit. The automated function of pumps and valves may be controlled by the control unit via a computer program to coordinate a user-defined perfusion and media replacement programs. The rate of perfusion may be programmed by altering the pump speed to change the perfusion flow rate. A perfusion program may use continuous perfusion, intermittent perfusion, or perfuse according to any other program. Media may be moved to waste, and fresh media brought into the system according to a user-defined protocol. A perfusion module may comprise sensors configured to provide real time feedback to the control unit to augment user-defined system controls, as shown in **FIG. 69**. For example, inline pH and dissolved oxygen sensors may be

used for real time feedback of the perfusion control to automatically trigger a change in perfusion rate or timing to achieve user-defined setpoints for pH and dissolved oxygen. Other real-time sensors, including, but not limited to, analyte sensors for IL-2, glucose, lactate, may be used to further permit automated control of media replacement functions to increase media utilization efficiency. Other real-time sensors, including, but not limited to, fluid flow rate, pressure, and fluid level or fill sensing, may be used to provide feedback control of volumetric dispense, media replacement, pump and valve, and other automated system operations.

**[00331]** A perfusion module may comprise more than one fluidic loop, as shown in **FIG. 68**. For example, a perfusion module may comprise two or more pumps configured to provide perfusion separate from media control functions (e.g., mixing). A first perfusion loop may use a pump to circulate media within a fluid loop that may include or exclude the active media reservoir based on the setpoint of the automated control of valves. A second media control loop may comprise a second pump, which may circulate media from the active media reservoir and/or perfusion loop through the gas exchanger to change the concentration of dissolved gasses. Through the automated control of system valves, the media control pump may direct media streams to a waste reservoir. Through the automated control of system valves, the media control pump may pump fresh bulk media from the chiller into the active media reservoir and/or perfusion loop. Reagents may be volumetrically dispensed into the first or second media control loops. In some embodiments, a perfusion module may use alternative fluidic manifold designs to achieve these system functions. For example, an alternative embodiment may utilize valves to direct the separate perfusion and media control functions and behavior of their respective fluidic loops using a single pump. For example, a pump may run perfusion, then automated valves may redirect the fluidic connections such that the same pump can run media replacement and gas-exchange functions instead of perfusing the bioreactor; after the media control operations are complete, the automated valves may redirect the fluidic connections such that the pump may resume performing bioreactor perfusion.

**[00332]** As described above, and shown in **FIG. 69**, the perfusion module may comprise a plurality of sensors. In an example, a perfusion system may comprise inline pH sensors, dissolved oxygen sensors, or both for real-time or near real-time feedback on biomass grown (e.g., via cellular metabolism) and gas exchange and/or perfusion parameters. Inline sensor may include sensors for fluid presence (e.g., bubble sensor), fluid flow rates, fluid pressure sensors, or any combinations thereof. A perfusion module may comprise inline analyte

sensors for real-time, or substantially real-time, monitoring of IL-2, glucose, lactate, or other analytes. A perfusion module may comprise an analyte measurement or sampling unit for directing a media stream to a separate analyte measurement module or user-facing sample collection port for off-instrument analysis. In an example, the reservoirs, such as reagent reservoirs and waste reservoirs, may comprise fill level sensors. A perfusion module may further comprise in situ sensors, such as, for example, dissolved oxygen tension (DOT) sensors for optical pH and DO measurements, in situ imaging sensors (e.g., cameras, microscopes) of the bioreactor for detection of bubbles, cells, or other aspects of the bioreactor, in situ biomass sensors (e.g., optical density sensors and in situ capacitance and dielectric spectroscopy sensors) for detection of biomass and viable biomass within the bioreactor, or any combinations thereof. The consumable may comprise a perfusion module or the perfusion module may be separate from the consumable. In an example, the perfusion module is a part of the consumable. The perfusion module sensors may be integrated within the consumable as single-use sterile components. Alternatively, the perfusion module sensors may be ex situ sensors within the cell processing system and configured to interface with the perfusion module of the consumable.

**[00333]** In an example, a perfusion module may comprise or otherwise be coupled to a gas exchange module. The gas exchange module may comprise or be coupled to one or more gas sources, such as tanks of compressed gas. A single gas mixture may be used to provide oxygen, nitrogen, carbon dioxide, or any combination thereof to the perfusion module. Alternatively, multiple separate gasses may be supplied independently, such as oxygen, carbon dioxide, nitrogen, an alternative balance gas, or any combinations thereof. The gas exchange module may comprise one or more pressure, gas flow, mass controller, sensors, or any combinations thereof to regulate (e.g., automatically or manually) volumetric flow of one or more supplied gases. In an example, the gas exchange module comprises a sparger. The gas exchange module may comprise one or more gas-permeable, liquid-impermeable transfer membranes (e.g., silicone membrane, silicone hollow fiber, etc.) to permit molecular gas to transfer between a gas flow and a fluid flow.

**[00334]** In an example, the consumable may comprise a loading and harvesting unit, as shown in **FIG. 70**. Alternatively, a loading and harvesting unit may be integrated within the cell processing system and may interface with the consumable. A loading and harvesting unit may comprise one or more filtration units. Input cell material and gel precursor solutions may be provided in a chiller. A pump may transfer the cell input material into a filtration unit (e.g., tangential flow filtration unit) and provided to a mixing chamber where the input cells

may be processed, for example, by washing, cell isolation, buffer replacement, concentration, or any combination thereof. The loading and harvesting unit may comprise a pump or may be configured to interface with a pump of the cell processing system. The pump may transfer a volume of gel precursor solution into the mixing chamber where it is combined with the processed cells. The pump may subsequently be used to transfer the cell-laden gel precursor solution to the bioreactor for polymerization and solid state bioprocessing. At the user-specified or automatically determined harvest time, the 3D matrix may be resolubilized and the cell-laden mixture may be transferred by a pump to a formulation filtration unit. A subset of the sample volume may be directed by the pump and automated valve control to an in-process quality testing and release testing subsystem for analysis, such as by cytometry, molecular assays, and other automated sample characterization methods. The sample may be automatically formulated by the system, such as by cell washing, cell isolation, buffer exchange, concentration adjustment, mixed with a formulation buffer, or any combinations thereof. A pump may transfer a subset of the formulated material to a release testing unit for analysis. A pump may transfer the formulated material into one or more product bags or other product sample vessels, where the material is stored at a given temperature or automatically cryopreserved. In an example, an in-process sampling unit is connected to the bioreactor and/or perfusion and media control system. The in-process sampling unit may perform in-process sample collection as described elsewhere herein. Samples may be routed to a user-facing sampling port or sample collection vessel(s). Alternatively, samples may be routed to the In-Process Quality Testing and Release Testing Assay Module for automated quality testing.

**[00335]** In an example, the consumable, the cell processing system, or both comprises one or more filtration modules, as shown in **FIGs. 71-74**. As shown in **FIG. 71**, a sample being filtered may be transferred to a retentate mixing chamber. During operation, the sample may be mixed to ensure homogeneity and prevent the settling-out of cells and other particles. Mixing may be performed by an integrated agitator or mixer element within the chamber, such as a magnetically actuated mixing bar, or alternatively the sample may be mixed using fluid motions driven by pumps within the filtration module (e.g., the retentate circulation pump, the wash buffer addition pump, or a separate pump loop). The filtration module may comprise a wash buffer pump that transfer wash buffer into the retentate mixing chamber. The filtration module may further comprise an inline flow sensor that may be used for real-time feedback control of the pump and volumetric dispense operations. Wash buffer may be added according to a user-determined program, such as to maintain a substantially steady-



state volume in the retentate mixing chamber during filtration operations while permeate volume is removed by the filtration module. The filtration module may further comprise a retentate circulation pump that creates a flow of the fluidic sample mixture within the retentate loop. An inline flow sensor may be used for real-time monitoring and feedback control of fluid velocity during filtration. The filtration module may comprise retentate outlet and permeate output proportional valves that may be controlled by a computer program to regulate the transmembrane pressure and/or transmembrane fluid flow across the permeable membrane or filtration membrane separating the retentate and permeate sides of the filtration flow cell. For example, closing down the retentate outlet proportional valve may increase the pressure and/or restrict flow of fluid exiting the retentate outlet of the filtration flow cell, causing fluid to pass through the membrane to the retentate side, and also transferring in such transmembrane fluid flow particles capable of passing through the membrane under a particular fluid dynamic and pressure regime (e.g., particles smaller than a pore size of the filter membrane will pass through the membrane, while particles larger than the pore size of the filter membrane will remain on the retentate side). A computer-controlled proportional valve control program may use real time sensor feedback from inline pressure and/or flow sensors, including but not limited to a retentate inlet flow sensor, a retentate inlet pressure sensor, a permeate outlet pressure sensor, a permeate outlet flow sensor, or any combination thereof. The filtration module may be controlled automatically via a computer-controlled control program. For example, the control program may be designed to operate the filtration module within a user-defined pressure or flow rate regime to optimize the viability of cells, filtration performance, or other metrics. As another example, the control program may detect or infer the membrane permittivity or membrane fouling state, such as by measuring changes in transmembrane pressure response to proportional valve control, such as for the purpose of automatically initializing backflush operations to reduce membrane fouling or sedimentation.

**[00336]** The retentate flow rate may be less than or equal to about 30 milliliters per minute (mL/min), 20 mL/min, 15 mL/min, 10 mL/min, 8 mL/min, 6 mL/min, 4 mL/min, 2 mL/min, 1 mL/min, 0.5 mL/min, 0.25 mL/min, or less. The retentate flow rate may be greater than or equal to about 0.25 mL/min, 0.5 mL/min, 1 mL/min, 2 mL/min, 4 mL/min, 6 mL/min, 8 mL/min, 10 mL/min, 15 mL/min, 20 mL/min, 30 mL/min or more. The retentate flow rate may be from about 0.25 to 0.5, 0.25 to 1, 0.25 to 2, 0.25 to 4, 0.25 to 6, 0.25 to 8, 0.25 to 10, 0.25 to 15, 0.25 to 20, 0.25 to 30, 0.5 to 1, 0.5 to 2, 0.5 to 4, 0.5 to 6, 0.5 to 8, 0.5 to 10, 0.5 to 15, 0.5 to 20, 0.5 to 30, 1 to 2, 1 to 4, 1 to 6, 1 to 8, 1 to 10, 1 to 15, 1 to 20, 1 to 30, 2 to 4, 2 to 6, 2 to 8, 2 to 10, 2 to 15, 2 to 20, 2 to 30, 4 to 6, 4 to 8, 4 to 10, 4 to 15, 4 to 20, 4 to 30, 6

to 8, 6 to 10, 6 to 15, 6 to 20, 6 to 30, 8 to 10, 8 to 15, 8 to 20, 8 to 30, 10 to 15, 10 to 20, 10 to 30, 15 to 20, 15 to 30, or 20 to 30 mL/min. The retentate flow may be configured to minimize membrane fouling by continuously washing away debris and sedimented cells from the membrane surface. The filtration module may be operated at any pressure configured to maintain cell viability. For example, the filtration module may be operated at a pressure of less than or equal to about 150 kilopascals (kPa), 125 kPa, 100 kPa, 75 kPa, 50 kPa, 40 kPa, 30 kPa, 20 kPa, 10 kPa, 5 kPa, or less.

**[00337]** In an example, the filtration unit may be configured to manually or automatically interrupt filtration to backflush the membrane(s). In such an operation, the wash buffer pump may be actuated with the retentate addition valve closed, the backflush valve open, the permeate outlet proportional valve closed, and the retentate outlet proportional valve open, such that wash buffer is transferred into the permeate side of the flow cell and forced across the membrane into the retentate side (e.g., in the opposite direction as typical filtration flow, for the purpose of washing debris and/or sedimented cells from the retentate side of the membrane surface). The wash buffer flow rate and backflush duration may be user defined and/or determined by the automated control system to transiently improve and/or prolong filter membrane performance by removing debris and sediment. Waste, wash buffer, and retentate mixing chambers or fluid vessels may be deformable or rigid. When using rigid fluid vessels, a vent, such as a connection to atmosphere via a sterile inline filter, may be used to accommodate changes in fluid volume without pressurizing the vessel.

**[00338]** In an example, a filtration module further comprises one or more filtration sensors. The one or more filtration sensors may be positioned and configured to measure fluid mixtures on the retentate side, permeate side, or both. The one or more filtration sensors may be configured to measure, for example, particle density, turbidity, color, electrical resistance or capacitance, or any combinations thereof, in real-time or substantially real-time. The automated control system may use real-time sensor feedback to control system behavior to achieve a user-defined setpoint of sample purity. For example, optical light scattering or optical density may be used to measure the density or concentration of cells to determine filtration efficiency and progress over time. As another example, imaging sensors may be used to measure the density or concentration of nucleated cells such as lymphocytes and anucleated cells such as red blood cells and platelets.

**[00339]** The filtration module may be configured to adjust or may adjust the concentration of samples prior to, during, or after filtration. The module may dilute the sample by adding wash buffer as described previously. The module may concentrate a cell sample by running a

filtration operation causing transmembrane fluid flow, which reduces the fluidic volume of the retentate sample, while retaining cells via the filter membrane and not adding wash buffer to replace the washed-out volume.

**[00340]** The filtration module may further comprise a flow loop configured to permit the filtration module to reverse the direction of flow (e.g., alternating flow) and the inlet and outlet side of the filtration module, as shown in **FIG. 72**. By permitting alternating retentate flow, the filtration module may more efficiently minimize membrane fouling by continuously washing away debris and sediment from the membrane surface. Alternatively, or in addition to, the filtration module may be configured to provide dual flow, as shown in **FIG. 73**. The filtration module may comprise a symmetric fluidic flow pathway on the retentate and permeate sides of the flow cell. A permeate inlet circulation pump may be used to create a fluid flow across the permeate side of the flow cell, in addition to the retentate fluid flow. Inline sensors and other components may be similarly replicated on the permeate side permitting precise automated control. Fluid flow across both the retentate and permeate side of the membrane may be more efficient in minimizing membrane fouling by continuously washing away debris and sedimented cells from the membrane surface. By regulating the retentate inlet and permeate inlet pump speeds and valve states of the retentate outlet and permeate outlet proportional valves, the automated control system may create variable transmembrane pressures, flows, or both, such that filtration occurs when the transmembrane fluid flow is from the retentate to the permeate direction, while backflush flow from the permeate to retentate side of the flow cell can be used to clear debris and sedimentation from the membrane. In another example, the filtration unit may provide alternating dual flow configurations, as shown in **FIG. 74**. This example may combine the flow paths of the dual flow and alternating flow configurations by adding a bridge to both the retentate and permeate fluid paths of the dual flow configuration. Under automated system control, this configuration may permit the module to independently reverse the direction of flow and the inlet and outlet of both the retentate and permeate side. Alternating flow may be more efficient in minimizing membrane fouling by continuously washing away debris and sedimented cells from the membrane surface.

**[00341]** The filtration module may comprise one or more flow cells. In an example, a filtration module comprises at least 1, 2, 4, 6, 8, 10, or more flow cells. A flow cell may comprise a membrane, as described elsewhere herein, a retentate side, and a permeate side. As described elsewhere herein, a flow cell may comprise more than one retentate compartments, more than one permeate compartment, or more than one of both retentate

compartments and permeate compartments. For example, a flow cell may comprise a sandwich configuration in which a permeate compartment is sandwiched between two retentate compartments or a retentate compartment is sandwiched between two permeate compartments. A membrane may be disposed between the permeate and retentate compartments such that the flow cell comprises more than one membrane, for example, two membranes. A flow cell may be configured to reduce cell trapping by eliminating or minimizing fluidic dead zones or fluid velocity drops which may cause cells to settle out or become trapped within the fluidic paths and compartments. Using flow channels with a single inlet and outlet and flow spreading configurations which provide substantially uniform fluid velocity across the width and length of the flow cell may minimize or eliminate fluidic dead zones or velocity drops. The surface area of the membrane may be large compared to the thickness of the membrane to maximize filtration area. For example, the surface area to thickness ratio may be greater than or equal to about 100 cm, 200 cm, 400 cm, 600 cm, 800 cm, 1000 cm, 2000 cm, 4000 cm, 5000 cm, 10000 cm, 50000 cm, 100000 cm, 500000 cm, or more centimeters. The membrane may further comprise surface texture, vibration transducers, or both that are configured to generate turbulent flow and promote interaction between fluid components and the membrane. Alternatively, or in addition to, the chamber walls of the flow cell may comprise surface texture, vibration transducers, or both that are configured to generate turbulent flow and promote interaction between fluid components and the membrane. Alternatively, or in addition to, ultrasonic or acoustic focusing may be used to promote cell mixing and interaction between the solution or components in the solution and the membrane. For example, ultrasonic or acoustic focusing may be used to direct trans-filter particle motion.

**[00342]** Prior to, during, or after cell processing the cells may be sampled and analyzed. The consumable may be configured to permit integrated quality testing, sample-based quality testing, or both. During cellular processing, in-process samples may be generated from the perfused media stream, from a liquid “sheath” that surrounds the 3D matrix within the 3D matrix compartment of the bioreactor, the 3D matrix may be directly sampled from the bioreactor (e.g., by removing a portion of the 3D matrix), or any combination thereof. For example, a syringe, punch, or other method or apparatus may be used to remove a segment of the 3D matrix for in-process testing. Cells may be tested prior to, during, or subsequent to cell processing for sterility, mycoplasma, replication-competent lentiviruses, replication-competent retroviruses, vector copy number, insoluble material, or any combinations thereof. Cell purity may be tested prior to, during, or subsequent to cell processing via T cell content,

Car-T cell content, contaminating cell content, or any combination thereof. Cell potency may be tested prior to, during, or subsequent to cell processing via CAR T cell content, in vitro cytotoxicity, cytokine secretion, or any combinations thereof. Quality and release testing may be completed within the consumable, within the cell processing system, or samples may be removed and quality testing may be performed outside the cell processing system.

#### *Scalability*

**[00343]** A solid-state bioreactor or cell processing system may comprise one or more cell processing units. A cell processing unit may comprise one or more cell compartment(s) configured to retain a plurality of cells, one or more additional compartment(s) in fluidic communication with the one or more cell compartment(s) (e.g., perfusion compartment(s) configured for fluidic flow independent of the cell compartment), one or more interface(s) between the two or more compartments, or any combinations thereof. The fluid flow path may be adjacent to the 3D substrate, but independent from the 3D substrate. The one or more interfaces may provide fluid communication between the two or more compartments (e.g., fluid compartment and cell compartment). Fluidic communication between compartment may permit materials present in a compartment to be moved to or otherwise conveyed to another compartment. Material(s) moved between compartments may be disposed in solution. Materials may be dissolved in a solution conveyed between compartments or may be in suspension in a solution. Material(s) may include, but are not limited to, dissolved gasses (e.g., [O<sub>2</sub>]), atoms, salts (e.g., [NaCl]), ions (e.g., [Ca<sup>2+</sup>]), nutrients, macromolecules (e.g., proteins, antibodies, complexes such as viral particles or lipid nanoparticles), or any combinations thereof. At least one of the compartments may be configured to retain a plurality of cells or a 3D substrate comprising a plurality of cells. A cell processing unit may include at least 2, 3, 4, 5, 6, 9, 10, or more compartments. In an example, a cell processing unit comprises at least two compartments. In another example, a cell processing unit comprises two compartments. In another example, a cell processing unit comprises at least three compartments. In another example, a cell processing unit may comprise three compartments. Each compartment may be separated from another compartment by an interface.

**[00344]** FIG. 25 shows an exploded view of an example cell processing unit. A cell processing unit may comprise a material stack. The material stack may include a gas-permeable membrane, in situ optical sensors (e.g., dissolved oxygen sensor, pH, etc.), perfusion compartment (e.g., or tangential flow compartment), semi-permeable membrane,

3D matrix compartment, viable biomass sensor, or any combination thereof. The in situ optical sensors and viable biomass sensor may monitor cell processing in real time.

**[00345]** A cell processing unit, or a plurality of cell processing units, may be provided to a cell processing system as a removable cartridge as shown in **FIG. 26**. In an example, the plurality of cell processing units may be a part of a solid-state bioreactor cartridge that is removably installed into a cell processing unit. The cartridge may be a fully enclosed consumable with no-touch sterile instrument interface connections. The cartridge may include a plurality of cell processing units each comprising a 3D matrix configured to mimic a tissue-like environment. The 3D matrix may support 10-100 times higher cell density compared to a comparable suspension culture. The cell processing units may comprise tangential or perfusion flow paths. In an example, a perfusion flow path with 3D matrix compartment may provide continuous circulation of media and precise control over the cellular environment. The cartridge may include integrated sensors for real-time monitoring of the cellular environment, cells, or both.

**[00346]** A cell processing unit may be configured as a consumable, as shown in **FIG. 27**. The cell processing unit may be single use consumable or a reusable consumable. The consumable may comprise a thermal interface configured to provide temperature control to the 3D matrix via thermal conduction. The consumable may comprise optical interfaces for in situ pH and dissolved oxygen sensors. The consumable may include additional optical interfaces for optical density, near-infrared, mid-infrared, and raman spectroscopy. The consumable may include a plurality of interconnects, for example, sterile cell processing system interconnects, perfusion and media inlets and outlets, 3D matrix and cell inlets and outlets, gas inlets and outlets, or any combinations thereof.

**[00347]** The cell processing units may be configured to permit scaling and multiplexing of cell processing operations. For example, scaling one or more dimensions of a cell processing unit, including the width or length, one of these being the long axis, or increasing or decreasing the thickness or depth of the 3D cell culture substrate or cell compartment (while still providing an effective mass transfer of materials to and from the cells in order to maintain a cellular state, given a cell type, cell density in the 3D substrate, 3D substrate composition, or perfusion method, e.g., rate of media replacement) can increase the overall volume of the cell compartment to increase the quantity of cells or cell byproducts manufactured per batch. Scaling up may comprise increasing the size of a cell processing unit. Alternatively, scaling up may comprise increasing a number of cell processing units. A cell processing system may comprise at least 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, or more cell

processing units. A cell processing system may comprise a plurality of cell processing units fluidically coupled to one another. Alternatively, or in addition to, a cell processing system may comprise a plurality of 3D substrates fluidically coupled to one another. Alternatively, a cell processing system may comprise a plurality of 3D matrices that are not fluidically coupled to one another. In an example, a subset of a plurality of 3D matrices are fluidically coupled together and another subset of a plurality of 3D matrices are not fluidically coupled together. A fluid flow path may be disposed between or adjacent to fluidically coupled 3D matrices. For example, a fluid flow path disposed between a first and second 3D matrix may provide fluidic material to the first, second, or both 3D matrices. The cell processing units may be configured to be similar (e.g., in number of compartments, volume, size, etc.) or some cell processing units in a cell processing system may be different (e.g., have a different number or configuration of compartments, volume, size, etc.). Flexibility is a benefit of the solid-state bioreactor design, as, for example, scaling one or more dimensions (e.g., width, length, height) of a cell processing unit (including the cell compartment) may increase the number of cells which can be cultured in the bioreactor without affecting other performance characteristics and attributes. In some embodiments, a solid-state bioreactor or cell processing system may be configured with two or more cell processing units, which are designed to operate in parallel. For example, a solid-state bioreactor or cell processing system may be configured to manufacture a certain cell product at a given scale, in which two or more cell processing units operate synchronously and identically. In another example, a solid-state bioreactor or cell processing system may be configured with two or more cell processing units for which the procedure, process, cellular material, or other aspect of the cell processing units are distinct, such as by the independent or asynchronous execution of operations in the separate cell processing units. Further according to this example, each cell processing units may independently operate procedures which are determined by real-time feedback from each cell processing units. In some embodiments, a plurality of cell processing units is configured for multiplex manufacturing within a solid-state bioreactor. For example, a plurality of cell processing units may be configured to execute independent procedures, such as by delivering a plurality of viral constructs to a plurality of independent cell processing units for the purpose of simultaneously manufacturing a plurality of cell products (also under which other cell processing unit operations may be the same or different, and operate synchronously or asynchronously, such as determined by real-time feedback).

**[00348]** In some embodiments, the aggregate total volume of the cell compartment(s) within a solid-state bioreactor or cell processing system may be pre-determined for a given

consumable, such volume being filled with 3D substrate during bioreactor operation, regardless of the number or density of cells loaded. In some embodiments where a plurality of cell processing units are provided, a subset may be populated with cells or operated at a given time, such as to achieve an initial seeding of a target cell number or density. For example, given a number of input cells, a suitable volume of 3D substrate may be determined, either automatically or by a user, and the bioreactor may be dynamically configured to operate a subset of a plurality of cell processing units corresponding to the target 3D substrate volume. In some embodiments, non-cell containing cell processing units may be loaded with empty 3D substrate or media, or fluidically isolated and unused during a particular seeding and culture operation (such “extra” cell processing units being available for later use, as in the case where cells are harvested and subsequently re-seeded for continued cell processing operations). For example, a solid-state bioreactor or cell processing system comprising one hundred cell processing units of identical volume may be configured to initially seed two of the one hundred provided cell processing units at a density 1 million cells per  $\text{cm}^3$  of 3D substrate. Upon expansion culture of the cell population to a density of 100 million cells per  $\text{cm}^3$  of 3D substrate (100-fold expansion), the cells may be harvested, and subsequently the harvested cells may be re-seeded into all 100 cell processing units at a density of 2 million cells  $\text{cm}^3$  of 3D substrate, suitable for further expansion culture.

**[00349]** Cells may be seeded onto or into a 3D matrix. Cells may be seeded at a cell density of at least about 10,000 cells per cubic centimeters ( $\text{cells}/\text{cm}^3$ ), 100,000  $\text{cells}/\text{cm}^3$ , 250,000  $\text{cells}/\text{cm}^3$ , 500,000 cells per cubic centimeters, 1 million  $\text{cells}/\text{cm}^3$ , 2 million  $\text{cells}/\text{cm}^3$ , 3 million  $\text{cells}/\text{cm}^3$ , 4 million  $\text{cells}/\text{cm}^3$ , 5 million  $\text{cells}/\text{cm}^3$ , 10 million  $\text{cells}/\text{cm}^3$ , 15 million  $\text{cells}/\text{cm}^3$ , 20 million  $\text{cells}/\text{cm}^3$ , 30 million  $\text{cells}/\text{cm}^3$ , 40 million  $\text{cells}/\text{cm}^3$ , 50 million  $\text{cells}/\text{cm}^3$ , 60 million  $\text{cells}/\text{cm}^3$ , 70 million  $\text{cells}/\text{cm}^3$ , 80 million  $\text{cells}/\text{cm}^3$ , 90 million  $\text{cells}/\text{cm}^3$ , 100 million  $\text{cells}/\text{cm}^3$ , or more. In an example, the seeding density is at least about 0.5 million  $\text{cells}/\text{cm}^3$ . In another example, the seeding density is at least about 5 million  $\text{cells}/\text{cm}^3$ . Cells may be seeded into or onto a 3D matrix at a cell density of less than or equal to about 100 million  $\text{cells}/\text{cm}^3$ , 90 million  $\text{cells}/\text{cm}^3$ , 80 million  $\text{cells}/\text{cm}^3$ , 70 million  $\text{cells}/\text{cm}^3$ , 60 million  $\text{cells}/\text{cm}^3$ , 50 million  $\text{cells}/\text{cm}^3$ , 40 million  $\text{cells}/\text{cm}^3$ , 30 million  $\text{cells}/\text{cm}^3$ , 20 million  $\text{cells}/\text{cm}^3$ , 15 million  $\text{cells}/\text{cm}^3$ , 10 million  $\text{cells}/\text{cm}^3$ , 5 million  $\text{cells}/\text{cm}^3$ , 4 million  $\text{cells}/\text{cm}^3$ , 3 million  $\text{cells}/\text{cm}^3$ , 2 million  $\text{cells}/\text{cm}^3$ , 1 million  $\text{cells}/\text{cm}^3$ , 0.5 million  $\text{cells}/\text{cm}^3$ , or less. The cells may be processed subsequent to seeding. In an example, cell processing may include cell expansion. In another example, cell processing does not include expansion such that harvested cell product comprises substantially the same



number of cells as are seeded. In another example, cell processing does not include expansion but includes a negative selection process, such that harvested cell product comprises substantially fewer cells than are seeded. At harvest, the cell density may be at least about 0.5 million cells per cubic centimeters (cells/cm<sup>3</sup>), 1 million cells/cm<sup>3</sup>, 2 million cells/cm<sup>3</sup>, 3 million cells/cm<sup>3</sup>, 4 million cells/cm<sup>3</sup>, 5 million cells/cm<sup>3</sup>, 10 million cells/cm<sup>3</sup>, 15 million cells/cm<sup>3</sup>, 20 million cells/cm<sup>3</sup>, 30 million cells/cm<sup>3</sup>, 40 million cells/cm<sup>3</sup>, 50 million cells/cm<sup>3</sup>, 60 million cells/cm<sup>3</sup>, 70 million cells/cm<sup>3</sup>, 80 million cells/cm<sup>3</sup>, 90 million cells/cm<sup>3</sup>, 100 million cells/cm<sup>3</sup>, or more. At harvest, the cell density may be less than or equal to about 100 million cells/cm<sup>3</sup>, 90 million cells/cm<sup>3</sup>, 80 million cells/cm<sup>3</sup>, 70 million cells/cm<sup>3</sup>, 60 million cells/cm<sup>3</sup>, 50 million cells/cm<sup>3</sup>, 40 million cells/cm<sup>3</sup>, 30 million cells/cm<sup>3</sup>, 20 million cells/cm<sup>3</sup>, 15 million cells/cm<sup>3</sup>, 10 million cells/cm<sup>3</sup>, 5 million cells/cm<sup>3</sup>, 4 million cells/cm<sup>3</sup>, 3 million cells/cm<sup>3</sup>, 2 million cells/cm<sup>3</sup>, 1 million cells/cm<sup>3</sup>, 0.5 million cells/cm<sup>3</sup>, or less. In an example, the cell density at harvest is at least about 10 million cells/cm<sup>3</sup>. In an example, the cell density at harvest is at least about 20 million cells/cm<sup>3</sup>. In an example, the cell density at harvest is at least about 100 million cells/cm<sup>3</sup>.

**[00350]** Because the cell processing units can be scaled as described above while maintaining select process characteristics, such as nutrient supply and aeration, process optimization may be done using a small-scale configuration, and an optimized process may subsequently be scaled up using the scaling strategies described above, without further process optimization. For example, select parameters, such as fluid perfusion rates, may be maintained upon scale-up.

**[00351]** The cell processing systems comprising one or more 3D matrices as described herein may be useful for efficient scale-up from multiplexed research and development to ultra-high capacity cell processing. The 3D matrix or 3D matrices may be provided in a microwell plate type configuration. A well of the microwell plate may comprise individually addressable 3D matrices. Alternatively, or in addition to, individual wells of a microwell plate may be individually addressable. Individually addressable wells or 3D matrices may comprise individual sensors and fluid flow pathways that permit the well or 3D matrices to be processed separately or individually from other wells or 3D matrices. The 3D matrices may be in fluid communication with a plurality of channels configured to provide media, wash solution, stimuli, or any combination thereof as described elsewhere herein.

**[00352]** The cell processing system may be configured for cell processing scale up. As shown in **FIG. 59A**, a cell processing system may comprise one or more 3D matrices. The plurality of 3D matrices may be configured within a fluid flow path or each 3D matrix may

be configured to be in fluid communication with an individual fluid flow path. The cell processing system may comprise an array of 3D matrices arranged adjacent to one another. An individual 3D matrix may be configured to process greater than or equal to 0.1 billion cells. A 3D matrix may span an approximate length and width of the cell compartment such that the 3D matrix is configured to process greater than or equal to 2 billion cells. A plurality of 3D matrices spanning the length and width of a cell compartment may be configured in a stacked arrangement to increase the number of cells processed. For example, a stack of five 3D matrices may be configured to process 10 billion cells. The cell processing system may be scaled by modifying or increasing the size of individual 3D matrices or increasing the number of 3D matrices. A plurality of stacked 3D matrices may be arrayed to generate ultra-high capacity cell processing, as shown in **FIG. 59B**. For example, one hundred stacked 3D matrices each configured to process 10 billion cells may be arrayed to process one trillion cells. A cell processing system configured for ultra-high capacity cell processing may comprise at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more stacks of 3D matrices. Each 3D matrix stack may be configured to process at least 1 billion, 2 billion, 3 billion, 4 billion, 5 billion, 6 billion, 7 billion, 8 billion, 9 billion, 10 billion, 12 billion, 15 billion, 20 billion, 30 billion, or more cells. The various scales of a cell processing system may be connected for process scale up. For example, cells may be produced in a system configured to process 0.1 billion cells. Cells produced from the 0.1 billion cell scale may be used to seed a system configured to process 10 billion cells. The cells obtained from the system configured to process 10 billion cells may be used to seed a system configured to process 1 trillion cells.

**[00353]** Each 3D matrix stack or array may be associated with various cell processing components, for example, mixing chambers, gas exchange units, thermal conditioning units, cell wash and concentration units, fluidic units, or any combination thereof. Each 3D matrix stack or array, or a plurality of 3D stacks or arrays, may further interface with one or more base plates, fluidic paths, mechanical interfaces, electrical interfaces, or any combination thereof. The various components may interface with a single 3D matrix stack or multiple 3D matrix stacks. The various components may interface with at least 1, 2, 3, 4, 5, 6, 8, 10, 15, or more 3D matrix stacks. The 3D matrix stacks may be configured or otherwise arranged in a cabinet-like configuration in which each 3D matrix stack is accessible on a planar surface. Alternatively, the 3D matrix stack(s) may be stacked or arranged in a static-position multiplex configuration, for example by stacking the 3D matrices in a deep, but not tall or

wide configuration. Alternatively, the 3D matrix stack(s) may be in a rack mounted configuration such that individual 3D matrix stacks may be easily accessed.

**[00354]** In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for the production of a single autologous or allogeneic therapeutic cell product or dose. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for production of a number of human doses of an allogeneic therapeutic cell product. The aggregate capacity may be suitable for the production of one human dose. In an example, the aggregate capacity of the cell processing system may be sufficient to produce at least 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, or more human dose equivalents. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for biobanking applications. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for producing a number of cells required for a cell or tissue replacement or organ printing application. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for viral particle manufacturing, such as for production of a gene therapy or for the production of viral particles used in cell therapy manufacturing. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for the manufacturing of biologic compounds, such as plasmids, proteins, or antibodies. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more cell processing units comprising an aggregate capacity suitable for the manufacturing of cultivated food products such as lab-grown meat.

#### *Clinical manufacturing*

**[00355]** In some embodiments, one or more solid-state bioreactor consumable(s) are provided in an internally sterile and closed configuration. The one or more solid-state bioreactors may each comprise a single cell processing unit or a plurality of cell processing units. The one or more solid-state bioreactor consumables may be provided to and integrated with a cell processing system. In some embodiments, a solid-state bioreactor consumable(s) may be configured to interface with a cell processing system via one or more sterile interconnect(s) to facilitate instrument loading and unloading, as well as the internal transfer of materials within the cell processing system to the one or more bioreactor consumables,

such as to or from the solid-state bioreactor apparatus, while maintaining sterility. In some embodiments, a solid-state bioreactor or cell processing system is configured with a sterile interconnect system, which can establish fluidic connections while maintaining an internally sterile environment. In some embodiments, a solid-state bioreactor is configured to be used within a cell processing system enclosure which is environmentally closed, permitting the system to be internally sterilized after consumables and reagents are loaded into the system, such that sterile interconnections can be automatically established among the plurality of closed sterile system components, one such component being the solid-state bioreactor. In some embodiments, the solid-state bioreactor or cell processing system may be configured to provide a sterilization routine or otherwise be compatible with a system and method of sterilization, including but not limited to, autoclaving, dry heat sterilization, filtration, chemical sterilization (e.g., via ethylene oxide, hydrogen peroxide vapor, peracetic acid, formaldehyde), gas plasma, radiation, gamma irradiation, ultraviolet (UV) irradiation, or any combinations thereof. The sterilization method and solid-state bioreactor or cell processing system may be mutually compatible. For example, such that bioreactor or cell processing system materials are not damaged or the properties of which are not negatively affected by the sterilization method(s) used. Sterilization processes may be validated to provide a targeted sterility assurance level (SAL). Regular monitoring and validation can be used to maintain the effectiveness of the sterilization process. Some sterilization methods, particularly chemical and radiation sterilization, may use specific safety measures and have environmental considerations to be considered during implementation.

**[00356]** In some embodiments, a solid-state bioreactor is configured to be loaded into a cell processing system or operate in a clean room environment. For example, a clean room or other type of controlled facility may be classified according to ISO classifications, USP (United States Pharmacopeia) classifications, or Good Manufacturing Practice (GMP) clean room standards. Such facilities may use high-efficiency particulate air (HEPA) or ultra-low particulate air (ULPA) filters to remove particles from the air, airflow management systems, including laminar flow systems which direct filtered air in a constant stream, for example, vertically from the ceiling to the floor, to prevent turbulence and mixing of air, controlled access, such that entry and exit are strictly controlled through airlocks, gowning procedures, or shower-in systems to minimize contamination, and regular monitoring, which ensures compliance with the specified clean room standards, for example, involving testing for particulate levels, microbial contamination, and other relevant parameters. Solid-state

bioreactors and cell processing systems may be configured for use in such clean room facilities to provide select product attributes and regulatory compliance.

**[00357]** In some embodiments, the solid-state bioreactor is configured to be loaded into a cell processing system or operate in a Controlled Not Classified (CNC) space, which may be controlled but does not meet the strict standards of formal cleanroom classifications under ISO 14644-1 or other regulatory standards like USP or EU GMP. In some embodiments, the solid-state bioreactor is configured to be loaded into a cell processing system or operate in a laboratory environment, in a hospital or other patient healthcare setting, in the field, or in another environment which is neither considered a CNC nor a clean room environment. CNC and non-controlled spaces may offer greater flexibility and reduced cost compared to other manufacturing environments and use cases. In such embodiments, solid-state bioreactors and cell processing systems may comply with broader regulatory guidelines for manufacturing practices, providing that such use does not compromise product quality or safety.

**[00358]** In some embodiments, one or more solid-state bioreactor consumable(s) or cell processing systems are provided for the purpose of clinical product manufacturing.

*Integrated cell processing systems and methods*

**[00359]** Various systems and methods may be functionally coupled with a bioreactor (e.g., solid-state bioreactor) or cell processing unit. The various systems or devices may not be integrated into the same housing as the bioreactor. Alternatively, or in addition to, one or more modules or units may be integrated into the same housing as the bioreactor (e.g., solid-state bioreactor) or cell processing unit. Such methods may be executed by integrated apparatus described herein using separate consumables. Alternatively, or in addition to, the consumables and apparatus used with coupled methods may be provided in the same consumable as the solid-state bioreactor or cell processing unit.

**[00360]** In an example, a compartment (e.g., cell compartment), fluid flow path, and analysis unit are integrated within (e.g., disposed within) a single housing. Other components, including preservation unit, control systems, thermal control units, one or more computer processors, or any combination thereof, may also be integrated within the same housing. In an example, a cell processing unit and one or more computer processors are integrated within a housing. The housing may have a volume of less than or equal to about 3 cubic meters ( $\text{m}^3$ ), 2.5  $\text{m}^3$ , 2  $\text{m}^3$ , 1.5  $\text{m}^3$ , 1.25  $\text{m}^3$ , 1  $\text{m}^3$ , 0.75  $\text{m}^3$ , 0.5  $\text{m}^3$ , 0.25  $\text{m}^3$ , 0.1  $\text{m}^3$ , 0.075  $\text{m}^3$ , 0.05  $\text{m}^3$ , 0.025  $\text{m}^3$ , 0.01  $\text{m}^3$ , or less. In an example, the housing has a volume of less than or equal to about 1  $\text{m}^3$ . In another example, the housing has a volume of less than or

equal to about  $0.5 \text{ m}^3$ . The housing may have a volume from about  $0.01 \text{ m}^3$  to  $0.025 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.05 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.075 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.1 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.25 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.01 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.05 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.075 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.1 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.25 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.025 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $0.075 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $0.1 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $0.25 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.05 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $0.1 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $0.25 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.075 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $0.25 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.1 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $0.5 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.25 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $0.5 \text{ m}^3$  to  $0.75 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $1 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $0.75 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $1 \text{ m}^3$  to  $1.25 \text{ m}^3$ ,  $1 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $1 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $1 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $1 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $1.25 \text{ m}^3$  to  $1.5 \text{ m}^3$ ,  $1.25 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $1.25 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $1.25 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $1.5 \text{ m}^3$  to  $2 \text{ m}^3$ ,  $1.5 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $1.5 \text{ m}^3$  to  $3 \text{ m}^3$ ,  $2 \text{ m}^3$  to  $2.5 \text{ m}^3$ ,  $2 \text{ m}^3$  to  $3 \text{ m}^3$ , or  $2.5 \text{ m}^3$  to  $3 \text{ m}^3$ . In an example, the housing has a volume from about  $0.01 \text{ m}^3$  to  $2 \text{ m}^3$ .

**[00361]** Integrated systems may comprise vessels and 3D matrices in various configurations. In some embodiments, a 3D matrix may be configured in contact with various fluidic volumes. As shown in **FIG. 28**, a 3D matrix may be disposed at a bottom of a reaction vessel. The reaction vessel may include a gas permeable and liquid impermeable membrane disposed adjacent to the 3D matrix. The vessel may be a flask, bag, or other type of vessel. The vessel may include one or more inputs and outputs. The inputs and outputs may be configured to add and remove the 3D matrix or polymer or gel precursors configured to generate the 3D matrix. The inputs and outputs may further comprise one or more media supplies for static batch or fed Batch modes. The vessel may, or may not, include a semi-permeable membrane that retains the 3D matrix. The 3D matrix may be configured in a variety of shapes or forms as described elsewhere herein, and as shown in **FIG. 29**.

*Integrated systems with functional modules*

**[00362]** The system may facilitate the movement of fluids to or between integrated apparatus, or processing modules, while maintaining a sterile environment through closed connections. For example, the system may be an integrated cell processing system that is aseptically closed. The system may ensure the integrity of the fluid and prevent contamination during transfer operations, thereby enhancing efficiency and reliability in various controlled or industrial applications. The closed sterile fluid transfer system may comprise multiple modules interconnected through sterile fluid pathways. When not provided in a single consumable, such modules may utilize sterile interconnects, which may be formed manually by the user at the time of system setup or formed automatically during system operation. Such sterile interconnects may include, but are not limited to, connection ports equipped with sealing mechanisms to ensure a closed and sterile environment during fluid transfer operations.

**[00363]** Integrated apparatus may be designed to perform specific bio or cell processing functions. These may include, but are not limited to, cell washing, cell concentration, cell retention, cell isolation, cell separation, dissociation of cell aggregates, transfection (including by use of viral and non-viral approaches, such as lipid nanoparticles (LNP) and electroporation, and for the purpose of delivering any suitable molecular payload, including DNA, RNA, protein, or any combination or plurality thereof), cell analysis, product analysis, product harvest and formulation, product preservation, buffer exchange, fluid mixing, fluid analysis, bioproduct separation, concentration, filtration (e.g., ultrafiltration and diafiltration), chromatography, for resource and waste management, or any combinations thereof.

**[00364]** In some embodiments, a single integrated apparatus is configured to perform multiple bioprocessing functions simultaneously or subsequently. For example, cell washing and cell concentration may be performed simultaneously. Alternatively, or in addition, cell washing and cell concentration may be performed sequentially. In some embodiments, a single integrated apparatus is configured to operate in a plurality of modes to perform multiple bioprocessing functions, such as a mode for cell washing and a mode for cell concentration. In an example, an integrated device for particle separation may be configured to perform a cell washing function under a certain flow configuration, and cell concentration under a separate flow configuration. In another example, an integrated device for particle separation may be configured to perform a cell washing function and a cell concentration function under the same or substantially the same flow configurations. In another example, an integrated device for product analysis may be configured to perform a protein assay under

a certain reagent and software configuration, and a nucleic acid assay under a separate reagent and software configuration.

**[00365]** The integrated cell processing system (e.g., bioprocessing system) may comprise one or more computerized control system(s), electronic sensors, actuators, software algorithms, or any combination thereof. In some embodiments, a computerized control unit serves as the brain of the system, overseeing and coordinating all operations. In some embodiments, a system of computerized or electronic control systems operate together to oversee and coordinate system operations. For example, a first microcontroller may be configured to control the operation of a fluidic system, and a second microcontroller may be configured to control the operation of a product analysis module. In some embodiments, the computerized control systems are integrated within the housing. In some embodiments, the bioprocessing system is connected to one or more separate computer systems, such as via a wired or wireless connection (e.g., a network connection). In some embodiments, a computerized control system executes control over a plurality of bioprocessing systems in series or in parallel. In an example, computerized control systems may record critical process data and other system and product characteristics, such as for the purpose of regulatory compliance.

*Fluid handling and analysis*

**[00366]** The cell processing system (e.g., bioprocessing system) may comprise integrated apparatuses and methods for fluid handling, mixing, and analysis to facilitate fluid transfer, mixing, and analysis within a closed sterile environment. The system may incorporate components such as pumps, pressure regulators, mixing chambers, and analytical sensors to enable precise control over fluid processing operations, including the measurement of various parameters. Fluid transfer mechanisms may include, but are not limited to, pumps, valves, conduits, and tubing to facilitate the movement of fluids between the interconnected modules. For example, high-precision pumps equipped with variable speed control mechanisms may facilitate accurate fluid transfer, while pressure regulators may provide consistent flow rates and pressure levels. In some embodiments, the fluid handling apparatus may be provided in the form of integrated microfluidic systems. In some embodiments, the fluid handling apparatus is configured to interface with integrated microfluidic systems, such as those provided by one or more single-use or reusable consumables.

**[00367]** Integrated apparatus and methods of mixing may provide continuous flow-based mixing approaches. Alternatively, or in addition to, integrated apparatuses and methods of



mixing may provide batch mixing methods. Flow-based mixing strategies may include, but are not limited to, laminar flow mixing, chaotic advection, use of passive mixing elements, or combinations thereof. In some embodiments, mixing chambers are provided with agitation mechanisms such as impellers, magnetic stirrers, baffles, static mixers, or combinations thereof, tailored to suit the specific fluid characteristics and process requirements. Such systems and methods may provide efficient mixing, reduce residence time variability, and provide consistent product quality throughout the cell processing operations. Control algorithms may be used to govern the parameters of continuous flow and non-continuous batch mixing and may incorporate real-time data feedback.

**[00368]** The system may integrate analytical sensors for real-time monitoring of various fluid characteristics, including but not limited to, pH, dissolved oxygen (DO), conductivity, temperature, and the concentration of other dissolved analytes. Sensors may provide feedback to the system's electronic control system(s), allowing for adjustments to maintain targeted process conditions. Sensors may also record various process characteristics, such as for the purpose of regulatory compliance. Additionally, the system may include sterile sampling ports for offline analysis of fluid samples, further permitting comprehensive process monitoring and quality control.

**[00369]** A cell processing system may be integrated with a variety of upstream and downstream processing modules, as shown in **FIG. 30**. Upstream and downstream processing modules may include a receiving unit configured to receive a cell source, such as a LeukoPak or other cell source. The receiving unit may be further configured to receive reagents, such as lysis buffers, washing buffers, gel precursors, or any combination thereof. The receiving unit may be coupled to a mixing and transfer unit, a functional module, or both. The mixing and transfer module may include or be coupled to a sensor module. The functional module (FX) may be configured for buffer exchange and other cell processing operations. The functional module may comprise one or more filters, fractionation equipment, acoustophoretics, magnetics, or any combination thereof. The functional module and mixing and transfer module(s) may be coupled to and integrated with a cell processing unit (SSB) or plurality of cell processing units.

**[00370]** A functional module may be configured to subject the plurality of cells or cell products to a plurality of different processes. A functional module may be usable for upstream processes, downstream processes, or both. A functional module may include one or more filters. A filter may be a tangential flow filter, alternating tangential flow filter, or any other type of filter. In an example, the functional module comprises a spiral inertial filtration

device. The functional module may be configured to reduce a fluid volume of a dilute cellular suspension. The functional module may reduce the volume of a dilute cellular suspension by a factor of at least about 2, 4, 6, 8, 10, 15, 20, 50, 100, 200, 300, 400, 500, or greater. The functional module may comprise a flow centrifuge configured to concentrate one or more components within the system.

**[00371]** Upstream processes may include an input module and a reagent module, as shown in **FIG. 31**. The input and reagent modules may be coupled to or integrated with a mixing and transfer unit. The input module may be a receiving unit configured to receive a plurality of cells. The plurality of cells may be loaded into the input or receiving module and transferred to the mixing and transfer module. Cells within the mixing and transfer module may be washed, undergo buffer exchange, volume reduction, or any combination thereof. Waste materials may be generated during washing, buffer exchange, etc. Generated waste may be removed from the unit via a waste module. In the mixing and transfer module, the cells may be mixed with polymer or gel precursors and loaded into one or more cell processing units.

**[00372]** A cell processing system may be integrated with a variety of upstream and downstream processing modules, as shown in **FIG. 32**. Upstream and downstream processing modules may include a mixing and transfer unit coupled to a reagent source, functional module, fill and finish module, waste module, or any combination thereof. The cell processing unit (SSB) may be configured to provide the 3D matrix comprising encapsulated cells or a solution comprising cells to the mixing and transfer unit. The mixing and transfer unit may be configured to provide a washed cell product to a fill and finish module to generate a cell product.

#### *Other processes*

**[00373]** The cell processing (e.g., bioprocessing) system may comprise one or more integrated apparatus configured to automatically perform or that automatically perform one or more methods of cell washing, cell concentration, cell retention, cell isolation, cell separation, or any combination thereof. Such methods may be performed using batch or continuous flow operations. In an example, the methods are performed in batch flow operation. In another example, the methods are performed in continuous flow operations. Methods and integrated apparatus may be used at the beginning of a cell processing workflow (e.g., prior to loading cells into a solid-state bioreactor), during cell processing (e.g., during a bioreactor re-seeding procedure), at the end of a cell processing workflow (e.g., after

harvesting cells from the solid-state bioreactor for formulation), or combinations thereof. The system may utilize particle-based methods, for example, using carrier particles to facilitate these processes. In an example, cells adhere to carrier particles, either specifically or non-specifically. In another example, a cell isolation method may utilize antibody-conjugated microbead particles which specifically bind a target moiety present on the surface of cells, such as for positive or negative cell selection. Cell-laden particles may be separated from a fluid, which may contain cells not bound to particles or other substances, by density (e.g., by centrifugation or by inertial flow), by magnetic fields (e.g., using paramagnetic particles), by acoustic fields (e.g., by using particles with certain acoustic properties, such as size, density, or compressibility), by using other methods, or combinations thereof. Other methods of cell washing, cell concentration, cell retention, cell isolation, cell separation, or combinations thereof may provide separation via physical characteristics of the cells. For example, methods that process cells via physical characteristics of the cells may include centrifugation, acoustic fields, magnetic fields, inertial flow, deterministic lateral displacement (DLD), or other particle separation methods. Cell processing may be provided via filtration. For example, tangential flow filtration (TFF), alternating tangential flow filtration (ATF), or a combination thereof may be used for cell washing, cell concentration, cell retention, cell isolation, cell separation, or combinations thereof. Prior to, during, or subsequent to any process described herein, cells may be separated from a first fluid and transferred to a second fluid, thereby providing a buffer exchange.

**[00374]** The cell processing system may comprise one or more integrated apparatus configured to automatically perform methods of dissociation of cell aggregates. Methods of dissociating cell aggregates may include, but are not limited to, using microfluidic flow (e.g., against microscale objects such as pillars, silica knives, or mesh), by tailoring mechanical shear forces and fluid jets or flow streams, or combinations thereof, which can disrupt cellular aggregates, using ultrasound frequency sonication, using physical disruption (e.g., trituration or mixing) or combinations thereof. Methods of dissociating cell aggregates may optionally involve treating cellular biomass with an agent to mediate cell dissociation, such as a protease (e.g., trypsin), a Dnase (e.g., Dnase I), a surfactant (e.g., Pluronic F-68, a non-toxic, non-ionic surfactant), other cell disaggregation agents for use with cell bioprocessing, or combinations thereof. In some embodiments, dissociation of cell aggregates is mediated by one or more integrated apparatus for cell washing, cell concentration, cell retention, cell isolation, cell separation, or combinations thereof. For example, a microfluidic deterministic lateral displacement (DLD) apparatus, which performs a method of particle separation based

on the interaction of suspended particles in a liquid flowing through an array of microposts under low Reynolds conditions, may be used to dissociate cell aggregates. In other embodiments, a separate apparatus is provided to dissociate cell aggregates.

**[00375]** In some embodiments, the cell processing system is configured to automatically perform biomaterials processing functions on perfused or spent media, such as for manufacturing cell-derived products, including but not limited to, viral vectors, antibodies, other biologics, or combinations thereof. The automated and integrated cell processing system may be designed to permit manufacturing of biologic products by automating and integrating select unit operations, from upstream cell culture (e.g., via a tangential flow perfusion bioreactor or 3D matrix cell culture system) through downstream purification and formulation. The system may provide seamless integration of automated processes for buffer exchange, product separation, concentration, filtration, chromatography processes, or combinations thereof for streamlining cell processing workflows and accelerating production of high-quality biologic products. An automated or integrated system may include automated fluidic sample handling, modular process units, real-time monitoring and control, comprehensive data management, or combinations thereof to permit biopharmaceutical manufacturers to meet increasing demands for scalable or personalized biologic therapeutics with enhanced efficiency, scalability, and regulatory compliance.

**[00376]** In some embodiments, automated liquid handling systems precisely handle samples, media, buffers, reagents, or combinations thereof throughout the cell processing workflow to minimize manual intervention and increase sample integrity. Sample transfer between process units, bioreactors, storage vessels, or combinations thereof may be performed with high accuracy and reproducibility, reducing the risk of contamination and error. Samples, such as fluid media, may comprise soluble biologic products generated by cultured cells.

**[00377]** In some embodiments, the system comprises modular process units for buffer exchange, product separation, concentration, filtration, chromatography, or combinations thereof. Modular process units may provide for flexible process design and optimization. Modular process units may or may not be integrated into the same housing as a bioreactor or other cell processing unit. Modular process units may be integrated with a bioreactor or other cell processing unit via aseptic couplings. In some embodiments, modular process units are provided in the form of microfluidic systems. A modular process unit, or each modular process unit, may comprise sensors, actuators, control systems, or combinations thereof to permit real-time monitoring and control of process parameters. Real-time monitoring and

control of process parameters may provide consistent product quality and yield. Automated buffer exchange functions may permit removal of unwanted components (e.g., media components, by-products, etc.) and exchange with buffer solutions, providing downstream purification and formulation. In some embodiments, ultrafiltration/diafiltration (UF/DF) membranes with defined molecular weight cutoffs are used to retain target molecules while permitting passage of buffer components. In some embodiments, tangential flow filtration (TFF) systems are employed for product separation and concentration, permitting the removal of impurities and concentration of target molecules. In some embodiments, hollow fiber membranes with varying pore sizes are utilized to retain target molecules while permitting passage of smaller impurities and obtaining high product recovery and purity. In some embodiments, depth filtration systems are integrated into the system for removal of particulate matter and large impurities from process streams, increasing product quality and filter longevity. In some embodiments, a chromatography system, such as a system comprising chromatography columns packed with affinity resins, ion exchange resins, size exclusion media, or combinations thereof are used for purification, or additional purification, and polishing of target molecule(s) to obtain high product purity and yield. In some embodiments, process control systems, including electronic and computerized systems, monitor and adjust process parameters, including flow rates, pressures, temperatures, pH, or combinations thereof in real time to improve process performance and ensure product quality. In some embodiments, feedback control loops based on sensor measurements and analytical data may permit dynamic process optimization and fault detection, reducing or minimizing process variability and downtime. Integrated data acquisition and management systems may collect, store, analyze, or combinations thereof process data, including sensor readings, analytical results, batch records, or combinations thereof. In some embodiments, data and records are electronically communicated to a separate computer system, database, or both. In some embodiments, data visualization tools and reporting functionalities, either provided on a screen of the system or provided via a remote interface such as a web browser, may permit operators and process engineers to visualize trends, analyze process performance, generate regulatory-compliant documentation, or combinations thereof for batch release.

**[00378]** In some embodiments, the system is configured for manufacturing viral vectors for applications including, but not limited to, research, gene therapy, vaccine production, or combinations thereof. In some embodiments, the system is configured for production of monoclonal antibodies (mAbs), bispecific antibodies, antibody-drug conjugates (ADCs), or combinations thereof for research or therapeutic use. In some embodiments, the system is

configured for manufacturing recombinant proteins, enzymes, other biologic products, or combinations thereof for pharmaceutical, biotechnology, and research applications.

*Product preservation and formulation*

**[00379]** In some embodiments, the cell processing system is configured to automatically perform product formulation and preservation, thereby improving stability, efficacy, and long-term storage of biologic products. These functions may include the formulation of drug products and the implementation of cryopreservation techniques for cell-based therapies and other sensitive biologics.

**[00380]** After downstream purification and concentration, the purified biologic, cell product, or both may undergo formulation to prepare the drug product for storage, distribution, administration, or combinations thereof. Formulation processes may involve mixing purified product(s) with excipients, stabilizers, buffers, other additives, or combinations thereof to improve product stability, solubility, and shelf life. Cryoprotective agents such as dimethyl sulfoxide (DMSO), glycerol, trehalose, or combinations thereof may be added to the product formulation to protect cells or biomolecules from damage during freezing and thawing. Robotic liquid handling systems or dispensers may accurately dispense formulation components into product containers, such as vials, syringes, or bags, ensuring uniformity and reproducibility. Robotic liquid handling system or dispensers may be integrated into the same housing as the cell processing system or coupled to the cell processing system via aseptic connections. In some embodiments, the system supports customizable formulation recipes to accommodate different product formulations, dosage forms, administration routes, or combinations thereof to provide for development and manufacturing of diverse biologic drugs.

**[00381]** A cell processing system may include a fill and finish module, as shown in **FIG. 33**. The fill and finish module may be integrated into the same housing as the cell processing unit. The fill and finish module may comprise a temperature probe configured to monitor and control cryopreservation of the cell product. The fill and finish module may comprise or otherwise be coupled with a thermal control module or preservation unit configured to provide controlled cryopreservation of the cell product. The fill and finish module may include a product bag with a traceability label (e.g., barcode, RFID, etc.) for digital tracking. The product bag may include a sterile weld or interface configured to seal the product bag. The fill and finish module may further comprise a weight sensor, flow sensor, or both to control fill volume or fill weight.

**[00382]** In some embodiments, an integrated preservation unit (e.g., cryopreservation unit) is included in the cell processing system to permit long-term storage and preservation of cell-based therapies, viral vectors, other biologic products, or combinations thereof that benefit from cryogenic storage conditions. In an example, the preservation unit may be configured to generate a cryopreserved cell product. A cryopreservation unit may be integrated into the same housing as the cell processing system or coupled to the cell processing system via aseptic connections. The preservation unit may be electronically coupled to or integrated with the cell processing system such that a single computer system controls the cell processing system and the preservation unit. The preservation unit may employ controlled freezing techniques (e.g., cryopreservation), such as slow-rate freezing or controlled-rate freezing, to minimize ice crystal formation and cell damage to provide high cell viability and product stability post-thaw. The preservation unit may be a cryopreservation unit configured to provide a temperature ramp or may provide a temperature ramp at a rate of at least about 0.1°C/minute, 0.25 °C/minute, 0.5°C/minute, 0.75°C/minute, 1 °C/minute, 1.25°C/minute, 1.5°C/minute, 1.75°C/minute, 2 °C/minute, or more. The cryopreservation unit may be configured to provide a temperature ramp or may provide a temperature ramp at a rate of less than about 2 °C/minute, 1.75°C/minute, 1.5°C/minute, 1.25°C/minute, 1°C/minute, 0.75°C/minute, 0.5°C/minute, 0.25°C/minute, 0.1°C/minute, or less. In some embodiments, the preservation unit may perform a controlled, closed-loop temperature ramp program. In an example ramp program, the preservation unit may alter a temperature of a drug product (e.g., cell product) from room temperature to 4°C at 1°C/minute, from 4°C to -35°C at 0.75°C/minute, holding the sample at -35°C for 30 minutes to allow for the whole sample to freeze after ice nucleation, and then from -35°C to -60°C at 0.75°C/minute. The temperature ramp program may be specified by a user, such as by using a user interface on the cell processing system or by remote access and control, such as by using a web browser. At the end of cryopreservation, the system may be configured to maintain or may maintain the sample in cryogenic storage until a user retrieves the material for transfer to long-term storage, shipping, or both.

**[00383]** A preservation unit may provide chemical or thermal preservation (e.g., cryopreservation). The preservation unit may comprise a thermal control system configured to control or alter a temperature of the population of cells, 3D matrix, fluids provided to the population of cells, biological product(s), cell product(s), or combinations thereof. The thermal control system may be configured to provide or may provide a temperature of less than or equal to about 60°C, 50°C, 40°C, 37°C, 35°C, 30°C, 25°C, 20°C, 15°C, 10°C, 5 °C,

0°C, -5°C, -10°C, -20°C, -30°C, -40°C, -50°C, -60°C, -70°C, -80°C, -90°C, -100°C, -120°C, -140°C, -160°C, -180°C, -196°C, -200°C, or less. The thermal control system may be configured to provide or may provide a temperature of greater than or equal to -200°C, -196°C, -180°C, -160°C, -140°C, -120°C, -100°C, -90°C, -80°C, -70°C, -60°C, -50°C, -40°C, -30°C, -20°C, -10°C, -5°C, 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 50°C, 60°C, or greater. In some embodiments, the preservation unit comprises a thermal system capable of maintaining a temperature, or ramping a temperature, of a population of cells or biological drug product between approximately -60°C and 37°C. In some embodiments, the preservation unit comprises a thermal system capable of maintaining a temperature, or ramping a temperature, of a population of cells or biological drug product between approximately -196°C and 37°C. In some embodiments, the preservation unit comprises a liquid nitrogen (LN2) cooling system. Liquid nitrogen (LN2) may be used as a cryogenic coolant in bioprocessing applications due to its low temperature (-196°C) and high cooling capacity. In such a configuration, LN2 may be stored in insulated dewars or tanks and delivered to the cell processing system via vacuum-insulated transfer lines or flexible hoses. In some embodiments, the preservation unit comprises electrical thermal systems utilizing electrical heating and cooling elements, such as resistive heaters, Peltier devices, refrigeration units, or combinations thereof, to control temperature within the preservation unit. Preservation unit(s) may be integrated with cell processing and controlled via programmable logic controllers (PLCs) or computerized control systems to maintain temperature setpoints, monitor temperature profiles, implement temperature ramping or cycling protocols, or combinations thereof. In an example, a preservation unit may be a cryopreservation unit comprising thermal insulation.

**[00384]** In some embodiments, the preservation unit (e.g., cryopreservation unit) comprises configurable holders for sample containers, such as cryovials and cryobags. In some embodiments, sample containers are loaded in a sterile, aseptic manner by a user, such as by using sterile tube welding. In some embodiments, sample containers are contained within one or more consumables configured to automatically form a sterile aseptic fluid interface with and by the cell processing system. In some embodiments, sample containers comprise cryobags with a fill volume between approximately 10 milliliter (mL) and 275 mL. A cryobag fill volume may be greater than or equal to about 1 mL, 10 mL, 20 mL, 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL, 100 mL, 125 mL, 150 mL, 175 mL, 200 mL, 225 mL, 250 mL, 275 mL, 300 mL, or greater. In some embodiments, the system may include an intuitive user interface that guides operators through the loading process. A user may be



prompted to place cryobags into designated loading areas or receptacles within the system. In some embodiments, the system prompts a user to provide, such as by a user interface, specifications about the sample carriers, such as target fill volumes. In some embodiments, the cell processing system automatically determines the specifications of loaded sample containers, such as by reading a barcode, RFID tag, or other data indicator on the container(s), and retrieving the specifications, such as via a database or network connection. In some embodiments, the bioprocessing system integrates sensors for container size determination, container fill, or both. Sensors integrated with the preservation unit may include sensor for determining mass or weight of a container.

**[00385]** In some embodiments, the cryopreservation system integrates sensors for temperature feedback. In some embodiments, one or more probe-based sensors are used to determine temperature. Probe-based sensors, such as thermocouples or resistance temperature detectors (RTDs), may be directly inserted into cryobags or storage vessels to accurately measure the temperature of the contents. Alternatively, or in addition to, sensors may be attached to the exterior surface of cryobags or storage vessels to indirectly monitor temperature changes. This approach may provide real-time feedback on temperature fluctuations without affecting or disturbing the cryobag or vessel contents. In some embodiments, probes may be integrated within cryo-containers or consumables comprising integrated cryo-containers. Probes may be configured to electronically interface or may electronically interface with the cell processing system. In some embodiments, probes may be integrated into the cell processing system and configured to thermally couple or may thermally couple with containers during system operation. In some embodiments, imaging-based temperature monitoring systems may be employed to provide non-invasive temperature feedback. Thermal imaging cameras or infrared sensors may capture temperature data from the surface of cryobags or storage vessels, allowing for continuous monitoring of temperature profiles.

**[00386]** In some embodiments, the system comprises functions for cryopreservation validation, including freeze-thaw cycles, cell viability assessment, product stability testing, or combinations thereof to improve or confirm integrity and potency of cryopreserved products. For example, automated analytical assays, such as cell counting, viability staining, and functional assays, may be performed on cryopreserved samples to assess product quality, purity, potency, or combinations thereof post-thaw. In some embodiments, the cryopreservation process complies with regulatory guidelines and standards, including Good Manufacturing Practice (GMP), Good Distribution Practice (GDP), International Conference

on Harmonisation (ICH) guidelines, or combinations thereof for biologic drug manufacturing and storage.

**[00387]** In some embodiments, the system software comprises functions for, or electronically communicates with systems which provide automated inventory management or Laboratory Information Management Systems (LIMS). Integrated inventory management software may track the location, quantity, expiration dates, or combinations thereof of cryopreserved products. In some embodiments, comprehensive documentation, including batch records, validation reports, certificates of analysis, or combinations thereof, are generated and stored electronically to demonstrate compliance with regulatory requirements and facilitate batch release and regulatory submissions.

**[00388]** In some embodiments, an integrated preservation unit is configured to provide a controlled rate thawing function, permitting loading of cryogenically frozen source materials. A preservation unit may provide programmable thawing protocols to permit users to define thawing parameters such as thawing rate, temperature setpoints, duration, or combinations thereof. The preservation unit may provide consistent and controlled thawing conditions for different types of frozen input materials. Sensors may be embedded within the preservation unit, sample containers, or both to continuously monitor temperature profiles and thawing progress in real time. This feedback may permit the system to adjust thawing parameters dynamically to maintain optimal conditions throughout the thawing process. The system may use a closed-loop control algorithms to regulate the flow of heat transfer fluid or electrical heating elements based on sensor feedback. This may permit precise temperature control and minimizes the risk of thermal shock or damage to the thawed materials. The thermal system(s) of the preservation unit may be designed to ensure uniform temperature distribution across the thawing or thawed materials, reducing, preventing, or substantially preventing uneven thawing and preserving product integrity. In some embodiments, the system supports single or batch thawing of frozen input materials, allowing flexibility in processing one or more small or large quantities of samples simultaneously or asynchronously during system operation. Upon completion of the thawing process, the system may automatically transfer thawed input materials into integrated cell processing workflows, such as cell culture, purification, or other downstream processing. Thawing parameters and thawing progress data may be automatically transferred to the cell processing system's software to provide operators with real-time information on the status of thawed materials and permitting timely decision-making. In some embodiments, thawing processes may adhere to regulatory requirements and

guidelines, including Good Manufacturing Practice (GMP) and Good Distribution Practice (GDP), to ensure product safety, efficacy, and compliance with regulatory standards.

**[00389]** An automated preservation unit within a cell processing system may be versatile and capable of processing a wide range of input materials, including various types of frozen cell sources, primary human materials, secondary materials, or combinations thereof. Primary human materials may include, but are not limited to, primary human materials used in cell therapy and bioprocessing applications, such as blood products, whole blood, leukopaks (leukapheresis products), cord blood, bone marrow suspensions, or combinations thereof. Secondary materials may include, but are not limited to, cell lines used in biologic production, drug screening, and research applications, such as established cell lines, including immortalized cell lines derived from various tissues or organisms. In some embodiments, the system's user interface permits operators to specify the type and quantity of frozen cell sources or secondary materials to be loaded into the preservation unit. In some embodiments, an input material is assigned a unique identifier or barcode to facilitate sample tracking and traceability throughout the preservation (e.g., cryopreservation) and cell processing workflows.

#### *Resource and waste management*

**[00390]** In some embodiments, the cell processing system is configured to automatically perform or automatically performs resource and waste management functions. The cell processing system may comprise sterile aseptic reagent loading modules, which may automatically transfer reagents into the cell processing system from sterile containers or reservoirs. The system may use sterile connectors, such as sterile tubing assemblies or disposable fluid paths, to maintain aseptic conditions during reagent loading and transfer. Automated pumps, valves, flow sensors, or combinations thereof may regulate the flow of reagents, providing precise and consistent dispensing into the cell processing vessels or equipment. In some embodiments, fluid or gas inlets are configured with inline sterilization filters, such as 0.2  $\mu\text{m}$  or 0.22  $\mu\text{m}$  filters, to remove microbial contaminants from gasses, liquids, or air entering the system. In some embodiments, ultraviolet (UV) irradiation systems are integrated for inline sterilization of gasses, air, or fluid streams, providing an additional layer of microbial control.

**[00391]** In some embodiments, the cell processing system may include or may be coupled to one or more refrigeration units. A refrigeration unit may be configured to maintain or may maintain reagents at a specific temperature during storage and system operation. A refrigeration unit may maintain reagents at a temperature of less than or equal to about 10°C,

8°C, 6°C, 4°C, 2°C, 0°C, -2 °C, -4°C, -6°C, -8°C, -10°C, or less. Refrigeration units may thermally interface with reagent containers and may be equipped with temperature sensors and control algorithms to maintain temperature setpoints. In some embodiments, a liquid cooling system is employed to maintain reagent temperatures.

**[00392]** In some embodiments, a cell processing unit may be integrated with or coupled to real-time volume monitoring systems to provide efficient and safe operation throughout the cell processing workflow. For example, fluid fill detectors, such as inline sensors or detectors integrated into the reagent storage system or fluid reservoirs or containers, may be used to monitor the volume of reagents or fluids in real time and provide continuous feedback on fluid levels within the system. In another example, weight sensors or load cells are integrated into the reagent storage system or fluid reservoirs or containers measure the mass of reagents, permitting accurate volume estimation and monitoring.

**[00393]** In some embodiments, the cell processing system comprises a waste management function. The system may include dedicated waste containers, including biohazard waste containers, non-biohazard waste containers, or both. Waste containers may be integrated into the system for the collection and disposal of waste generated during cell processing operations. In some embodiments, software algorithms and control logic routes waste streams to appropriate waste vessels based on predefined criteria, such as waste type, volume, and biohazard classification. In some embodiments, biohazardous waste containers may be subjected to sterilization cycles to deactivate pathogens and ensure safe disposal. For example, the waste management system may automatically perform a method of chemical disinfection. Chemical disinfection may include using liquid or gaseous disinfectants to inactivate pathogens present in biohazardous waste. Biohazardous waste can be treated with liquid disinfectants such as sodium hypochlorite (bleach), hydrogen peroxide, phenolic compounds, or combinations thereof. Gaseous disinfection may use ethylene oxide (ETO), hydrogen peroxide vapor (HPV), or both and may be suitable for treating biohazardous waste in sealed containers or chambers. Automated sterilization of biohazardous waste may provide streamlined and regulatory-compliant options for healthcare facilities, research laboratories, and biopharmaceutical manufacturers seeking to provide the safe disposal of infectious materials generated by the cell processing system. In some embodiments, the system incorporates automated loading and unloading mechanisms of vessels or containers comprising liquid cell processing system resources or waste.

*Aseptic interconnections*

**[00394]** A cell processing system may include aseptic interconnections configured to couple cartridges, consumables, reagent containers, cell sources, or any combinations thereof. Aseptic interconnections may comprise aseptic fluidic interfaces or may be sterilized in place. As shown in **FIG. 34**, an aseptic fluidic interface may comprise an actuator. The actuator may be a male or genderless actuator. The connector may include an inner compartment that is sterile or configured to be sterilized in situ. Interconnect may comprise a cover configured to maintain the sterility of the inner compartment and to move aside upon coupling of one interconnect with another interconnect. The cover may be mechanically actuated, a pull-trip membrane, a puncturable membrane, or any combination thereof. The interconnect interface may be coupled to a fluid path, such as a tube, conduit, or other flow path. The fluidic connector may have one or more locking or engagement mechanisms such as threaded, pressure fit, clips, or any combination thereof. The interconnect may comprise a valve (e.g., a mechanical or pneumatic valve) configured to block, meter, or open the fluid flow path.

**[00395]** An aseptic interconnection may be generated by coupling interfaces from matching aseptic fluidic interfaces, as shown in **FIG. 35**. Outer connections from two different interconnects may be mated. Each connection may include a removable cover that is actuated to expose a sterilized inner chamber. A valve may be used to move two fluid paths together and mated to form an internal, aseptic fluid flow path.

**[00396]** A cell processing system may include aseptic or non-aseptic connections, as shown in **FIG. 36**. Non-aseptic connections may comprise one or more non-sterile interfaces that may be mated to form a fluidic connection. Alternatively, a cell processing system may comprise a plurality of nonsterile connections that may be sterilized in situ. A nonsterile connection may comprise a non-sterile compartment and a closed, sterile fluid flow path. The compartment may be sterilized prior to generating a connection between two, opposing closed, sterile fluid flow paths.

**[00397]** In an example, an aseptic interconnection may be sterilized in situ as shown in **FIG. 37**. Connections with closed valves may be mated to form an outer connection (1). The cell processing system may include an internal sterilization compartment configured to sterilize the inner connection space of an interconnection. The internal sterilization compartment may be configured to provide a chemical, gas, vapor, or any combination thereof to sterilize an inner chamber of an interconnection (2). An actuation mechanism may be used to mate internal components to generate a sterile fluidic pathway (3). Valves

configured to seal each side of the fluidic connection may be opened to permit flow of fluid through the interconnection.

**[00398]** In an example, aseptic interconnections may be generated by hybrid interconnects as shown in **FIG. 38**. Hybrid interconnects may include mating an open, non-sterile interconnection with an internally sterile interconnection. Prior to mating, the non-sterile interconnection may be sterilized in situ. The outer housing of the interconnection may be mated. Subsequent to mating outer housings of the interconnections, the inner connections may be mated and, if present, valves may be opened to provide a sterile fluid flow path. Hybrid interconnections may include a male connector to puncture a female connection, and actuatable interface, or combination thereof.

**[00399]** A cell processing system may include a variety of different sterile fluid interconnects configured to generate a closed, aseptic fluid flow path. A cell processing system may include an access door configured to permit consumables or cartridges to be input into the cell processing system, as shown in **FIG. 39**. Once the access door is closed, the system may be configured to provide in situ sterilization of consumables, cartridges, interconnections, or any combination thereof. In situ sterilization may include use of hydrogen peroxide vapors or other chemicals, gases, vapors, or any combination thereof that may be used for sterilization. In situ sterilization may comprise sterilizing connection interfaces of non-sterile interconnections or non-sterile chambers of a partially sterile interconnection.

#### *Multiple configurations*

**[00400]** The integrated cell processing system may provide flexibility in configuration to accommodate the manufacturing of a single product or a plurality of products. Additionally, the cell processing system may provide options for controlling systems locally, within a cluster of interconnected systems, or through distributed networks. In a single product configuration, the cell processing system may be configured to produce or may produce a specific product or therapeutic. System components and consumables may be configured to specifically for a target product. Alternatively, or in addition to, system components and consumables may be configured for specific processes. The manufacturing process may be configured for efficiency and consistency, with minimal reconfiguration between batches. This setup may be beneficial products with consistent manufacturing requirements and high production volumes. The cell processing system may be designed with modular components that can be easily reconfigured to produce multiple products with different specifications. Reconfiguration may include the use of interchangeable bioreactors and other functional

modules described elsewhere herein. The manufacturing process may be adaptable to accommodate variations in product formulations, cell lines, process parameters, or any combination thereof. Operators can adjust system settings and workflows to meet the unique processes for producing each product. To prevent cross-contamination between products, the system may incorporate measures such as dedicated single-use consumables, segregated processing areas, robust automated cleaning and sterilization protocols, or combinations thereof.

**[00401]** In some embodiments, an individual processing system is equipped with onboard control units, such as programmable logic controllers (PLCs) or microcontrollers, for local operation and control. Local control interfaces may permit operators to monitor system status, adjust parameters, initiate processes, or combinations thereof directly from the equipment. In some embodiments featuring a clustered control setup, multiple systems within a localized area are interconnected and controlled by a central master controller. An individual system may function as a slave unit, executing commands and receiving feedback from the master controller. Reagents, waste, power, and other bioprocessing system resources may also be distributed or shared within the cluster. In some embodiments, distributed control systems utilize network communication protocols, such as Ethernet or Wi-Fi, to connect individual systems across different locations or facilities. A central server or cloud-based platform may serve as the control hub, allowing operators to remotely monitor and manage system operations from any location with internet access. Distributed control systems may support scalable integration of additional systems or facilities, permitting centralized oversight and control of multi-site operations.

**[00402]** By providing versatile configuration options and flexible control systems, the integrated cell processing system may provide efficient and scalable production of cell and biologic products while providing operators with the flexibility to adapt to changing manufacturing and operational processes. The system can be tailored to meet the demands of diverse bioprocessing applications, including producing single or varied products.

#### **In situ cell processing systems and methods**

**[00403]** In another aspect, the present disclosure provides methods for cell processing. Methods for cell processing may include a variety of in situ cell processing process, for example, in situ cell selection, transfection, transduction, electroporation, gene delivery, genome engineering, multiplex genome engineering, or combinations thereof. The systems and methods described herein may be useful for clinical cell manufacturing (e.g., Car-T,

hematopoietic stem cells, mesenchymal stem cells, stem-derived cells, etc.) and versatile cell manufacturing (e.g., primary cells, secondary cells, cell lines, stem cells, etc.).

**[00404]** In another aspect, the present disclosure provides methods for cell processing, including cell selection. A method for cell processing, such as cell selection, may include providing a plurality of cells *ex vivo* to a cell processing unit. In the cell processing unit, the plurality of cells may be contacted with a selection reagent. The selection reagent, upon contacting the plurality of cells, may couple with cells of a sub-population of the plurality of cells and perturb a state of one or more cells of the sub-population of the plurality of cells to produce a population of perturbed cells and a population of non-perturbed cells. The method may include processing either the population of perturbed cells or the population of non-perturbed cells into a cell therapy product.

**[00405]** The systems and methods described herein may be configured to provide or may provide a variety of *in situ* processes to a plurality of cells. The plurality of cells may be subjected to *in situ* processes in a compartment or external to a compartment. The plurality of cells may be subjected to *in situ* processes while encapsulated within the 3D matrix or substrate. Alternatively, the plurality of cells may be subjected to *in situ* processing prior to or subsequent to encapsulation within the 3D matrix or substrate. In an example, the plurality of cells may be subjected to *in situ* activation. In another example, the plurality of cells may be subjected to *in situ* activation via membrane signal transduction in the 3D matrix. In another example, the plurality of cells may be subjected to *in situ* electroporation in the 3D matrix. In another example, the plurality of cells may be subjected to *in situ* electroporation external to the 3D matrix. In another example, the plurality of cells may be subjected to *in situ* cell culture or expansion within the 3D matrix. In another example, the plurality of cells may be subjected to *in situ* activation within the 3D matrix to generate or obtain a cell product. In another example, the plurality of cells may be subjected to *in situ* transduction within the 3D matrix to generate or obtain a cell product. In another example, the plurality of cells may be subjected to *in situ* transfection within the 3D matrix to generate or obtain a cell product.

*In situ cell selection*

**[00406]** The systems and methods described elsewhere herein may use *in situ* cell selection to select a cell or population of cells for processing. A cell population provided to a cell processing system may be a heterogeneous population of cells. A heterogeneous population of cells may include cells of different genotypes, phenotypes, cell types, morphologies, functions, or any combination thereof. *In situ* cell selection may selectively remove a cell population, selectively enhance a population, or a combination thereof. The



method may use a selection reagent. The selection reagent may selectively target and perturb a state of select or non-select cells. A selection reagent may be selected or designed to target and eliminate non-select cells or modulate proliferation rates of select or non-select cells to enrich a select population of cells. In an example, the selection reagent may perturb cells (e.g., a population of cells) to have an altered growth rate or viability as compared to a population of non-perturbed cells. In an example, the population of perturbed cells comprises cells with arrested cell cycles, cells with irreversibly arrested cell cycles, pre-apoptotic cells, cells irreversibly committed to apoptosis, apoptotic cells, dead cells, or cell degradation products. In another example, the population of perturbed cells may have an altered cell morphology as compared to the population of non-perturbed cells. In another example, the perturbed cells or the non-perturbed cells may be subjected to processing such as cell culture, transduction, transfection, transformation, expansion, in situ electroporation, gene delivery, or any combination thereof. The cell population may be subjected to in situ cell selection and cell processing simultaneously. Alternatively, or in addition to, the cell population may be subjected to in situ cell selection and subsequently to cell processing via cell culture, transduction, transfection, transformation, expansion, in situ electroporation, gene delivery, or any combination thereof.

**[00407]** The described cell selection methods may be performed in the presence or absence of beads. In an example, cell selection or cell enrichment may utilize beads. In another example, cell selection or cell enrichment may be performed in absence of or without beads. Beads may be magnetic or non-magnetic. Beads may be degradable or may not be degradable. Beads may be configured for non-specific or targeted binding of cells or cell degradation products.

**[00408]** As shown in **FIG. 40**, the negative cell selection workflow described herein may provide for efficient and effective cell selection. In an example, cells may be contacted with a plurality of selection agents. A selection agent may be an antibody-drug conjugate. An antibody-drug conjugate may be configured to alter a growth rate of a population of cells. Alternatively, an antibody-drug conjugate may prevent cell growth or cause cell death. The cells may be incubated with the antibody-drug conjugate to eliminate a population of cells. Negative cell selection processes may use fewer operations and reagents than magnetic-type cell selection. As shown in **FIG. 40**, magnetic-type cell selection may include contacting a population of cells with magnetic beads comprising a binding agent. In an example, a binding agent may be an antibody. A subset of the population of cells may bind to the magnetic beads. The magnetic beads may be subjected to a magnetic field. Subjecting the

magnetic beads to a magnetic field may separate the magnetic beads and thus separate one subset of cells from another subset of cells.

**[00409]** The method may utilize one or more selection reagents to mediate a cell selection bioprocessing function. A selection reagent may be a targeted cell selection agent (TCSA). A selection reagent may comprise one, two, three, four, five, six, eight, ten, or more components. In an example, the selection reagent comprises at least two components. In another example, the selection reagent comprises at least three components. The selection reagent may comprise a binding agent, a selection agent, a linker, or any combination thereof. In an example, the selection reagent comprises a binding agent. In another example, the selection reagent comprises a selection agent. In another example, the selection reagent comprises a binding agent and a selection agent. In another example, the selection reagent comprises a binding agent, a selection agent, and a linker.

**[00410]** The selection reagent may comprise a binding agent. Alternatively, the selection reagent may comprise a plurality of binding agents. For example, the selection reagent may comprise at least 2, 3, 4, 5, 6, 8, 10, or more binding agents. In an example, the selection reagent comprises a single binding agent. In another example, the selection reagent comprises at least two binding agents. In another example, the selection reagent comprises at least three binding agents. In another example, the selection reagent comprises at least four binding agents. The plurality of binding agents may be the same or the selection reagent may comprise multiple different types of binding agents. In an example, the selection reagent comprises a plurality of binding agents and the plurality of binding agents are selective to different targets. The binding agent may bind a target analyte and may be selected for affinity and specificity for a target antigen on a surface of a target cell. In an example, the target-binding moiety comprises an antibody, such as a monoclonal antibody (mAb). In another example, the target-binding moiety comprises a binding moiety other than a monoclonal antibody, including but not limited to, nanobodies, single-domain antibodies, single-chain variable fragments (scFvs), and aptamers, which offer advantages in terms of size, stability, and ease of modification. In an example, the selection reagent may comprise a plurality of antibody binding agents such as CD14, CD16(a/b), CD19, CD36, CD56, CD123, CD235a (glycophorin A), CD34, CD15 or any combination thereof. The selection reagent may be configured to remove or otherwise eliminate monocytes, macrophages, dendritic cells, myeloid cells (e.g., neutrophils and eosinophils), NK cells, B cells, HSCs, adipocytes, basophils, erythrocytes, or any combination thereof.

[00411] The selection reagent may comprise a selection agent (e.g., payload). Alternatively, the selection reagent may comprise a plurality of selection agents. For example, the selection reagent may comprise at least 2, 3, 4, 5, 6, 8, 10, or more selection agents. In an example, the selection reagent comprises a single selection agent. In another example, the selection reagent comprises at least two selection agents. In another example, the selection reagent comprises at least three selection agents. In another example, the selection reagent comprises at least four selection agents. The plurality of selection agents may be the same or the selection reagent may comprise multiple different types of selection agents. The selection agent may be configured to mediate or may mediate one or more cellular processes. In an example, the selection reagent comprises a plurality of different selection agents that mediate different cellular processes. In another example, the selection reagent comprises a plurality of selection agents that are the same or mediate the same cellular process. For a negative selection process, a selection agent (e.g., payload) may be selected to mediate cell death, quiescence, senescence, cell cycle arrest, inhibit cellular proliferation, or combinations thereof. In an example negative selection process, the selection agent is a cytotoxic agent that is responsible for inducing cell death once internalized by the target cell. The selection agent may comprise one or more of Tubulin inhibitors (e.g., auristatins (MMAE, MMAF), maytansinoids (e.g. DM1, DM4)), DNA damaging agents (e.g., pyrrolobenzodiazepines (PBD), duocarmycins, Calicheamicans), Topoisomerase I Inhibitors (e.g., Camptothecins, SN-38, deruxtecans (Dxd), etc.) RNA metabolism/processing targets (e.g., Thailanstatins, amatoxins), or any combination thereof. Payloads may be selected for activity against cell types, including efficacy based on cell division rate (e.g., can kill slowly dividing cells or non-dividing cells). For a positive selection process, a selection agent may be selected to mediate cell viability or cellular proliferation, or to inhibit quiescence, senescence, or cell death. In an example positive selection process, the selection agent is a growth factor. The choice of selection agent (e.g., payload) depends on potency, mechanism of action, and type of cell being targeted. In some embodiments, the mechanism of action is mediated by internalization of the selection reagent (e.g., TSCA). In some embodiments, the mechanism of action is mediated by cell membrane signal transduction. In some embodiments, the mechanism of action of the selection agent is dependent on other aspects of the system, for example, the cellular genotype, culture conditions, or combination thereof. For example, a particular selection agent (e.g., payload) may counteract the effect of one or more factor(s) provided in media, which may otherwise (in the absence of the selection agent) prevent cellular proliferation, cause cell death, or both.

In an example, a particular selection agent (e.g., payload) may counteract the effect of one or more genotype(s) provided by the cells, which may otherwise (in the absence of the selection agent) prevent cellular proliferation, cause cell death, or both.

**[00412]** The selection reagent (e.g., TSCA) may further comprise a linker that connects the binding agent (e.g., target-binding moiety) to the selection agent (e.g., payload(s)). The linker may be configured to couple or may couples the binding agent to the selection agent. A linker's primary function may be to provide stability to the selection reagent (e.g., TSCA) in use, to prevent premature release of the selection agent (e.g., payload), and to enable the release of the selection agent (e.g., payload) once the selection reagent has bound the target cell, has been internalized, or both. Linkers can be cleavable or non-cleavable. A selection reagent may comprise a single linker or a plurality of linkers. A plurality of linkers may be cleavable, non-cleavable, or a combination thereof. Cleavable linkers may be designed to be stable in media culture conditions, but to release the selection agent (e.g., payload) in response to specific conditions within the target cell, such as low pH in lysosomes, high concentrations of glutathione, or the presence of certain enzymes. For selection reagents comprising non-cleavable linkers, the entire selection reagent or significant portion thereof may be degraded to release the selection agent (e.g., payload) within the target cell. For example, the selection reagent may comprise a cytotoxic selection agent and the selection reagent may be degraded by lysosomal enzymes to release the cytotoxic selection agent into the target cell. The kinetics and efficiency of cell selection may be modulated by the linker type, selection agent, or combination thereof. For example, an efficient selection reagent may remain stable during culture, reducing off-target effects on non-target cells and increasing delivery of selection agents to the target cells.

**[00413]** In an example, cell processing may begin with an initial population or plurality of cells that exhibit heterogeneity in at least one cellular marker, cells are contacted with one or more selection (e.g., TSCA) reagents, which may bind the one or more cellular targets and mediate the one or more cellular processes, resulting in positive or negative selection (e.g., a population or plurality of cells may be enriched or depleted if the cells express the target cellular marker). The target cells may comprise suspension, adherent, or other cell types, which may be cultured in 2D, 2.5D, 3D culture systems, or combinations thereof. In some embodiments, excess selection (e.g., TSCA) reagent is diluted, washed, or removed from the cell population or growth media after a period of incubation sufficient to mediate selection. In some embodiments, a pre-enrichment procedure is performed prior to cell selection using a selection reagent (e.g., TSCA). For example, a label-free separation procedure may be used to

enrich a whole blood source material for nucleated cells (e.g., a peripheral blood mononuclear cell (PBMC) or total nucleated cell (TNC) population), such as by using a cell selection apparatus integrated within a bioprocessing system. In the same or a separate example, a label-free separation procedure may be used to deplete a whole blood source material of red blood cells (RBCs), platelets, granulocytes, or any combinations thereof, which differ in their size and other physical properties from T cells, and which may be the primary source material for CAR-T and T cell therapies. In some embodiments, the starting material is a primary human cellular product, including but not limited to, whole blood, an enriched blood product (e.g., leukopak, apheresis product), or derived from a tissue sample (e.g., a tumor resection).

**[00414]** When performed as part of a complex bioprocessing workflow, in situ cell selection may be performed separately, or concurrently, with one or more other bioprocessing (e.g., cell processing) processes. For example, cell selection may be performed concurrently, substantially concurrently, or partially overlapping with, procedures for cell engineering, including but not limited to, viral transduction, or cell stimulation (e.g. immune cell stimulation, as by using anti-CD3/CD28 antibodies). **FIG. 41** shows example comparison between in situ cell stimulation within a 3D matrix and suspension culture. Performing multiple bioprocessing processes concurrently may reduce manufacturing time, cost, and complexity. Dead cells may be substantially removed at any time during bioprocessing. In some embodiments, an in-situ cell selection method is used for selection prior to product formulation, such as to remove a contaminating cellular species from a therapeutic preparation (e.g., to deplete Tregs from a CAR-T preparation, which may otherwise inhibit the CAR-T mechanism of action, cancer cell killing, by immune tolerogenic mechanisms).

**[00415]** In some embodiments, a plurality of selection reagents (e.g., TSCAs) are used for the purpose of pan-T cell selection. Non-T cell target cells, for example, monocytes, neutrophils, eosinophils, B cells, stem cells, dendritic cells, NK cells, granulocytes, erythroid cells, or combinations thereof are labeled by using a cocktail of selection reagents (e.g., TSCAs) comprising antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (Glycophorin A) conjugated to a cytotoxic small molecule. Subsequently, non-target cells may be depleted, resulting in a highly pure T cell population. In some embodiments, a selection reagent (e.g., TSCA) is used to deplete a primary T cell source of Tregs prior to T cell activation using an anti-CD25 antibody selection reagent. As the presence and level of contaminating Tregs in CAR-T therapeutic products is correlated

with poorer clinical outcomes, the method of Treg depletion by in situ cell selection may result in higher quality clinical outcomes and reduced variability.

**[00416]** A method of process development may be used to optimize the procedure of in situ cell selection. Process development may involve a series of optimization experiments, including titration to determine the ideal selection reagent concentration based on reaction volume and cell count, and time-course experiments to fine-tune the exposure time to selection reagent selection efficiency. To achieve a population-level enrichment or depletion of target cells the process parameters (e.g., selection reagent concentration, exposure time, cell count, etc.) may be adjusted based on factors such as reduction levels or remaining target cell percentages, and to minimize off-target effects. The cells may be contacted with the selection reagent at least 1, 2, 3, 4, 5, 6, 8, 10, or more times. In some embodiments, the cells may be contacted with selection reagent two or more times during bioprocessing. In such cases, serial challenge optimization experiments in which the cell population is exposed to multiple doses of selection reagent over a period of time may be used to determine the number and timing interval of selection reagent treatments to achieve the selection. Determination of in situ cell selection procedures, including selection reagent concentration, exposure duration, interval of multiple exposures, or any combination thereof, may be performed by monitoring the population-level enrichment or depletion, as an enrichment factor (e.g., 2x enrichment), as a percent (e.g., <x% of target cells remain), as a receiver-operator curve optimization (e.g., to optimize selection reagent action on target cells vs. off-target effects on non-target cells), or by any other process development procedure using these and other metrics.

**[00417]** In some embodiments, the method of in situ cell selection is used during multiplex genome engineering process. For example, the in situ cell selection method may be used for negative or positive selection of a library of cells subjected to genetic perturbation, such as by using a CRISPR or viral transgene library, to select for genetic factors that result in gain or loss of expression of a cellular marker, for example, as a proxy for selection of a cellular phenotype, state, or cell type.

**[00418]** As shown in **FIG. 42**, negative cell selection may be useful for eliminating one or more non-targeted cell types from a homogenous population to enrich a target cell without affecting or without substantially affecting the viability or expansion of the targeted cells. Selection reagents may be configured to modulate an ability of a subset of a population of cells to expand. Cell selection using the selection reagents described herein may be useable for cell selection in suspension cultures, adherent cultures, 3D cultures, or any combination

thereof. As shown in **FIG. 43**, cell viability of a non-target cell may vary as a function of selection reagent concentration. Selection reagents may be highly selective such that a selection reagent that reduces viability of a non-target cell population to zero or near zero does not affect or substantially does not affect viability or expansion of the targeted cells.

**[00419]** In some embodiments, the method of in situ cell selection is automatically performed by a bioprocessing system (e.g., cell processing system). In some embodiments, the method of in situ cell selection is automatically performed using a solid-state bioreactor. The solid-state bioreactor may comprise a 3D matrix as described elsewhere herein. In an example, the 3D matrix encapsulates the plurality of cells subjected to the cell selection process and the plurality of cells may be contacted with the selection reagent within the 3D matrix. In some embodiments, the method of in situ cell selection is automatically performed in a static bioreactor (e.g., a flask, vessel, or bag), rocking bioreactor, shaking flask bioreactor, perfusion bioreactor, or other cell culture apparatus. In some embodiments, the method of in situ cell selection is automatically performed as a process in the manufacturing of a therapeutic cell product. The therapeutic cell product may be produced by the system at a scale of at least one human dose equivalent. The therapeutic cell product may be produced by the system at a scale of at least 1, 2, 3, 4, 5, 6, 8, 10, 20, or more human dose equivalence.

#### *Transfection*

**[00420]** The 3D matrix may be configured to permit a variety of in situ cell processes as shown in **FIG. 44**. Cells may be activated and expanded within a 3D matrix. For example, cytokines and antibodies may be added to the 3D substrate via media, via functionalization, or both to provide cell activation. Cells may be subjected to viral transduction within the 3D matrix. High cell concentration within the 3D matrix may increase efficiency of cell transduction at reduced multiplicity of infection (MOI). The 3D matrix may also be configured to permit electroporation of cells within the 3D matrix. The 3D matrix may be configured with one or more sets of electrode pairs configured to provide an electric field to the 3D matrix and cells encapsulated therein.

**[00421]** The system may comprise one or more integrated apparatus configured to automatically perform or that automatically perform one or more methods of cell transfection or transduction. Cell transfection may facilitate efficient cell manipulation, including genome editing, across various cell types and applications. Methods of cell transfection may include, but are not limited to, viral transduction (e.g., by using a viral vector such as adeno-associated viral, adenoviral, lentiviral, retroviral vectors, or combinations thereof) and non-viral transduction. Non-viral transduction methods may include, but are not limited to, use of

lipid nanoparticles (LNP), electroporation, sonoporation, laser poration, mechanical cell membrane disruption (e.g., by using microfluidic membrane deformation, nanowires, nanoneedles, microinjection, etc.), or combinations thereof. In some embodiments, a flow-based electroporation function is provided by an integrated electroporation apparatus. For example, suspended cells may be suspended in an electroporation buffer within a channel and genetic payloads may be provided in a continuous flow configuration or pulse-flow configuration. In an example, a continuous flow configuration may comprise subjecting the cells to steady state flow with continuous electrical pulses. In another example, a pulse-flow configuration may comprise subjecting cells to alternating flow and electrical pulses. The channel may comprise integrated electrode pair(s) configured to subject the cells to one or more electric fields. The one or more electric fields may provide electroporation and transfection with the genetic payload. In some embodiments, a viral transduction function is provided by an integrated flow chamber, hollow fiber chamber, or combination thereof which is configured to retain or which retains cells in a high surface-area-to-volume format for the purpose of enhancing viral transduction efficiency.

**[00422]** The transfection system may be configured to perform transient or permanent cell manipulations. Transient transduction of exogenous protein, non-integrating plasmid deoxyribonucleic acid (DNA), or ribonucleic acid (RNA, including messenger RNA, mRNA), without creating permanent genome modifications or genomic integration, may be used for short-term gene expression studies, protein production, and functional assays. For example, transient transfection of nucleic acid payloads may encode genes of interest, RNA interference (RNAi) constructs, or genome editing tools like CRISPR-Cas9 components. Alternatively, or in addition to, genome engineering may be used to modify the cellular genome, leading to permanent alterations in gene sequences and functions. Transposase-based systems, such as the Sleeping Beauty transposon system or piggyBac transposon system, may be employed to deliver DNA payloads containing genetic sequences into the host cell's genome. Other genome editing approaches, including, but not limited to, CRISPR-Cas9, TALENs, and zinc finger nucleases (ZFNs), may enable precise manipulation of the genome. These systems may enable stable gene editing or stable integration of transgenes into the cellular genome, allowing for long-term expression and heritable modifications.

**[00423]** Transfection payloads may include DNA, RNA, protein, or any combination or plurality thereof. Genetic payloads may include, but are not limited to, chimeric antigen receptors (CAR), T cell receptors (TCR), genes encoding soluble proteins including antibodies, major histocompatibility complex (MHC) genes, antigens, and other transgenes



useful for cell line creation, cell engineering, cell therapy, in the manufacturing of virus and other biologics, or combinations thereof.

**[00424]** Cell transduction within a 3D matrix may provide comparable efficiency to transduction in a cell suspension, as shown in **FIG. 45**. **FIG. 45** shows an example comparison of cell transduction as a function of cell viability between a cell suspension and a 3D matrix. Within a cell suspension, the transduction rate may be 70% of viable cells. In comparison, in situ transduction within a 3D matrix may achieve a transduction rate of 57% of viable cells. Lentiviral transduction within a 3D matrix may be comparably efficient as lentiviral transduction in cell suspensions, as shown in **FIG. 46**. **FIG. 46** shows an example comparison between lentiviral transduction in suspended cells (left bar in each pair of bars) and lentiviral transduction in a collagen-based 3D matrix (right bar in each pair of bars) at the same MOI.

#### *Genetic engineering*

**[00425]** In some embodiments, a solid-state bioreactor or cell processing system is configured to perform in situ genetic engineering on cells cultured within a 3D substrate. Genetic engineering of cells may involve modifying their genetic material, either transiently or permanently, to achieve specific biological properties, such as to introduce new functions or correct genetic disorders. Genetic engineering modalities that may comprise a genetic payload and delivery mechanism. Genetic payloads can result in transient or permanent changes to a cell's genetic material. For example, recombinant DNA technology can be used to splice genetic material or insert synthetic genetic material into a plasmid or other vector, which is subsequently introduced to a cell. Methods of genetic engineering may involve the generation of breaks in a cell's genome for the purpose of perturbing a genetic sequence or inserting exogenous DNA. For example, CRISPR-Cas9, Transcription Activator-Like Effector Nucleases (TALENs), and Zinc Finger Nucleases (ZFNs) can be used to facilitate targeted DNA editing by creating DNA breaks at specific genomic locations. As such breaks are repaired by a cell's natural or engineered DNA repair mechanisms, the original genetic sequence can be perturbed, such as by insertions and deletions that arise from non-homologous end joining, or exogenous genetic sequences can be inserted, such as through homologous recombination with a donor payload sequence. Other genome engineering modalities include transposon-mediated gene transfer and viral vector-mediated gene transfer, which can insert genes of interest into a cellular genome via the activity of transposases or viral processes. Other genome engineering modalities include RNA interference (RNAi) and related transcriptional modalities, which may use single stranded RNA, double stranded

RNA, or nucleic acid analogs to specifically translationally silence or target for degradation complementary messenger RNA (mRNA) molecules, thereby preventing the mRNA from producing proteins.

**[00426]** In some embodiments, a solid-state bioreactor or cell processing system is configured to perform transient in situ genetic engineering, which involves the temporary introduction of genetic material into cells. The introduced DNA or RNA may not integrate into the host genome and is eventually degraded or lost during cell division. Transient expression systems may use plasmids, viral vectors, or RNA genetic payloads. These methods may permit a gene of interest to be expressed for a limited period, for example, ranging from a few hours to several days. Transient genetic engineering may be useful for: short-term experiments to study gene function, protein interactions, or cellular responses without permanently altering the cell's genetic makeup; RNA-based genetic payloads and modalities, where the effect is achieved through transient expression of proteins from mRNA or knock-down such as by RNAi; or the production of recombinant proteins and viral particles, where cells are engineered to produce a product of interest temporarily. In some embodiments, a solid-state bioreactor or cell processing system is configured to perform stable in situ genetic engineering, which may involve permanent modification of a cell's genetic material or the integration of new genetic material into the genome of the host cell, permitting genetic changes to be maintained over the long term, including through cell divisions.

**[00427]** In some embodiments, a solid-state bioreactor is configured to deliver a genetic engineering composition to cells. In some embodiments, a solid-state bioreactor is configured to deliver a soluble genetic engineering composition to cells. Soluble genetic engineering compositions may include, but are not limited to, molecular compositions or reagents comprising one or more genetic engineering payload(s), a cellular delivery mechanism, or any combination thereof. Cellular delivery mechanisms for soluble genetic engineering compositions may include, but are not limited to, viral vectors (e.g., Adenovirus, Adeno-associated Virus (AAV), Herpes virus, Lentivirus, etc.), viral-like particles, protein-based carriers, lipid nanoparticles (LNP), cationic lipids (e.g., Lipofectamine, 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dimyristyloxy-propyl-3-dimethyl-hydroxy ethyl ammonium bromide and cholesterol (DMRIE-C), etc.), polymers (e.g., Hexadimethrine bromide (Polybrene), Polyethylenimine (PEI), poly-L-lysine, diethylaminoethyl (DEAE)-dextran, etc.), lipopolyplexes, dendrimers (e.g., Polyamidoamine (PAMAM)), inorganic compounds (e.g., calcium phosphate), microparticles, nanoparticles, other gene-delivery

particles, or any combinations thereof. Genetic payload(s) may include nucleic acids such as DNA and RNA. Genetic engineering compositions may further include proteins, such as CRISPR-Cas9 proteins, including in the form of ribonucleoprotein (RNP) complexes. Genetic engineering compositions may be delivered to the cell compartment, such as via a perfusion mechanism, fluid flow, other mass transfer systems and methods described herein, or any combination thereof to contact and transfect or transduce cells.

**[00428]** In some embodiments, a solid-state bioreactor or cell processing system is configured to deliver a genetic engineering composition to cells in the form of a soluble genetic payload via in situ membrane electroporation, electroporation, or both. Genetic payloads may be delivered to the cell compartment to provide contacting and transfecting cells upon permeabilization. The solid-state bioreactor may be configured to deliver a conductive solution or buffer suitable for electroporation to the cell compartment, such as via a perfusion mechanism, fluid flow, a buffer exchange, or any combination thereof as described elsewhere herein. In the presence of or upon the introduction of a genetic payload and cells in the 3D substrate, and optionally an electroporation buffer or other conductive solution with properties conducive to cell electroporation, an electrical circuit may be closed such that an electrical pulse at a given voltage and duration (e.g., a few microseconds to a millisecond) is discharged through the cell-matrix, as by the configuration of embedded electrodes, electrodes in communication with the conductive environment of the 3D substrate, or both embedded and non-embedded electrodes. In an example, a 3D substrate comprises embedded electrodes. The 3D substrate may comprise at least 2, 3, 4, 5, 6, 8, 10, 15, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more embedded electrodes. The electrical pulse may disturb the phospholipid bilayer of the cell membrane and results in the formation of temporary pores. The electric potential across the cell membrane simultaneously rises to allow charged molecules like DNA to be driven across the membrane through the pores in a manner similar to electrophoresis. Factors such as strength of the electrical field, shape of the pulse, number of pulses applied, composition of the buffer, or conductivity of the 3D substrate may be tuned, individually or collectively, to modulate transfection efficiency. In some embodiments, a solid-state bioreactor is configured with one or more electrode(s) designed to provide a spatially uniform pulse, a spatially uniform electric field across the cells, or both within the 3D substrate or cell compartment. Electrode arrangements, buffers, 3D substrate compositions, electrode pulse configurations, or any combinations thereof may be configured to prevent or substantially reduce gas bubble evolution and/or Joule heating during electric pulsing, which may otherwise disrupt the

structure of the 3D substrate or damage cells. Cells electroporated in situ within a 3D matrix may be comparable to CAR gene expression in suspension electroporation samples, as shown in **FIG. 47**.

**[00429]** The 3D matrix or cell compartment may comprise two or more electrodes configured to provide or that provide electroporation of cells within or adhered to the 3D matrix. As shown in **FIGs. 60A – 60C**, the electrodes may be configured to span a long axis of the 3D matrix or cell compartment. The electrode(s) may be in direct contact with the 3D matrix, may be disposed adjacent to the 3D matrix and separated from the 3D matrix by a conductive fluid, may be partially or fully embedded within the 3D matrix, or any combination thereof. Each electrode may form an electrode pair with another electrode (e.g., negative electrode may pair with a positive electrode). Alternatively, an electrode may pair with multiple other electrodes (e.g., a negative electrode may pair with multiple positive electrodes or vice versa). In an example, the 3D matrix comprises two or more electrodes spanning a long axis of the 3D matrix, as shown in **FIG. 60A**. The electrodes may comprise a plate-like configuration and be disposed along one or more edges of the 3D matrix such that the largest surface area of the electrode is disposed adjacent to an edge of the 3D matrix. In an example, and as shown in **FIG. 60B**, the 3D matrix may comprise a plurality of needle or rod-like electrodes disposed parallel to a long dimension of the 3D matrix. The electrodes may be disposed along a surface of the 3D matrix, may be separated from the 3D matrix by a conductive fluid, may be embedded within the 3D matrix, or any combination thereof. The 3D matrix may comprise at least 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more needle or rod-like electrodes. In another example, the electrodes may be disposed in a single plane or in multiple planes, as shown in **FIG. 60C**. The electrodes may be configured with a first conductive portion connected to one or more second conductive portions extending away from the first conductive portion. The second conductive portion of a first and a second electrode may be disposed in an interdigitized configuration. The 3D matrix may comprise at least 1, 2, 3, 4, 5, 6, 8, 10, or more planes of interdigitized electrodes. The interdigitized electrodes may be disposed on or near a surface of the 3D matrix, may be embedded within the 3D matrix, or both. In an example, the 3D matrix or cell compartment comprises a plurality of electrodes that are individually addressable such that the electrodes are configured to apply localized electroporation.

**[00430]** The 3D matrix may comprise two or more electrodes disposed to span a short axis of the 3D matrix or cell compartment, as shown in **FIGs. 61A and 61B**. The electrode may be

configured as two plates or a plurality of plates disposed across the 3D matrix or compartment from each other, as shown in **FIG. 61A**. The 3D matrix or compartment may comprise at least 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more electrodes. Each electrode may pair with one other electrode (e.g., a negative electrode may pair with one positive electrode). Alternatively, an electrode may pair with multiple other electrodes (e.g., a negative electrode may pair with multiple positive electrodes). The 3D matrix or cell compartment may comprise a plurality of needle or rod-like electrodes configured to be disposed through a short axis of the 3D matrix or cell compartment, as shown in **FIG. 61B**. In an example, the 3D matrix or cell compartment comprises a plurality of electrodes that are individually addressable such that the electrodes are configured to apply localized electroporation.

**[00431]** In some embodiments, a solid-state bioreactor or cell processing system is configured to deliver a genetic engineering composition to cells via membrane sonopermeabilization, sonoporation, or both, which may use ultrasonic vibrations to transiently disrupt cell membranes to permit entry of genetic material. The efficiency of sonoporation may be influenced by factors such as cell type, viscosity of the medium, presence of cavitation enhancers (microbubbles), parameters including the frequency, intensity, or duration of ultrasound exposure, the type and nature of molecule(s) being delivered, or any combination thereof. In some embodiments, a solid-state bioreactor or cell processing system is configured with one or more transducer(s) designed to provide a spatially uniform acoustic pulse, spatially acoustic field, or both across the cells within the cell compartment or 3D substrate.

**[00432]** In some embodiments, genetic material and other reagents and compositions for gene delivery and cell engineering may be delivered to the cell compartment via the transfer of a fluid, such as by fluid flow. For example, aqueous gene engineering compositions may be delivered to the cell compartment via fluid flow in the perfusion compartment (e.g., fluid flow path). For example, the aqueous gene engineering compositions may contact cells via diffusion, fluid flow, active molecular transport under an applied electric field (electrophoresis), or any combination thereof. Perfusion culture and in situ washing and buffer exchange may provide delivery of gene engineering compositions to cells for transduction, transfection, delivery via electroporation, delivery via sonoporation, or any combination thereof. For example, genetic engineering compositions may be delivered to cells via mixing with perfused media and subsequent fluid flow via a perfusion compartment in fluidic communication with a cell compartment. Such a configuration may simplify the

solid-state bioreactor or cell processing system. In some embodiments, one or more gene engineering compositions may be delivered to the cell compartment via a separate fluidic compartment or solid-state bioreactor or cell processing function than those used for media perfusion. For example, a solid-state bioreactor or cell processing system may be configured with specialized fluid delivery paths, fluidic compartments, or both in fluid communication with the cell compartment. The specialized fluid delivery paths or fluid compartments may be configured to provide or may provide high-efficiency transfer of genetic engineering constructs. High efficiency transfer of genetic engineering constructs may provide genetic engineering compositions at high concentration, in a reduced volume, or both as compared to a culture media approach which may increase the overall efficiency of genetic engineering.

*Modulating cell environment*

**[00433]** In some embodiments, a solid-state bioreactor or cell processing system is configured to provide a variable or programmable, or both, 3D cell culture environment. In some embodiments, a solid-state bioreactor may be configured with a programmable 3D substrate, which may change state or exhibit a variety of physical properties via altering the composition of the substrate (e.g., adding additional matrix material, crosslinkers, etc. to a 3D substrate), using a stimulus (e.g., temperature, chemical(s), enzyme(s), light, electricity, or any combination thereof), or both. For example, a solid-state bioreactor or cell processing system may be configured with a 3D substrate composition comprising a crosslinking chemistry in a given crosslinking state, and further configured to deliver a stimulus, such as an aqueous crosslinking or chemical cleavage composition, which may modulate crosslinking state (e.g., density) within the 3D substrate, thereby altering physical properties of the 3D substrate such as stiffness, modulus, elasticity, porosity, or any combination thereof. A stimulus which alters the physical state of a 3D substrate may be delivered at any time, or at more than one time, during operation of the solid-state bioreactor or cell processing system. For example, a stimulus may be delivered upon a setup or harvest procedure to facilitate the loading or retrieval of cells. In another example, a stimulus may be delivered during cell culture within the 3D substrate. In some embodiments, a solid-state bioreactor or cell processing system may be configured to physically deform a 3D substrate, such as by applying a force. For example, a solid-state bioreactor or cell processing system may be configured to apply a force to the 3D substrate, such as by physically reducing (e.g., compression), increasing (e.g., expansion, rarefaction, stretching), or altering (e.g., shear) the physical dimensions of the cell compartment through the use of motion systems, motors, pressure, or any combination thereof. By physically deforming the 3D substrate, physical

forces may be communicated to cells via physical changes in the 3D substrate, fluid dynamics, or both. Such a solid-state bioreactor configuration may aid in the culture or phenotypic determination of certain cell types, such as those comprising cartilage tissue, which may respond to physiological compression.

#### *Concurrent processes*

**[00434]** In some embodiments, a solid-state bioreactor or cell processing system is configured to execute two or more methods or processes sequentially, concurrently, or partially concurrently. For example, in a certain configuration a solid-state bioreactor or cell processing system is configured to perform a membrane signal transduction operation, such as T cell activation by introduction of soluble or 3D substrate-linked activation factors, concurrently with another operation, such as media perfusion, gene delivery, or in situ negative selection. In some embodiments, a solid-state bioreactor or cell processing system is configured for CAR-T manufacturing or another application which uses concurrent processes. In some embodiments, a solid-state bioreactor or cell processing system is configured to enable arbitrary combinations and time ordering of operations. Fluid flow path, compartment, cell processing unit, control systems, and other components of the bioreactor or cell processing system may be configured to provide or may provide sequential, concurrent, or partially concurrent processing.

#### *Multiplexing*

**[00435]** In some embodiments, the integrated bioprocessing system incorporates a plurality of parallel miniaturized systems to enable high-throughput screening of various processes. For example, the integrated bioprocessing system may include a plurality of miniaturized solid-state bioreactors arranged in a parallel configuration, allowing simultaneous processing of multiple experimental conditions. In some embodiments, each miniaturized bioreactor utilizes channels or chambers to provide precise control over fluid flow, mixing, and cell culture conditions. The system may include a bioreactor array that includes at least 2, 3, 4, 5, 6, 12, 24, 96, 384, or more individual bioreactors. An individual bioreactor may comprise a 3D matrix or a plurality of 3D matrices. An individual bioreactor may comprise at least 2, 3, 4, 5, 6, 12, 24, 96, 384, or more 3D matrices. ] The bioreactor array may be modular and scalable, accommodating different sizes and configurations to meet specific experimental conditions. Other functional modules comprising the integrated bioprocessing system, described herein, may be flexibly configured to operate by various process parameters, including variations in reagents, concentrations, flow rates, and other conditions and configurations specific to the module and apparatus. Operators can design

experimental layouts to systematically evaluate the impact of these parameters on product quality and performance. Integrated control systems may automate the manipulation of process parameters within each bioreactor, ensuring consistent and reproducible experimental conditions across the array. Sensors and integrated assay technologies may provide real-time feedback on cell viability, metabolic activity, protein expression, and other relevant parameters, allowing for continuous monitoring of experimental outcomes. The system may thereby facilitate rapid iteration and optimization of bioprocessing protocols. Operators may quickly evaluate multiple process conditions and iterate on experimental designs to identify parameters for product production. Statistical design of experiment methodologies can be applied to systematically explore and analyze the effects of multiple variables on process outcomes. The integrated bioprocessing system may support the execution of design of experiments (DoE) studies, enabling efficient optimization of complex bioprocesses. Software tools and data analytics algorithms may be integrated into the system to facilitate data analysis and visualization. Operators can interpret experimental results, identify trends, and generate insights to guide process optimization efforts.

**[00436]** In some embodiments, the system comprises a procedure for screening a plurality of 3D matrix conditions using a plurality of solid-state bioreactors. In some embodiments, the system comprises a procedure for screening a plurality of perfusion culture conditions using a plurality of solid-state bioreactors. In some embodiments, the system comprises a procedure for screening a plurality of bioprocessing workflows by variously utilizing or differentially configuring the functions or procedures of one or more integrated bioprocessing apparatus. For example, the system may automatically collect data relevant to various processes and product attributes for each of a plurality of screened conditions, such as by using integrated analytical apparatus and methods described elsewhere herein.

**[00437]** Configuring parallel miniaturized systems within the integrated bioprocessing system may enable high-throughput screening of process conditions, optimization of bioprocessing protocols, and preparation of variated cell products for testing and evaluation. The system may permit researchers and bioprocess engineers to accelerate innovation, improve productivity, and advance the development of biologic products and cell therapies.

#### *Multiplexed genome engineering*

**[00438]** In some embodiments, the integrated bioprocessing system is configured to perform or performs a method of automated multiplex genome engineering. Multiplex genome engineering may be a genome editing technique that enables quick editing of an organism's deoxyribonucleic acid (DNA) to produce multiple changes across the genome. By



leveraging the integrated capabilities of the bioprocessing system, researchers can iteratively introduce, select, and refine genome edits in a controlled and efficient manner. This iterative approach may enable the stepwise modification of cellular genomes to achieve targeted phenotypic outcomes, such as enhanced productivity, improved therapeutic properties, or increased resistance to environmental stressors. In some embodiments, the system enables a method of producing cell products with extensively engineered genomes.

**[00439]** For example, researchers may design a first genome edit(s) to be introduced into the target cell population. This may involve the knockout of specific genes, insertion of transgenes, modification of regulatory elements, or genome edits for any other purpose. The target cells are cultured within the bioreactor under conditions conducive to genome editing. A plurality of bioreactors within the system may contain different experimental conditions, such as varying concentrations of CRISPR components or guide RNAs targeting different genomic loci. The first genome edit(s) may be introduced into the target cell population, resulting in cells with the targeted genetic modifications. Following the introduction of the first genome edits, an optional selection process may be included to enrich or deplete specific cell populations within the culture. This process may involve the automated use of selectable markers, integrated cell sorting techniques, or metabolic selection strategies to enrich or isolate cells with the targeted genetic modifications. The cell product with the first edit(s) may be analyzed, cryopreserved, or stored, or the user may proceed with a subsequent edit(s). Based on the outcomes of the first round of genome editing and any optional selection processes, researchers may design the second genome edit(s) to be introduced into the edited cell population. This may involve introducing additional modifications to further enhance or refine the cellular phenotype. The edited cell population may be cultured once again within the bioreactor(s). If a plurality of bioreactors are used, each bioreactor may contain a different experimental condition corresponding to the second genome edit(s). This may allow for parallel testing of various editing strategies and conditions. The second round of genome editing may be performed on the cultured cells, resulting in cells with additional genetic modifications or alterations. Integrated assay technologies may provide monitoring of editing efficiency and cell viability. The cell product with the second edit(s) may be analyzed, cryopreserved, or stored, or the user may proceed with a subsequent edit(s).

**[00440]** In some embodiments, the optional cell selection process utilizes machine learning (ML) or artificial intelligence (AI) for the purpose of screening and selecting one or more cells or plurality of cells for subsequent processing. Integrating ML and AI into the analysis and selection processes of the bioprocessing workflow can create a closed-loop AI

cell engineering platform. Throughout the bioprocessing workflow, various data points may be collected, including genomic data, transcriptomic data, proteomic data, and phenotypic data. This data can include information on gene expression levels, cellular morphology, metabolic activity, and other relevant parameters. ML algorithms may aggregate and integrate data from multiple sources, creating a comprehensive dataset that captures the characteristics of the engineered cell population. ML techniques are applied to extract relevant features or patterns from the data, identifying key biomarkers or genomic signatures associated with targeted cellular phenotypes or functions. AI algorithms, such as neural networks or random forests, may be trained on the integrated dataset to build predictive models of cellular behavior. These models can predict the effects of specific genetic modifications or environmental conditions on cellular phenotypes. AI-driven optimization algorithms, such as genetic algorithms or reinforcement learning, can be used to iteratively explore the design space to identify optimal combinations of genome edits, culture conditions, and selection strategies for achieving targeted cellular outcomes. AI systems may provide decision support to researchers by recommending experimental interventions or process adjustments based on real-time data analysis and predictive modeling. In some embodiments, ML algorithms continuously analyze the evolving cellular population and adjust selection criteria in real time to favor cells exhibiting targeted phenotypic traits. This adaptive selection process may maximize the efficiency of enrichment or depletion strategies. ML/AI systems may provide feedback to the bioprocessing system, directing subsequent experimental interventions or modifications based on the outcomes of other selections. This closed-loop feedback mechanism may ensure that the system iteratively refines and optimizes the engineered cell population over time. The closed-loop AI cell engineering system may learn from each iteration of the bioprocessing workflow, incorporating new data and insights to improve predictive accuracy and decision-making capabilities. Through continuous experimentation and analysis, the system may iteratively refine its models and strategies, driving ongoing innovation and improvement in cell engineering outcomes. By leveraging ML/AI in the engineering, analysis, and selection processes of the bioprocessing workflow, the closed-loop AI cell engineering platform may offer a powerful approach for systematically designing, optimizing, and controlling cellular phenotypes with improved precision and efficiency. This integrated system may have the potential to improve the field of synthetic biology and accelerate the development of cell-based therapies, bioproducts, and biotechnologies.

#### **Analysis systems and methods**

**[00441]** Cell processing systems may comprise one or more analysis systems or units. The analysis systems or units may be configured to monitor, or may monitor, one or more of the fluids within the fluid flow path (e.g., perfusion compartment or other flow path), 3D substrate, cell compartment, cells within the cell compartment, or any combination thereof. A cell processing system may comprise a plurality of analysis systems or units. Different components of the cell processing system may be associated with a different analysis unit. Alternatively, or in addition to, multiple components may be associated with a single analysis unit. In an example, cell processing system comprises an analysis unit configured to monitor the plurality of cells within the compartment (e.g., cell compartment). The analysis unit may be configured to monitor the fluid flow path (e.g., perfusion or fluid flow compartment, 3D substrate, cell compartment, cells within the cell compartment, or any combination thereof in real time. In an example, the analysis unit is configured to monitor the plurality of cells in real time. The analysis unit may monitor or detect one or more properties of the cells or cellular environment. In an example, the analysis may monitor or detect one or more of marker expression, phenotype, viability, concentration, potency, sterility, endotoxin, safety, or any combination thereof. The analysis unit may comprise one or more sensor configured to monitor an environment comprising a plurality of cells. The environment may be a 3D substrate, a solution comprising 3D substrate/matrix precursors, a solution comprising degradation products of the 3D substrate or matrix, or a solution comprising cells in absence of the 3D substrate or matrix precursors. In an example, the one or more sensors may include metabolite sensors, dissolved gas sensors, pH sensors, temperature sensors, viscosity sensors, pressure sensors, or any combinations thereof.

**[00442]** The analysis unit may further, or alternatively, comprise one or more sensors configured to analyze the cells product. Sensors configured to or that analyze the cell product may include Raman spectroscopy, infrared (IR) absorption spectroscopy, near-infrared (NIR) spectroscopy, mid-infrared spectroscopy, Fourier Transform infrared (FTIR) spectroscopy, optical coherence tomography, confocal microscopy, multiphoton microscopy, microwave impedance microscopy, ultrasound imaging, photoacoustic microscopy, elastography, computed tomography, conductance sensor, radio-frequency impedance sensor, dielectric spectroscopy, permittivity sensor, fluorescence imaging, mass spectroscopy, optical density, magnetic resonance angiography, or any combination thereof. The analysis unit may be configured to provide temporal or real time sensing or detection of one or more cell properties or cell product properties. In an example, the analysis unit is configured to provide or provides in situ, real time detection or monitoring of properties related to cell culture,

membrane transduction, expansion, gene delivery, cell selection, or any combination thereof. Properties detected or monitored by the analysis unit may include cell viability, density, concentration, cytometry, pH, dissolved oxygen, metabolites, or any combination thereof. In an example, the analysis unit is configured to provide or provides release testing of the cell or cell product. Release testing of the cell or cell product may include imaging cytometry, quantitative polymerase chain reaction (qPCR), digital polymerase chain reaction (dPCR), enzyme-linked immunosorbent assay (ELISA), cell-based assays, cell count, viable cell count, imaging bead count, or any combination thereof.

**[00443]** In an example, an analysis unit may provide for optical density measurements within the 3D matrix. **FIG. 48** shows example optical density measurements as a function of cell density within a 3D matrix and linearity between measurements taken within a 3D culture versus measurements taken in a suspension. As shown in **FIG. 49**, optical density measurements taken from cells within a 3D matrix as compared to measurements taken of a cell suspension.

**[00444]** The optical density unit may be configured to measure absorbance at a wavelength of about 430 nanometers (nm), 560 nm, 600 nm, 850 nm, or any combination thereof. In an example, the optical density unit may measure an absorbance wavelength within about 1%, 2%, 3%, 5%, or 10% of the target wavelength. For example, the optical density unit may be configured to measure a wavelength range of a 600 nm target wavelength from about 594 to 606 nm, 588 to 612 nm, 582 to 618 nm, 570 to 630 nm, or 540 to 660 nm. Alternatively, or in addition to, the optical density unit may be configured to measure multiple different wavelengths for example, wavelengths in the ultraviolet spectrum, visible light spectrum, infrared spectrum, or any combination thereof. The optical density unit may be configured to linearly measure the optical density within the 3D matrix or cell compartment. The optical density unit may be configured to measure scattering, absorbance, backscattering, or any combination thereof. The optical density unit may comprise a photodiode based detector. The photodiode detector may be an integrated photodiode, an integrated 6-band photodiode, an integrated 11-band photodiode, or any combination thereof.

**[00445]** The cell processing system may comprise a unit for measuring optical density of cells within the 3D matrix or cell compartment, as shown in **FIGs. 62A** and **62B**. The optical density unit may comprise one or more of a light source (e.g., light-emitting diode). The light source may comprise a constant current driver (CC) for temperature compensation and emission stability. The optical density unit may comprise one or more detectors. The detector may comprise a photodiode. The detector may comprise a transimpedance amplifier

(TIA) configured to convert impedance signals to voltage signals. The optical density unit may further comprise one or more of anti-aliasing filter, lock-in amplifier to reduce ambient noise, analog to digital converter (A/D), digital to analog converter (D/A), microcontroller unit, or any combination thereof.

**[00446]** In an example, the light path for the optical density may be directed through the 3D matrix comprising cells and media, as shown in **FIG. 62A**. The 3D matrix comprising cells and media (e.g., media comprising phenol) may comprise red spectra overlap. It may be challenging to measure absorbance at discrete wavelengths via the same light path through the 3D matrix and media and the spectra for each may evolve independently. In another example, a light path through the 3D matrix may be separate from the light path through the media, as shown in **FIG. 62B**. The media channel may bypass the gel (e.g., media channel may be measured separately) or the 3D matrix channel may bypass the media (e.g., gel channel may be measured separately). **FIG. 62B** shows an example of the media channel bypassing the 3D matrix channel. Alternatively, the optical density unit may comprise a plurality of sensor spots, as shown in **FIG. 63**. The sensor spots may comprise electronic circuitry, for example TIA, CC, A/D, D/A, MCU, or any combination thereof. The optical density unit comprising a plurality of spot sensors may be further configured to measure backscattering of optical signals.

**[00447]** An analysis unit may be configured to monitor cell expansion in situ. As shown in **FIG. 50**, cell expansion within a 3D matrix may closely track corresponding cell expansion within a suspension culture. Additionally, as shown in **FIG. 51**, cell viability within a 3D matrix may be comparable to cell viability within a suspension culture.

#### *Cell and product analysis*

**[00448]** The bioprocess system (e.g., cell process system) may comprise one or more integrated apparatus (e.g., analysis unit) configured to automatically perform one or more methods of cell analysis, product analysis, or both. An analysis unit may be integrated into the cell processing system via incorporation into the same housing as the cell processing system. Alternatively, or in addition to, an analysis system may be integrated with a cell processing system via aseptic interconnections. The analysis unit may be configured for single mode cell analysis or multi-modal cell or product analysis. An integrated multi-modal cell analysis unit may permit comprehensive characterization of cellular products for various applications, including cell therapy release testing, cell line creation and validation, process development, assessment of other critical product characteristics, or combinations thereof. The analysis unit may integrate multiple analytical techniques, including cytometry (such as

by imaging, flow, or both), polymerase chain reaction (PCR) methodologies such as digital droplet PCR (ddPCR) and quantitative PCR (qPCR), as well as other assays for nucleic acid detection, protein detection, sterility determination, mycoplasma detection, endotoxin detection, viral or transposon copy number determination, determination of cellular identity, phenotype, genotype, cell viability, concentration, other cell-based assays, or combinations thereof. By combining these analytical modalities, the analysis unit may provide a versatile platform for in-depth cellular analysis, quality assessment, or validation across different stages of cell-based product development and manufacturing processes, including automated therapeutic product release testing.

#### *Cytometry*

**[00449]** The analysis unit may comprise one or more integrated apparatus configured to automatically perform one or more cytometry methods. Cytometry, the quantitative analysis of cells and cellular components, may include a diverse array of techniques including, but not limited to, flow cytometry, imaging, optical detection, fluorescence detection, Raman spectroscopy, or combinations thereof. These techniques may provide complementary advantages in terms of resolution, sensitivity, and the types of cellular parameters that can be assessed. In some embodiments, the analysis unit comprises one or more modules for automated determination of cell viability and concentration to provide quantitative measurements of cell viability, proliferation rates, cell concentrations, or combinations thereof. In some embodiments, one or more integrated cytometry modules may additionally be used to determine cellular identity, including immunophenotyping and genetic profiling for regulatory compliance in manufacturing cell lines and therapeutic products.

**[00450]** In an example, a cell processing system may have a sampling loop or sampling port fluidically connected to cell compartment. Alternatively, or in addition to, cell processing system may comprise a sampling or analysis compartment. The sampling compartment may, or may not, be in communication with a cell compartment. In an example, the sample compartment may not be in communication with the cell compartment. In another example, the sample compartment may be in communication with the cell compartment. The sample compartment may provide for real time assays in a surrogate cell-matrix without contaminating the cells within a cell compartment. The sample compartment may provide for a cytometry zone. The sample or analysis compartment may permit viability dyes, fluorescent probes, or other potentially contaminating reagents to be contacted with cells and imaged without contaminating the cells within the cell compartment.

**[00451]** In some embodiments, the analysis unit comprises an optical cytometry apparatus configured to measure cellular properties, analyze cellular properties, or both. Optical cytometry approaches may provide simplicity, speed, and cost-effectiveness for cellular analysis. Optical cytometry apparatus may include, but are not limited to, instrumentation configured for absorbance cytometry, which measures light absorption by cells to assess cell density and viability, phase contrast microscopy, which provides contrast enhancement for the visualization of cellular structures without staining, or fluorescence cytometry, which may be used to detect specific cellular components or biomolecules. In some embodiments, fluorescent dyes, fluorescent proteins, and fluorophore-conjugated antibodies are used for labeling or detecting cells, cellular structures, proteins, or nucleic acids of interest. For example, antibodies conjugated with fluorophores may permit specific binding to target molecules, allowing for the detection and quantification of specific cellular markers. Additionally, fluorescent dyes, such as propidium iodide, Hoechst dyes, and SYTO dyes, may be used to stain nucleic acids, membranes, or organelles for cell viability assays, cell cycle analysis, or subcellular localization studies. Fluorescence cytometry may permit multiplexed analysis of multiple targets within a single cell, providing insights into cellular function, signaling pathways, or biomarker expression patterns.

**[00452]** In some embodiments, the analysis unit comprises an apparatus configured for other cytometry methods, including but not limited to, Raman spectroscopy. Raman spectroscopy may be used as a label-free technique to provide chemical and molecular information about cellular components based on their characteristic vibrational spectra. By irradiating cells with a laser beam and analyzing the scattered light, Raman spectroscopy can identify and quantify biomolecules such as proteins, lipids, nucleic acids, and metabolites within cells. In some embodiments, Raman spectroscopy may be used in a label-based application for detection of Raman dyes or other chemical moieties with specific detectable Raman signals, which may be conjugated to molecular probes such as antibodies. Raman imaging approaches may provide non-destructive, non-invasive, and highly specific analysis of cellular composition and biochemistry. In some embodiments, the analysis unit comprises apparatus configured to perform other label-free cytometry techniques, including impedance cytometry, mass cytometry (CyTOF), and interferometric scattering microscopy (iSCAT), which permit the analysis of cells without exogenous labels or stains. These methods may measure intrinsic cellular properties such as size, shape, density, refractive index, or electrical impedance to characterize cell populations and assess their physiological status.

**[00453]** In some embodiments, the analysis unit is configured to perform cytometry methods in a flow configuration. For example, a cell analysis unit configured for flow cytometry may hydrodynamically focus cells or otherwise convey cells using a fluid flow such that the cells pass through a laser, where they scatter light and emit fluorescence signals. By measuring the intensity of scattered light and fluorescence emitted by fluorophore-labeled cells, flow cytometers can quantify various cellular properties, such as size, granularity, surface marker expression, and intracellular signaling events. In some embodiments, the analysis unit is configured to perform microscopy-based imaging cytometry, which may be performed in a flow or non-flow configuration. Microscopy-based cytometry may acquire high-resolution images of individual cells using a digital camera, permitting detailed morphological analysis, subcellular localization studies, or multiparametric imaging of cellular events at the single-cell level.

**[00454]** In some embodiments, the bioprocessing system (e.g., cell processing system) comprises an integrated, automated cytometry function. In some embodiments, one or more samples are dispensed into one or more microplate wells using automated liquid handlers. In some embodiments, one or more samples are introduced into one or more microfluidic channels or chambers via automated syringe pumps or microfluidic valves. One or more fluorescent dyes, antibodies, or other molecular probes may be added to the samples to label specific cellular markers or structures. Automated dispensers may accurately dispense one or more staining reagents into the samples, providing uniform labeling and reproducibility. Samples may be automatically incubated to allow for binding of fluorescent probes to target molecules. For example, in a microfluidic system, samples may flow through the microfluidic channels or chambers, allowing for passive or active mixing and incubation. Automated integrated fluid handling apparatus may perform one or more cycles of washing. For example, in a microfluidic system, washing may be achieved by flowing a wash buffer through the microfluidic channels or chambers to remove unbound fluorescent probes. Passive or active mixing may be used to enhance washing efficiency. For detection, microplates may be configured for detection or automatically transferred to an automated imaging system equipped with fluorescent microscopy or other signal detectors. The analysis unit may automatically capture images or fluorescence signals from each well, allowing for the visualization and analysis of stained cells or particles. In another example, imaging may be performed directly in the chambers, flow channels, or both using integrated microscopy or imaging components configured to interface with or that interface with a microfluidic consumable. In a microfluidic flow configuration, fluorescence signals from stained cells or



particles may be captured as they flow through the microfluidic channels or chambers, permitting high-throughput cell analysis.

**[00455]** In some embodiments, an automated cytometry function is performed. A cell source contained within the system, such as a suspension of cells, may be sampled by collecting one or more volumes of the cell suspension and automatically transferring the material into the analysis unit. The amount of cell suspension being sampled for assay may be determined by a volumetric amount (e.g., 10 microliters) or a target number of cells (e.g., approximately 10,000 cells). The cell suspension volume may be less than or equal to about 10 milliliters (mL), 8 mL, 6 mL, 5 mL, 4 mL, 3 mL, 2 mL, 1000 microliters ( $\mu$ L), 750  $\mu$ L, 500  $\mu$ L, 400  $\mu$ L, 300  $\mu$ L, 200  $\mu$ L, 100  $\mu$ L, 80  $\mu$ L, 60  $\mu$ L, 50  $\mu$ L, 40  $\mu$ L, 30  $\mu$ L, 20  $\mu$ L, 10  $\mu$ L, 5  $\mu$ L, or less. The target number of cells may be less than or equal to about 10 million cells, 8 million cells, 6 million cells, 5 million cells, 4 million cells, 3 million cells, 2 million cells, 1 million cells, 500,000 cells, 100,000 cells, 75,000 cells, 50,000 cells, 40,000 cells, 30,000 cells, 20,000 cells, 10,000 cells, 5,000 cells, 2,500 cells, 1000 cells, or less. The analysis unit may be configured to perform or may perform additional analysis of control samples, such as compensation beads for analysis unit configuration and data analysis. For example, the analysis unit may be configured to run control compensation beads in parallel to the sample(s) or for instrument configuration and validation separate from sample analysis. Compensation beads may provide a standardized reference for quantifying and compensating for spectral overlap in multi-color flow cytometry experiments, permitting accurate and reproducible compensation calculations, leading to improved data quality and interpretation. Other control samples, depending on the analysis mode being used, may be provided and automatically processed by the system for the purpose of system validation, system configuration, assay validation, data analysis, or combinations thereof.

**[00456]** As shown in **FIG. 52**, flow cytometry measurements for cultures expanded in 3D matrixes show similar canonical scatter profiles and CD4/CD8 T cell identity as cells expanded in suspension cultures. In this example, flow cytometry shows 77.1% lymphocytes in the suspension culture as compared to 73.6% in the comparable 3D matrix culture. Additionally, the lymphocyte composition for the suspension culture shows 99.9% T-cells in the suspension culture compared to 98.8% T-cells in the 3D matrix.

**[00457]** Cytometry data may be analyzed using various metrics and algorithms to extract quantitative information about cellular properties. Cellular properties may include measures of cell size, granularity (forward scatter), complexity (side scatter), fluorescence intensity, and the expression levels of specific markers or biomolecules. Data analysis may include

computational techniques for signal processing, such as background correction, compensation, feature extraction, quantitative analysis, or combinations thereof. Data analysis may involve gating strategies to identify cell populations of interest, normalization techniques to correct for instrument variability, or statistical methods to compare experimental conditions or treatment groups. In some embodiments, cytometry data may be analyzed, either automatically or with the aid of one or more human users, on the bioprocessing system (e.g., cell processing system). In some embodiments, cytometry data are communicated to a separate analysis unit for analysis, either automatically or with the aid of one or more human users.

*Nucleic acid and protein analysis*

**[00458]** The analysis system or unit may comprise one or more integrated apparatus configured to automatically perform one or more biomolecular assays, such as nucleic acid assays, protein assays, or both. Biomolecular assays may encompass a diverse array of techniques including, but not limited to, polymerase chain reaction (PCR) -based methods, loop mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assay (ELISA), and ELISA-like methods. In some embodiments, nucleic acid, protein analysis, or detection function is automated. A cell source contained within the system, such as a suspension of cells, may be sampled by collecting one or more volumes of the cell suspension and automatically transferring the material into the analysis unit. The amount of cell suspension being sampled for assay may be determined by a volumetric amount (e.g., 10 microliters) or a target number of cells (e.g., approximately 10,000 cells). The cell suspension volume may be less than or equal to about 10 milliliters (mL), 8 mL, 6 mL, 5 mL, 4 mL, 3 mL, 2 mL, 1000 microliters ( $\mu$ L), 750  $\mu$ L, 500  $\mu$ L, 400  $\mu$ L, 300  $\mu$ L, 200  $\mu$ L, 100  $\mu$ L, 80  $\mu$ L, 60  $\mu$ L, 50  $\mu$ L, 40  $\mu$ L, 30  $\mu$ L, 20  $\mu$ L, 10  $\mu$ L, 5  $\mu$ L, or less. The target number of cells may be less than or equal to about 10 million cells, 8 million cells, 6 million cells, 5 million cells, 4 million cells, 3 million cells, 2 million cells, 1 million cells, 500,000 cells, 100,000 cells, 75,000 cells, 50,000 cells, 40,000 cells, 30,000 cells, 20,000 cells, 10,000 cells, 5,000 cells, 2,500 cells, 1000 cells, or less. Cell lysis may be automatically performed to disrupt cellular membranes to release nucleic acids, proteins, or both for subsequent amplification or detection. Automated cell lysis methods, such as heat-based lysis, chemical lysis, or mechanical disruption, may be integrated into the module to efficiently lyse cells within the process or therapeutic product. The cell lysis method may be selected for sample type, cell density, or downstream applications. The nucleic acid module, protein analysis module, or both may further comprise a biomolecular extraction or purification function. Alternatively,

the nucleic acid module or protein analysis unit may use or comprise a biomolecular extraction or purification function. In an example, following cell lysis, nucleic acids (DNA or RNA), protein, or both may be extracted and purified from the lysate to remove contaminants and inhibitors that may interfere with downstream analysis. This process may comprise methods such as column-based purification, magnetic bead-based purification, organic extraction, or combinations thereof. Alternatively, these processes may not use or comprise column-based purification, magnetic bead-based purification, or organic extraction methods.

**[00459]** The nucleic acid or protein analysis module may automatically load samples with reaction master mixtures containing primers, probes, or both, and the reaction mixtures may be aliquoted into reaction vessels or microfluidic devices for amplification and detection. In some embodiments, one or more samples are dispensed into one or more microplate wells using automated liquid handlers. In an embodiment, one or more samples are introduced into one or more microfluidic channels or chambers via automated syringe pumps or microfluidic valves. One or more reaction buffers, reaction master mixes, fluorescent dyes, antibodies, or other molecular probes may be added to the samples to label, amplify, or detect specific biomolecular targets. Automated dispensers may accurately dispense one or more staining reagents into the samples to provide uniform labeling and reproducibility. Samples may be automatically incubated. The module(s) may further comprise a thermal cycling functionality. In some embodiments, a thermal cycling element is provided in the analysis unit or system and configured to thermally interface with an analysis consumable for the purpose of permitting analysis methods utilizing thermal cycling.

**[00460]** In some embodiments, an automated nucleic acid analysis module may be equipped with high-throughput PCR instrumentation capable of performing one or more single-plex or multiplexed PCR reactions, assay endpoint detection or real-time monitoring of amplification kinetics, and automated data analysis. In some embodiments, an analysis module integrates PCR methodologies, including but not limited to digital droplet PCR (ddPCR) and quantitative PCR (qPCR), for precise quantification of nucleic acids within cellular samples.

**[00461]** In some embodiments, an automated nucleic acid analysis method other than PCR (including droplet-based and quantitative methods) is performed. Other nucleic acid assays may include, but are not limited to, CRISPR-based sequence detection, Loop-Mediated Isothermal Amplification (LAMP), and isothermal nucleic acid assays. For example, CRISPR-based detection methods leverage the programmable nature of CRISPR-Cas systems

for nucleic acid detection. These methods, such as CRISPR-Cas-based nucleic acid detection (CRISPR-CasND) and specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), may utilize Cas enzymes, such as Cas12 and Cas13, to target and cleave specific nucleic acid sequences. Upon target recognition, the Cas enzymes may undergo collateral cleavage of nearby reporter molecules, generating detectable signals such as fluorescence or colorimetric changes. CRISPR-based detection may offer high sensitivity, specificity, and multiplexing capabilities for nucleic acid detection in various sample types, including clinical specimens, environmental samples, and cell-based products. In a separate example, a Loop-mediated isothermal amplification (LAMP) method may be used, which operates at a constant temperature, for example 60-65°C, without thermal cycling equipment. LAMP may use a DNA polymerase with strand displacement activity and a set of four to six primers targeting multiple regions of the target sequence. The amplification reaction may produce large amounts of DNA within a short time frame through a series of strand displacement and loop formation operations, resulting in a characteristic ladder-like amplification product. LAMP may provide rapid, sensitive, and specific amplification of target nucleic acids and may be suitable for point-of-care testing. Other isothermal nucleic acid assays may be used including, but are not limited to, Recombinase Polymerase Amplification (RPA), Nucleic Acid Sequence-Based Amplification (NASBA), Helicase-Dependent Amplification (HDA), Rolling Circle Amplification (RCA), Hyperbranched Rolling Circle Amplification (HRCA), or any combination thereof.

**[00462]** In some embodiments, the cell processing system is configured to automatically perform nucleic acid analysis to permit accurate determination of viral copy numbers, gene expression levels, genetic modifications, other product attributes, or combinations thereof to provide quality control and validation of cell-based products. The automated nucleic acid analysis module may employ various assays to assess various parameters in cell therapy products, including, for example, viral contaminant detection (VCD), assays targeting viral genomes, such as adenovirus, retroviruses, or lentiviruses, that may be used to screen for potential viral contaminants in cell therapy products. For example, a principal concern regarding the safety of cell-based therapies manufactured utilizing lentiviral vectors may be replication competent lentivirus (RCL). qPCR-based assays may provide a rapid alternative to cell-based assays for assessment of RCL in products intended for fresh infusion. For example, RCL may be measured using a qPCR assay based on detection of envelope gene sequences (vesicular stomatitis virus G glycoprotein [VSV-G]), in accordance with Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.

These assays may be highly sensitive and specific, reproducible, and have low false-positive rate to permit detection of low levels of viral nucleic acids that may compromise the safety and efficacy of the therapeutic product. The automated nucleic acid analysis module may use genetic modification detection in which assays designed to amplify and detect specific transgenes or gene editing events may be used to confirm the presence, population-level editing frequency, and per-cell copy number of genetic modifications in cell therapy products. These assays may assess the integrity and stability of engineered cells, ensuring that the intended genetic modifications are present, exhibit vector copy number (VCN) within the targeted range, and that modified cells are present at the targeted frequency or abundance. The automated nucleic acid analysis module may provide gene expression analysis in which quantitative assays targeting mRNA transcripts of specific genes may be employed to quantify gene expression levels in cell therapy products. These assays may provide insights into the functionality and potency of therapeutic cells, permitting the assessment of targeted therapeutic gene expression and the evaluation of cell phenotype and differentiation status. Nucleic acid detection assays may also be used for determination of protein expression levels, such as by detection of nucleic acid tags present on antibodies used to label one or more target moieties. The automated nucleic acid analysis module may perform assays for assessing sterility, including mycoplasma contamination, in cellular samples. These tests may ensure the safety and purity of cell-based products, meeting regulatory requirements for clinical applications and research use.

**[00463]** In these examples, reaction master mixtures containing buffers, enzymes such as DNA polymerase, dNTPs (deoxynucleotide triphosphates), and other components may be prepared and used by the system according to specific assay. These master mixtures may also include primers, probes, fluorescent dyes, or any combination thereof for target amplification and detection. The nucleic acid analysis module may utilize pre-designed or custom-designed PCR primer sets targeting specific genomic regions or gene sequences relevant to the therapeutic product.

**[00464]** Controls may be used to confirm the accuracy and reliability of analysis for each assay. These controls may include: (a.) Positive Controls: Synthetic DNA or RNA templates containing the target sequences of interest may be used as positive controls to validate the performance of the assay. Positive controls may permit verification of sensitivity, specificity, and efficiency of the amplification reaction and ensure that the assay is capable of detecting the target sequences within the expected dynamic range. (b.) Negative Controls: Non-template controls, containing no nucleic acid template, may be included to monitor for

potential contamination and false-positive results. Negative controls may assess the specificity of the assay and permit identification of background amplification or contamination issues that may affect the accuracy of the results. (c.) Standard Curve Controls: Two or more control samples (for example, at least five control data points over several orders of magnitude) with different, known quantities of template target sequences of interest. Standard curve controls, also known as calibration controls, may be used to determine either the absolute quantity (AQ) or the relative quantity (RQ) a target sequence, characterize the linearity and other performance characteristics of an assay, or both.

**[00465]** In some embodiments, the cell processing system is configured to automatically perform one or more integrated protein analysis functions, such as, for example, an automated protein analysis function capable of performing enzyme-linked immunosorbent assay (ELISA) or other protein assays. The protein analysis functions may automatically aliquot samples into microplate wells or other assay formats, including microfluidic formats, providing uniform distribution and consistent assay performance. Automated systems may accurately provide or dispense reagents, including antibodies, detection reagents, blocking buffers, or any combination thereof into the assay wells, chambers, or other sample format. Capture antibodies, ligands, or antigens specific to the protein of interest may be provided via a solid or semi-solid substrate, including but not limited to one or more flow cells, plates, wells, chambers, or beads for the purpose of immobilizing target proteins and facilitating subsequent detection. The system may incubate samples and controls with the assay reagents, allowing target proteins to bind to the capture antibodies or other binding moiety. In some embodiments, automated fluidic system washers may rinse the substrate to remove unbound proteins and contaminants, ensuring specificity and reducing background noise in the assay. The system may further automatically incubate the sample-bound substrate with additional antibodies or secondary antibodies conjugated to enzymes or fluorophores, which may bind to the target proteins or primary antibodies bound to target proteins as captured by the capture antibodies, thereby permitting detection. In some embodiments, the ELISA method may be a form of direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, or other proteomics applications, such as Luminex. In some embodiments, enzymatic detection systems such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) catalyze the conversion of chromogenic substrates into colored products. In some embodiments, fluorescent detection systems utilize fluorophores to generate fluorescent signals, with such fluorophores being provided by the one or more assay antibodies or upon enzymatic reaction. The system may further measure the generated signals, such as through measuring

absorbance or fluorescence, using integrated detectors or optical sensors. These measurements may correlate with the amount of target protein present in the samples. The system may automatically generate standard curves using given concentrations of protein standards or controls, or otherwise use reference values for the purpose of quantitation. In some embodiments, the system utilizes standard curves to allow for the quantification of target proteins in the samples based on their absorbance or fluorescence readings. In such a configuration, the system may automatically analyze the acquired data to calculate protein concentrations in the samples.

**[00466]** The integrated module for nucleic acid analysis, protein analysis, or both may perform a series of processes including cell lysis, biomolecular extraction, biomolecular purification, preparation of reaction master mixtures, addition of primers and probes, reaction incubation, amplification, real-time monitoring, analysis of data, or any combination thereof.

#### *Cell-based assays*

**[00467]** In some embodiments, the cell processing system is configured to automatically perform one or more cell-based assays. Leveraging automation and integrated workflows, this system may streamline assay execution, provide reproducibility, and accelerate manufacturing and validation timelines. The system may include automated sample handling, precise reagent dispensing, multiplexed assay capabilities, real-time data analysis, or any combination thereof. Sample aliquoting and distribution into microplates or microfluidic devices may be performed with high throughput, permitting parallel processing of multiple samples, multiple assays, or both. Cell-based assays may include, but are not limited to, proliferation assays, cytokine release assays, cytotoxicity assays, phenotypic characterization assays, or any combination thereof. Cell-based assay reagents may include, but are not limited to, antibodies (including tetramers and other antibody configurations), cytokines, growth factors, inhibitors, cells, beads, proteins, small molecules, or any combination thereof. In some embodiments, the cell-based assay unit utilizes integrated detection systems, including cytometry, imaging, ELISA, real-time cell analysis (RTCA), or any combination thereof to permit flexible assay design and data acquisition. Performance of cell-based assays may utilize the same or separate analysis units or systems from those used for cytometry, nucleic acid, or protein analysis. In an example, the cell-based assay module performs a cytokine release assay on a therapeutic T cell product, such as by automatically incubating cells with antigen multimers, and detecting and quantifying secreted cytokines by automatically performing an ELISA function.

#### *Endotoxin Assay*

**[00468]** In some embodiments, the cell processing system comprises a module or unit for automatically performing an endotoxin test. In some embodiments, an automated endotoxin testing system is specifically configured to automate the Limulus Amebocyte Lysate (LAL) assay for endotoxin detection in pharmaceutical products. The cell processing system may integrate automation technologies with standardized LAL assay protocols to provide a reliable, efficient, and compliant solution for endotoxin testing. Automated fluidic systems may precisely handle samples, controls, and reagents, for example, to accurately dispense LAL reagents, endotoxin standard, and chromogenic substrate, providing consistent and reliable assay setup. Reagent dispensing parameters may be customizable to accommodate various sample volumes, assay formats, and endotoxin detection methods (e.g., chromogenic, turbidimetric). Signal detectors may provide data for kinetic analysis software, which monitors the reaction kinetics of the LAL assay in real time to allow for continuous monitoring of endotoxin levels and calculation of reaction rates. In some embodiments, endpoint and kinetic reading modes are available to accommodate different assay processes and endotoxin detection limits to provide flexibility in assay design and data analysis. By integrating an automated LAL assay function, this system may provide enhanced efficiency, reduced variability, and timely release of endotoxin-free products.

*Software, Regulatory compliance, integrated release testing*

**[00469]** A cell processing system may comply with regulatory guidelines such as those provided by regulatory bodies like the Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The system may be configured with software and hardware components which can perform, for example, upon an initial configuration, or periodic, automated validation procedures to ensure accuracy, reliability, and compliance with regulatory requirements. The system may generate comprehensive documentation, including electronic records and audit trails, to track testing processes and provide traceability and accountability. Automated data acquisition and documentation may provide compliance with regulatory standards, including US Pharmacopeia (USP) and European Pharmacopoeia (EP) guidelines. Audit trails, electronic signatures, access controls, data encryption, and secure data storage functionalities may be integrated into the system to maintain data integrity and traceability throughout the testing process.

**[00470]** In some embodiments, the system utilizes standardized protocols for product release testing, including assays for potency, purity, identity, safety, or combinations thereof. Automated systems may accurately handle samples, reagents, and assay consumables to



minimize human error and increase or provide consistency. Automated software may control the execution of assays, including sample preparation, assay setup, incubation, and data acquisition, streamlining the testing process and reducing turnaround time. In some embodiments, the system may be configured to perform or may perform real-time data analysis during testing, allowing for immediate decision-making and release of test results. In some embodiments, the system may be in data communication with a separate computer system, which may provide analysis, decision-making, record keeping, release of test results, or combinations thereof. In some embodiments, the system automatically determines, based on the results of integrated product testing, whether or not a product is suitable for release, stores the results, transmits results to a data storage system, displays results on a user interface (either on the system directly, or remotely, such as by communicating or publishing results to a web-based system), notifies a human operator or physician, or any combination thereof.

**[00471]** In some embodiments, system software features an intuitive user interface that guides users through setup, execution, and data analysis, minimizing extensive training. For example, the system may comprise a protocol templating system which includes predefined protocol templates for common workflows within the software, allowing users to quickly set up and execute assays with minimal effort. In an example, an integrated analysis unit may provide for release testing of a cell product, as shown in **FIG. 53**. For example, users may customize assay parameters, including sample types, assay conditions, and data analysis settings, to meet specific application requirements. During assay execution, real-time assay progress and results may be displayed on a screen, allowing users to monitor the testing process and identify any issues or anomalies. Assay results, including graphical representations of data such as dose-response curves, histograms, and scatter plots, may be displayed on a screen for easy interpretation and analysis. For example, assay results may be electronically communicated to a separate computer system. At the end of each procedure, a summary of results may be displayed on a screen, highlighting key findings and any deviations from acceptance criteria. The software may further generate comprehensive reports summarizing assay results, including assay parameters, sample information, test outcomes, or combinations thereof, facilitating regulatory submissions and documentation.

#### **Systems and methods for generating cell products**

**[00472]** Systems and methods described herein may be useful for generating cell products. Cell products may comprise whole cell usable as a cell therapy or an expression product produced from a cell. The systems and methods may produce a cell product, or a product

usable to generate a cell product, within less than or equal to about 10 days, 8 days, 6 days, 5 days, 4 days, 3 days, 48 hours (hrs), 24 hrs, 12 hrs, 6 hrs, or less. In an example, the systems and methods may produce a cell product, or a product usable to generate a cell product in less than 48 hrs. Generating a cell product may comprise cell expansion. Cell expansion may comprise increasing a number of cells in a cell population by at least about 25%, 50%, 75%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, or more.

**[00473]** The cell processing systems and methods described herein may be useful for cell and gene therapy manufacturing. As shown in **FIG. 54**, the cell processing system may be useful for generating viral vectors. The viral vectors may be generated in cells what are expanded within one or more cell processing units described herein. The cells may be transfected, harvested, and formulated. Target cells may be isolated an enriched. The isolated and enriched cells may be activated, modified, or both by the harvested and formulated viral vectors. The activated cells may be expanded, harvested, and formulated into a therapy product. The process may be fully automated such that the system may generate the viral vectors and use the viral vectors to modify the target cells with little or no human intervention. In an example, modification and activation may be performed concurrently.

#### *Clinical cell manufacturing*

**[00474]** The cell processing systems and methods described herein may permit cells to be subjected to multiple different operations to generate a cell product. For an example CAR-T product, as shown in **FIG. 55**, a fresh or frozen cell source may be provided to the cell processing system. The cells may be washed, concentrated, isolated, or any combination thereof upstream of a solid-state bioreactor. Within the solid-state bioreactor (e.g., within the 3D matrix), the cells may be subjected to perfusion culture, membrane transduction, in situ selection, viral transduction, or any combination thereof. Downstream of the solid-state bioreactor, the cells may be washed, concentrated, isolated, or any combination thereof and fresh or frozen cells may be output. In another example, and for a viral vector product, the input may be a fresh or frozen cell source. Upstream of the solid-state bioreactor, the cells may be washed, concentrated, or both. Within the solid-state bioreactor the cells may be subjected to perfusion culture, transduction, transfection, or any combination thereof. Downstream of the solid-state bioreactor the cells and/or permeate may be subjected to viral vector purification and fresh or frozen product may be output.

[00475] In another example, the systems and methods described here may provide for CAR-T manufacturing as shown in **FIG. 56**. A cell processing system may be loaded with cells from a Leukopak or other cell source. Targeted cells may be enriched, sorted, or both. The targeted cells may be loaded into a 3D matrix, activated, subjected to gene transfer, cultured, or any combination thereof. The cells may be released from the 3D matrix, formulated, cryopreserved, or any combination thereof.

[00476] In some embodiments, the 3D substrate(s), solid-state bioreactor(s), cell processing system(s), or any combination thereof described herein are configured for clinical cell manufacturing, including but not limited to the production of chimeric antigen-receptor T cells (CAR-T), hematopoietic stem and progenitor Cells (HSPCs), mesenchymal stem cells (MSCs), somatic cell types, cells derived from stem cells and induced pluripotent stem cells (iPSCs), or any combinations thereof.

[00477] Another CAR-T cell manufacturing workflow is shown in **FIG. 57**. A Leukopak, reagents, consumables, cartridges, or any combination thereof may be loaded into a cell processing system. Cells from the Leukopak may be isolated and enriched to remove red blood cells and platelets. The cells may be added to a 3D matrix prior to or after enrichment removal of red blood cells and platelets. The cells may be loaded into a 3D matrix with activation reagents (e.g., antibodies, cytokines, etc.), lentiviruses for gene transfer, selection reagents (e.g., antibody-drug conjugates), or any combinations thereof. The cells may be subjected to perfusion expansion culture to produce activated and transduced cells. Cells that are not activated and transduced may be eliminated. The generated cells may be released from the 3D matrix, formulated, and cryopreserved.

[00478] In some embodiments, the 3D substrate(s), solid-state bioreactor(s), cell processing system(s), or any combination thereof described herein are configured for versatile cell manufacturing workflows, including but not limited to the production of primary cells, secondary cells, cell lines, stem cells, or any combination thereof. For example, the systems and methods described herein may be configured for the establishment, maintenance, or both of cell lines for a variety of purposes. In another example, the systems and methods described herein may be configured for the production of cells as a source of biomass for tissue and organ printing applications.

#### *Cell byproduct manufacturing*

[00479] In some embodiments, the 3D substrate(s), solid-state bioreactor(s), cell processing system(s), or any combination thereof described herein are configured for bioproduction, including but not limited to, biopharmaceutical manufacturing processes for

the production of monoclonal antibodies, recombinant proteins, viral vectors for gene therapies, or any combination thereof. For example, the systems and methods described herein may be configured for bioproduction workflows utilizing non-mammalian expression systems (e.g., bacterial, yeast, plant, insect, or combination thereof) or mammalian expression systems (e.g., mammalian Chinese hamster ovary cells, murine myeloma (NS0, Sp2/0) cell lines, human bioproduction cell lines, etc.). In some embodiments, the systems and methods described herein are configured for bioproduction systems in which secreted products are manufactured (e.g., not requiring cell lysis for product extraction). For example, a continuous bioproduction system as described herein may be configured to continuously produce a secreted product from a 3D culture system.

### **Computer systems**

**[00480]** The present disclosure provides computer systems that are programmed to implement methods of the disclosure. **FIG. 58** shows a computer system **5801** that is programmed or otherwise configured to implement methods of processing cells, analyzing cell products, and release testing. The computer system **5801** can regulate various aspects of cell processing systems of the present disclosure, such as, for example, bioreactors, preservation units, thermal control systems, analysis units, fluidic control systems, sensors, feedback systems, or any combination thereof. The computer system **5801** can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device. Alternatively, the computer system may be an integrated component of a cell processing system. The computer system may or may not be disposed in the same housing as one or more components of a cell processing system.

**[00481]** The computer system **5801** includes a central processing unit (CPU, also “processor” and “computer processor” herein) **5805**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **5801** also includes memory or memory location **5810** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **5815** (e.g., hard disk), communication interface **5820** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **5825**, such as cache, other memory, data storage or electronic display adapters. The memory **5810**, storage unit **5815**, interface **5820** and peripheral devices **5825** are in communication with the CPU **5805** through a communication bus (solid lines), such as a motherboard. The storage unit **5815** can be a data storage unit (or data repository) for storing data. The computer system **5801** can be operatively coupled to a computer network

(“network”) **5830** with the aid of the communication interface **5820**. The network **5830** can be the Internet, an internet or extranet, or an intranet or extranet that is in communication with the Internet. The network **5830** in some cases is a telecommunication or data network. The network **5830** can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network **5830**, in some cases with the aid of the computer system **5801**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **5801** to behave as a client or a server.

[00482] The CPU **5805** can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory **5810**. The instructions can be directed to the CPU **5805**, which can subsequently program or otherwise configure the CPU **5805** to implement methods of the present disclosure. Examples of operations performed by the CPU **5805** can include fetch, decode, execute, and writeback.

[00483] The CPU **5805** can be part of a circuit, such as an integrated circuit. One or more other components of the system **5801** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00484] The storage unit **5815** can store files, such as drivers, libraries and saved programs. The storage unit **5815** can store user data, e.g., user preferences and user programs. The computer system **5801** in some cases can include one or more additional data storage units that are external to the computer system **5801**, such as located on a remote server that is in communication with the computer system **5801** through an intranet or the Internet.

[00485] The computer system **5801** can communicate with one or more remote computer systems through the network **5830**. For instance, the computer system **5801** can communicate with a remote computer system of a user (e.g., laboratory technician, researcher, doctor, or any combination thereof). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system **5801** via the network **5830**.

[00486] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system **5801**, such as, for example, on the memory **5810** or electronic storage unit **5815**. The machine executable or machine readable code can be provided in the form of software.

During use, the code can be executed by the processor **5805**. In some cases, the code can be retrieved from the storage unit **5815** and stored on the memory **5810** for ready access by the processor **5805**. In some situations, the electronic storage unit **5815** can be precluded, and machine-executable instructions are stored on memory **5810**.

**[00487]** The code can be pre-compiled and configured for use with a machine having a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

**[00488]** Aspects of the systems and methods provided herein, such as the computer system **5801**, can be embodied in programming. Various aspects of the technology may be thought of as “products” or “articles of manufacture” typically in the form of machine (or processor) executable code or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. “Storage” type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

**[00489]** Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media

include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

**[00490]** The computer system **5801** can include or be in communication with an electronic display **5835** that comprises a user interface (UI) **5840** for providing, for example, cell processing system controls, system status, analytical results, or any combination thereof. Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

**[00491]** Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit **5805**. The algorithm can, for example, provide automated cell processing for generation of a cell product.

### EXAMPLES

#### **Example 1: Automated cell input processing**

**[00492]** In an example, a container of primary patient whole blood, such as a Vacutainer containing 10-200 mL of whole blood, or bag containing 50-500 mL of whole blood, is connected to the cell processing system via an aseptic fluid connector, such as a single-use sterile interconnect. The sample may be automatically transferred from the input container to the consumable via the fluid connection by a pump. In another example, a container of primary patient apheresis or leukopheresis product, such as a bag containing 20-500 mL of said product, may be connected to the cell processing system via an aseptic fluid connector, such as a single-use sterile interconnect. The sample may be automatically transferred from the input container to the consumable via the fluid connection by a pump.

[00493] The consumable may be configured with a suitable wash buffer, such as Dulbecco's phosphate-buffered saline (PBS), optionally supplemented with Fetal bovine serum (FBS), Human serum (huS), or human platelet lysate (hPL), for example, at a final 2% concentration, and ethylenediaminetetraacetic acid (EDTA), for example, at 1 millimolar (mM) concentration for processing leukapheresis product, or at a higher concentration, such as greater than 50 mM concentration for processing whole blood. The consumable may be optionally configured with a separate source of EDTA at higher concentration, such as between 50 and 500 mM, which may be used to adjust the final concentration of EDTA to at least 6 mM final concentration for processing whole blood.

[00494] The consumable may be optionally configured with one or more additional bead isolation reagents for positive cell selection, negative cell selection, or both. Selection bead may comprise paramagnetic beads for magnetic cell sorting or beads larger than 5 micrometers ( $\mu\text{M}$ ) in diameter for size-based cell sorting. The beads may be modified with a moiety that binds an extracellular factor, either directly (e.g., an anti-CD3 antibody which binds to the extracellular domain of the CD3 protein) or indirectly (e.g., biotin-modified beads, which bind a streptavidin-modified antibody bound to the extracellular domain of the CD3 protein).

[00495] Within the consumable, the sample may be automatically transferred via a pump to a processing vessel, which may be a rigid or flexible vessel such as a chamber or bag. The vessel may be configured for mixing, for example, using an agitator or impeller, or by the process of transferring fluid to and from the vessel by a pump. In some examples, the vessel contains a fixed agitator or impeller which is actuated by a rotor or magnet. A volume of wash buffer may be automatically transferred from a wash buffer reagent vessel within the consumable to the processing vessel to dilute the sample. The volume of wash buffer may be a fixed amount, such as 10 mL, or determined as a function of the sample volume, such as 0.5-2X the sample volume. The mixing vessel may be configured to automatically mix the sample with the buffer to generate a homogenous, diluted sample mixture. Optionally, before or after the addition of wash buffer, the selection reagent(s) (e.g., comprising beads and/or one or more antibodies) may be automatically transferred to the vessel from the selection reagent vessel within the consumable. The automatic fluid transfer may be accomplished via the action of a pump. The sample may be mixed automatically and incubated with the selection reagent(s). The one or more antibodies may be added prior to the beads, if provided separately, or at the same time as the beads if provided separately or together.



**[00496]** The sample may be transferred to the cell processing module, either in bulk, in batches over time, or in a continuous fluid flow over time. In an example, the sample is transferred in bulk or in batches over time to an automated magnetic cell isolation module, which uses a magnetic field to separate paramagnetic beads from the mixture. The unbound fraction of the mixture may be removed from the automated magnetic cell isolation module. Wash buffer may be automatically transferred from the wash buffer reagent vessel to the automated magnetic cell isolation module and the magnetic field may be removed, forming a suspension mixture of paramagnetic particles with cells bound thereto. In positive isolation examples, the fraction of cells bound to the paramagnetic beads are subsequently transferred to the mixing vessel of the consumable for further processing. In negative isolation examples, the unbound fraction of the mixture is subsequently transferred to the mixing vessel of the consumable for further processing. In some examples, the sample is transferred in a continuous fluid flow through a continuous flow magnetic cell isolation module comprising two or more outlet streams. A magnetic field may be applied to the fluid flow, thereby separating the paramagnetic particles and cells bound thereto into one or more fluid streams, and the unbound fraction of the mixture into one or more fluid streams, at least one of which is a separate fluid stream from the stream containing the paramagnetic particles.

**[00497]** In some examples, the sample is transferred via flowing the sample through a filtration module, such as a tangential flow filtration (TFF) module. The TFF module may be configured to separate particles in fluid flows by size. The module may be configured to separate nucleated cells such as lymphocytes from anucleated cells such as red blood cells and platelets. The module may be configured to separate cells bound to beads from cells not bound to beads for positive or negative cell separation.

**[00498]** For bead or particle-based separation methods (e.g., magnetic- or size-based separation methods), the sample may be further processed to remove the separation particles or beads. For example, cell separation particles may bind target cells via a cleavable linker, which may be subsequently cleaved. The resulting isolated cells and beads or other separation particles may be subsequently separated from each other by subsequent magnetic isolation or size-based separation methods as described herein.

**[00499]** The concentration of the sample may be adjusted. By measuring the cell or particle density of the processed sample prior to, during, after concentration adjustment, or any combination thereof, for example, using optical methods such as cell counting and inline sensing approaches, the cell processing system may automatically dilute or concentrate the cells to a target concentration, such as between 1 and 10 million cells per mL. Concentration

functions may be performed by or in a TFF filtration module. The input sample may be mixed with a gel precursor solution and loaded into the 3D bioreactor. The gel precursors may be polymerized, and the cells may be cultured or otherwise processed. **FIG. 75** shows example cell expansion for donor cells in 3D cell culture and suspension. Cells cultured in a 3D matrix show greater expansion and viability than comparable suspension cultures. As shown in **FIG. 76**, cells transduced and expanded in 3D culture show high viability and expansion rates. An example image of an example 3D matrix prior to cell harvest is shown in **FIG. 77**. **FIG. 78** shows an example 3D bioreactor during cell expansion. **FIG. 79** shows example sensors used during 3D cell culture. **FIG. 80** shows example phenotype quality of cells subjected to 3D culture versus suspension culture.

**Example 2: Use of functional 3D matrices**

**[00500]** In an example, the 3D matrix may be functional gel usable for T cell activation and CAR-T manufacturing. Functional 3D matrices may provide activation, differentiation, and proliferation signals to T cells in a more physiological 3D structural context compared to using soluble antibody factors, functionalized 2D rigid substrates (e.g. coated plastic well), or beads. Indirect synthesis method may use asymmetrical conjugations reagents to functionalize the collagen in and antibodies separately in optimized conditions, and gentle bioconjugation may be used to preserve antibody function.

**[00501]** The 3D matrices may be generated using a biotin-modified collagen solution. The collagen solution (10 mg/mL, Type I Atelocollagen) may be adjusted to neutral pH and diluted to 6.4 mg/mL and mixed with biotinylation reagent used to attach biotin to primary amines (N-hydroxysuccinimide (NHS)-biotin reagent). The collagen solution may be buffered first with PBS at a neutral pH. NHS-biotin dilution may be prepared fresh from 100 mg/ml frozen stock in dimethyl sulfoxide (DMSO), and added 1:5000 volume to volume (v/v) to the PBS-buffered collagen solution (final concentration 0.2 mg/ml). The NHS-amine reaction may occur concomitant with polymerization at 37 °C for 2 hours (hrs).

**[00502]** The polymerized biotin-labeled collagen may be re-solubilized with acetic acid chilled to 4 °C. One mL of 500 mM acetic acid dilution may be added to each 1 mL of polymerized biotin-labeled collagen gel. After pipette mixing and confirming the collagen is fully dissolved, the labeled collagen solution may be transferred into a dialysis cassette (20,000 MW cutoff). Dialysis may be performed in 0.1x PBS overnight at 4 °C, followed by exchange of the 0.1x PBS in the morning and additional 4 or more hours of incubation. The dialyzed and labeled collagen solution may be collected from the dialysis chamber, and

stored at 4 °C, and it is ready for use, such as by dilution 1:20 in normal collagen to form a 5% functionalized gel.

**[00503]** The 3D matrix may be functionalized with one or more antibodies via a streptavidin -biotin interaction. Antibody conjugation to streptavidin may be performed using proprietary streptavidin conjugation kit (Abcam, Streptavidin Conjugation Kit – Lightning-Link™), following the manufacturer's protocol. The conjugated antibody may be mixed with the modified collagen in an buffered solution. The antibody may strongly bind the collagen via the biotin-streptavidin linkage. The biotinylated collagen solution may be mixed in given ratio (e.g., 5%) with buffered unlabeled collagen (e.g., Type I Collagen Solution, 3.2 mg/ml). Streptavidin-tagged antibodies may be added into the collagen mix at user-specified ratios (e.g., 0.5-10 mg/mL). The prepared mix may be left to polymerize for 2 h at 37 °C. Quality control may be performed using fluorescently tagged secondary antibodies which specifically stains the collagen with linked streptavidin tagged antibodies.

**[00504]** This example may be extended to other conjugation chemistries, such as click chemistry (e.g., prepare azide-modified collagen solution using an NHS-PEG–Azide and prepare dibenzocyclooctyne (DBCO)-modified antibody solution using an NHS-PEG-DBCO or Malimide-PEG-DBCO reagent) and when mixed conjugation chemistries may permit collagen functionalization. In an example, the linkage chemistry may be a cleavable linkage chemistry, for example, desthiobiotin or disulfide linkers. Collagen polymers may be functionalized with desthiobiotin and used to generate a functional 3D matrix with streptavidin-conjugated antibodies, such that the antibody linkage may be displaced by the addition of excess biotin to the sample (e.g., a solution of complete media supplemented with 4 mM biotin). Displaced antibodies may be subsequently washed from the 3D culture matrix. Collagen polymers may be functionalized with cleavable disulfide linkers and used to generate functional 3D matrix. Antibodies may be cleaved from the collagen hydrogel matrix by the addition of reducing agents compatible with cell culture, such as 20-500 mM tris(2-carboxyethyl)phosphine (TCEP) or 0.05mM beta-mercaptoethanol (BME).

**[00505]** In some examples, conjugation may be performed in a single vessel, for example, using a NHS-Ester bifunctional PEG or otherwise multifunctional (e.g., 4-arm), or other reactive groups (e.g., maleimide) in a mixture containing collagen polymers and antibody factors. Direct crosslinks may form among the collagen polymers forming a collagen gel during functionalization, which is resistant to re-solubilization due to the formation of the chemical crosslinks and therefore may not be easily used as a liquid matrix for cell embedding for 3D culture. In some methods, the collagen may be functionalized in a liquid

form for direct use for cell embedding (or may be further purified, e.g. by dialysis or precipitation, and reformulated as a liquid mixture capable of forming a cell-gel for 3D culture) or it may be functionalized in a gel form. In examples, without chemical crosslink formation between collagen polymers (e.g., a collagen gel) re-solubilization (e.g., by acid treatment) and re-purification (e.g., by dialysis or precipitation) may generate a liquid gel mixture capable of forming a cell-gel for 3D culture. This example may be extended to other classes of polymers suitable for 3D cell culture, including other natural polymers such as alginate, and other synthetic polymers, such as thermo-responsive PEG hydrogel-forming polymers. **FIG. 81** shows example T cell activation and expansion using functionalized matrices as compared to an ImmunoCult™ activator. **FIG. 82** shows example antibody conjugation to a functionalized 3D matrix. Normal collagen may be used as a negative control and biotin-modified collagen may bind the activating antibodies for structural presentation to T cells. Disulfide cleavable-biotin collagen and desthiobiotin collagen may be used to provide T cell activation. The activation period may be from 24-72 hours and, subsequent to activation, the antibodies may be cleaved by reducing agent or biotin displacement and the activation signal may be washed from the 3D matrix. The activation time may be selected to permit T cell expansion without activation-induced apoptosis or exhaustion due to prolonged activation signal.

### **Example 3: Cell isolation**

**[00506]** Functional collagen may be used to create antibody-conjugated collagen beads for use in size-based cell isolation using the filtration module (e.g., TFF module). Cells binding the beads may be retained by the TFF filter membrane, while cells not bound to beads may be separated. Collagen beads may be created by controlled dropping of collagen solution into a stirred vessel of neutral oil, such as olive oil, with or without the addition of a detergent, such as SPAN™-80, to create a dispersion of collagen microdroplets. By increasing the temperature to 37 °C, the collagen may gel and form functional collagen beads. The beads may be isolated and washed, such as by acetone and/or detergent solutions, pelleted by centrifugation or filtration, and size-selected for the appropriately sized distribution by filtration. **FIG. 83** shows example filtration of cells subjected to antibody modified beads and non-modified control beads. Collagen-based beads used for cell isolation may be loaded directly into the soluble 3D matrix precursor solution and gelled into the resulting 3D matrix. Collagen-based beads may be subsequently solubilized during cell harvest by addition of collagenase solution. Other types of functionalized beads may be used, including

biodegradable beads such as agarose, alginate, and other polymer matrix beads, and rigid beads, such as polystyrene and/or glass beads.

**Example 4: Filtration**

**[00507]** A cell processing system may comprise a bioreactor compartment and a number of additional cell processing modules with a filtration module (e.g., TFF module). The separate modules may be configured to perform specific cell processing functions, including peripheral blood mononuclear cells (PBMC) or total nucleated cell (TNC) enrichment from whole blood or leukopak (apheresis or leukapheresis product) with removal of hematocrit, red blood cells, platelets, and/or other solutes; marker-based cell isolation, either positive or negative, for example, using paramagnetic beads or size-exclusion beads; buffer exchange and concentration adjustment; cell washing, for example, to remove liquified 3D matrix; and final formulation. Function-specific configurations may include module TFF flow cell geometry (e.g., channel width, height, and length dimensions of retentate and permeate compartments, flow configurations, such as number of inputs and outputs, etc.) and filter membrane (pore size, porosity, material, etc.) as shown in **FIGs. 84** and **85**. The TFF module may be used for filtration and separation of cells from other components within the system, including cell isolation, selection, and purification. **FIG. 86** shows example filtration of 1  $\mu\text{m}$  beads from formalin-fixed T cells using a 3  $\mu\text{m}$  pore filter. Washed cells show a low amount of residual beads while wash-out (e.g., waste) shows an absence of cells. **FIG. 87** shows example of filtering red blood cells (RBCs) and platelets from primary T cells. Flow cytometry of the input, waste, and retentate shows enrichment of lymphocytes in retentate relative to initial input and specific removal of RBCs and platelets in the waste after a short period of filtration with a TFF module using a 5  $\mu\text{m}$  pore filter.

**[00508]** While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in

practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

## CLAIMS

WHAT IS CLAIMED IS:

1. A system for manufacturing a cell product, comprising:
  - a compartment configured to retain a plurality of cells;
  - a fluid flow path in fluid communication with said compartment, wherein said fluid flow path is configured to (i) flow a fluid along a first direction having an angle with respect to a second direction of flow within said compartment, wherein said angle is greater than zero degrees, and (ii) provide material from said fluid to said compartment to thereby generate said cell product; and
  - an analysis unit coupled to and within a same housing as said compartment and said fluid flow path, wherein said analysis unit is configured to monitor said plurality of cells or said fluid as said materials are provided to said compartment during generation of said cell product.
2. The system of claim 1, wherein said angle is greater than 45 degrees.
3. The system of any one of claims 1 or 2, wherein said cell product is a cell therapy product.
4. The system of claim 3, wherein said system is configured to generate said cell therapy product at a scale of at least one human dose equivalent.
5. The system of any one of claims 1-4, wherein said analysis unit is configured to monitor said plurality of cells in said compartment.
6. The system of any one of claims 1-5, system further comprising a cell processing unit in fluid communication with said compartment.
7. The system of any one of claims 1-6, wherein said compartment is configured to provide to said plurality of cells one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.
8. The system of any one of claims 1-7, wherein said housing has a volume of about 0.01 cubic meters (m<sup>3</sup>) to about 2 m<sup>3</sup>.
9. The system of any one of claims 1-8, wherein said analysis unit comprises one or more sensors configured to monitor an environment comprising said plurality of cells.
10. The system of any one of claims 1-9, wherein said analysis unit comprises one or more sensors configured to analyze said cell product.
11. A system for cell processing, comprising:
  - a cell processing unit configured to retain and process a plurality of cells to generate a cell product;

a preservation unit coupled to said cell processing unit, wherein said preservation unit is configured to preserve said cell product; and

an analysis unit coupled to said cell processing unit or said preservation unit, wherein said analysis unit is configured to detect or monitor one or more properties of said plurality of cells or said cell product.

12. The system of claim 11, wherein said cell product is a cell therapy product.

13. The system of claim 11 or claim 12, further comprising a receiving unit configured to receive said plurality of cells.

14. The system of claim 13, wherein said plurality of cells is a plurality of cryopreserved cells.

15. The system of claim 14, wherein said receiving unit is configured to subject said plurality of cryopreserved cells to controlled thawing to generate a plurality of thawed cells.

16. The system of claim 15, wherein said receiving unit is configured to automatically transfer said plurality of thawed cells to said cell processing unit.

17. The system of any one of claims 11-16, wherein said preservation unit is configured to generate a cryopreserved cell product.

18. The system of any one of claims 11-17, further comprising a bioreactor coupled to said cell processing unit, said preservation unit, or said analysis unit.

19. The system of claim 18, wherein said bioreactor is a solid-state bioreactor.

20. The system of claim 18, wherein said bioreactor is configured to provide to said plurality of cells one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.

21. The system of any one of claims 11-20, wherein said analysis unit is configured to provide in situ, real-time detection or monitoring of said one or more properties of said plurality of cells or said cell product.

22. The system of any one of claims 11-21, wherein said analysis unit is configured to provide release testing of said cell product.

23. The system of any one of claims 11-22, wherein said cell processing unit, said analysis unit, and said preservation unit are integrated withing a housing.

24. The system of claim 23, wherein said housing has a volume of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ .

25. The system of any one of claims 11-24, wherein said analysis unit comprises one or more sensors configured to monitor an environment comprising said plurality of cells.



26. The system of any one of claims 11-25, wherein said analysis unit comprises one or more sensors configured to analyze said cell product.
27. A method for manufacturing a cell product, comprising:
- (a) providing a plurality of cells to a system comprising (i) a compartment that retains said plurality of cells, (ii) a fluid flow path in fluid communication with said compartment, and (iii) an analysis unit coupled to and within a same housing as said compartment and said fluid flow path;
  - (b) providing material from said a fluid in said fluid flow path to said compartment to generate said cell product, wherein said fluid flow path flows said fluid along a first direction having an angle with respect to a second direction of flow within said compartment, wherein said angle is greater than zero degrees; and
  - (c) using said analysis unit to monitor said plurality of cells or said fluid as said materials are provided to said compartment during generation of said cell product.
28. The method of claim 27, wherein said angle is greater than 45 degrees.
29. The method of any one of claims 27 or 28, wherein said cell product is a cell therapy product.
30. The method of claim 29, wherein said cell therapy product is generated at a scale of at least one human dose equivalent.
31. The method of any one of claims 27-30, wherein said analysis unit monitors said plurality of cells in said compartment.
32. The method of any one of claims 27-31, wherein said analysis unit monitors said plurality of cells in real time.
33. The method of any one of claims 27-32, further comprising using a cell processing unit to generate said cell product.
34. The method of any one of claims 27-33, further comprising subjecting said plurality of cells to one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.
35. The method of any one of claims 27-34, wherein said housing has a volume of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ .
36. A method for cell processing, comprising:
- (a) providing a plurality of cells to a system comprising (i) a cell processing unit that retains said plurality of cells, (ii) a preservation unit coupled to said cell processing unit, and (iii) an analysis unit coupled to said cell processing unit or said preservation unit;

- (b) processing said plurality of cells in said cell processing unit to generate a cell product;
  - (c) preserving said cell product in said preservation unit; and
  - (d) detecting or monitoring one or more properties of said plurality of cells or said cell product during said processing or said preserving.
37. The method of claim 36, wherein said cell product is a cell therapy product.
38. The method of claim 36 or 37, further comprising providing said plurality of cells to receiving unit coupled to said cell processing unit, said preservation unit, or said preservation unit.
39. The method of claim 38, wherein said plurality of cells provided to said receiving unit is a plurality of cryopreserved cells.
40. The method of claim 39, wherein said receiving unit subjects said plurality of cryopreserved cells to controlled thawing to generate a plurality of thawed cells.
41. The method of claim 40, wherein said receiving unit automatically transfers said plurality of thawed cells to said cell processing unit.
42. The method of any one of claims 36-41, wherein said preservation unit generates a cryopreserved cell product.
43. The method of any one of claims 36-42, further comprising a bioreactor coupled to said cell processing unit, said preservation unit, or said analysis unit.
44. The method of claim 43, wherein said bioreactor is a solid-state bioreactor.
45. The method of claim 43, further comprising using said bioreactor to subject said plurality of cells to one or more processes selected from the group consisting of cell culture, membrane transduction, expansion, gene delivery, and selection.
46. The method of any one of claims 36-45, wherein said analysis unit provides in situ, real-time detection or monitoring of said one or more properties of said plurality of cells or said cell product.
47. The method of any one of claims 36-46, further comprising using said analysis unit for release testing of said cell product.
48. A system of cell processing, comprising:
  - a plurality of three-dimensional (3D) matrices, wherein a 3D matrix of said plurality of 3D matrices is configured to contain or encapsulate a population of cells; and
  - a plurality of fluid flow paths, wherein a fluid flow path of said plurality of fluid flow paths is independent from and adjacent to said 3D matrix, and wherein said fluid flow path is

configured to deliver a fluidic material to said 3D matrix to process said population of cells into a cell product.

49. The system of claim 48, wherein said cell product is a cell therapy product.

50. The system of claim 48 or claim 49, wherein said 3D matrix and said fluid flow path are separated by a membrane.

51. The system of any one of claims 48-50, wherein said 3D matrix is configured as a slab comprising a first dimension and a second dimension that are larger than a third dimension.

52. The system of claim 51, wherein said third dimension is less than or equal to about 10 millimeters (mm).

53. The system of any one of claims 48-52, wherein said 3D matrix is configured as a fiber comprising a diameter that is smaller than said long dimension.

54. The system of claim 53, wherein said diameter is less than or equal to about 10 mm.

55. The system of any one of claims 48-54, wherein said 3D matrix is configured as a sphere and wherein said tangential flow of said fluid flows along an outer circumference of said sphere.

56. The system of any one of claims 48-55, wherein said plurality of 3D matrices are fluidically coupled to one another.

57. The system of any one of claims 48-56, wherein said fluid flow path is disposed between a first 3D matrix and a second 3D matrix.

58. The system of claim 57, wherein said fluid flow path is configured to deliver said fluidic material to said first 3D matrix and said second 3D matrix.

59. The system of any one of claims 48-58, wherein said 3D matrix comprises a pore size of less than or equal to about 400 micrometers ( $\mu\text{m}$ ).

60. The system of any one of claims 48-59, wherein said 3D matrix comprises a porosity of greater than or equal to about 80%.

61. The system of any one of claims 48-60, wherein an interfacial area between said fluid flow path and said 3D matrix is greater than or equal to about 5 square centimeters ( $\text{cm}^2$ ).

62. The system of any one of claim 48-61, wherein said 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within said 3D matrix.

63. The system of any one of claims 48-62, wherein said 3D matrix is a programmable 3D matrix.

64. The system of any one of claims 48-63, wherein said 3D matrix has a surface area to volume ratio of greater than or equal to about 0.1.
65. The system of any one of claims 48-64, wherein said 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m.
66. The system of any one of claims 48-65, wherein said 3D matrix is configured to provide activation of said plurality of cells via membrane signal transduction in said 3D matrix.
67. A method for cell processing, comprising:
- (a) providing (i) a population of cells, (ii) a plurality of three-dimensional (3D) matrices, and (iii) a plurality of fluid flow paths, wherein said population of cells is contained or encapsulated within a 3D matrix of said plurality of 3D matrices, and wherein a fluid flow path of said plurality of fluid flow paths is independent from and adjacent to said 3D matrix;
  - (b) using said fluid flow path to deliver fluidic material to said 3D matrix; and
  - (c) in a presence of said fluidic material delivered to said 3D matrix, processing said population of cells into a cell product.
68. The method of claim 67, wherein said cell product is a cell therapy product.
69. The method of claim 68, wherein said cell therapy product is produced at a scale of at least one human dose equivalent.
70. The method of claim 67 or claim 68, further comprising functionalizing said 3D matrix to generate a functionalized 3D matrix.
71. The method of claim 70, wherein said functionalizing comprises coupling one or more biomolecules to said 3D matrix.
72. The method of claim 71, wherein said one or more biomolecules are usable for membrane signal transduction.
73. The method of claim 70, further comprising polymerizing pre-functionalized polymer precursors to generate said 3D matrix.
74. The method of claim 71, further comprising releasing said one or more biomolecules from said 3D matrix or replacing said one or more biomolecules with additional or different biomolecules.
75. The method of any one of claims 67-74, wherein said 3D matrix and said fluid flow path are separated by a membrane, and wherein said fluidic material is transported through said membrane.

76. The method of any one of claims 67-75, where said 3D matrix has a dimension less than or equal to about 10 millimeters (mm).
77. The method of any one of claims 67-76, wherein said plurality of 3D matrices are fluidically coupled to one another.
78. The method of any one of claims 67-77, wherein said fluid flow path is disposed between a first 3D matrix and a second 3D matrix of said plurality of 3D matrices.
79. The method of claim 78, wherein said fluid flow path delivers said fluidic material to said first 3D matrix and said second 3D matrix.
80. The method of any one of claims 67-79, wherein said 3D matrix comprises a pore size of less than or equal to about 400 micrometers ( $\mu\text{m}$ ).
81. The method of any one of claims 67-80, wherein said 3D matrix comprises a porosity of greater than or equal to about 80%.
82. The method of any one of claims 67-81, wherein an interfacial area between said fluid flow path and said 3D matrix is greater than or equal to about 5 square centimeters ( $\text{cm}^2$ ).
83. The method of any one of claims 67-82 wherein said 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within said 3D matrix.
84. The method of any one of claims 67-83, wherein said 3D matrix has a surface area to volume ratio of greater than or equal to about 0.1.
85. The method of any one of claims 67-84, wherein said 3D matrix is a programmable 3D matrix.
86. A method of cell processing, comprising:
- (a) providing a three-dimensional (3D) matrix containing a plurality of cells in a cell processing unit;
  - (b) in said cell processing unit, automatically:
    - (i) processing said plurality of cells within said 3D matrix to produce a plurality of processed cells; and
    - (ii) separating said plurality of processed cells from said 3D matrix; and
  - (c) collecting said plurality of processed cells.
87. The method of claim 86, wherein said plurality of cells are encapsulated in said 3D matrix or immobilized to the 3D matrix.
88. The method of claim 86 or 87, wherein said plurality of processed cells is a cell therapy product.

89. The method of claim 88, wherein said cell therapy product is produced at a scale of at least one human dose equivalent.
90. The method of any one of claims 86-89, further comprising providing a mixture comprising a matrix precursor and said plurality of cells to said cell processing system.
91. The method of claim 90, further comprising, in said cell processing unit, applying a first stimulus to said mixture comprising said matrix precursor and said plurality of cells to generate said 3D matrix encapsulating said plurality of cells.
92. The method of claim 91, wherein said first stimulus is a thermal stimulus.
93. The method of claim 91, wherein said first stimulus is a chemical stimulus.
94. The method of claim 91, wherein said first stimulus is an enzymatic stimulus.
95. The method of claim 91, further comprising functionalizing said 3D matrix with one or more biomolecules concurrently with said applying said first stimulus to generate said 3D matrix.
96. The method of claim 91, wherein (ii) comprises applying a second stimulus to said 3D matrix to release said cell product from said 3D matrix.
97. The method of claim 96, wherein said second stimulus is a thermal stimulus.
98. The method of claim 96, wherein said second stimulus is a chemical stimulus.
99. The method of claim 96, wherein said second stimulus is an enzymatic stimulus.
100. The method of any one of claims 86-99, further comprising, prior to (a), generating said 3D matrix in presence of said plurality of cells such that said 3D matrix encapsulates said plurality of cells.
101. The method of claim 100, wherein (a) comprises loading said 3D matrix encapsulating said plurality of cells is loaded into a compartment in said cell processing unit.
102. The method of any one of claims 86-101, wherein (a) comprises separately providing said plurality of cells and said 3D matrix to said cell processing unit.
103. The method of claim 102, wherein said 3D matrix is generated within a compartment in said cell processing unit.
104. The method of claim 102, wherein said 3D matrix is generated external to said cell processing system and subsequently provided to said cell processing unit.
105. The method of any one of claims 86-104, further comprising functionalizing said 3D matrix to generate a functionalized 3D matrix.
106. The method of claim 105, wherein said functionalizing comprises coupling one or more biomolecules to said 3D matrix.

107. The method of claim 106, wherein said one or more biomolecules are usable for membrane signal transduction.
108. The method of claim 105, further comprising releasing said one or more biomolecules from said 3D matrix or replacing said one or more biomolecules with additional biomolecules.
109. The method of any one of claims 86-108, wherein (i) comprises activating said plurality of cells within said 3D matrix to obtain said cell product.
110. The method of any one of claims 86-108, wherein (i) comprises transducing said plurality of cells within said 3D matrix to obtain said cell product.
111. The method of any one of claims 86-108, wherein (i) comprises transfecting said plurality of cells within said 3D matrix to obtain said cell product.
112. The method of any one of claims 86-111, wherein said 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m.
113. The method of any one of claims 86-112, further comprising activating said plurality of cells via membrane signal transduction in said 3D matrix.
114. A cell processing system, comprising:  
a cell processing unit configured to retain a three-dimensional (3D) matrix, wherein said 3D matrix is configured to contain or encapsulate a plurality of cells; and  
one or more computer processors operatively coupled to said cell processing unit, wherein said one or more computer processors are individually or collectively programmed to automatically (i) process said plurality of cells within said 3D matrix to produce a plurality of processed cells, (ii) direct said cell processing unit to separate said plurality of processed cells from said 3D matrix, and (iii) direct said cell processing unit to collect said plurality of processed cells.
115. The system of claim 114, further comprising a receiving unit configured to receive said plurality of cells from a user.
116. The system of claim 115, wherein said one or more computer processors is operatively coupled to said receiving unit and individually or collectively programmed to automatically direct said receiving unit to provide said plurality of cells to said cell processing unit.
117. The system of claim 115, wherein said receiving unit is configured to receive said plurality of cells in a cryopreserved state, and wherein said receiving unit is configured to provide controlled thawing to said plurality of cells in said cryopreserved state.

118. The system of any one of claims 114-117, further comprising an analysis unit configured to monitor said plurality of cells during processing of said plurality of cells.
119. The system of claim 118, wherein said analysis unit comprises one or more sensors configured to monitor an environment comprising said plurality of cells.
120. The system of claim 118, wherein said analysis unit comprises one or more sensors configured to analyze said cell product.
121. The system of any one of claims 114-120, further comprising a housing configured to retain said cell processing unit and said one or more computer processors.
122. The system of claim 121, wherein said housing has a footprint of about 0.01 cubic meters ( $\text{m}^3$ ) to about 2  $\text{m}^3$ .
123. The system of any one of claims 114-122, wherein said cell processing unit is configured to generate said 3D matrix in presence of said plurality of cells.
124. The system of any one of claims 114-123, wherein said cell processing unit is configured to receive a pre-formed 3D matrix.
125. The system of claim 124, wherein said cell processing unit is configured to receive said pre-formed 3D matrix comprising said plurality of cells.
126. The system of claim 125, wherein said cell processing unit is configured to encapsulate said plurality of cells in said pre-formed 3D matrix.
127. The system of any one of claims 114-126, wherein said 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m.
128. The system of any one of claims 114-127, wherein said 3D matrix is configured to provide activation of said plurality of cells via membrane signal transduction in said 3D matrix.
129. A method for cell processing, comprising:
- (a) providing a plurality of cells ex vivo to a cell processing unit;
  - (b) in said cell processing unit, contacting said plurality of cells with a selection reagent, wherein, upon said contacting, said selection reagent (i) couples with cells of a sub-population of said plurality of cells and (ii) perturbs a state of one or more cells of said sub-population to product (A) a population of perturbed cells and (B) a population of non-perturbed cells; and
  - (c) processing either said population of perturbed cells or said population of non-perturbed cells into a cell therapy product.



130. The method of claim 129, wherein said cell therapy product is produced at a scale of at least one human dose equivalent.
131. The method of claim 129 or claim 130, wherein said population of perturbed cells have an altered growth rate or viability as compared to said population of non-perturbed cells.
132. The method of any one of claims 129-131, wherein said cell processing unit comprises a three-dimensional (3D) matrix encapsulating said plurality of cells.
133. The method of claim 132, further comprising contacting said plurality of cells with said selection reagent within said 3D matrix.
134. The method any one of claims 129-133, wherein said cell processing unit comprises a vessel comprising said plurality of cells.
135. The method of claim 134, further comprising contacting said plurality of cells with said selection reagent within said vessel.
136. The method of any one of claims 129-135, wherein said selection reagent comprises a binding agent and a selection agent.
137. The method of claim 136, wherein said selection agent is a cytotoxic agent.
138. The method of claim 136, wherein said binding agent is coupled to said selection agent via a linker.
139. The method of claim 138, wherein said linker is a cleavable linker.
140. The method of claim 136, wherein said binding agent is an antibody.
141. The method of claim 136, wherein said binding agent is an aptamer.
142. The method of any one of claims 129-141, wherein said population of perturbed cells comprises dead cells or cell degradation products.
143. The method of any one of claims 129-142, wherein said population of perturbed cells have an altered cell morphology as compared to said population of non-perturbed cells.
144. A system for manufacturing a cell therapy product, comprising:  
a three-dimensional (3D) matrix configured to contain or encapsulate a population of cells at a density of greater than about 80 million cells per cubic centimeter (cell/ cm<sup>3</sup>), wherein said system is capable of processing cells of said population of cells, with aid of said 3D matrix, into said cell therapy product.
145. The system of claim 144, wherein said density is at least 100 million cells/ cm<sup>3</sup>.
146. The system of claim 144 or claim 145, wherein said 3D matrix has a surface area to volume ratio greater than or equal to 0.1.
147. The system of any one of claim 144-146, wherein said 3D matrix has a porosity of at least about 80%.

148. The system of any one of claim 144-147, further comprising a plurality of 3D matrices comprising said 3D matrix.
149. The system of any one of claim 144-148, further comprising a fluid flow path in fluid communication with said 3D matrix, wherein said fluid flow path is adjacent to and independent from said 3D matrix.
150. The system of claim 149, further comprising a membrane disposed between said fluid flow path and said 3D matrix.
151. The system of any one of claim 144-150 wherein said 3D matrix is programmable.
152. A method for manufacturing a cell therapy product, comprising (i) providing a population of cells at a density of greater than about 80 million cells per centimeters (cells/cm<sup>3</sup>) retained within a three-dimensional (3D) matrix and (ii) with aid of said 3D matrix, processing cells of said population of cells into said cell therapy product.
153. The method of claim 152, wherein said processing of said cells comprises culturing said population of cells.
154. The method of claim 152 or 153, wherein said processing of said cells comprises in situ electroporation of said cells.
155. The method of claim 154, wherein said 3D matrix has a conductivity from about 10<sup>-5</sup> Siemens per m (S/m) to about 10<sup>-1</sup> S/m.
156. The method of any one of claims 152-155, wherein said processing of said cells comprises activation of said cells in said 3D matrix.
157. A bioreactor for processing a population of cells comprising a three-dimensional (3D) matrix configured to retain said population of cells, wherein said bioreactor has a volume of at most about 2 cubic meters (m<sup>3</sup>) and is configured to, with aid of said 3D matrix and at high reproducibility, processes said population of cells into a cell product.
158. The bioreactor of claim 157, wherein said cell product has a viability of greater than or equal to about 75%.
159. The bioreactor of claim 157 or 158, wherein said volume is of at most 1 m<sup>3</sup>.
160. A method for processing a population of cells comprising (i) providing a population of cells retained within three-dimensional (3D) matrix of a bioreactor, wherein said bioreactor has a volume of at most 2 cubic meters (m<sup>3</sup>) and (ii) processing, with aid of said 3D matrix and at high reproducibility, said population of cells into a cell product.
161. The method of claim 160, wherein said cell product has a viability of greater than or equal to about 75%.

162. The method of claim 160 or 161, further comprising, prior to (i), generating said 3D matrix in presence of said population of cells.
163. The method of any one of claims 160-162, further comprising, subsequent to (ii), dissolving said 3D matrix and collecting said population of cells to generate said cell product.
164. A system for cell therapy manufacturing, comprising:  
a three-dimensional (3D) matrix configured to retain a population of cells at a density of at least about 5 million cells per cubic centimeters (cells/cm<sup>3</sup>), wherein said system is capable of processing cells of said population within said 3D matrix for a time period of at most about 48 hours to generate a cell product, and wherein said cell product is usable to generate a cell therapy.
165. The system of claim 164, wherein said density is at least 10 million cells/ cm<sup>3</sup>.
166. The system of any one of claims 164 or 165, wherein said 3D matrix has a surface area to volume ratio greater than or equal to 0.1.
167. The system of any one of claims 164-166, wherein said 3D matrix has a porosity of at least about 80%.
168. The system of any one of claims 164-167, wherein said 3D matrix has a dimension of less than about 200 micrometers (μm).
169. The system of any one of claims 164-168, wherein said 3D matrix comprises a porous, solid matrix having interconnected pores with an average pore size sufficient to permit growth of lymphocyte cells within said 3D matrix.
170. The system of any one of claims 164-169, further comprising a plurality of 3D matrices comprising said 3D matrix.
171. The system of any one of claims 164-170, further comprising a fluid flow path in fluid communication with said 3D matrix, wherein said fluid flow path is adjacent to and independent from said 3D matrix.
172. The system of claim 171, further comprising a membrane disposed between said fluid flow path and said 3D matrix.
173. A method for manufacturing a cell therapy, comprising (i) providing a population of cells at a density of at least about 5 million cells per centimeters (cells/ cm<sup>3</sup>) retained within a three-dimensional (3D) matrix; (ii) processing said cells of said population of cells within said 3D matrix for a time period of at most about 48 hours to generate a cell product; and (iii) using said cell product to generate said cell therapy.
174. The method of claim 173, wherein said processing of said cells comprises culturing said population of cells.

175. The method of claim 173 or 174, wherein said processing of said cells comprises in situ electroporation of said population of cells.

176. The method of claim 174, wherein said 3D matrix has a conductivity from about  $10^{-5}$  Siemens per m (S/m) to about  $10^{-1}$  S/m.

177. The method of any one of claims 173-176, wherein said processing of said cells comprises activation via membrane signal transduction in said 3D matrix.