

BIOPRINTING METHODS AND SYSTEMS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of US Provisional Application No. 63/449,291, filed March 1, 2023, entitled BIOPRINTING METHODS AND SYSTEMS, and incorporates the entire contents of this application by reference herein.

TECHNICAL FIELD OF DISCLOSURE

[0002] Aspects of the present invention relate to quality control methods and apparatus for monitoring a three-dimensional (3D) bioprinting system. More particular aspects relate to methods and apparatus for monitoring different stages of the 3D bioprinting system. Yet more particular aspects relate to a computer vision and machine-learning system providing real time visual images of a plurality of cells at different locations in the bioprinting platform, in order to assess various characteristics of the plurality of cells, including but not limited to a quantity, distribution, concentration, quality, and/or morphology of the plurality of cells.

BACKGROUND

[0003] A 3D bioprinting process has a number of stages, including a so-called pre-print stage, a printing stage, and a post-print stage. It would be desirable to provide a system that is capable of monitoring different stages of the 3D bioprinting process, to assess how operation of the various stages can affect print quality. Such a system can enable improvement of operation at each of the various stages, and thereby promote the production of suitable 3D-bioprinted tissues for therapeutic use.

SUMMARY

[0004] The present invention addresses the foregoing problems in the art by integrating computer vision and deep learning into a three-dimensional (3D) bioprinting system, thereby enabling direct monitoring of operation and flow of a plurality of different materials at different stages within the bioprinting system. In embodiments, the materials may include, *e.g.*, cell-laden hydrogels and other cross-linkable materials. In

embodiments, the different stages of the bioprinting system may include one or more reservoirs, a printhead, and a print surface.

[0005] Embodiments of the invention enable contactless sensing of the characteristics of a plurality of cells as they flow through the bioprinting system, before, during, and/or after printing. Embodiments also enable automated analyses, which in turn enable derivation of quantitative measures of properties of the plurality of cells. These measures can serve as quality control and/or quality assurance parameters. In some embodiments, such quantitative measures include real-time high-level quantitation of biological material (e.g., cells) before, during, and/or after printing thereof, for qualitative assessment of cell quality in terms of the consistency of biological material. In some embodiments, such quantitative measures include real-time high-level quantitation of other objects within a material flow.

[0006] In embodiments, the material flow comprises at least one cross-linkable material, and preferably at least one hydrogel, optionally further comprising at least one biological material, *e.g.* a plurality of cells in a biocompatible material. In embodiments, the plurality of cells is selected from the group comprising or consisting of a single-cell suspension, cell aggregates, cell clusters, cell agglomerates, cell spheroids, cell organoids, or combinations thereof. In embodiments, the material flow comprises micro-particles.

[0007] In embodiments, the plurality of cells comprises cells from endocrine and exocrine glands selected from the group consisting of pancreas, liver, thyroid, parathyroid, pineal gland, pituitary gland, thymus, adrenal gland, ovary, testis, enteroendocrine cells, stem cells, stem cell-derived cells or cells engineered to secrete a biologically active agent of interest. In embodiments, the plurality of cells releases cell-derived extracellular vesicles in the form of exosomes containing a therapeutic protein or nucleic acid. In embodiments, the biocompatible material is selected from alginate, collagen, decellularized extracellular matrices, hyaluronic acid, PEG, fibrin, gelatin, GEL-MA, silk, chitosan, cellulose, PCL, PLA, POEGMA, and combinations thereof.

[0008] In embodiments, the printhead may comprise one or more transparent channels, and an optical system may monitor material flow through at least one of the one or more

transparent channels, preferably wherein the printhead comprises a transparent nozzle or dispensing channel. In embodiments, the printhead may be an extrusion printhead or, a co-extrusion printhead, including but not limited to multi-material extrusion, multi-axial extrusion, or melt-extrusion, an inkjet printhead, an electrowriting printhead, or a microfluidic printhead, including but not limited to on-chip crosslinking, microfluidic stereolithography or microfluidic spinning.

[0009] Generally, the printhead will comprise one or more inputs fluidically connected to one or more reservoirs, one or more dispensing channels for depositing the material flow onto the print surface, and preferably one or more transparent channels or regions. The cross-linkable material within the material flow may be cross-linked within the printhead, while dispensing from the printhead, and/or after deposition on the print surface, as is known in the art. The print surface may further comprise, e.g. a cross-linker bath.

[0010] In embodiments, the optical system may comprise one or more microscopes, one or more still cameras, and/or one or more video cameras. There may be an optical system at each stage of the bioprinting system, from the reservoir to the printhead to the print surface.

[0011] In an exemplary embodiment, the printhead may comprise a plurality of transparent channels and the optical system may comprise an equal plurality of microscopes, still cameras, and/or video cameras.

[0012] In embodiments, the machine learning system identifies one or more deviations in the flow of a plurality of cells from the user-established material flow parameters at each of a plurality of stages as the plurality of cells pass through the bioprinting system. In embodiments, and responsive to the identified one or more deviations, the machine learning system identifies whether adjusting the material flow parameters is necessary. In embodiments, the machine learning system may adjust the material flow parameters in response to cumulative deviations exceeding a predetermined amount. In embodiments, the machine learning system adjusts the material flow parameters in order to maintain suitable physical properties of the plurality of cells within a predetermined tolerance.

[0013] In embodiments, the machine learning system performs panoptic segmentation and/or semantic and/or instance segmentation of the streaming images of the flow of the plurality of cells through the bioprinting system. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables detection of location of one or more objects within the material flow. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables visual estimation of a shape and/or size of the one or more objects within the material flow. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables visual estimation of a general amount and/or distribution of biological material (e.g., plurality of cells) within the material flow.

[0014] In embodiments, the device comprises a three-dimensional (3D) bioprinting printhead. In embodiments, the 3D bioprinting printhead comprises a plurality of channels to selectively provide a respective plurality of materials for the material flow.

[0015] In embodiments, the at least one cross-linkable material comprises a hydrogel. In embodiments, the material flow further comprises at least one biological material; preferably wherein said at least one biological material comprises a plurality of cells. In embodiments, the plurality of cells is selected from the group comprising or consisting of a single-cell suspension, cell aggregates, cell clusters, cell agglomerates, cell spheroids, cell organoids, or combinations thereof. In embodiments, the material flow further comprises microparticles. In embodiments, the material flow further comprises dyes, pigments or colloids. In embodiments, the presence of cells in the material flow acts as a contrast agent to facilitate measurement of physical properties of the bioprinted fibers.

[0016] In another embodiment, the computing system uses the results of the comparison to control the material flow by adjusting pressure of material flow within the bioprinting system. In embodiments, the system further comprises a pressure controller responsive to the results of the comparison to control the material flow and pressures through the printhead during printing of the printed fiber.

[0017] In embodiments, the machine learning system may comprise a neural network selected from the group consisting of a convolutional neural network (CNN), a fully convolutional network (FCN), a region-based CNN (R-CNN), a Mask R-CNN, a you

only look once (YOLO) based model, and a transformer-based instance segmentation model. In addition, in embodiments, flow estimation networks, including but not limited to flow estimation networks in the FlowNet family (e.g. FlowNet, FlowNet 2), Recurrent All-Pairs Field Transforms (RAFT), and Self-Teaching Multi-Frame Unsupervised RAFT with Full-Image Warping (SMURF) may be used. Still further, in embodiments, a technique known as SegFlow may be used for joint prediction of cell segmentation and flow estimation.

[0018] In embodiments, the determining further comprises, using the machine learning system, performing panoptic segmentation and/or semantic and/or instance segmentation of the streaming images of the material flow. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables identification of cells, fragments, and aggregates within the material flow. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables visual estimation of a shape and/or size of the one or more objects within the material flow. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables visual estimation of a general amount and/or distribution of biological material (e.g., plurality of cells) within the material flow.

[0019] In embodiments, the obtaining comprises obtaining streaming images through one or more transparent channels in a three-dimensional (3D) bioprinting printhead in the device, the 3D bioprinting printhead producing bioprinted fibers. In embodiments, the monitoring comprises monitoring a plurality of channels within the 3D bioprinting printhead, the plurality of channels to selectively provide a respective plurality of materials for the material flow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The accompanying drawings, which are incorporated herein and form a part of the specification, illustrate embodiments of the present disclosure and, together with the description, further serve to explain the principles of the disclosure and to enable a person skilled in the relevant art to make and use the disclosure.

[0021] FIG. 1 is a high level diagram of a bioprinting system according to an embodiment;

- [0022] FIG. 2 is a high level flowchart showing different stages before, during, and after passage of a plurality of cells through different stages of a bioprinting system according to an embodiment;
- [0023] FIGS. 3A-3F depict different geometric and feature characterizations of cells, fragments, and aggregates according to an embodiment;
- [0024] FIG. 4 is a flowchart showing how to differentiate among cells, fragments, and aggregates according to an embodiment;
- [0025] FIG. 5 shows a plurality of microwells for characterization of a plurality of cells before printing according to an embodiment;
- [0026] FIGS. 6A-6D show application of characterization of cells, fragments, and aggregates according to an embodiment;
- [0027] FIG. 7A shows detection of aggregates according to one embodiment, and FIG. 7B shows detection of both cells and aggregates according to another embodiment;
- [0028] FIGS. 8A and 8B show characterizations of cells, fragments, and aggregates before printing, according to an embodiment;
- [0029] FIGS. 9A and 9B show characterizations of cells, fragments, and aggregates during printing, according to an embodiment;
- [0030] FIGS. 10A-10H show characterizations of cells, fragments, and aggregates after printing, according to embodiments;
- [0031] FIGS. 11A-11C show characterizations of cells, fragments, and aggregates according to an embodiment;
- [0032] FIGS. 12A and 12B show characterizations of cells, fragments, and aggregates after printing, according to an embodiment;
- [0033] FIGS. 13A and 13B show identification of cells, fragments, and aggregates according to an embodiment;
- [0034] FIGS. 14A and 14B demonstrate detection of cells, fragments, and aggregates according to an embodiment;

- [0035] FIGS. 15A and 15B demonstrate detection of cells, fragments, and aggregates according to an embodiment;
- [0036] FIGS. 16A and 16B demonstrate detection of cells, fragments, and aggregates according to an embodiment;
- [0037] FIGS. 17A and 17B demonstrate detection of cells, fragments, and aggregates according to an embodiment;
- [0038] FIGS. 18A and 18B demonstrate detection of cells, fragments, and aggregates according to an embodiment;
- [0039] FIGS. 19A and 19B show identifications of cells, fragments, and aggregates after printing, according to an embodiment;
- [0040] FIGS. 20A and 20B show identifications of cells, fragments, and aggregates after printing, according to an embodiment;
- [0041] FIGS. 21A, 21D, 21G, and 21J are input images, FIGS. 21B, 21E, 21H, and 21K show detected cells according to an embodiment, and FIGS. 21C, 21F, 21I, and 21L show bar charts of distributions of cell characteristics.
- [0042] FIGS. 22A-22F show identification of cells and aggregates before printing, according to an example;
- [0043] FIGS. 23A-23F show identification of cells and aggregates during printing, according to an example
- [0044] FIGS. 24A-24P show identification of cells and aggregates after printing, according to an example;
- [0045] FIGS. 25A-25F show identification of cells and aggregates delineating beads in a printed fiber, according to an example;
- [0046] FIG. 26 shows a bead quantification model integrated into the print analysis software.

DETAILED DESCRIPTION

[0047] Certain illustrative aspects of the systems, apparatuses and methods according to the present invention are described herein in connection with the following description and the accompanying figures. These aspects are indicative, however, of but a few of the various ways in which the principles of the invention may be employed and the present invention is intended to include all such aspects and their equivalents. Other advantages and novel features of the invention may become apparent from the following detailed description when considered in conjunction with the figures.

[0048] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. In other instances, well known structures, interfaces and processes have not been shown in detail in order not to unnecessarily obscure the invention. However, it will be apparent to one of ordinary skill in the art that those specific details disclosed herein need not be used to practice the invention and do not represent a limitation on the scope of the invention, except as recited in the claims. It is intended that no part of this specification be construed to effect a disavowal of any part of the full scope of the invention. Although certain embodiments of the present disclosure are described, these embodiments likewise are not intended to limit the full scope of the invention.

[0049] FIG. 1 shows a high level diagram of a bioprinting system 100 according to an embodiment. The bioprinting system 100 includes a plurality of stages, labeled for ease of reference as reservoir 110 (which may be one or more reservoirs), printhead 120, and print surface 130 (which may be any surface on which material from printhead 120 may be deposited). In embodiments, the printhead may be an extrusion or co-extrusion printhead, including but not limited to multi-material extrusion (*e.g.*, Liu et al. *Adv Mater.* 2017 Jan;29(3):10.1002), multi-axial extrusion, or melt-extrusion (*e.g.*, Loewner et al., *Frontiers in Bioengineering and Biotechnology* 2022 DOI 10.3389/fbioe.2022.896719), , an inkjet printhead (*e.g.* Lal Roy, *Lab on a Chip* 2021 DOI 10.1039/d1lc00524c), an electrowriting printhead (*e.g.*, Kade and Dalton, *Advanced Healthcare Materials* 2020 DOI 10.1002/adhm.202001232), or a microfluidic printhead, including but not limited to on-chip crosslinking, microfluidic stereolithography (*e.g.*, Miri et al., *Adv Mater.* 2018 Jul; 30(27): e1800242) or microfluidic spinning (*e.g.*, Lee et al. *Nature Materials* 2011 10:877-883). In preferred embodiments, the bioprinting system employs on-chip crosslinking as disclosed in U.S.

Patent No. 11,046,930, PCT/CA2014/050556, PCT/CA2018/050315, PCT/CA2018/050315, PCT/CA2018/050315 and PCT/CA2022/051855, the disclosures of which are expressly incorporated by reference herein.

[0050] Each of the stages has associated with it respective optical systems which provide image data for cells and cell aggregates at each stage. For example, reservoir 110 may have associated with it optical system 112, which may comprise microscopes, still cameras, and/or video cameras to obtain images, at appropriate angles, of a plurality of cells in the reservoir, to characterize that plurality of cells before printing. Those cells may be individual cells; they may be what are termed fragments; or they may be cell aggregates. Similarly, printhead 120 may have associated with it optical system 122, which also may comprise microscopes, still cameras, and/or video cameras to obtain images, at appropriate angles, of cells in the printhead, for example, in a nozzle of the printhead as described in the above-referenced US provisional and PCT applications, to characterize that plurality of cells during printing. Those cells similarly may be individual cells; they may be what are termed fragments; or they may be cell aggregates. Likewise, print surface 130 may have associated with it optical system 132, which likewise may comprise microscopes, still cameras, and/or video cameras to obtain images, at appropriate angles, of cells on the print surface, to characterize that plurality of cells after printing. Those cells likewise may be individual cells; they may be what are termed fragments; or they may be cell aggregates.

[0051] In embodiments, bioprinting system 100 may be under control of computing system 150, which may include one or more central processing units (CPUs), volatile memory, non-volatile memory, and non-transitory non-volatile storage. Computing system 150 may connect with one or more of optical systems 112, 122, and 132 through a variety of connections, either direct, or over a local area network (LAN), or through the cloud.

[0052] The computing system 150 may include a machine-learning system 160. In embodiments, the machine learning system 160 may employ one or a plurality of graphical processing units (GPUs) with pluralities of cores to facilitate the necessary calculations for a machine-learning system to perform rapid computational analysis of data streams or captured images, and training of the model that provides feedback to control material flow in the bioprinting system. In embodiments, the GPUs of machine

learning system 160 may be part of computing system 150. In embodiments, the machine learning system 160 may use the memory and storage that computing system 150 has. In other embodiments, the machine learning system 160 may have its own volatile memory, non-volatile memory, and/or non-transitory non-volatile storage. In embodiments, the model training may involve testing and validation to facilitate optimization and inference by the model being trained.

[0053] In embodiments, machine learning system 160 may comprise a neural network selected from the group consisting of a convolutional neural network (CNN), a region-based CNN (R-CNN), a Mask R-CNN, a you only look once (YOLO) based model, and a transformer-based instance segmentation model to perform image segmentation on the imaging data received from optical systems 112, 122, and 132. In an embodiment, machine learning system 160 may interact with computing system 150 to perform a number of user-interactive functions and also system-interactive functions. For user interaction, the computing system 150 may provide an appropriate graphical display and graphical user interface for interaction with various other components such as the optical systems, the machine learning system 160, and the rest of bioprinting system 100.

[0054] In a cloud environment, machine-learning system 160 and its user interface may be deployed as a cloud application, for example, as a login-accessible website, through which the users can view and interact with print videos online and see results of machine learning analysis. In such embodiments, the computing system 150 and machine learning system 160 may be hosted in the cloud.

[0055] Depending on the embodiment, computing system 150 may control the optical systems 112, 122, and 132 through one or more graphical user interfaces on one or more displays. Depending on the embodiment, optical systems 112, 122, and 132 may have their own control apparatus, whereby these optical systems, responsive to commands from the computing system 150, may position themselves appropriately relative to reservoir 110, printhead 120 and its associated feeds and nozzle(s), and print surface 130, respectively.

[0056] Part of the control that computing system 150 performs involves monitoring of access to control systems in the bioprinting system to adjust material flow, whether by

control of pressure in various printhead channels (including, for example, microfluidic printhead channels) to be discussed below, or by control of displacement of material through the channels. In embodiments, control may involve toggling on or off or otherwise adjusting opening and closing of pneumatic valves on a printhead. In embodiments, part of the control that computing system 150 performs involves panoptic segmentation and/or semantic and/or instance segmentation. In embodiments, the panoptic segmentation and/or semantic and/or instance segmentation enables visual estimation of a general amount and/or distribution of biological material (e.g., plurality of cells) within a material flow.

[0057] Computing system 150 also may enable reading of the respective feeds from one or all of the optical systems to enable recording and/or loading of one or both of the feeds, and adjust viewing parameters such as contrast, tint, brightness, and sharpness, among others.

[0058] Depending on the embodiment, machine learning system 160 may characterize particles in bioprinting system 100, in a manner to be prescribed. Alternatively or in addition, machine learning system 160 may help provide focus to images from optical systems 112, 122, and 132 to aid in characterizing the particles. Alternatively or in addition, machine learning system 160 may provide outputs which can be fed back in real-time to the 3D bioprinting system in order to adjust pressure and/or displacement and subsequent flow of materials from the reservoir 110, to the printhead 120, to the print surface 130, to enable consistent production of quality tissues and minimize loss of expensive biomaterial and cell inputs.

[0059] FIG. 2A shows a very high level view of the flows involved in the operation of bioprinting system 100 in conjunction with computing system 150 and machine learning system 160 according to an embodiment. At 210, cell quantification and characterization may be carried out before printing, for example, in a reservoir 110 of bioprinting system 100. FIG. 5, discussed below, shows cells in a plate of microwells, with an aggregate contained in each microwell. Processes discussed below with respect to FIG. 4 may be used to quantify the aggregate in each microwell.

[0060] At 212, cells may optionally be pre-printed on a plate, *e.g.* to prompt aggregation. At this stage as well, the cells may be quantified and characterized. 212 is

not essential, and may be omitted. At 214, the cells are in a printhead, to be printed. Here, too, using the optical system, computing system, and machine learning system in FIG. 1, the cells in the printhead may be characterized. In an embodiment, comparisons may be made between the cells in the printhead and the cells in the microwells. At 216, the cells may be deposited into a printed device, for example, on print surface 130 in FIG. 1. At 218, the device structure is characterized. At 220, before the resulting tissue is implanted, cell analysis may be done. Depending on the embodiment, the same machine learning system 160 in FIG. 1, or different machine learning systems, may be applied at each stage 210-220, and the results at a given stage compared to results at other stages. In an embodiment, the results at a given stage may be compared to a reference to enable a determination of whether and/or how further training of the machine learning system should be carried out.

[0061] FIG. 2B is a high level flow chart for the implementation and use of computer vision and machine-learning tools to provide automated feedback control. At 230, the imaging devices in optical systems 112, 122, and 132 are controlled to record video and/or still images of material passing through the respective stages of bioprinting system 100. These images are obtained through various types of operation of the bioprinting system. To generate data for training sets for the machine-learning model, for example, the system may be operated with known materials and parameters to obtain known results. Other data sets, to be used in testing and evaluation of the trained system, also may be generated. In an embodiment, existing data sets may be used for training.

[0062] At 235, the collected data may be separated in the aforementioned training sets and testing/evaluation sets. At 240, focusing on the training sets, features of interest may be labeled, to facilitate focusing on those during training. At 245, appropriately labeled training sets may be applied to a machine learning model of the types mentioned above, to train the model, evolving the model to have it produce correct results from the training data. In some machine learning models, forward propagation of results may be used. In other models, back propagation may be used.

[0063] At 250, training results may be analyzed to identify various features of interest, for example, a quantity, distribution, concentration, quality, and/or morphology of the plurality of cells passing through the particular stage of bioprinting system 100. In an

embodiment, the morphology may comprise at least one characteristic selected from the group consisting of cell count, area, perimeter, ellipsoidal volume, circularity, smoothness, compactness, circle diameter, mean diameter, min diameter, max diameter, mean intensity, and cell density in fiber. The results then may be classified according to the features of interest, level of accuracy, etc. At 255, the testing/validation sets may be used to evaluate performance of the trained network. At 260, if the model is satisfactory, then at 270 the completed trained model may be deployed. If the model is not satisfactory, then at 265 additional training samples may be added, and/or the model otherwise adjusted. Control then returns to 240 for further training. In an embodiment, additions to training sets may be informed by the nature of the results obtained with the previous training sets.

[0064] FIGS. 3A-3F aid in determining how to characterize a plurality of cells passing through the bioprinting system 100 according to an embodiment. In an embodiment, the morphology of the plurality of cells may be characterized. FIG. 3A shows a aggregate 300, comprising one or more cells, which is to be characterized. The aggregate 300 may be approximated as a polygon. The polygon will have a centroid, and will have area. FIG. 3B shows a circle 310 with approximately the same area and centroid as the polygon. FIG. 3B shows areas 311, 313, 315, and 317 where the aggregate 300 is outside of the circumference of the circle 310. FIG. 3B also shows areas 312, 314, 316, and 318 where the circle 310 is outside of the aggregate 300. The circle will have an area $A = \pi r^2$. Solving the equation for r enables determination of the circle diameter.

[0065] FIG. 3C shows an ellipsoidal volume 320, with a long diameter D_L and a short diameter D_S . The ellipsoidal volume 320 is drawn to have the same volume as a sphere. To an approximation, $\sqrt{D_L D_S}$ equals the diameter D of the circle.

[0066] With the foregoing in mind, a value for circularity of the aggregate 300 may be obtained. Circularity is a ratio of the area of the circle 310 to the area of the aggregate 300, as seen in FIG. 3D, which shows the same areas 311, 313, 315, 317 where the aggregate 300 is outside of the circumference of the circle 310, and the same areas 312, 314, 316, 318 where the circle 310 is outside of the aggregate 300. The more circular the aggregate 300, the closer the circularity will be to 1. The less circular the shape (e.g. the more elliptical), the closer the circularity will be to 0.

[0067] FIG. 3E is a diagram to help characterize the surface complexity of aggregate 300. The more convex the aggregate 300, the smoother it is. FIG. 3E shows aggregate 300 with a convex hull 330 surrounding it. Convex hull 330 is the smallest convex polygon that contains the aggregate 300. Smoothness (or convex hull compactness) of aggregate 300, then, is a ratio of the area of the aggregate 300 to the area of the convex hull 330. Smoothness will be 1 for a completely convex polygonal shape of aggregate 300. The greater the number of concavities in aggregate 300, and the larger the concavities, the closer the smoothness gets to 0.

[0068] FIG. 3F shows an alternative approach to determine area and/or diameter of aggregate 300. In FIG. 3F, circle 350 surrounds aggregate 300. In an embodiment, circle 350 has the same centroid as aggregate 350. Several diameters 360, 365, and 370 of the aggregate 300 are shown, with diameter 360 being the longest and diameter 365 being the shortest. Diameter 370 represents a mean of diameters 360 and 365.

[0069] The following table summarizes the metrics that FIGS. 3A-3F describe:

Metric	Description
Aggregate Area	Internal area of the aggregate polygon
Aggregate Perimeter	Perimeter of the aggregate exterior
Circle Diameter	Diameter of a circle with the same area as the polygon
Ellipsoidal Volume	Volume of an ellipsoid with the same cross-sectional area as the aggregate but a depth given by the Circle Diameter
Circularity	Proportion of the aggregate area within the Circle Radius of the aggregate centroid
Smoothness	Ratio of the aggregate area to the convex hull area
Compactness	Ratio of the Approximate Circle perimeter to the Aggregate Perimeter
Mean Diameter	Diameter of the angle bisector between the min/max diameters
Min Diameter	Shortest diameter of the aggregate through its centroid

Max Diameter	Longest diameter of the aggregate through its centroid
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[0070] FIG. 4 is a flowchart for characterizing a group of cells among the plurality of cells that pass through the bioprinting system 100. At 410, a determination is made whether a diameter of the group of cells is greater than 40 μm . If it is, then at 412 the group of cells is determined to be an aggregate. If it is not, then at 420 a determination is made whether a diameter of the group of cells is greater than 25 μm , but less than 40 μm . If it is, then at 422 it is determined whether a ratio of the long axis of the group of cells to the short axis of the group of cells is greater than or equal to 1.3. If it is, then at 424 the group of cells is determined to be a fragment. If it is not, then at 430 a determination is made whether the just-mentioned ratio of long axis to short axis is less than or equal to 1.3. If it is, then at 432 a determination is made whether the diameter of the group of cells is less than 30 μm . If it is, then at 434 the group of cells is determined to be a single cell. If it is not, then at 440 a determination is made whether the diameter of the group of cells is greater than 30 μm and less than 40 μm . If it is, then at 442 a determination is made whether the circularity of the group of cells is greater than or equal to 0.70. If it is, then at 444 the group of cells is determined to be a fragment. If it is not, then at 450 a determination is made whether the circularity of the group of cells is greater than 0.70. If it is, then at 452 the group of cells is determined to be an aggregate. Finally, looking back up to the top of FIG. 4, if at 420 it is determined that the diameter of the group of cells is not greater than 25 μm and less than 40 μm , then at 460 a determination is made whether the diameter of the group of cells is less than 25 μm . If it is, then at 462 the group of cells is determined to be a single cell.

[0071] FIG. 5 shows a plurality of microwells, each with a plurality of cells contained therein. The numbers in the figure show the smoothness and circularity of the plurality of cells in each microwell.

[0072] FIG. 6A shows pre-print cells. FIG. 6B is a slightly enlarged view of a portion of FIG. 6C, FIGS. 6B and 6C containing color coding to characterize the plurality of cells shown. FIG. 6D is a set of bar charts corresponding to the color coding in FIGS.

6C and 6D. The first bar chart shows the area of the various aggregates congregating around a relatively low number. The second bar chart, consistent with the first, shows radii of the various aggregates congregating around a relatively low number. The third and fourth bar charts show that the various aggregates tend to have a high degree of circularity and a high degree of smoothness.

[0073] FIG. 7A shows an example of cell detection according to one embodiment. FIG. 7B shows an example of both cell detection and aggregate detection according to another embodiment at a pre-print stage. Among other things, the comparison shows that the latter embodiment identifies a significantly larger number of cells than the former embodiment.

[0074] FIG. 8A shows a plurality of cells coded in different colors to signify differently sized structures, as determined by a machine learning system according to an embodiment. FIG. 8B shows what the different colors signify, and contains a table categorizing the various structures in FIG. 8A as cells, fragments, or cell aggregates.

[0075] FIGS. 9A and 9B show cell quantification at the printhead, during printing. Similarly to FIGS. 6B, 6C, FIG. 9B shows a categorization of the plurality of cells in the printhead.

[0076] FIG. 10A shows cells after printing, and FIG. 10B shows quantification of cells according to an embodiment. Similarly, FIG. 10C shows cells after printing, and FIG. 10D shows cell quantification after printing.

[0077] FIGS. 10E-10H show detection of aggregates and cells in post-print images. According to an embodiment, the cell and aggregates are located in a shell region of the printed device. FIG. 10E shows cells after printing, and FIG. 10F shows cell quantification after printing according to an embodiment. Similarly, FIG. 10G shows cells after printing, and FIG. 10H shows cell quantification after printing according to an embodiment.

[0078] FIG. 11A shows post-print cells, and FIG. 11B shows quantification of those post-print cells. Similarly to FIG. 6D, FIG. 11C shows generally small area and small radius of the cells being sampled, and high degrees of circularity and smoothness.

[0079] FIGS. 12A and 12B, along with a number of subsequent figures, show another task that a machine learning system can be trained to do according to an embodiment. FIG. 12A shows cell detection after printing, and provides a border around each identified cell. FIG. 12B identifies which cells are in focus, and provides a border around each of those. From these figures, ordinarily skilled artisans will appreciate that a machine learning system according to embodiments can distinguish between in-focus and out-of-focus images from optical systems, and work just with the cells that are in focus in the in-focus image.

[0080] FIGS. 13A and 13B are similar to FIGS. 12A and 12B in that FIG. 13A provides a border around detected cells after printing, while FIG. 13B shows a determination as to which of the detected cells actually are in focus. FIG. 13B has fewer borders relative to FIG. 13A, similarly to FIGS. 12A and 12B, because FIG. 13B places borders only around in-focus cells.

[0081] FIG. 14A shows a picture of post-print cells. FIG. 14B makes more clear the post-print cells that are in focus. A machine learning system according to an embodiment can be trained to identify cells, fragments, and aggregates, and further can discern which of the detected cells, fragments, and aggregates are in focus, and highlight them accordingly, in this case by contrast. Similarly to FIGS. 14A and 14B, FIG. 15A shows a picture of post-print cells, while FIG. 15B makes more clear the post-print cells that are in focus.

[0082] FIGS. 16A and 16B describe yet another task that a machine learning system according to an embodiment can perform. FIG. 16A shows a picture of a plurality of cells, fragments, and aggregates in a fiber. The cells, fragments, and aggregates are in various degrees of focus. FIG. 16B shows a picture of the same plurality of cells, fragments, and aggregates, but with more of them in focus. The machine learning system according to an embodiment can not only identify cells, fragments, and aggregates, but also can sharpen their focus.

[0083] FIG. 17A shows post-print cells before the machine learning system has detected them. FIG. 17B shows detected cells that are both in focus and out of focus. These Figures thus demonstrate that a machine learning system according to an embodiment can detect cells, whether in focus or out of focus, and highlight them

appropriately. This detection facilitates quantification and quality review. FIGS. 18A and 18B, FIGS. 19A and 19B, and FIGS. 20A and 20B are to similar effect to FIGS. 17A and 17B, respectively. FIGS. 20A and 20B show cells in a fiber according to an embodiment.

[0084] FIGS. 21A, D, G, and J are examples of input Z-stack images, taken at different times. These images show detection of in-focus cells and aggregates in different Z-stack layers of a printed fiber at different times. In an embodiment, the model detects in-focus cells and aggregates. As a user changes focus of the microscope to image different layers, in-focus cells and aggregates in different the Z-stack layers may be quantified. FIGS. 21B, E, H, and K show cell detection in the images of FIGS. 21A, D, G, and J, respectively. FIGS. 21C, F, I, and L show bar charts which indicate a wider distribution of characteristics (area, radius, circularity, and smoothness) than is indicated in FIGS. 6A-6D and 11A-11C. These Figures thus indicate that a machine learning system according to an embodiment can be trained to detect and quantify cells, fragments, and aggregates even when the shapes are irregular.

[0085] Material Flows:

[0086] Aspects of the invention include material flows that can be used for printing structures for advantageous use as biomaterials. “Biomaterial” as used herein refers to a natural or synthetic substance that is useful for constructing or replacing tissue, *e.g.* human tissue with or without living cells. In the field of bioprinting, the term “biomaterial” is often synonymous with the term “bioink.”

[0087] The material flow will generally comprise at least one cross-linkable material, *e.g.*, hydrogels including but not limited to, alginate, chitosan, PEGDA, PEGTA, Hyaluronic acid (HA), HAMA, collagen, CollMA, gelatin, gelMA, agarose, gellan, fibrin (fibrinogen), PVA, and the like, or any combination thereof, as well as non-hydrogels including but not limited to, PCL, PLGA, PLA, and the like, or any combination thereof. In preferred embodiments the material flow comprises at least one hydrogel. Non-limiting examples of hydrogels include alginate, agarose, collagen, fibrinogen, gelatin, chitosan, hyaluronic acid-based gels, or any combination thereof. A variety of synthetic hydrogels are known and can be used in embodiments of the systems and methods provided herein. For example, in some embodiments, one or more

hydrogels form at least part of the structural basis for three dimensional structures that are printed. In some embodiments, a hydrogel has the capacity to support growth and/or proliferation of one or more cell types, which may be dispersed within the hydrogel or added to the hydrogel after it has been printed in a three dimensional configuration.

[0088] In embodiments, a hydrogel is cross-linkable by a chemical cross-linking agent. For example, a hydrogel comprising alginate may be cross-linkable in the presence of a divalent cation such as calcium chloride (CaCl_2), a hydrogel containing chitosan may be cross-linked using a polyvalent anion such as sodium tripolyphosphate (STP), a hydrogel comprising fibrinogen may be cross-linkable in the presence of an enzyme such as thrombin, and a hydrogel comprising collagen, gelatin, agarose or chitosan may be cross-linkable in the presence of heat or a basic solution.

[0089] In some embodiments, a hydrogel comprises alginate. Alginate forms solidified colloidal gels (high water content gels, or hydrogels) when contacted with divalent cations. Any suitable divalent cation can be used to form a solidified hydrogel with an input material that comprises alginate. In the alginate ion affinity series $\text{Cd}^{2+} > \text{Ba}^{2+} > \text{Cu}^{2+} > \text{Ca}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$, Ca^{2+} is the best characterized and most used to form alginate gels (Ouwerx, C. et al., *Polymer Gels and Networks*, 1998, 6(5):393-408). Studies indicate that Ca-alginate gels form via a cooperative binding of Ca^{2+} ions by poly G blocks on adjacent polymer chains, the so-called “egg-box” model (ISP Alginates, Section 3: Algin-Manufacture and Structure, in *Alginates: Products for Scientific Water Control*, 2000, International Specialty Products: San Diego, pp. 4-7). G-rich alginates tend to form thermally stable, strong yet brittle Ca-gels, while M-rich alginates tend to form less thermally stable, weaker but more elastic gels. In some embodiments, a hydrogel comprises a depolymerized alginate.

[0090] In some embodiments, a hydrogel is cross-linkable using a free-radical polymerization reaction to generate covalent bonds between molecules. Free radicals can be generated by exposing a photoinitiator to light (often ultraviolet), or by exposing the hydrogel precursor to a chemical source of free radicals such as ammonium peroxodisulfate (APS) or potassium peroxodisulfate (KPS) in combination with N,N,N,N-Tetramethylethylenediamine (TEMED) as the initiator and catalyst respectively. Non-limiting examples of photo cross-linkable hydrogels include: methacrylated hydrogels, such as hyaluronic acid methacrylate (HAMA), gelatin

methacrylate (GEL-MA) or polyethylene (glycol) acrylate-based (PEG-Acrylate) hydrogels, which are used in cell biology due to their inertness to cells. Polyethylene glycol diacrylate (PEG-DA) is commonly used as scaffold in tissue engineering, since polymerization occurs rapidly at room temperature and requires low energy input, has high water content, is elastic, and can be customized to include a variety of biological molecules.

[0091] In embodiments, the material flow comprises a non-biodegradable polymer. In examples the input material may be a synthetic polymer, for example polyvinyl acetate (PVA). In embodiments, the material flow may comprise hyaluronic acid (HA).

[0092] In embodiments, the material flow comprises microparticles, “Microparticles” as used herein refers to immiscible particles in the range of about 0.1 μm to about 100 μm that are typically composed of a polymer, a metal, or other inorganic material. They can be symmetrical (*e.g.* spherical, cubic, etc) although this is not a requirement. Microparticles having an aspect ratio of 2:1 or greater may be considered a microrod or microfibre.

[0093] Additional Components:

[0094] Material flows in accordance with embodiments of the invention can comprise any of a wide variety of natural or synthetic polymers that support the viability of living cells, including, *e.g.*, alginate, laminin, fibrin, hyaluronic acid, poly(ethylene) glycol based gels, gelatin, chitosan, agarose, or combinations thereof. In embodiments, the subject compositions are physiologically compatible, *i.e.*, conducive to cell growth, differentiation and communication. In certain embodiments, an input material comprises one or more physiological matrix materials, or a combination thereof. By “physiological matrix material” is meant a biological material found in a native mammalian tissue. Non-limiting examples of such physiological matrix materials include: fibronectin, thrombospondin, glycosaminoglycans (GAG) (*e.g.*, hyaluronic acid, chondroitin-6-sulfate, dermatan sulfate, chondroitin-4-sulfate, or keratin sulfate), deoxyribonucleic acid (DNA), adhesion glycoproteins, and collagen (*e.g.*, collagen I, collagen II, collagen III, collagen IV, collagen V, collagen VI, or collagen XVIII).

[0095] Collagen gives most tissues tensile strength, and multiple collagen fibrils approximately 100 nm in diameter combine to generate strong coiled-coil fibers of

approximately 10 μm in diameter. Biomechanical function of certain tissue constructs is conferred via collagen fiber alignment in an oriented manner. In some embodiments, an input material comprises collagen fibrils. An input material comprising collagen fibrils can be used to create a fiber structure that is formed into a tissue construct. By modulating the diameter of the fiber structure, the orientation of the collagen fibrils can be controlled to direct polymerization of the collagen fibrils in a desired manner.

[0096] In embodiments, the plurality of cells is selected from the group comprising or consisting of a single-cell suspension, cell aggregates, cell spheroids, cell organoids, or combinations thereof. Flow materials in accordance with embodiments of the invention can incorporate any mammalian cell type, including but not limited to stem cells (e.g., embryonic stem cells, adult stem cells, induced pluripotent stem cells), germ cells, endoderm cells (e.g., lung, liver, pancreas, gastrointestinal tract, or urogenital tract cells), mesoderm cells (e.g., kidney, bone, muscle, endothelial, or heart cells), ectoderm cells (skin, nervous system, pituitary, or eye cells), stem cell-derived cells, or any combination thereof.

[0097] For example, a flow material can comprise cells from endocrine and exocrine glands including pancreas (alpha, beta, delta, epsilon, gamma), liver (hepatocyte, Kupffer, stellate, sinusoidal endothelial cells, cholangiocytes), thyroid (Follicular cells), pineal gland (pinealocytes), pituitary gland (somatotropes, Lactotropes, gonadotropes, corticotropes, and thyrotropes), thymus (thymocytes, thymic epithelial cells, thymic stromal cells), adrenal gland (cortical cells, chromaffin cells), ovary (granulosa cells), testis (Leydig cells), gastrointestinal tract (enteroendocrine cells - intestinal, gastric, pancreatic), fibroblasts, chondrocytes, meniscus fibrochondrocytes, bone marrow stromal (stem) cells, embryonic stem cells, mesenchymal stem cells, induced pluripotent stem cells, differentiated stem cells, tissue-derived cells, smooth muscle cells, skeletal muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, myoblasts, chondroblasts, osteoblasts, osteoclasts, and any combinations thereof.

[0098] Cells can be obtained from donors from the same species as the recipient (allogenic), from a different species to the recipient (xenogeneic) or from recipients (autologous). Specifically, in embodiments, cells can be obtained from a suitable donor, such as a human or animal, or from the subject into which the cells are to be implanted. Mammalian species include, but are not limited to, humans, monkeys, dogs, cows,

horses, pigs, sheep, goats, cats, mice, rabbits, and rats. In one embodiment, the cells are human cells. In other embodiments, the cells can be xenogeneic, *e.g.*, derived from animals such as dogs, cats, horses, monkeys, or any other mammal.

[0099] In some embodiments, the at least one biological material comprises a plurality of cells expressing/secreting one or more endogenous biologically active agent(s), *e.g.*, insulin, glucagon, ghrelin, pancreatic polypeptide, Factor VII, Factor VIII, Factor IX, alpha-1-antitrypsin, an angiogenic factor, a growth factor, a hormone, an antibody, an enzyme, a protein, an exosome, and the like. Discussed herein, endogenous biologically active agents comprise those agents that the cell naturally produces in a biological context (*e.g.*, insulin release in response to elevated glucose concentrations). An endogenous biologically active agent can constitute a therapeutic agent in the context of the present disclosure.

[0100] In some embodiments, a flow material can comprise genetically engineered cells that secrete specific factors. It is within the scope of this disclosure that a plurality of cells as discussed above can comprise, in embodiments, engineered cells (*e.g.*, genetically engineered cells) that secrete specific factors. Cells can also be from established cell culture lines, or can be cells that have undergone genetic engineering and/or manipulation to achieve a desired genotype or phenotype. In some embodiments, pieces of tissue can also be used, which may provide a number of different cell types within the same structure.

[0101] Genetic engineering techniques applicable to the present disclosure can include but are not limited to recombinant DNA (rDNA) technology (Stryjewska et al., Pharmacological Reports. 2013; 65: 1075), cell-engineering based on use of targeted nucleases (*e.g.*, meganuclease, zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), clustered regularly interspaced short palindromic repeat-associated nuclease Cas9 (CRISPR-Cas9), etc. (Lim et al., Nature Communications. 2020; 11: 4043; Stoddard BL, Structure. 2011; 19(1): 7-15; Gaj et al., Trends Biotechnol. 2013; 31(7): 397-405; Hsu et al., Cell. 2014; 157(6): 1262; Miller et al., Nat Biotechnol. 2010; 29(2): 143-148), cell-engineering based on use of site-specific recombination using recombinase systems (*e.g.*, Cre-Lox) (Osborn et al., Mol Ther. 2013; 21(6): 1151-1159; Hockemeyer et al., Nat Biotechnol. 2009; 27(9): 851-857; Uhde-Stone et al., RNA. 2014; 20(6): 948-955; Ho et al., Nucleic Acids Res. 2015;

43(3): e17; Sengupta et al., Journal of Biological Engineering. 2017; 11(45): 1-9), and the like. In some embodiments, some combination of the above-mentioned techniques for cell-engineering may be used.

[0102] Encompassed by the present disclosure are engineered cells capable of producing one or more therapeutic agents, including but not limited to proteins, peptides, nucleic acids (e.g., DNA, RNA, mRNA, siRNA, miRNA, nucleic acid analogs), peptide nucleic acids, aptamers, antibodies or fragments or portions thereof, antigens or epitopes, hormones, hormone antagonists, growth factors or recombinant growth factors and fragments and variants thereof, cytokines, enzymes, antibiotics or antimicrobial compounds, anti-inflammation agent, antifungals, antivirals, toxins, prodrugs, small molecules, drugs (e.g., drugs, dyes, amino acids, vitamins, antioxidants) or any combination thereof.

EXAMPLE

[0103] An accumulated dataset of 660 images with cell, aggregate, and bead annotations gathered from pre-, during-, and post-print phases of the bioprinting pipeline was gathered. The choice for model architecture was an adapted Torchvision implementation of a Mask R-CNN instance segmentation model with a ResNet-50-FPN backbone, pre-trained on the Common Images in COntext (COCO) dataset. The images were fed into the model by tiling through a moving window slid over each image. Varied on-the-fly data augmentation methods were utilized to improve model generalizability including random rotation, scale, shift, color jitter altering brightness, contrast, saturation, and hue, addition of Gaussian noise, random perspective transform, random CLAHE and Gamma transform, and random image sharpening and blurring. For model optimization, the stochastic gradient descent with a step decay policy was used. The method hyper-parameters were adjusted and fine-tuned per down-stream tasks. FIGS. 22A-22F, 23A-23F, 24A-24P, 25A-25F, and 26 show sample segmentation and quantification results in various print stages. Following is a description of those results.

- FIGS. 22A-22F (Pre-Print-Cell):
 - These Figures show print cell quantification examples delineating aggregates, cells, and fragments. The cell quality features are calculated

based on the annotations and are stored. FIGS. 22B and 22E are bar charts corresponding to FIGS. 22A and 22D, respectively, and showing distributions of values for area, diameter, perimeter, circularity, smoothness, and compactness, which are among the characteristics discussed previously.

- FIGS. 23A-23F (During-Print-Cell):
 - These Figures show cell quantification examples delineating fiber, aggregates, and cells during print, using a camera trained on a printhead nozzle. The cell quality features are calculated based on the annotations and are stored. FIGS. 23B and 23E show bar charts, corresponding to FIGS. 23A and 23D, respectively, and showing distributions of values for area, diameter, perimeter, circularity, smoothness, and compactness, which are among the characteristics discussed previously.
- FIGS. 24A-24P (Post-Print-Cell):
 - For post-print measurement, the same aggregate and cell detection and morphology as in pre- and during-print analysis are provided. In the post-print instance, allowance also is made for measurement of linear density, defined as an expected number of aggregates per unit length of fiber. To avoid the added complexity of regions where fibers overlap or meet the image edge, "Segment" and "Midline" annotations are provided, to allow for targeted measurement densities of aggregates. FIGS. 24B and 24C, 24F and 24G, 24J and 24K, and 24N and 24O show bar charts, corresponding to FIGS. 24A, 24D, 24I, and 24M respectively, and showing distributions of values for area, diameter, perimeter, circularity, smoothness, and compactness, which are among the characteristics discussed previously, as well as core diameter.
- FIGS. 25A-25F (During-Print-Bead):
 - These Figures show during-print bead quantification examples delineating beads in a printed fiber. FIGS. 25B and 25E show bar charts, corresponding to FIGS. 25A and 25D, respectively, and showing distributions of values for

area, diameter, perimeter, circularity, smoothness, and compactness, which are among the characteristics discussed previously.

- FIG. 26 (Software-Integration):
 - This Figure shows a bead quantification model integrated in Aspect's print analysis software. The software depicts bead quantification and relative distribution across the length of the print, as shown on various plots. Fibre diameter across the length of the print is also shown. Several video feeds of the print process from the perspectives of the nozzle and print surface can be seen, as well as a representation of the toolpath that defined the printed pattern.

[0104] All patent and non-patent references cited in the present specification are hereby incorporated by reference in their entirety and for all purposes.

[0105] Aspects of the present invention are set out in the following clauses:

CLAUSE 1. A bioprinting system comprising:

a bioprinter, optionally comprising one or more reservoirs, a printhead, and a print surface on which the bioprinting is dispensed;

an optical system to collect imaging data of a plurality of cells within a material flow comprising a cross-linkable material before, during and/or after passage of the plurality of cells through the bioprinting system; and

a computing system to process the imaging data to determine a quantity, distribution, concentration, and/or morphology of the plurality of cells;

wherein the computing system comprises a machine learning system that:

compares the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system to user-established cell morphologies corresponding to physical properties of cells and/or cell aggregates within a predetermined tolerance, and/or

compares the quantity, distribution, concentration, and/or morphology of the plurality of cells at one of before, during and/or after passage of the plurality of cells through the bioprinting system to the quantity, distribution, concentration, and/or

morphology of the plurality of cells at another of before, during and/or after the plurality of cells pass through the bioprinting system, and

records the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system, and results of the comparison.

CLAUSE 2. The system of clause 1, wherein the cross-linkable material is cross-linked to form a printed microcapsule or fiber; preferably wherein the cross-linkable material is cross-linked to form a printed fiber.

CLAUSE 3. The system of clause 1, wherein the machine learning system performs image segmentation on the imaging data to determine the cell morphology.

CLAUSE 4. The system of clause 3, wherein the machine learning system comprises a neural network selected from the group consisting of a convolutional neural network (CNN), a fully convolutional network (FCN), a region-based CNN (R-CNN), a Mask R-CNN, a you only look once (YOLO) based model, a transformer-based instance segmentation model, a flow estimation network selected from the group consisting of flow estimation networks in the FlowNet family, Recurrent All-Pairs Field Transforms (RAFT), and Self-Teaching Multi-Frame Unsupervised RAFT with Full-Image Warping (SMURF) to perform the image segmentation on the imaging data.

CLAUSE 5. The system of clause 3 or clause 4, wherein the image segmentation comprises panoptic segmentation and/or semantic segmentation and/or instance segmentation.

CLAUSE 6. The system of any preceding clause, wherein, responsive to the comparison, the computing system adjusts one or more parameters of the bioprinting system to alter a flow of the plurality of cells through the printhead.

CLAUSE 7. The system of any preceding clause, wherein the image data comprises a plurality of image frames of portions of a printed fiber, and the computing system assembles the plurality of image frames to provide an image of an entire length of the printed fiber.

CLAUSE 8. The system of clause 6, wherein the computing system uses the image of the entire length of the printed fiber to obtain a measurement of a number of cells inside the printed fiber.

CLAUSE 9. The system of clause 7, wherein the computing system uses the measurement of the number of cells inside the printed fiber, and/or a comparison of the quantity, distribution, concentration, and/or morphology of the cells before, during, and/or after passage of the plurality of cells through the bioprinting system to calculate a therapeutic dose.

CLAUSE 10. The system of any preceding clause, wherein the printhead has one or more transparent channels, wherein the optical system collects imaging data of the plurality of cells through at least one of the one or more transparent channels, preferably wherein the printhead comprises a transparent nozzle or dispensing channel.

CLAUSE 11. The system of any preceding clause, wherein the optical system comprises one or more imaging devices selected from the group consisting of microscopes, still cameras, and video cameras, the optical system positioned at different positions in the bioprinting system to collect the imaging data before, during, and/or after passage of the plurality of cells through the bioprinting system.

CLAUSE 12. The system of clause 10, wherein the optical system comprises one or more imaging devices positioned at each of the input reservoir, the printhead, and the print surface.

CLAUSE 13. The system of any preceding clause, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and performs the comparing and recording on the in focus imaging data.

CLAUSE 14. The system of any preceding clause, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and improves the focus on the out of focus imaging data to provide further in focus imaging data, so as to performs the comparing and recording on both the in focus imaging data and the further in focus imaging data.

CLAUSE 15. The system of any preceding clause, wherein the imaging data includes a series of Z-stack images for a printed fiber, wherein the machine learning model detects in-focus cells and aggregates in different Z-stack layers of the printed fiber, such that a change in focus of the optical system to image different Z-stack layers enables quantification of the in-focus cells and aggregates in the different Z-stack layers .

CLAUSE 16. The system of clause 1, wherein the printhead comprises a printhead selected from the group consisting of an extrusion printhead, a co-extrusion printhead, an inkjet printhead, an electrowriting printhead, and a microfluidic printhead.

CLAUSE 17. The system of any preceding clause, further comprising determining a quality of the plurality of cells responsive to determination of quantity, distribution, concentration, and/or morphology.

CLAUSE 18. The system of any preceding clause, wherein the morphology comprises at least one characteristic selected from the group consisting of cell count, area, perimeter, ellipsoidal volume, circularity, smoothness, compactness, circle diameter, mean diameter, minimum diameter, maximum diameter, mean intensity, and cell density in fiber.

CLAUSE 19. A bioprinting method comprising:

collecting imaging data of a plurality of cells within a material flow comprising a cross-linkable material before, during and/or after passage of the plurality of cells through a bioprinting system, the bioprinting system comprising a bioprinter, optionally comprising one or more input reservoirs, a printhead, and a print surface on which the bioprinting is dispensed;

processing the imaging data with a computer to determine a quantity, distribution, concentration, and/or morphology of the plurality of cells, the processing comprising;

comparing, with a machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system to user-established cell morphologies corresponding to physical properties of cells and/or cell aggregates within a predetermined tolerance, and/or

comparing, with the machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells at one of before, during and/or after cross-linking in the bioprinting system to the quantity, distribution, concentration, quality, and/or morphology of the plurality of cells at another of before, during and/or after passage of the plurality of cells through the bioprinting system, and

recording, with the machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system, and results of the comparison.

CLAUSE 20. The method of clause 19, wherein the cross-linkable material is cross-linked to form a printed microcapsule or fiber; preferably wherein the cross-linkable material is cross-linked to form a printed fiber.

CLAUSE 21. The method of clause 19, further comprising performing, with the machine learning system, image segmentation on the imaging data to determine the cell morphology.

CLAUSE 22. The method of clause 19, wherein the machine learning system comprises a neural network selected from the group consisting of a convolutional neural network (CNN), a region-based CNN (R-CNN), a Mask R-CNN, a you only look once (YOLO) based model, a transformer-based instance segmentation model, a flow estimation network selected from the group consisting of flow estimation networks in the FlowNet family, Recurrent All-Pairs Field Transforms (RAFT), and Self-Teaching Multi-Frame Unsupervised RAFT with Full-Image Warping (SMURF) to perform the image segmentation on the imaging data.

CLAUSE 23. The method of clause 21 or clause 22, wherein the image segmentation comprises panoptic segmentation and/or semantic segmentation and/or instance segmentation.

CLAUSE 24. The method of any of clauses 19 to 23, further comprising, responsive to one or both of the comparing, adjusting, with the computer, one or more parameters of the bioprinting system to alter a flow of the cross-linkable material through the printhead.

CLAUSE 25. The method of any of clauses 19 to 24, wherein the image data comprises a plurality of image frames of portions of a printed fiber, the method further comprising assembling, with the computer, the plurality of image frames to provide an image of an entire length of the printed fiber.

CLAUSE 26. The method of clause 25, further comprising obtaining, with the computer, a measurement of a number of cells inside the printed fiber using the image of the entire length of the printed fiber.

CLAUSE 27. The method of clause 26, further comprising, responsive to the obtaining, and/or comparing the quantity, distribution, concentration, quality, and/or morphology of the cells before, during, and/or after cross-linking in the bioprinting system, calculating a therapeutic dose.

CLAUSE 28. The method of clause 19, wherein the printhead has one or more transparent channels, the method further comprising collecting, with the optical system, imaging data of the plurality of cells through at least one of the one or more transparent channels, preferably wherein the printhead comprises a transparent nozzle or dispensing channel.

CLAUSE 29. The method of clause 19, wherein collecting the image data comprises providing, in the optical system, one or more imaging devices selected from the group consisting of microscopes, still cameras, and video cameras, and positioning the optical system at different positions in the bioprinting system to collect the imaging data before, during, and/or after passage of the plurality of cells through the bioprinting system.

CLAUSE 30. The method of clause 29, wherein the positioning comprises positioning one or more imaging devices at each of the input reservoir, the printhead, and the print surface.

CLAUSE 31. The method of any of clauses 19 to 30, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and performs the comparing and recording on the in focus imaging data.

CLAUSE 32. The method of any of clauses 19 to 31, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and improves the focus on the out of focus imaging data to provide further in focus imaging data, so as to performs the comparing and recording on both the in focus imaging data and the further in focus imaging data.

CLAUSE 33. The method of any of clauses 19 to 32, wherein the imaging data includes a series of Z-stack images for a printed fiber, wherein the machine learning model detects in-focus cells and aggregates in different Z-stack layers of the printed fiber, such that a change in focus of the optical system to image different Z-stack layers enables quantification of the in-focus cells and aggregates in the different Z-stack layers.

CLAUSE 34. The method of any of clauses 19 to 33, wherein the printhead comprises a printhead selected from the group consisting of an extrusion printhead, a co-extrusion printhead, an inkjet printhead, and a microfluidic printhead.

CLAUSE 35. The method of any of clauses 19 to 34, further comprising determining a quality of the plurality of cells responsive to determination of quantity, distribution, concentration, and/or morphology.

CLAUSE 36. The method of any of clauses 19 to 35, wherein the morphology comprises at least one characteristic selected from the group consisting of cell count, area, perimeter, ellipsoidal volume, circularity, smoothness, compactness, circle diameter, mean diameter, minimum diameter, maximum diameter, mean intensity, and cell density in fiber.

CLAIMS

1. A bioprinting system comprising:
 - a bioprinter, optionally comprising one or more reservoirs, a printhead, and a print surface on which the bioprinting is dispensed;
 - an optical system to collect imaging data of a plurality of cells within a material flow comprising a cross-linkable material before, during and/or after passage of the plurality of cells through the bioprinting system; and
 - a computing system to process the imaging data to determine a quantity, distribution, concentration, and/or morphology of the plurality of cells;wherein the computing system comprises a machine learning system that:
 - compares the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system to user-established cell morphologies corresponding to physical properties of cells and/or cell aggregates within a predetermined tolerance, and/or
 - compares the quantity, distribution, concentration, and/or morphology of the plurality of cells at one of before, during and/or after passage of the plurality of cells through the bioprinting system to the quantity, distribution, concentration, and/or morphology of the plurality of cells at another of before, during and/or after the plurality of cells pass through the bioprinting system, and
 - records the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system, and results of the comparison.
2. The system of claim 1, wherein the cross-linkable material is cross-linked to form a printed microcapsule or fiber; preferably wherein the cross-linkable material is cross-linked to form a printed fiber.
3. The system of claim 1, wherein the machine learning system performs image segmentation on the imaging data to determine the cell morphology.
4. The system of claim 3, wherein the machine learning system comprises a neural network selected from the group consisting of a convolutional neural network (CNN), a fully convolutional network (FCN), a region-based CNN (R-CNN), a Mask R-CNN, a you only look once (YOLO) based model, a transformer-based instance segmentation model, a flow estimation network selected from the group consisting of flow estimation networks in the

FlowNet family, Recurrent All-Pairs Field Transforms (RAFT), and Self-Teaching Multi-Frame Unsupervised RAFT with Full-Image Warping (SMURF) to perform the image segmentation on the imaging data.

5. The system of claim 3 or claim 4, wherein the image segmentation comprises panoptic segmentation and/or semantic segmentation and/or instance segmentation.

6. The system of any preceding claim, wherein, responsive to the comparison, the computing system adjusts one or more parameters of the bioprinting system to alter a flow of the plurality of cells through the printhead.

7. The system of any preceding claim, wherein the image data comprises a plurality of image frames of portions of a printed fiber, and the computing system assembles the plurality of image frames to provide an image of an entire length of the printed fiber.

8. The system of claim 6, wherein the computing system uses the image of the entire length of the printed fiber to obtain a measurement of a number of cells inside the printed fiber.

9. The system of claim 7, wherein the computing system uses the measurement of the number of cells inside the printed fiber, and/or a comparison of the quantity, distribution, concentration, and/or morphology of the cells before, during, and/or after passage of the plurality of cells through the bioprinting system to calculate a therapeutic dose.

10. The system of any preceding claim, wherein the printhead has one or more transparent channels, wherein the optical system collects imaging data of the plurality of cells through at least one of the one or more transparent channels, preferably wherein the printhead comprises a transparent nozzle or dispensing channel.

11. The system of any preceding claim, wherein the optical system comprises one or more imaging devices selected from the group consisting of microscopes, still cameras, and video cameras, the optical system positioned at different positions in the bioprinting system to collect the imaging data before, during, and/or after passage of the plurality of cells through the bioprinting system.

12. The system of claim 10, wherein the optical system comprises one or more imaging devices positioned at each of the input reservoir, the printhead, and the print surface.

13. The system of any preceding claim, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and performs the comparing and recording on the in focus imaging data.

14. The system of any preceding claim, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and improves the focus on the out of focus imaging data to provide further in focus imaging data, so as to performs the comparing and recording on both the in focus imaging data and the further in focus imaging data.

15. The system of any preceding claim, wherein the imaging data includes a series of Z-stack images for a printed fiber, wherein the machine learning model detects in-focus cells and aggregates in different Z-stack layers of the printed fiber, such that a change in focus of the optical system to image different Z-stack layers enables quantification of the in-focus cells and aggregates in the different Z-stack layers .

16. The system of claim 1, wherein the printhead comprises a printhead selected from the group consisting of an extrusion printhead, a co-extrusion printhead, an inkjet printhead, an electrowriting printhead, and a microfluidic printhead.

17. The system of any preceding claim, further comprising determining a quality of the plurality of cells responsive to determination of quantity, distribution, concentration, and/or morphology.

18. The system of any preceding claim, wherein the morphology comprises at least one characteristic selected from the group consisting of cell count, area, perimeter, ellipsoidal volume, circularity, smoothness, compactness, circle diameter, mean diameter, minimum diameter, maximum diameter, mean intensity, and cell density in fiber.

19. A bioprinting method comprising:

collecting imaging data of a plurality of cells within a material flow comprising a cross-linkable material before, during and/or after passage of the plurality of cells through a bioprinting system, the bioprinting system comprising a bioprinter, optionally comprising one or more input reservoirs, a printhead, and a print surface on which the bioprinting is dispensed;

processing the imaging data with a computer to determine a quantity, distribution, concentration, and/or morphology of the plurality of cells, the processing comprising;

comparing, with a machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system to user-established cell morphologies corresponding to physical properties of cells and/or cell aggregates within a predetermined tolerance, and/or

comparing, with the machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells at one of before, during and/or after cross-linking in the bioprinting system to the quantity, distribution, concentration, quality, and/or morphology of the plurality of cells at another of before, during and/or after passage of the plurality of cells through the bioprinting system, and

recording, with the machine learning system, the quantity, distribution, concentration, and/or morphology of the plurality of cells before, during and/or after passage of the plurality of cells through the bioprinting system, and results of the comparison.

20. The method of claim 19, wherein the cross-linkable material is cross-linked to form a printed microcapsule or fiber; preferably wherein the cross-linkable material is cross-linked to form a printed fiber.

21. The method of claim 19, further comprising performing, with the machine learning system, image segmentation on the imaging data to determine the cell morphology.

22. The method of claim 19, wherein the machine learning system comprises a neural network selected from the group consisting of a convolutional neural network (CNN), a region-based CNN (R-CNN), a Mask R-CNN, a you only look once (YOLO) based model, a transformer-based instance segmentation model, a flow estimation network selected from the group consisting of flow estimation networks in the FlowNet family, Recurrent All-Pairs Field Transforms (RAFT), and Self-Teaching Multi-Frame Unsupervised RAFT with Full-Image Warping (SMURF) to perform the image segmentation on the imaging data.

23. The method of claim 21 or claim 22, wherein the image segmentation comprises panoptic segmentation and/or semantic segmentation and/or instance segmentation.

24. The method of any of claims 19 to 23, further comprising, responsive to one or both of the comparing, adjusting, with the computer, one or more parameters of the bioprinting system to alter a flow of the cross-linkable material through the printhead.

25. The method of any of claims 19 to 24, wherein the image data comprises a plurality of image frames of portions of a printed fiber, the method further comprising assembling, with the computer, the plurality of image frames to provide an image of an entire length of the printed fiber.

26. The method of claim 25, further comprising obtaining, with the computer, a measurement of a number of cells inside the printed fiber using the image of the entire length of the printed fiber.

27. The method of claim 26, further comprising, responsive to the obtaining, and/or comparing the quantity, distribution, concentration, quality, and/or morphology of the cells before, during, and/or after cross-linking in the bioprinting system, calculating a therapeutic dose.

28. The method of claim 19, wherein the printhead has one or more transparent channels, the method further comprising collecting, with the optical system, imaging data of the plurality of cells through at least one of the one or more transparent channels, preferably wherein the printhead comprises a transparent nozzle or dispensing channel.

29. The method of claim 19, wherein collecting the image data comprises providing, in the optical system, one or more imaging devices selected from the group consisting of microscopes, still cameras, and video cameras, and positioning the optical system at different positions in the bioprinting system to collect the imaging data before, during, and/or after passage of the plurality of cells through the bioprinting system.

30. The method of claim 29, wherein the positioning comprises positioning one or more imaging devices at each of the input reservoir, the printhead, and the print surface.

31. The method of any of claims 19 to 30, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and performs the comparing and recording on the in focus imaging data.

32. The method of any of claims 19 to 31, wherein the machine learning system differentiates between in focus imaging data and out of focus imaging data, and improves the focus on the out of focus imaging data to provide further in focus imaging data, so as to performs the comparing and recording on both the in focus imaging data and the further in focus imaging data.

33. The method of any of claims 19 to 32, wherein the imaging data includes a series of Z-stack images for a printed fiber, wherein the machine learning model detects in-focus cells and aggregates in different Z-stack layers of the printed fiber, such that a change in focus of the optical system to image different Z-stack layers enables quantification of the in-focus cells and aggregates in the different Z-stack layers.

34. The method of any of claims 19 to 33, wherein the printhead comprises a printhead selected from the group consisting of an extrusion printhead, a co-extrusion printhead, an inkjet printhead, and a microfluidic printhead.

35. The method of any of claims 19 to 34, further comprising determining a quality of the plurality of cells responsive to determination of quantity, distribution, concentration, and/or morphology.

36. The method of any of claims 19 to 35, wherein the morphology comprises at least one characteristic selected from the group consisting of cell count, area, perimeter, ellipsoidal volume, circularity, smoothness, compactness, circle diameter, mean diameter, minimum diameter, maximum diameter, mean intensity, and cell density in fiber.