be decoupled from a resolution when using a holographic printing projection such as the one used by the 3D holographic printing methods and systems provided herein. A printing speed may be volume dependent, and the print volume may be dictated by static optical components, when using the 3D holographic printing methods and systems provided herein.

[0014] Thus far, the field of tissue engineering may have failed to produce responsive, biologically-active vascularized tissues that behave as native tissue in large enough structures be useful for transplantation, systems-integrative drug testing, and/or development of biologic therapeutics, such as human antibodies.

[0015] Two-photon lasers may provide a low cellular toxicity profile based on: 1) pinpoint sites of two-photon excitation that fall off as a function of the square of the distance from the focal point in the x, y, and z dimensions such that peak laser power may not be spread throughout the sample; 2) rapid x, y scanning of the excitation point minimizing the time a cell may be exposed to the peak laser power at the point of excitation; and 3) ultra-short, sub-picosecond pulse widths that may allow for gaps in time where few to no photons are engaging the material or cells.

[0016] In one aspect, the present disclosure refers to a method for printing a three-dimensional (3D) biological material, comprising: providing a media chamber comprising a medium comprising one or more polymer precursors; and directing at least one energy beam to said medium in said media chamber along at least one energy beam path that is patterned into a simultaneous 3D projection in accordance with computer instructions for printing said 3D biological material in computer memory, to form at least a portion of said 3D biological material comprising a polymer formed from said one or more polymer precursors. In an example, the present disclosure provides a method for printing a three-dimensional (3D) biological material, comprising: (a) providing a media chamber comprising a medium comprising (i) a plurality of cells and (ii) one or more polymer precursors; and (b) directing at least one energy beam to the medium in the media chamber along at least one energy beam path that is patterned into a 3D projection in accordance with computer instructions for printing the 3D biological material in computer memory, to form at least a portion of the 3D biological material comprising (i) at least a subset of the plurality of cells, and (ii) a polymer formed from the one or more polymer precursors.

[0017] In some examples, the biological material develops into a biologically functional tissue. In some examples, the method further comprises prior to (b), generating a point-cloud representation or lines-based representation of the 3D biological material in computer memory, and using the point-cloud representation or lines-based representation to generate the computer instructions. In some examples, the method further comprises converting the point-cloud representation or lines-based representation into an image.

[0018] In some examples, the image is projected in a holographic manner. In some examples, the image is deconstructed and reconstructed prior to projection in a holographic manner. In some examples, the point-cloud representation or the lines-based representation comprises multi-dimensional structural elements corresponding to the 3D biological material. In some examples, the point-cloud representation or the lines-based representation comprises structural elements in two dimensions, wherein the structural elements are associated with tissue function and/or cellular segregation. In some examples, point-cloud representation or the lines-based representation comprises structural elements in three dimensions, wherein the structural elements are associated with tissue function and/or cellular segregation.

[0019] In some examples, the at least one energy beam comprises coherent light. In some examples, the at least one energy beam is generated by a laser. In some examples, the at least one energy beam is phase modulated. In some examples, the one or more polymer precursors comprise at least two different polymeric precursors.

[0020] In some examples, the method further comprises repeating (b) along one or more additional energy beam paths to form at least another portion of the 3D biological material. In some examples, the at least another portion of the 3D biological material is linked to the 3D biological material formed in (b). In some examples, the at least another portion of the 3D biological material is not linked to the 3D biological material formed in (b). In some examples, (b) further comprises directing at least two energy beams to the medium in the media chamber along at least two energy beam paths in accordance with the computer instructions, to permit multiple portions of the medium in the media chamber to simultaneously form at least a portion of the 3D biological material.

[0021] In some examples, the at least two energy beams are of identical wavelengths. In some examples, the at least two energy beams are of different wavelengths. In some examples, the at least the portion of the 3D biological material comprises microvasculature for providing one or more nutrients to the plurality of cells. In some examples, the microvasculature is a blood microvasculature, a lymphatic microvasculature, or any combination thereof. In some examples, the microvasculature has a cross-section from about 1 μm to about 20 μm. In some examples, the 3D biological material has a thickness or diameter from about 100 μm to about 5 cm.

[0022] In some examples, the medium further comprises a plurality of beads, and wherein in (b) the at least the portion of the 3D biological material, as formed, includes the plurality of beads. In some examples, the beads further comprise signaling molecules or proteins. In some examples, the signaling molecules or proteins promote formation of the 3D biological material to permit organ function. In some examples, the at least the portion of the 3D biological material comprises a cell-

containing scaffold, which cell-containing scaffold comprises at least a subset of the plurality of cells. In some examples, the 3D biological material comprises cell-containing scaffolds.

[0023] In some examples, the cell-containing scaffolds are coupled together. In some examples, the cell-containing scaffolds are cohesively or mechanically coupled together. In some examples, the cell-containing scaffolds are mechanically coupled together through one or more members selected from the group consisting of joints, hinges, locking joints and hinges, Velcro-like elements, springs, coils, points of stretch, interlocking loops, sockets, gears, ratchets, screw, and chain links. In some examples, the cell-containing scaffolds comprise a network, wherein the network comprises a plurality of strands. In some examples, the plurality of strands forms a mesh structure, a grid structure, a sheet structure, or a tube structure. In some examples, the individual strands of the plurality of strands have a thickness from about 0.1 nm to about 5 cm.

[0024] In some examples, subsequent to (b), the at least another portion of the 3D biological material is formed within the at least the portion of the 3D biological material. In some examples, the computer instructions comprise a set of images corresponding to the 3D biological material. In some examples, the computer instructions direct adjustment of at least (i) one or more parameters of the at least one energy beam as a function of time during formation of the 3D biological material, and/or (ii) location of a stage upon which the 3D biological material is formed.

[0025] In some examples, the method further comprises subjecting at least a portion of the at least the subset of the plurality of cells to differentiation to form the cells of the at least two different types. In some examples, the at least the subset of the plurality of cells comprises cells of at least two different types. In some examples, in (b), the plurality of cells comprises the cells of the at least two different types. In some examples, the at least one energy beam is a multi-photon energy beam. In some examples, the multi-photon energy beam is a two-photon energy beam.

[0026] In another example, the present disclosure provides a method of printing a three-dimensional (3D) biological material, comprising: (a) providing a media chamber comprising a first medium, wherein the first medium comprises a first plurality of cells and a first polymeric precursor; (b) directing at least one energy beam to the first medium in the media chamber along at least one energy beam path in accordance with computer instructions for printing the 3D biological material in computer memory, to subject at least a portion of the first medium in the media chamber to form a first portion of the 3D biological material; (c) providing a second medium in the media chamber, wherein the second medium comprises a second plurality of cells and a second polymeric precursor, wherein the second plurality of cells is of a different type than the first plurality of cells; and (d) directing at least one energy beam to the second medium in the media chamber along at least one energy beam path in accordance with the computer instructions, to subject at least a

portion of the second medium in the media chamber to form at least a second portion of the 3D biological material.

[0027] In some examples, the biological material is a biologically functional tissue. In some examples, the method further comprises, subsequent to (d), removing a remainder of the first medium from the media chamber to leave the first portion of the 3D biological material in the media chamber. In some examples, the first portion of the 3D biological material left in the medium chamber is removably fixed to the media chamber. In some examples, the method further comprises prior to (b), generating a point-cloud representation or lines-based representation of the 3D biological material in computer memory, and using the point-cloud representation or lines-based representation to generate the computer instructions.

[0028] In some examples, the method further comprises converting the point-cloud representation or lines-based representation into an image or image set that is used to spatially modulate an incoming coherent light source such that biological structures are projected in one dimension. In some examples, the method further comprises converting the point-cloud representation or lines-based representation into an image or image set that is used to spatially modulate an incoming coherent light source such that biological structures are projected in two dimensions. In some examples, the method further comprises converting the point-cloud representation or lines-based representation into an image or image set that is used to spatially modulate an incoming coherent light source such that biological structures are projected in three dimensions.

[0029] In some examples, the method further comprises converting the point-cloud representation or lines-based representation into an image or image set that is used to spatially modulate at least one incoming coherent light source such that biological structures are projected in a mixture of one-dimensional, two-dimensional and/or three-dimensional structures. In some examples, the image or image set is projected in a holographic manner. In some examples, the image or image set is deconstructed and reconstructed into partial elements or representative structures prior to projection in a holographic manner.

[0030] In some examples, the point-cloud representation or the lines-based representation comprises multi-dimensional structural elements corresponding to the 3D biological material. In some examples, the point-cloud representation or the lines-based representation comprises structural elements in two dimensions, wherein the structural elements are associated with tissue function and/or cellular segregation. In some examples, the point-cloud representation or the lines-based representation comprises structural elements in three dimensions, wherein the structural elements are associated with tissue function and/or cellular segregation.

[0031] In some examples, the at least one energy beam comprises coherent light. In some examples, the at least one energy beam is generated by a laser. In some examples, the at least one energy beam is phase modulated. In some examples, the at least one energy beam is phase modulated and raster-scanned throughout the sample medium. In some examples, the at least a portion of the 3D biological material comprises microvasculature for providing one or more nutrients to the plurality of cells. In some examples, the microvasculature is a blood microvasculature, a lymphatic microvasculature, or any combination thereof. In some examples, the microvasculature has a cross-section from about 1 μm to about 20 μm. In some examples, the 3D biological material has a thickness or diameter from about 100 μm to about 5 cm.

[0032] In some examples, the first medium and/or the second medium further comprise a plurality of beads, and wherein in (b) the at least the portion of the 3D biological material, as formed, includes the plurality of beads. In some examples, the beads further comprise signaling molecules or proteins. In some examples, the signaling molecules or the proteins promote formation of the 3D biological material to permit organ function. In some examples, the 3D biological material is printed in a time period of at most about 350 hours. In some examples, the 3D biological material is printed in a time period of at most about 72 hours. In some examples, the 3D biological material is printed in a time period of at most about 12 hours.

[0033] In some examples, the at least the portion of the 3D biological material comprises a cell-containing scaffold, which cell-containing scaffold comprises at least a subset of the plurality of cells. In some examples, the 3D biological material, as formed, includes a plurality of cell-containing scaffolds. In some examples, the plurality of cell-containing scaffolds is coupled together. In some examples, the plurality of cell-containing scaffolds are coupled together to form a cohesive structure. In some examples, the plurality of the cell-containing scaffolds is mechanically coupled together. In some examples, the plurality of cell-containing scaffolds are mechanically coupled together through one or more members selected from the group consisting of joints, hinges, locking joints and hinges, Velcro-like elements, springs, coils, points of stretch, interlocking loops, sockets, gears, ratchets, screw, and chain links.

[0034] In some examples, the cell-containing scaffolds comprise a network, wherein the network comprises a plurality of strands. In some examples, the plurality of strands forms a mesh structure, a grid structure, a sheet structure, or a tube structure. In some examples, the plurality of strands has a thickness from about 0.1 nm to about 5 cm. In some examples, subsequent to (d), the at least another portion of the 3D biological material is formed within the first portion of the 3D biological material and/or the second portion of the 3D biological material. In some examples, the first medium and/or said second medium further comprise glutathione or a functional variant thereof.

[0035] In another example, the present disclosure provides a system for printing a three-dimensional (3D) biological material, comprising: (a) a media chamber configured to contain a medium comprising a plurality of cells comprising cells of at least two different types and one or more polymer precursors; (b) at least one energy source configured to direct at least one energy beam to the media chamber; and (c) one or more computer processors operatively coupled to the at least one energy source, wherein the one or more computer processors are individually or collectively programmed to (i) receive computer instructions for printing the 3D biological material from computer memory; and (ii) direct the at least one energy source to direct the at least one energy beam to the medium in the media chamber along at least one energy beam path in accordance with the computer instructions, to subject at least a portion of the polymer precursors to form at least a portion of the 3D biological material.

[0036] In some examples, the one or more computer processors are individually or collectively programmed to generate a point-cloud representation or lines-based representation of the 3D biological material in computer memory, and use the point-cloud representation or lines-based representation to generate the computer instructions for printing the 3D biological material in computer memory. In some examples, the one or more computer processors are individually or collectively programmed to convert the point-cloud representation or lines-based representation into an image. In some examples, the one or more computer processors are individually or collectively programmed to project the image in a holographic manner.

[0037] In some examples, the at least one energy source is a plurality of energy sources. In some examples, the plurality of energy sources directs a plurality of the at least one energy beam. In some examples, the at least one energy source is a laser. In some examples, the at least one energy source is derived from a coherent light source. In some examples, the coherent light source comprises a wavelength from about 300 nm to about 5 mm. In some examples, the one or more computer processors are individually or collectively programmed to direct the at least one energy source to direct the at least one energy beam along one or more additional energy beam paths to form at least another portion of the 3D biological material. In some examples, the one or more additional energy beam paths are along an x axis, an x and y plane, or the x, y, and z planes.

[0038] In some examples, the method further comprises at least one objective lens for directing the at least one energy beam to the medium in the media chamber. In some examples, the at least one objective lens comprises a water dipping objective lens. In some examples, the one or more computer processors are individually or collectively programmed to receive images of the edges of the 3D biological material. In some examples, the one or more computer processors are individually or collectively programmed to direct linking of the 3D biological material with other

tissue, which linking is in accordance with the computer instructions. In some examples, the plurality of cells comprises cells of at least two different types. In some examples the medium further comprises glutathione or a functional variant thereof.

[0039] In another example, the present disclosure provides a system for printing a three-dimensional (3D) biological material, comprising: (a) a media chamber configured to contain a medium comprising a plurality of cells and a plurality of polymer precursors; (b) at least one energy source configured to direct at least one energy beam to the media chamber; and (c) one or more computer processors operatively coupled to the at least one energy source, wherein the one or more computer processors are individually or collectively programmed to (i) receive computer instructions for printing the 3D biological material from computer memory; (ii) direct the at least one energy source to direct the at least one energy beam to the medium in the media chamber along at least one energy beam path in accordance with the computer instructions, to subject at least a portion of the polymer precursors to form at least a portion of the 3D biological material; and (iii) direct the at least one energy source to direct the at least one energy beam to a second medium in the media chamber along at least one energy beam path in accordance with the computer instructions, to subject at least a portion of the second medium in the media chamber to form at least a second portion of the 3D biological material, wherein the second medium comprises a second plurality of cells and a second polymeric precursor, wherein the second plurality of cells is of a different type than the first plurality of cells.

[0040] In some examples, the one or more computer processors are individually or collectively programmed to generate a point-cloud representation or lines-based representation of the 3D biological material, and use the point-cloud representation or lines-based representation to generate the computer instructions for printing the 3D biological material in computer memory. In some examples, the one or more computer processors are individually or collectively programmed to convert the point-cloud representation or lines-based representation into an image. In some examples, the one or more computer processors are individually or collectively programmed to project the image in a holographic manner. In some examples, the at least one energy source is a plurality of energy sources. In some examples, the plurality of energy sources directs a plurality of the at least one energy beam. In some examples, the at least one energy source is a laser. In some examples, the at least one energy source is derived from a coherent light source.

[0041] In some examples, the coherent light source comprises a wavelength from about 300 nm to about 5 mm. In some examples, the one or more computer processors are individually or collectively programmed to direct the least one energy source to direct the at least one energy beam along one or more additional energy beam paths to form at least another portion of the 3D biological material. In some examples, the one or more additional energy beam paths are along an x axis, an x and y plane, or the x, y, and z planes. In some examples, the system further comprises at least one objective lens for directing the at least one energy beam to the medium in the media chamber. In some examples, the at least one objective lens comprises a water dipping objective lens. In some examples, the one or more computer processors are individually or collectively programmed to receive images of the edges of the 3D biological material. In some examples, the one or more computer processors are individually or collectively programmed to direct linking of the 3D biological material with other tissue, which linking is in accordance with the computer instructions. In some examples, the medium further comprises glutathione or a functional variant thereof.

[0042] In another example, the present disclosure provides a method for printing a three-dimensional (3D) object, comprising: directing at least one energy beam into a medium comprising one or more precursors, to generate the 3D object comprising a material formed from the one or more precursors, wherein the at least one energy beam is directed into the medium as a 3D projection corresponding to the 3D object.

[0043] In some examples, the material is a polymeric material. In some examples, the medium comprises cells or cellular constituents. In some examples, the one or more precursors are polymeric precursors. In some examples, the one or more precursors include one or more metals. In some examples, the 3D projection is a hologram. In some examples, the medium further comprises glutathione or a functional variant thereof.

[0044] In another example, the present disclosure provides a method for printing a three-dimensional (3D) biological material, comprising: (a) directing at least a first energy beam into a media chamber comprising a first medium comprising (i) a first plurality of cells and (ii) a first polymeric precursor, to generate a first portion of the 3D biological material, and; (b) directing at least a second energy beam into the media chamber comprising a second medium comprising (i) a second plurality of cells and (ii) a second polymeric precursor, to generate a second portion of the 3D biological material adjacent to the first portion of the 3D biological material.

[0045] In some examples, the at least first energy beam and the at least second energy beam are from the same energy source. In some examples, the at least first energy beam and the at least second energy beam are laser beams. In some examples, the cells of the first plurality of cells and the cells of the second plurality of cells are of different types. In some examples, the cells of the first plurality of cells and the cells of the second plurality of cells are of the same type. In some examples, the first polymeric precursor and the second polymeric precursor are different. In some examples, the first polymeric precursor and the second polymeric precursor are the same. In some examples, the first medium and/or second medium further comprise glutathione or a functional variant thereof.

[0046] The present disclosure provides methods and systems for rapid generation of multilayered vascularized tissues using spatial light modulation of multi-photon excitation sources. Using this approach, a method for rapid creation of cell-containing structures is provided by layering cell-size specific nets with embedded mechanical and, or biological elements such as microvasculature. The deposition of cells contained in nets of collagen or another biologically compatible, or inert material, is a rapid, iterative, process based on a three dimensional (holographic) projection, a two-dimensional projection, and/or in any planar axis such as x,y, x, z, or y, z, which may be combined with scanning of the multi-photon laser excitation. Three dimensional scanning, two-dimensional scanning, and raster scanning may be used simultaneously in various combinations to achieve rapid creation of a complete structure. The dynamic shifts between modes of laser projection allows for rapid generation of complex structures in a large field of view, while maintaining fine micrometer to nanometer resolution. This method allows for rapid production of large (e.g., up to about 5 centimeters (cm)) multi-layered and small vasculature (e.g. 1-10 micrometers (μm)) single-cell layered vasculature.

[0047] The present disclosure permits layering of multiple cell types in two dimensions and/or three dimensions such that tissue may be constructed in a manner that is not limited by multiple cell types, sizes, or complexities. In some cases, this is achieved using multiphoton (e.g., two-photon) excitation light, as may be provided, for example, by a laser.

[0048] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0049] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0050] 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 examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, 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.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for printing a three-dimensional (3D) biological material, comprising:
   (a) providing a media chamber comprising a medium comprising one or more polymer precursors; and
   (b) directing at least one energy beam to said medium in said media chamber along at least one energy beam path that is patterned into a simultaneous 3D projection in accordance with computer instructions for printing said 3D biological material in computer memory, to form at least a portion of said 3D biological material comprising a polymer formed from said one or more polymer precursors.
2. The method of claim 1, wherein said biological material develops into a biologically functional tissue.
3. The method of claim 1, further comprising prior to (b), generating a point-cloud representation or lines-based representation of said 3D biological material in computer memory, and using said point-cloud representation or lines-based representation to generate said computer instructions.
4. The method of claim 3, further comprising converting said point-cloud representation or lines-based representation into an image.
5. The method of claim 4, wherein said image is projected in a holographic manner.
6. The method of claim 5, wherein said image is deconstructed and reconstructed prior to projection in a holographic manner.
7. The method of claim 3, wherein said point-cloud representation or said lines-based representation comprises multi-dimensional structural elements corresponding to said 3D biological material.
8. The method of claim 7, wherein said point-cloud representation or said lines-based representation comprises structural elements in two dimensions, wherein said structural elements are associated with tissue function and/or cellular segregation.
9. The method of claim 7, wherein said point-cloud representation or said lines-based representation comprises structural elements in three dimensions, wherein said structural elements are associated with tissue function and/or cellular segregation.
10. The method of claim 1, wherein said at least one energy beam comprises coherent light.
11. The method of claim 1, wherein said at least one energy beam is generated by a laser.
12. The method of claim 1, wherein said at least one energy beam is phase modulated.

13. The method of claim 1, wherein said one or more polymer precursors comprise at least two different polymeric precursors.
14. The method of claim 1, further comprising repeating (b) along one or more additional energy beam paths to form at least another portion of said 3D biological material.
15. The method of claim 14, wherein said at least another portion of said 3D biological material is linked to the 3D biological material formed in (b).
16. The method of claim 14, wherein said at least another portion of said 3D biological material is not linked to the 3D biological material formed in (b).
17. The method of claim 1, wherein (b) further comprises directing at least two energy beams to said medium in said media chamber along at least two energy beam paths in accordance with said computer instructions, to permit multiple portions of said medium in said media chamber to simultaneously form at least a portion of said 3D biological material.
18. The method of claim 17, wherein said at least two energy beams are of identical wavelengths.
19. The method of claim 17, wherein said at least two energy beams are of different wavelengths.
20. The method of claim 1, wherein said at least said portion of said 3D biological material comprises microvasculature for providing one or more nutrients to a plurality of cells.
21. The method of claim 20, wherein said microvasculature is a blood microvasculature, a lymphatic microvasculature, or any combination thereof.
22. The method of claim 20, wherein said microvasculature has a cross-section from 1 μm to 20 μm.
23. The method of claim 1, wherein said 3D biological material has a thickness or diameter from 100 μm to 5 cm.
24. The method of claim 1, wherein said medium further comprises a plurality of beads, and wherein in (b) said at least said portion of said 3D biological material, as formed, includes said plurality of beads.
25. The method of claim 24, wherein said beads further comprise signaling molecules or proteins.
26. The method of claim 25, wherein said signaling molecules or proteins promote formation of said 3D biological material to permit organ function.
27. The method of claim 1, wherein said at least said portion of said 3D biological material comprises a cell-containing scaffold, which cell-containing scaffold comprises at least a plurality of cells.

28. The method of claim 1, wherein said 3D biological material comprises cell-containing scaffolds.
29. The method of claim 28, wherein said cell-containing scaffolds are coupled together.
30. The method of claim 29, wherein said cell-containing scaffolds are cohesively or mechanically coupled together.
31. The method of claim 30, wherein said cell-containing scaffolds are mechanically coupled together through one or more members selected from the group consisting of joints, hinges, locking joints and hinges, Velcro-like elements, springs, coils, points of stretch, interlocking loops, sockets, gears, ratchets, screw, and chain links.
32. The method of claim 28, wherein said cell-containing scaffolds comprise a network, wherein said network comprises a plurality of strands.
33. The method of claim 32, wherein said plurality of strands forms a mesh structure, a grid structure, a sheet structure, or a tube structure.
34. The method of claim 32, wherein said individual strands of said plurality of strands have a thickness from 0.1 nm to 5 cm.
35. The method of claim 1, wherein subsequent to (b), at least another portion of said 3D biological material is formed within said at least said portion of said 3D biological material.
36. The method of claim 1, wherein said computer instructions comprise a set of images corresponding to said 3D biological material.
37. The method of claim 1, wherein said computer instructions direct adjustment of at least (i) one or more parameters of said at least one energy beam as a function of time during formation of said 3D biological material, and/or (ii) location of a stage upon which said 3D biological material is formed.
38. The method of claim 1, wherein said medium further comprises a plurality of cells and wherein said at least said portion of said 3D biological material comprises at least a subset of said plurality of cells.
39. The method of claim 38, wherein said at least said subset of said plurality of cells comprises cells of at least two different types.
40. The method of claim 39, wherein in (b), said plurality of cells comprises said cells of said at least two different types.
41. The method of claim 1, wherein said at least one energy beam is a multi-photon energy beam.
42. The method of claim 41, wherein said multi-photon energy beam is a two-photon energy beam.

43. The method of claim 1, wherein said medium further comprises glutathione or a functional variant thereof.

44. The method of claim 38, further comprising subjecting at least a portion of said at least said subset of said plurality of cells to differentiation to form said cells of said at least two different types.