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## (71) Applicant: ASPECT BIOSYSTEMS LTD. [CA/CA];

Lower Mall Research Station, 359-2259 Lower Mall, Vancouver, British Columbia V6T 1Z4 (CA).

## (72) Inventors: BEYER, Simon Travis; c/o Aspect Biosystems

Ltd., Lower Mall Research Station, 359-2259 Lower Mall, Vancouver, British Columbia V6T 1Z4 (CA). WALUS, Konrad; c/o Aspect Biosystems Ltd., Lower Mall Research Station, 359-2259 Lower Mall, Vancouver, British Columbia V6T 1Z4 (CA). MOHAMED, Tamer; c/o Aspect Biosystems Ltd., Lower Mall Research Station, 359-

2259 Lower Mall, Vancouver, British Columbia V6T 1Z4 (CA). BSOUL, Anas Amjad Mohammed; c/o Aspect Biosystems Ltd., Lower Mall Research Station, 359-2259 Lower Mall, Vancouver, British Columbia V6T 1Z4 (CA).

(74) Agents: CHARL, Santosh K. et al.; Blake, Cassels &amp; Graydon LLP, 199 Bay Street, Suite 4000, Box 25, Commerce Court West, Toronto, Ontario M5L 1A9 (CA).

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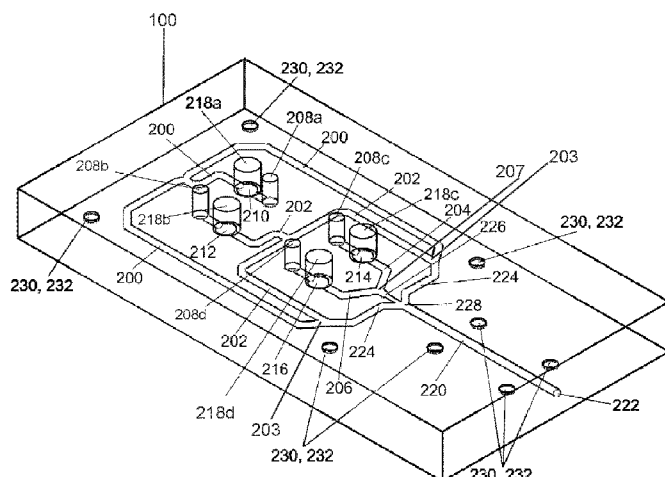


FIG. 3

(57) Abstract: A system and method for additive manufacturing of three-dimensional structures, including three-dimensional cellular structures, are provided. The system comprises at least one print head for receiving and dispensing materials, the materials comprising a sheath fluid and a hydrogel, the print head comprising an orifice for dispensing the materials, microfluidic channels for receiving and directing the materials, fluidic switches corresponding to one of the microfluidic channels in the print head and configured to allow or disallow fluid flow in the microfluidic channels; a receiving surface for receiving a first layer of the materials dispensed from the orifice; a positioning unit for positioning the orifice of the print head in three dimensional space; and a dispensing means for dispensing the materials from the orifice of the print head.



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**SYSTEM FOR ADDITIVE MANUFACTURING OF THREE-DIMENSIONAL STRUCTURES  
AND METHOD FOR SAME**

**CROSS REFERENCE TO PRIOR APPLICATIONS**

**[0001]** This application claims priority under the Paris Convention from US Application Number 61/834,420, filed on June 13, 2013, the entire contents of which are incorporated herein by reference.

**FIELD OF THE INVENTION**

**[0002]** The present invention relates generally to three-dimensional (3D) printing and generation of three-dimensional biological structures from digital files. Specifically, the invention relates to a system, apparatus and method for fabricating 3D cell-laden hydrogel structures.

**BACKGROUND OF THE INVENTION**

**[0003]** 3D printing, a form of additive manufacturing (AM), is a process for creating three-dimensional objects directly from digital files. Software is used to slice a computer aided design (CAD) model or a 3D scan of an object into a multitude of thin cross-sectional layers. This collection of layers is sent to the AM system where the system builds the three-dimensional object layer by layer. Each layer is deposited on top of the previous layer until the object has been fully constructed. Support material can be used to support overhanging and complex features of the object. Various AM processes exist that can build parts in plastic, metal, ceramic and/or biological materials.

**[0004]** Additive manufacturing could have applications in biological systems. For example, until recently, most cell culture studies were performed on 2-dimensional (2D) surfaces, such as micro-well plates and Petri dishes. However, 2D culture systems do not mimic the 3D environment in which cells exist *in vivo*. Researchers have found that 3D cell cultures behave more like natural biological tissue than 2D cell cultures at least in part because the 3D arrangement of cells in natural tissue influences cell-cell interactions, which in turn influences cell growth and physiology.

**[0005]** Additive manufacturing devices and systems for fabricating cellular constructs are known. For example, known fused fiber deposition techniques have been applied to biological materials. In fused fiber deposition, high viscosity liquids are dispensed from a relatively narrow orifice and then rapidly solidified by a variety of means. Biocompatible

1 plastics, thermal gelling hydrogels, UV-cross-linkable polymers and high concentration  
2 alginates have been used as scaffolds for 3D cellular structures, wherein cells are added to  
3 the scaffold after it has solidified. A draw back to these techniques is that they require cells  
4 to be added to the scaffold after printing, making it difficult to control cell placement. Further,  
5 the composition of the scaffold substrates may not be appropriate for facilitating cell  
6 proliferation and growth.

7 **[0006]** Systems for printing 3D structures that comprise direct printing of cellular  
8 materials are known and desired, at least in part, because they may allow cells to be  
9 deposited within a 3D scaffold. For example, ink jet printing technology has been used to  
10 print biological materials. However, the shear force involved with propelling droplets of fluid  
11 onto a substrate can damage cells dispersed in the fluid. Further, ink jet printing is a slow  
12 process, which makes it challenging to adapt to biological materials, which require specific  
13 environmental conditions for survival.

14 **[0007]** Other systems for directly printing cells within a 3D structure include US Patent  
15 No: 8,639,484, which relates to use of a CAD model and a 3D positioning unit to deposit  
16 cellular materials through a multitude of nozzles, layer by layer, to create a 3D object.  
17 Multiple nozzles allow for multiple different materials to be included in the 3D object. US  
18 Patent Application Publication No: 2012/0089238 discloses a multi cartridge print system for  
19 producing composite organic 3D structures, whereby the structure is built using at least two  
20 syringes, one comprising a structural support polymer and another comprising a living cell  
21 composition, that iteratively deposit the structural support polymer and living cell composition  
22 on a surface. US Patent Application Publication No: 2014/0012407 discloses a device  
23 comprising one or more print heads, each configured to receive and hold one or more  
24 cartridges. Each cartridge comprises a fluid, such as a bio-ink comprising cells or support  
25 material, and an orifice wherefrom the fluid can be dispensed from the cartridge.

26 **[0008]** The prior art methods generally require requires multiple nozzles and/or cartridge  
27 orifices in order to facilitate printing of multiple different materials (i.e., one material is  
28 dispensed by one nozzle or cartridge orifice). Use of multiple nozzles for dispensing  
29 different materials requires a corresponding increase in movement of the printing system in  
30 order to position the appropriate nozzle or cartridge orifice in a controlled sequence to  
31 dispense a sequence of different materials. Such increased movement decreases speed  
32 and efficiency of printing.

33 **[0009]** It is desirable to obviate or mitigate one or more of the above deficiencies.

1   **[0010]**     SUMMARY OF THE INVENTION

2   **[0011]**     In a first aspect, a system for additive manufacturing of three-dimensional  
3   structures is provided. The system comprises at least one a print head for receiving and  
4   dispensing materials, the materials comprising a sheath fluid and a hydrogel. In one  
5   embodiment, the print head comprises an orifice for dispensing the materials; microfluidic  
6   channels comprising one or more first channels for receiving and directing the sheath fluid  
7   and one or more respective second channels for receiving and directing the hydrogel, the  
8   second channels intersecting at a first intersection point with the first channels, the second  
9   and first channels joining together at the first intersection point to form a dispensing channel  
10   which extends to the orifice; and fluidic switches, each fluidic switch corresponding to one of  
11   the microfluidic channels in the print head and configured to allow or disallow fluid flow in the  
12   microfluidic channels of the print head when actuated. In one embodiment, the system  
13   further comprises a receiving surface for receiving a first layer of the materials dispensed  
14   from the orifice; a positioning unit for positioning the orifice of the print head in three  
15   dimensional space, the positioning unit operably coupled to the print head; and a dispensing  
16   means for dispensing the materials from the orifice of the print head.

17   **[0012]**     In one embodiment of the first aspect, the system comprises a programmable  
18   control processor for controlling the positioning unit and for controlling dispensing of the  
19   materials from the print head onto the receiving surface.

20   **[0013]**     In one embodiment of the first aspect, the one or more first channels comprise at  
21   least two channels, the one or more first channels being configured to flank respective  
22   second channels at the first intersection point.

23   **[0014]**     In one embodiment of the first aspect, the sheath fluid comprises a cross-linking  
24   agent for solidifying the hydrogel upon contact therewith at the intersection point and/or in  
25   the dispensing channel.

26   **[0015]**     In one embodiment of the first aspect, each second channel has a diameter less  
27   than that of the first channels and the dispensing channel, whereby flow from the first  
28   channels forms a coaxial sheath around the hydrogel in the dispensing channel.

29   **[0016]**     In one embodiment of the first aspect, the hydrogel comprises living cells.

30   **[0017]**     In one embodiment of the first aspect, the system further comprises a fluid  
31   removal feature for removing excess sheath fluid from dispensed from the print head.

1   **[0018]**     In one embodiment of the first aspect, the receiving surface comprises a porous  
2   membrane comprising pores sized to permit passage of the excess sheath fluid there  
3   through.

4   **[0019]**     In one embodiment of the first aspect, the fluid removal feature comprises  
5   absorbent material or a vacuum for drawing the excess sheath fluid away from the receiving  
6   surface.

7   **[0020]**     In one embodiment of the first aspect, the absorbent material or vacuum is  
8   applied below a porous membrane. In one embodiment of the first aspect, the vacuum is  
9   applied above the receiving surface.

10   **[0021]**    In one embodiment of the first aspect, the vacuum is applied through one or more  
11   vacuum channels provided on the print head, the one or more vacuum channels having an  
12   orifice situated near the orifice of the print head.

13   **[0022]**    In one embodiment of the first aspect, the system further comprises reservoirs for  
14   containing the materials, the reservoirs being fluidly coupled respectively to the microfluidic  
15   channels in the print head.

16   **[0023]**    In one embodiment of the first aspect, the print head further comprises at least  
17   two inlets for receiving the materials from the reservoirs, each of the inlets being in fluid  
18   communication with respective microfluidic channels and the respective reservoirs.

19   **[0024]**    In one embodiment of the first aspect, the dispensing means comprises a  
20   pressure control unit.

21   **[0025]**    In one embodiment of the first aspect, the fluidic switches comprise valves.

22   **[0026]**    In one embodiment of the first aspect, the print head further comprises a hollow  
23   projection configured to extend from the orifice toward the receiving surface.

24   **[0027]**    In one embodiment of the first aspect, the print head comprises two second  
25   channels, each of the second channels being adapted to convey respective hydrogels, the  
26   two second channels intersecting at a second intersection and joining together at the second  
27   intersection to form a third channel which extends to the first intersection point.

28   **[0028]**    In a second aspect, a system for additive manufacturing of three-dimensional  
29   structures is provided, the system comprising at least one a print head for receiving and  
30   dispensing materials, the materials comprising a sheath fluid and a hydrogel. In one

embodiment, the print head comprises an orifice for dispensing the materials; microfluidic channels for receiving and directing the materials to the orifice; and fluidic switches, each fluidic switch corresponding to one of the microfluidic channels in the print head and configured to allow or disallow fluid flow in the microfluidic channels in the print head when actuated. In one embodiment, the system further comprises a receiving surface for receiving the materials dispensed from the orifice; a fluid removal feature for removing excess sheath fluid dispensed from the orifice; a positioning unit for positioning the orifice of the print head in three dimensional space, the positioning unit operably coupled to the print head; and a dispensing means for dispensing the materials from the orifice of the print head.

**[0029]** In one embodiment of the second aspect, the fluid removal feature comprises a vacuum for drawing the excess sheath fluid away from or through the receiving surface and/or from the hydrogel dispensed on the receiving surface.

**[0030]** In one embodiment of the second aspect, the receiving surface comprises a porous membrane comprising pores sized to permit passage of the excess sheath fluid there through.

**[0031]** In one embodiment of the second aspect, the vacuum is applied below the porous membrane. In one embodiment of the second aspect, the vacuum is applied above the receiving surface.

**[0032]** In one embodiment of the second aspect, the vacuum is applied through one or more vacuum channels provided on the print head, the one or more vacuum channels having an orifice situated near the orifice of the print head.

**[0033]** In one embodiment of the second aspect, the fluid removal feature comprises an absorbent material for drawing away from the receiving surface the excess sheath fluid.

**[0034]** In one embodiment of the second aspect, the system further comprises a programmable control processor for controlling the positioning unit and for controlling dispensing of the materials from the print head onto the receiving surface.

**[0035]** In one embodiment of the second aspect, the print head further comprises a hollow projection configured to extend from the orifice toward the receiving surface.

**[0036]** In one embodiment of the second aspect, the print head comprises one or more first channels for receiving and directing the sheath fluid and one or more respective second channels for receiving and directing the hydrogel, the second channels intersecting at a first

1 intersection point with the first channels, the second and first channels joining together at the  
2 first intersection point to form a dispensing channel which extends to the orifice.

3 **[0037]** In one embodiment of the second aspect, the print head comprises two second  
4 channels, each of the second channels being adapted to convey respective hydrogels, the  
5 two second channels intersecting at a second intersection and joining together at the second  
6 intersection to form a third channel which extends to the first intersection point

7 **[0038]** In a third aspect, a method of printing a three-dimensional (3D) structure is  
8 provided, the method comprising providing a 3D printer, the printer comprising: a print head  
9 comprising an orifice for dispensing materials; a receiving surface for receiving a first layer of  
10 the materials dispensed from the orifice of the print head; and a positioning unit operably  
11 coupled to the print head, the positioning unit for positioning the print head in three  
12 dimensional space. In one embodiment, the method comprises providing the materials to be  
13 dispensed, the materials to be dispensed comprising a sheath fluid and one or more  
14 hydrogels; encoding the printer with a 3D structure to be printed; dispensing from the print  
15 head orifice the materials to be dispensed; depositing a first layer of the dispensed materials  
16 on the receiving surface; repeating the depositing step by depositing subsequent dispensed  
17 material on the first and any subsequent layers of deposited material, thereby depositing  
18 layer upon layer of dispensed materials in a geometric arrangement according to the 3D  
19 structure; and removing excess sheath fluid dispensed by the print head orifice at one or  
20 more time point during or between depositing steps.

21 **[0039]** In one embodiment of the third aspect, the sheath fluid comprises a cross-linking  
22 agent suitable for cross-linking and solidifying the hydrogel upon contact therewith, the  
23 contact creating a hydrogel fiber.

24 **[0040]** In one embodiment of the third aspect, the sheath fluid and the hydrogel are  
25 dispensed in a coaxial arrangement, wherein the sheath fluid envelops the hydrogel.

26 **[0041]** In one embodiment of the third aspect, the depositing step and the removing step  
27 are carried out continuously, thereby continuously removing the excess sheath fluid as the  
28 layers of dispensed materials are deposited.

29 **[0042]** In one embodiment of the third aspect, the removing step is carried out  
30 intermittently between and/or at the same time as the depositing step, thereby intermittently  
31 removing the excess sheath fluid as the layers of dispensed materials are deposited.



1   **[0043]**     In one embodiment of the third aspect, the one or more hydrogels are adapted for  
2   supporting growth and/or proliferation of living cells dispersed therein.

### 3   BRIEF DESCRIPTION OF THE DRAWINGS

4   **[0044]**     The features of the invention will become more apparent in the following detailed  
5   description in which reference is made to the appended drawings wherein:

6   **[0045]**     Figure 1 is a perspective view of one embodiment of the printing system of the  
7   present invention.

8   **[0046]**     Figure 2 is a perspective view of software-designed objects and corresponding  
9   objects printed using one embodiment of the printing system of the present invention.

10  **[0047]**     Figure 3 is a perspective view of one embodiment of the print head of the present  
11  invention.

12  **[0048]**     Figure 4 is a cross-section of a valve in the print head of Figure 3, including  
13  deflection of a valve membrane when the valve is actuated.

14  **[0049]**     Figure 5 is a cross-section of an alternate embodiment of the print head of Figure  
15  3.

16  **[0050]**     Figure 6 is a top view of an alternate embodiment of the print head of Figure 3.

17  **[0051]**     Figure 7 is an exploded perspective view of one embodiment of the print-bed  
18  assembly of the present invention.

19  **[0052]**     Figure 8 is a cross-section of the assembled print-bed of Figure 9.

20  **[0053]**     Figure 9 is a cross-section of an alternate embodiment of the print-bed of Figure  
21  9.

22  **[0054]**     Figure 10 is a perspective view of one embodiment of the print head of the  
23  present invention.

### 24  DETAILED DESCRIPTION OF THE INVENTION

25  **[0055]**     The definitions of certain terms as used in this specification are provided below.  
26  Unless defined otherwise, all technical and scientific terms used herein generally have the  
27  same meaning as commonly understood by one of ordinary skill in the art to which this  
28  invention belongs.

1   **[0056]**     As used herein, the term "about" will be understood by persons of ordinary skill in  
2   the art and will vary to some extent depending upon the context in which it is used. If there  
3   are uses of the term which are not clear to persons of ordinary skill in the art, given the  
4   context in which it is used, "about" will mean up to plus or minus 10% of the enumerated  
5   value.

6   **[0057]**     As used herein, the term "hydrogel" refers to a composition comprising water and  
7   a network or lattice of polymer chains that are hydrophilic. Examples of natural hydrogels  
8   include, for example, alginate, agarose, collagen, fibrinogen, gelatin, chitosan, hyaluronic  
9   acid based gels or any combination thereof. A variety of synthetic hydrogels are known and  
10   could be used in embodiments of the systems and methods provided herein. For example,  
11   in embodiments of the systems and method provided herein, one or more hydrogels form the  
12   structural basis for three dimensional structures printed. In some embodiments, the hydrogel  
13   has the capacity to support growth and/or proliferation of one or more cell types, which may  
14   be dispersed within the hydrogel or added to the hydrogel after it has been printed in a three  
15   dimensional configuration. In some embodiments, the hydrogel is cross-linkable by a  
16   chemical cross-linking agent. For example, a hydrogel comprising alginate may be cross-  
17   linkable in the presence of a divalent cation, a hydrogel comprising fibrinogen may be cross-  
18   linkable in the presence of thrombin, and a hydrogel comprising collagen or chitosan may be  
19   cross-linkable in the presence of heat or a basic solution. Cross-linking of the hydrogel will  
20   increase the hardness of the hydrogel, in some embodiments allowing formation of a  
21   hydrogel that behaves like a solid.

22   **[0058]**     As used herein, the term "sheath fluid" refers to a liquid that is used, at least in  
23   part, to envelope or "sheath" a material to be dispensed, such as, for example, a hydrogel.  
24   In some embodiments, the sheath fluid comprises one or more of an aqueous solvent, for  
25   example water or glycerol, and a chemical cross-linking agent, for example materials  
26   comprising divalent cations (e.g.  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ , etc.), thrombin, or pH modifying chemicals  
27   such as sodium bicarbonate.

28   **[0059]**     As used herein, the term "excess sheath fluid" refers to a portion of the sheath  
29   fluid that is dispensed from the print head orifice and does not form part of a three  
30   dimensional structure printed using one or more embodiments of the systems or methods  
31   provided herein. For example, the excess sheath fluid may be useful in lubricating passage  
32   of the hydrogel through a dispensing channel in the print head and through the print head  
33   orifice. Once dispensed from the print head orifice the excess sheath fluid may run off of the

1 surface of a layer of dispensed hydrogel and onto a receiving surface, where it may collect  
2 or pool.

3 **[0060]** As used herein, the term “receiving surface” refers to the surface upon which a  
4 first layer of material dispensed from a print head orifice is deposited. The receiving surface  
5 also receives excess sheath fluid that is dispensed from the print head orifice and that runs  
6 off of one or more layers of material dispensed from the print head orifice. In some  
7 embodiments, the receiving surface is made of a solid material. In some embodiments, the  
8 receiving surface is made of a porous material. For example, in some embodiments, the  
9 porosity of the porous material is sufficient to allow passage of the sheath fluid there  
10 through. In some embodiments, the receiving surface is substantially planar, thereby  
11 providing a flat surface upon which a first layer of dispensed material can be deposited. In  
12 some embodiments, the receiving surface has a topography that corresponds to the three  
13 dimensional structure to be printed, thereby facilitating printing of a three dimensional  
14 structure having a non-flat first layer.

15 **[0061]** In one aspect, the present invention generally relates to an apparatus, system  
16 and method for additive manufacturing of three-dimensional (3D) biological structures.

17 **[0062]** GENERAL DESCRIPTION OF THE PRINTING SYSTEM

18 **[0063]** In an aspect, the invention provides a system for additive manufacturing of three-  
19 dimensional structures (also referred to herein as a “printer”, a “3D printer” or a “printing  
20 system” or “the system”). The system comprises a microfluidic print head, which is a  
21 microfluidic liquid handling device comprising one or more microfluidic channels for receiving  
22 and directing materials to be dispensed, fluidic switches corresponding to the microfluidic  
23 channels for regulating flow of the materials to be printed, and a single orifice for dispensing  
24 the materials to be dispensed.

25 **[0064]** The materials to be dispensed comprise a sheath fluid and at least one hydrogel.  
26 In a preferred embodiment, the sheath fluid comprises a chemical cross-linking agent  
27 suitable for solidifying the hydrogel upon contact therewith. In a preferred embodiment, the  
28 sheath fluid also serves as a lubricant for the solidified hydrogel.

29 **[0065]** The microfluidic channels serve as conduits for directing and combining the  
30 materials to be dispensed in a controlled manner. The microfluidic channels are arranged  
31 within the print head such that one or more first channels for receiving and directing the  
32 sheath fluid and a second channel for receiving and directing the hydrogel intersect at a first  
33 intersection point and join together to form a dispensing channel which extends to the orifice

1 of the print head. In one preferred embodiment, the first channels are configured to flank the  
2 second channel at the first intersection point. In this way, the sheath fluid is directed to flow  
3 along either side of the hydrogel in the dispensing channel.

4 **[0066]** In a preferred embodiment, materials in the dispensing channel are directed  
5 coaxially, the hydrogel being focussed to the center of the dispensing channel and the  
6 sheath fluid surrounding the hydrogel fluid, thereby forming a sheath around the hydrogel. In  
7 preferred embodiments where the sheath fluid also comprises a chemical cross-linking agent  
8 suitable for cross-linking the hydrogel, a solidified hydrogel fiber is formed in the dispensing  
9 channel and dispensed from the orifice of the print head.

10 **[0067]** In one aspect, the system further comprises a receiving surface for receiving a  
11 first layer of the materials dispensed from the orifice and a positioning unit for positioning the  
12 orifice of the print head in three dimensional space, the positioning unit operably coupled to  
13 the print head. For example, the print head can be coupled to a commercially available  
14 motorized positioning system with three degrees of motion so that the print head can be  
15 positioned above the receiving surface and oriented to direct dispensed material downward  
16 towards the receiving surface.

17 **[0068]** In one aspect, the system comprises a means for dispensing the materials from  
18 the print head orifice and may further comprise and/or be in data communication with a  
19 programmable control processor for regulating positioning of the print head orifice. The  
20 programmable control processor may also be used for regulating dispensing of the materials  
21 to be dispensed from the print head orifice.

22 **[0069]** Figure 1 shows a schematic perspective view of one embodiment of the 3D  
23 printing system provided herein.

24 **[0070]** Referring to Figure 1, the system comprises a microfluidic print head [100], which  
25 comprises a print head orifice [114] and at least one inlet for receiving material to be  
26 dispensed from the print head [100]. The material to be dispensed is stored in printed  
27 material reservoirs [110] and delivered to the print head through respective first connecting  
28 tubes [122], which provide fluid communication between the print head and the printed  
29 material reservoirs. In the illustrated embodiment, the means for dispensing the material to  
30 be dispensed from the print head orifice is a pressure control unit [112], which is fluidly  
31 coupled to the printed material reservoirs [110] by respective second connecting tubes [120].  
32 The pressure control unit is a means for providing a force to dispense the materials to be  
33 dispensed. The pressure control unit supplies pneumatic pressure to the printed material

reservoirs [110] via respective second connecting tubes [120]. The pressure applied to the printed material reservoirs forces fluid out of the reservoirs and into the print head via respective first connecting tubes [122]. Alternative means for dispensing the material to be dispensed could be used in the illustrated embodiment. For example, a series of electronically controlled syringe pumps could be used to provide force for dispensing the material to be dispensed from the print head orifice.

**[0071]** Referring to Figure 1, the microfluidic print head [100] is coupled to a 3D motorized stage comprising three arms [102, 103 and 104] for positioning the print head [100] and the print head orifice [114] in three dimensional space above a print bed [108], which comprises a surface [109] for receiving printed material. In one embodiment, the 3D motorized stage (i.e., the positioning unit) can be controlled to position a vertical arm [104], which extends along the z-axis of the 3D motorized stage such that the print head orifice [114] is directed downward. A first horizontal arm [102], which extends along the x-axis of the motorized stage is secured to an immobile base platform [116]. A second horizontal arm [103], which extends along the y-axis of the motorized stage is moveably coupled to an upper surface of the first horizontal arm [102] such that the longitudinal directions of the first and second horizontal arms [102 and 103] are perpendicular to one another. It will be understood that the terms “vertical” and “horizontal” as used above with respect to the arms are meant to describe the manner in which the print head is moved and do not necessarily limit the physical orientation of the arms themselves.

**[0072]** In the embodiment illustrated in Figure 1, the print-bed [108] is positioned on top of a platform [118], the platform being coupled to an upper surface of the second horizontal arm [103]. In the embodiment, the 3D motorized stage arms [102, 103 and 104] are driven by three corresponding motors [105, 106 and 107], respectively, and controlled by a programmable control processor, such as a computer (not shown). In a preferred embodiment, the print head [100] and print-bed [108] are collectively moveable along all three primary axes of a Cartesian coordinate system by the 3D motorized stage and movement of the stage is defined using computer software.

**[0073]** It will be understood that the invention is not limited to only the described positioning system and that other positioning systems are known in the art.

**[0074]** In the embodiment illustrated in Figure 1, as material is dispensed from the print head orifice [114], the positioning unit is moved in a pattern controlled by software, thereby creating a first layer of the dispensed material on the receiving surface [109]. Additional layers of dispensed material are stacked on top of one another such that the final 3D

1 geometry of the dispensed layers of material is generally a replica of the 3D geometry  
2 design provided by the software. The 3D design may be created using typical 3D CAD  
3 (computer aided design) software or generated from digital images, as known in the art.  
4 Further, if the software generated geometry contains information on specific materials to be  
5 used, it is possible, according to one embodiment of the invention, to assign a specific  
6 material type to different geometrical locations. For example, Figure 2 shows three 3D  
7 structures printed using one embodiment of the system provided herein: a cube [128], a  
8 hollow cylinder [129] and a hollow coaxial cylinder [130]. Software was used to generate  
9 cube, hollow cylinder and hollow coaxial cylinder designs ([125], [126] and [127],  
10 respectively), each design comprising two different types of materials (dyed alginate), which  
11 were dyed different colors to provide visual clarity of the materials used to generate the  
12 printed cube and hollow cylinder.

13 **[0075]** Any software, application or module referred to herein may be implemented using  
14 computer readable/executable instructions that may be stored or otherwise held by such  
15 computer readable media.

16 **[0076]** PRINT HEAD

17 **[0077]** Figure 3 shows a schematic perspective view of one embodiment of a  
18 microfluidic print head [100] for use in the system provided.

19 **[0078]** Referring to Figure 3, the illustrated embodiment depicts a microfluidic print head  
20 [100] comprising microfluidic channels for carrying various fluids. In the illustrated  
21 embodiment, the microfluidic channels have a cylindrical shape. However, channel shapes  
22 other than cylindrical could also be used in the print head provided herein. Channel [200] is a  
23 conduit for a cross-linking agent, channel [202] is a conduit for water. In the illustrated  
24 embodiment, the cross-linking agent and water, separately or together serve as the “sheath  
25 fluid”. Channel [204] is a conduit for a first hydrogel composition (referred to as “hydrogel  
26 A”), and channel [206] is a conduit for a second hydrogel composition (referred to as  
27 “hydrogel B”). In a preferred embodiment, one or more living cell types are compatible with  
28 and optionally dispersed within hydrogels A and/or B. In the illustrated embodiment, each  
29 microfluidic channel comprises a fluid inlet [208a, 208b, 208c, 208d], which allows fluid  
30 contained in the connecting tubes [122] to pass into the respective channels of the print  
31 head [100]. Downstream of the fluid inlets [208a, 208b, 208c, 208d] are valves [210, 212,  
32 214, 216] corresponding to each channel. In the illustrated embodiment, the valves serve as  
33 “fluidic switches”, which can be actuated to allow and disallow flow of fluid through a  
34 channel, each valve having a corresponding inlet [218, 218a, 218b, 218c, 218d], which

1 facilitates actuation and de-actuation of the valve. In one embodiment, the valves [210, 212,  
2 214, 216] can be electronically actuated. In another embodiment, the valves [210, 212, 214,  
3 216] can be actuated by a change in applied pressure, for example, by way of solenoid  
4 pistons. Electronic or pressure actuation of different valves facilitates rapid change of the  
5 material dispensed, thereby allowing the materials dispensed to be composed of a controlled  
6 sequence of different materials.

7 **[0079]** Referring further to Figure 3, in the illustrated embodiment, the crosslinking agent  
8 channels [200] and water channels [202] intersect at intersection points [203], such as in a  
9 "y-shaped" configuration, joining together to form channels referred to herein as "sheath flow  
10 channels" [224] immediately downstream of the crosslinking agent and water channels [200,  
11 202]. The hydrogel A and hydrogel B channels [204, 206] intersect at an intersection point  
12 [207], such as in a "y-shaped" configuration, joining together to form a channel referred to  
13 herein as a "focussing channel" [226] immediately downstream of the two hydrogel channels.  
14 The sheath flow channels [224] and the focussing channel [226] intersect at an intersection  
15 point [228] in a three-pronged configuration, for the described embodiment, wherein the  
16 focussing channel [226] is flanked by the sheath flow channels [224], joining together to form  
17 a channel referred to herein as a dispensing channel [220]. The dispensing channel [220]  
18 terminates in the dispensing orifice [222]. In a preferred embodiment illustrated in Figure 1,  
19 the dispensing channel projects from the print head [100] terminating in the dispensing  
20 orifice [114].

21 **[0080]** Referring further to Figure 3, in the illustrated embodiment, the sheath flow  
22 channels [224] and the dispensing channel [220] have larger diameters than the focussing  
23 channel [226]. When hydraulic pressure is applied to the sheath flow [224] and focussing  
24 channels [226], liquid in the focussing channel [226] is compressed laterally and "focussed"  
25 into a narrow stream along the central axis of the focussing channel [226]. Upon intersection  
26 with the focussing channel [226] at the intersection point [228], fluid from the larger diameter  
27 sheath flow channels [224] surrounds and envelopes the narrower focussed stream of  
28 hydrogel dispensed from the focussing channel [226].

29 **[0081]** In a preferred embodiment, liquid in the sheath flow channels [224] comprises a  
30 chemical cross-linking agent and liquid in the focussing channel [226] comprises one or  
31 more chemically cross-linkable hydrogels comprising one or more living cell types. When  
32 the one or more chemically cross-linkable hydrogels are focussed into a narrow stream in  
33 the focussing channel [226] and then enveloped by the cross-linking agent in the dispensing  
34 channel [220], at least the exterior surface of the one or more chemically cross-linkable

1 hydrogels is solidified in the dispensing channel [220], thereby creating a cross-linked or  
2 “solid” hydrogel fiber. The hydrogel fiber is then dispensed from the dispensing orifice [222]  
3 onto the receiving surface in a controlled manner, building a 3D structure, layer by layer.

4 **[0082]** In a particularly preferred embodiment, the sheath fluid surrounding the hydrogel  
5 fiber may also act to lubricate passage of the hydrogel fiber through the dispensing channel  
6 [220] until it is dispensed from the print head orifice [222].

7 **[0083]** In an embodiment, the sheath fluid comprises a chemical cross-linking agent,  
8 water or a combination thereof. In embodiments where the sheath fluid lacks a chemical  
9 cross-linking agent the hydrogel will not be solidified and would be dispensed as a liquid. In  
10 order to adjust the composition of the sheath fluid and start and/or stop solidification of the  
11 hydrogel, a crosslinking agent channel valve [210] and water channel valve [212] may be  
12 actuated. It is contemplated that dispensing a liquid rather than a solid hydrogel, or  
13 dispensing sheath fluid alone, may be desirable in order to construct some aspects of  
14 various three dimensional objects.

15 **[0084]** In an embodiment, the print head [100] may be configured to receive and  
16 dispense only one hydrogel material. In one embodiment, the print head may be configured  
17 to receive and dispense two or more hydrogel materials. For example, in an embodiment  
18 where the print head [100] is configured to receive two hydrogel materials, each, for  
19 example, comprising a different cell type, the system provided herein can be programed to  
20 dispense a heterogeneous cellular structure, wherein first and second cell types can be laid  
21 down in controlled patterns within and among layers, alone and/or in combination with one  
22 another. Boundaries between the two materials are controlled, e.g., by software, and the  
23 programmable control processor is used to instruct fluidic switched (e.g., one or more of  
24 valves [210], [212], [214], [216]) to change the flow of material within one or more  
25 microfluidic channels, thereby changing the content of the material being dispensed from the  
26 print head orifice. The number of hydrogel materials that can be received by and dispensed  
27 from the print head provided herein is limited only by the size of the print head that the user  
28 deems practical.

29 **[0085]** Referring to Figure 4, in one embodiment, the fluidic switch is a valve comprising  
30 a membrane [332] disposed over a bowl-shaped feature [318] formed in a microfluidic  
31 channel [308]. Upon application of pneumatic pressure (represented by arrows in Figure 4)  
32 to the exposed surface of the valve membrane [332], the valve membrane [332] will be  
33 deflected into the bowl shaped feature [318], thereby blocking passage of fluid through  
34 microfluidic channel [308]. In one preferred embodiment, the thickness of the valve



1 membrane [332] is about 150  $\mu\text{m}$ . In embodiments where the valve membrane thickness is  
2 increased, a skilled person would understand that the applied pneumatic valve actuation  
3 pressure must be increased accordingly. Similarly, a valve membrane formed of less  
4 resilient material will require a higher actuation pressure. A skilled person would understand  
5 how to adjust the actuation pressure to suit the specific material of the valve membrane.

6 **[0086]** In one embodiment, the print head comprises alternative fluidic switches for  
7 regulating materials to be dispensed from the print head orifice. For example, rather than  
8 using valves, a mechanism for engaging or disengaging the pressure applied to each  
9 channel could be used to regulate material flow in the microfluidic channels.

10 **[0087]** In one embodiment, the print head further comprises an extension tip comprising  
11 an orifice for dispensing materials from the print head. Such an extension tip facilitates  
12 precision dispensing of materials and deposition thereof in confined areas such as, for  
13 example, a well in a multi-well plate (e.g., a standard microtitre plate, microwell plate or  
14 microplate having 6, 24, 96 etc. wells) or a petri dish. Referring to the embodiment  
15 illustrated in Figure 5, a portion [500] of the dispensing channel [220] nearest to the  
16 dispensing orifice [222] has a larger diameter than the upstream portion of the dispensing  
17 channel [220]. The extension tip [502] comprises a tube (e.g., made of plastic, glass or  
18 metal) having an exterior configured to fit into the large-diameter portion [500] of the  
19 dispensing channel and an inner surface (defining a hollow space in the tube) configured to  
20 align with the dispensing channel [220]. The extension tip [502] can be inserted into the  
21 large-diameter portion [500] of the dispensing channel, thereby, extending the length of the  
22 dispensing channel [220], which facilitates deposition of material dispensed from an orifice  
23 [503] in the extension tip [502] into confined spaces, such as a well plate insert [504] or petri  
24 dish (not shown).

25 **[0088]** Referring to the embodiment illustrated in Figure 1, the extension tip [130] is a  
26 projection extending from the print head [100], the extension tip [130] terminating in the print  
27 head orifice [114]. In this embodiment, the extension tip [130] is integral with the print head.

28 **[0089]** In one embodiment, two or more hydrogel materials can be arranged coaxially in  
29 a hydrogel fiber dispensed from the system provided herein. Referring to Figure 6, in the  
30 illustrated embodiment, the print head [100] comprises microfluidic channels arranged to  
31 produce a coaxial hydrogel fiber comprising a hydrogel core material and hydrogel shell  
32 material. In the illustrated embodiment, the shell material, carried in channels [508], is a  
33 rapidly gelling hydrogel, such as alginate, and the core material, carried in channel [506], is a  
34 different hydrogel chosen by the user (e.g. collagen or fibrinogen). Channels [508] and

channel [506] intersect at a hydrogel focussing intersection point [510], for example in a “y-shaped” configuration (similar to intersection [528] shown in Figure 3) joining together to form a focussing channel [226] downstream of channels [506] and [508]. At the hydrogel focussing intersection [510], the shell material focusses the core material coaxially such that the shell material forms a sheath around the core material. In preferred embodiments, channels [508] and [226] have a larger diameter than channel [506] to facilitate coaxial focussing of the core and shell materials. In a preferred embodiment, the purpose of the shell material is to provide the core material with physical structural support so that it may be formed into a 3D geometry. The core may be solidified after the material is deposited, the precise method of solidification being specific to different core materials. For example, the core may comprise a material that solidifies very slowly. In another embodiment, the core and shell materials comprise the same materials. In yet another embodiment, the shell material comprises a hydrogel that rapidly solidifies and the core material comprises a material that will not gel, thereby facilitating generation of a hollow fiber.

**[0090]** In one embodiment, the print head [100] depicted in Figure 6 could further comprise additional core material channels, each with a corresponding fluidic switch, for example a valve, for regulating flow of the material therein. The fluidic switch facilitates rapid and frequent adjustments to the composition of the core material in the fiber being dispensed, for example, by commands provided by the programmable control processor.

**[0091]** In one embodiment, several print heads could be arranged, for example in parallel, to allow simultaneous printing of multiple structures. This would increase throughput production.

**[0092]** In some embodiments the print head is disposable. Use of disposable print heads can reduce the likelihood of contamination of materials used in different print jobs.

**[0093]** The print head can be fabricated, for example, using known microfluidics molding techniques (e.g., casting, imprinting or injection molding) and one or more moldable polymers, for example, polydimethylsiloxane (PDMS). Alternatively, commercially available 3D printing technology could be used to fabricate the print head.

#### **[0094]** FLUIDIC REMOVAL FEATURE

**[0095]** In an aspect, the invention provides a system for additive manufacturing of three-dimensional structures that comprises a feature for removing excess sheath fluid from the receiving surface where a first layer of material dispensed from the orifice of the print head is deposited and optionally from a surface of dispensed hydrogel. During printing, it is possible

1 that excess sheath fluid will collect or “pool” on the receiving surface or on a surface of  
2 dispensed hydrogel. Such pooling can interfere with deposition of hydrogel dispensed from  
3 the print head orifice onto the receiving surface and/or onto one or more layers of dispensed  
4 hydrogel. For example, pooled sheath fluid may cause a dispensed hydrogel fiber to slip  
5 from its intended position in the 3D structure being printed. Therefore, in embodiments of  
6 the system, removal of excess sheath fluid from the receiving surface and optionally from a  
7 surface of the dispensed hydrogel by way of a fluidic removal feature may improve additive  
8 manufacturing of three-dimensional structures.

9 **[0096]** Excess sheath fluid may be removed from the receiving surface or from a surface  
10 of one or more layers of dispensed hydrogel by drawing the fluid off of those surfaces, by  
11 allowing or facilitating evaporation of the sheath fluid from those surfaces or, in embodiments  
12 where the receiving surface is porous, excess sheath fluid may be removed by drawing it  
13 through the porous surface.

14 **[0097]** In a preferred embodiment, the receiving surface comprises a porous material,  
15 the pores being sized to facilitate passage of sheath fluid there through and sized to support  
16 one or more layers of hydrogel deposited thereon.

17 **[0098]** Referring to figures 7 and 8, in the illustrated embodiments, a print bed [108]  
18 comprises a porous membrane [400], which serves as the surface for receiving a first layer  
19 of dispensed material (i.e., the receiving surface). The porous membrane [400] is held in  
20 place in the print bed [108] between a box piece [408] and a lid piece [402]. The box piece  
21 [408] is a container, which can be any shape suitable for receiving and containing liquid  
22 (e.g., square, round). The space inside of the box piece [408] is referred to as a chamber  
23 [404]. The box piece [408] has an upper surface [409] comprising a recessed lip [412]  
24 extending the perimeter of the upper surface [409] of the box piece [408]. The upper surface  
25 [409] comprises an aperture defined by one or more walls [410], the aperture being  
26 surrounded by the recessed lip [412] and extending into the box piece [408].

27 **[0099]** Referring further to the embodiments illustrated in figures 7 and 8, the lid piece  
28 [402] comprises an upper surface [403] having an aperture [416] that extends therethrough  
29 and sidewalls [418] configured to fit around the recessed lip [412] of the box piece [408],  
30 thereby facilitating placement of the lid piece [402] on the upper surface [409] of the box  
31 piece [408]. When the lid piece [402] is placed on the box piece [408] apertures in the box  
32 and the lid piece [416] align. In operation, the porous membrane [400] is placed on the  
33 upper surface [409] of the box piece [408] such that it extends over the aperture in the upper  
34 surface [409] of the box piece [408], the lid piece [402] is then placed on top of the box piece

1 [408] and pressed downward. The downward pressure of the lid piece [402] stretches the  
2 porous membrane [400] over the aperture in the upper surface [409] of the box piece [408],  
3 thereby retaining the porous membrane [400] between the box piece [408] and the lid piece  
4 [402]. In preferred embodiments, the lid piece [402] and box piece [408] fit together snugly,  
5 thereby providing a connection that will remain secure during operation of the system  
6 provided herein.

7 **[00100]** Referring further to the embodiments illustrated in figures 7 and 8, the box piece  
8 [408] comprises a solid base [414] and at least one outlet duct [406] for directing fluid away  
9 from the chamber [404], and a vacuum source (not shown) in fluid communication with the  
10 outlet duct [406] of the chamber [404]. The porous membrane [400] comprises pores sized  
11 to facilitate passage of sheath fluid. The vacuum source (not shown) coupled to the outlet  
12 duct [406] may be actuated to draw the excess sheath fluid collected on the porous  
13 membrane [400] through the porous membrane [400] into the chamber [404] and from the  
14 chamber [404] through the outlet [406], leaving the hydrogel fiber in its dispensed  
15 configuration on top of the porous membrane [400].

16 **[00101]** In a preferred embodiment, a feature for removing excess sheath fluid from the  
17 receiving surface and optionally from a surface of dispensed hydrogel can be included in a  
18 system configured to dispense materials into a multiwall plate or petri dish. For example,  
19 referring to Figure 9, in the illustrated embodiment, a commercially available well-plate insert  
20 [504], is placed on top of the box piece [408]. Some well-plate inserts [504] have a basket  
21 shape with a base made out of a porous membrane material [512]. In the illustrated  
22 embodiment, a gasket [514] is placed between the well-plate insert [512] and the box piece  
23 [408] to improve sealing between the two pieces [504 and 408]. In such embodiments, the  
24 porous membrane [512] of the well-plate inset [504] would serve as the “receiving surface”  
25 and any excess sheath fluid could be removed therefrom using a vacuum coupled to the  
26 outlet duct [406], as described above, or using one of the other fluidic removal features  
27 described below.

28 **[00102]** In one embodiment (not shown), the receiving surface on the print bed comprises  
29 or is placed adjacent to an absorptive material, which facilitates absorption of excess sheath  
30 fluid from the receiving surface. For example, a well-plate insert having a base made out of a  
31 porous membrane material (for example, as shown in Figure 9), or any other porous  
32 membrane substrate, could be placed on top of or adjacent to an absorptive material, such  
33 as, for example, a sponge. The absorptive material would act to draw away from the  
34 receiving surface excess sheath fluid. In embodiments where the absorbent material is

1 disposed below a porous receiving surface, excess sheath fluid on the receiving surface  
2 would be drawn through the porous receiving surface and into the absorptive material,  
3 thereby preventing pooling of excess sheath fluid on the receiving surface. In embodiments  
4 where the absorbent material is disposed immediately beside or on top of a portion of the  
5 receiving surface (e.g., on the periphery of the receiving surface so as not to interfere with  
6 deposition of dispensed material) excess sheath fluid would be drawn off of the receiving  
7 surface and into the absorbent material.

8 **[00103]** In one embodiment (not shown), rather than using one of the print beds  
9 described above, one or more tubes may be provided in an area near the receiving surface  
10 and near the print head orifice. The one or more tubes may be fluidly coupled to a vacuum  
11 source (not shown), which can provide suction for removing excess sheath fluid from the  
12 receiving surface and optionally from a surface of dispensed hydrogel. In such  
13 embodiments, a solid or porous receiving surface may also be used.

14 **[00104]** In one embodiment, illustrated in Figure 10, the print head is configured to further  
15 comprise one or more vacuum channels [700a, 700b], the one or more vacuum channels  
16 each having an orifice [702a, 702b] situated near the print head orifice [222]. The one or  
17 more vacuum channels [700a, 700b] each have an inlet [704a, 704b] configured to facilitate  
18 fluid communication with one or more vacuums (not shown). When the print head [100] is in  
19 fluid communication with a vacuum, the one or more vacuum channels [702a, 702b] direct  
20 negative pressure to an area of the receiving surface where materials are being dispensed  
21 or have been dispensed from the print head orifice [222] and/or to a portion of the surface  
22 area of the dispensed hydrogel, thereby drawing up excess sheath fluid from the receiving  
23 surface and optionally from a surface of the dispensed hydrogel, thereby eliminating pooling  
24 of sheath fluid on the receiving surface and/or the dispensed hydrogel.

25 **[00105]** In one embodiment, the one or more vacuum tubes are provided, at least in part,  
26 in one or more extensions projecting from the print head, the extensions projecting in the  
27 same general direction as the extension comprising the print head orifice and dispensing  
28 channel (see, for example, Figure 10). In such embodiments, the one or more extensions  
29 comprising vacuum tubes do not extend further than the extension comprising the print head  
30 orifice and dispensing channel so as not to interfere with dispensed and deposited hydrogel.

31 **[00106]** It is contemplated that in some embodiments, the fluid removal feature may be a  
32 feature of the sheath fluid composition itself. For example, the sheath fluid composition may  
33 be designed to evaporate after it is dispensed from the print head orifice, thereby eliminating  
34 pooling of excess sheath fluid on the receiving surface or on surfaces of dispensed hydrogel.

1 For example, the sheath fluid may have a boiling point that results in evaporation after being  
2 dispensed, while remaining in a liquid state prior to being dispensed.

3 **[00107]** METHOD OF PRINTING A THREE DIMENSIONAL STRUCTURE

4 **[00108]** In an aspect, a method of printing a three-dimensional (3D) structure is provided.

5 **[00109]** The method first comprises providing a design for a 3D structure to be printed.  
6 The design may be created using commercially available CAD software. In one embodiment,  
7 the design comprises information regarding specific materials (e.g., for heterogeneous  
8 structures comprising multiple materials) to be assigned to specific geometrical locations in  
9 the design.

10 **[00110]** The method comprises the use of a 3D printer, the printer comprising: a print  
11 head, a receiving surface for receiving material dispensed by the print head; and a  
12 positioning unit operably coupled to the receiving surface, the positioning unit for positioning  
13 the print head at a location in three dimensional space above the receiving surface. For  
14 example, various embodiments of the printing system provided herein may be used in the  
15 method of printing a 3D structure.

16 **[00111]** The method comprises providing at least two materials to be dispensed by the  
17 print head, such as a sheath fluid and a hydrogel fluid. In preferred embodiments, one or  
18 more cell types are compatible with, and optionally dispensed within, the hydrogel. In a  
19 preferred embodiment, the sheath fluid serves as a lubricating agent for lubricating  
20 movement of the hydrogel within and from the print head. In a preferred embodiment, the  
21 sheath fluid comprises a cross-linking agent for solidifying at least a portion of the hydrogel  
22 before or while it is dispensed from the print head.

23 **[00112]** The method comprises communicating the design to the 3D printer.  
24 Communication can be achieved, for example, by a programmable control processor.

25 **[00113]** The method comprises controlling relative positioning of the print head and the  
26 receiving surface in three dimensional space and simultaneously dispensing from the print  
27 head the sheath fluid and the hydrogel, alone or in combination. In preferred embodiments,  
28 the materials dispensed from the print ahead are dispensed coaxially, such that the sheath  
29 fluid envelopes the hydrogel. Such coaxial arrangement allows the cross-linking agent to  
30 solidify the hydrogel, thereby resulting in a solid hydrogel fiber, which is dispensed from the  
31 printer head.

1   **[00114]**   The method comprises depositing a first layer of the dispensed materials on the  
2   receiving surface, the first layer comprising an arrangement of the material specified by the  
3   design and iteratively repeating the depositing step, depositing subsequent material onto the  
4   first and subsequent layers of material, thereby depositing layer upon layer of dispensed  
5   materials in a geometric arrangement specified by the design to produce the cell-laden 3D  
6   structure.

7   **[00115]**   In preferred embodiments, a plurality of materials, for example multiple  
8   hydrogels, at least some of which comprise one or more cell types, are deposited in a  
9   controlled sequence, thereby allowing a controlled arrangement of hydrogels and cell types  
10  to be deposited in a geometric arrangement specified by the design.

11  **[00116]**   In preferred embodiments, the method comprises removing excess sheath fluid  
12  from the receiving surface and optionally from the surface of the dispensed hydrogel. For  
13  example, the step of removing the excess sheath fluid can be done continuously throughout  
14  the printing process, thereby removing excess fluid that may otherwise interfere with layering  
15  the dispensed materials in the geometric arrangement provided by the design. Alternatively,  
16  the step of removing excess sheath fluid may be done intermittently throughout the printing  
17  process in sequence with or simultaneously with one or more depositing steps. In some  
18  embodiments, removal of excess sheath fluid is achieved by drawing the fluid off of the  
19  receiving surface and optionally off of a surface of the dispensed hydrogel. In another  
20  embodiment removal of excess sheath fluid is achieved by drawing excess fluid through the  
21  receiving surface, the receiving surface comprising pores sized to allow passage of the  
22  sheath fluid. In another embodiment removal of excess sheath fluid is achieved by providing  
23  a sheath fluid that evaporates after being dispensed from the print head orifice.

24  **[00117]**   EXEMPLARY USES OF EMBODIMENTS OF THE SYSTEM AND METHOD OF  
25  PRINTING CELL-LADEN THREE DIMENSIONAL STRUCTURES

26  **[00118]**   In some embodiments, structures generated using the system and method  
27  provided herein can be useful in the field of drug discovery, where, for example, determining  
28  cellular responses to various chemical compounds and compositions are of interest. Use of  
29  3D cell cultures fabricated using embodiments of the systems and methods provided herein  
30  may provide experimental conditions that more closely resemble *in vivo* cellular and tissue  
31  conditions relative to 2D cell cultures. 3D arrangement of the cells may more closely mimic  
32  *in vivo* cell-cell interactions and responses to external stimuli and the heterogeneous nature  
33  of the 3D structures that can be generated using the apparatus and methods provided permit  
34  study of tissues and potentially organs. It is contemplated that 3D cell-laden structures

1 fabricated using embodiments of the systems and methods provided herein may provide a  
2 similar benefit to the cosmetics industry by offering an alternative means to testing cosmetic  
3 products.

4 **[00119]** In some embodiments, various embodiments of the system and method provided  
5 herein are compatible with standard well-plate technology. Well-plates or well-plate inserts  
6 may be used with or as part of the print bed in the methods and systems provided herein.  
7 Various embodiments of the system and method provided herein are thus compatible with  
8 instruments and practices that utilize well-plates, allowing them to be readily integrated into  
9 existing process streams.

10 **[00120]** In some embodiments, the microfluidic channels within the print head are  
11 compatible with other microfluidic modules. For example, known microfluidic modules may  
12 be included in the print head of the systems provided herein upstream of the print head  
13 orifice. Such modules may include, for example, cell counting, cell sorting, cell analyzing,  
14 and/or concentration gradient generating modules.

15 **[00121]** In some embodiments, throughput of 3D printing may be increased by adding to  
16 the system additional print heads in parallel. Each print head comprising all of the elements  
17 required to print a multi-material structure, thus allowing several 3D structures to be printed  
18 simultaneously by including additional print heads in the system.

19 **[00122]** Although the invention has been described with reference to certain specific  
20 embodiments, various modifications thereof will be apparent to those skilled in the art  
21 without departing from the purpose and scope of the invention as outlined in the claims  
22 appended hereto. Any examples provided herein are included solely for the purpose of  
23 illustrating the invention and are not intended to limit the invention in any way. Any drawings  
24 provided herein are solely for the purpose of illustrating various aspects of the invention and  
25 are not intended to be drawn to scale or to limit the invention in any way. The disclosures of  
26 all prior art recited herein are incorporated herein by reference as if set forth in their entirety.

## 27 **[00123]** REFERENCES

28 **[00124]** The following references are provided as examples of the known art relating to  
29 the present invention. The following listing is not intended to comprise a comprehensive list  
30 of all relevant art. The entire contents of all references listed in the present specification,  
31 including the following documents, are incorporated herein by reference as if set forth in their  
32 entirety.



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**WE CLAIM:**

1. A system for additive manufacturing of three-dimensional structures, the system comprising:
  - at least one print head for receiving and dispensing materials, the materials comprising a sheath fluid and a hydrogel, the print head comprising:
    - an orifice for dispensing the materials;
    - microfluidic channels comprising one or more first channels for receiving and directing the sheath fluid and one or more respective second channels for receiving and directing the hydrogel, the second channels intersecting at a first intersection point with the first channels, the second and first channels joining together at the first intersection point to form a dispensing channel which extends to the orifice; and
    - fluidic switches, each fluidic switch corresponding to one of the microfluidic channels in the print head and configured to allow or disallow fluid flow in the microfluidic channels of the print head when actuated;
  - a receiving surface for receiving a first layer of the materials dispensed from the orifice;
  - a positioning unit for positioning the orifice of the print head in three dimensional space, the positioning unit operably coupled to the print head; and
  - a dispensing means for dispensing the materials from the orifice of the print head.
2. The system of claim 1, further comprising a programmable control processor for controlling the positioning unit and for controlling dispensing of the materials from the print head onto the receiving surface.
3. The system of claim 1 or 2, wherein the one or more first channels comprise at least two channels, the one or more first channels being configured to flank respective second channels at the first intersection point.
4. The system of any one of claims 1 to 3, wherein the sheath fluid comprises a cross-linking agent for solidifying the hydrogel upon contact therewith at the intersection point and/or in the dispensing channel.

5. The system any one of claims 1 to 4, wherein each second channel has a diameter less than that of the first channels and the dispensing channel, whereby flow from the first channels forms a coaxial sheath around the hydrogel in the dispensing channel.
6. The system of any one of claims 1 to 5, wherein the hydrogel comprises living cells.
7. The system of any one of claims 1 to 5, further comprising a fluid removal feature for removing excess sheath fluid from dispensed from the print head.
8. The system of claim 7, wherein the receiving surface comprises a porous membrane comprising pores sized to permit passage of the excess sheath fluid there through.
9. The system of claim 8, wherein the fluid removal feature comprises absorbent material or a vacuum for drawing the excess sheath fluid away from the receiving surface.
10. The system of claim 9, wherein the absorbent material or vacuum is applied below a porous membrane.
11. The system of claim 9, wherein the vacuum is applied above the receiving surface.
12. The system of claim 11, wherein the vacuum is applied through one or more vacuum channels provided on the print head, the one or more vacuum channels having an orifice situated near the orifice of the print head.
13. The system of any one of claims 1 to 12, further comprising reservoirs for containing the materials, the reservoirs being fluidly coupled respectively to the microfluidic channels in the print head.
14. The system of claim 13, wherein the print head further comprises at least two inlets for receiving the materials from the reservoirs, each of the inlets being in fluid communication with respective microfluidic channels and the respective reservoirs.
15. The system of any one of claims 1 to 14, wherein the dispensing means comprises a pressure control unit.

16. The system of any one of claims 1 to 15, wherein the fluidic switches comprise valves.
17. The system of any one of claims 1 to 16, wherein the print head further comprises a hollow projection configured to extend from the orifice toward the receiving surface.
18. The system of any one of claims 1 to 17, wherein the print head comprises two second channels, each of the second channels being adapted to convey respective hydrogels, the two second channels intersecting at a second intersection and joining together at the second intersection to form a third channel which extends to the first intersection point.
19. A system for additive manufacturing of three-dimensional structures, the system comprising:
- at least one print head for receiving and dispensing materials, the materials comprising a sheath fluid and a hydrogel, the print head comprising:
    - an orifice for dispensing the materials;
    - microfluidic channels for receiving and directing the materials to the orifice;
  - and
  - fluidic switches, each fluidic switch corresponding to one of the microfluidic channels in the print head and configured to allow or disallow fluid flow in the microfluidic channels in the print head when actuated;
  - a receiving surface for receiving the materials dispensed from the orifice;
  - a fluid removal feature for removing excess sheath fluid dispensed from the orifice;
  - a positioning unit for positioning the orifice of the print head in three dimensional space, the positioning unit operably coupled to the print head; and
  - a dispensing means for dispensing the materials from the orifice of the print head.
20. The system of claim 19, wherein the fluid removal feature comprises a vacuum for drawing the excess sheath fluid away from or through the receiving surface and/or from the hydrogel dispensed on the receiving surface.
21. The system claim 20, wherein the receiving surface comprises a porous membrane comprising pores sized to permit passage of the excess sheath fluid there through.

22. The system of claim 21, wherein the vacuum is applied below the porous membrane.
23. The system of claim 20, wherein the vacuum is applied above the receiving surface.
24. The system of claim 23, wherein the vacuum is applied through one or more vacuum channels provided on the print head, the one or more vacuum channels having an orifice situated near the orifice of the print head.
25. The system of claim 19, wherein the fluid removal feature comprises an absorbent material for drawing away from the receiving surface the excess sheath fluid.
26. The system of any one of claims 21 to 25, further comprising a programmable control processor for controlling the positioning unit and for controlling dispensing of the materials from the print head onto the receiving surface.
27. The system of any one of claims 21 to 26, wherein the print head further comprises a hollow projection configured to extend from the orifice toward the receiving surface.
28. The system of any one of claims 21 to 27, wherein the print head comprises one or more first channels for receiving and directing the sheath fluid and one or more respective second channels for receiving and directing the hydrogel, the second channels intersecting at a first intersection point with the first channels, the second and first channels joining together at the first intersection point to form a dispensing channel which extends to the orifice.
29. The system of claim 28, wherein the print head comprises two second channels, each of the second channels being adapted to convey respective hydrogels, the two second channels intersecting at a second intersection and joining together at the second intersection to form a third channel which extends to the first intersection point
30. A method of printing a three-dimensional (3D) structure, the method comprising:
- providing a 3D printer, the printer comprising:
    - at least one print head comprising an orifice for dispensing materials;
    - a receiving surface for receiving a first layer of the materials dispensed from the orifice of the print head; and

- a positioning unit operably coupled to the print head, the positioning unit for positioning the print head in three dimensional space;
- providing the materials to be dispensed, the materials to be dispensed comprising a sheath fluid and one or more hydrogels;
- encoding the printer with a 3D structure to be printed;
- dispensing from the print head orifice the materials to be dispensed;
- depositing a first layer of the dispensed materials on the receiving surface;
- repeating the depositing step by depositing subsequent dispensed material on the first and any subsequent layers of deposited material, thereby depositing layer upon layer of dispensed materials in a geometric arrangement according to the 3D structure; and
- removing excess sheath fluid dispensed by the print head orifice at one or more time point during or between depositing steps.

31. The method of claim 30, wherein the sheath fluid comprises a cross-linking agent suitable for cross-linking and solidifying the hydrogel upon contact therewith, the contact creating a hydrogel fiber.

32. The method of claim 31, wherein the sheath fluid and the hydrogel are dispensed in a coaxial arrangement, wherein the sheath fluid envelops the hydrogel.

33. The method of any one of claims 30 to 32, wherein the depositing step and the removing step are carried out continuously, thereby continuously removing the excess sheath fluid as the layers of dispensed materials are deposited.

34. The method of any one of claims 30 to 32, wherein the removing step is carried out intermittently between and/or at the same time as the depositing step, thereby intermittently removing the excess sheath fluid as the layers of dispensed materials are deposited.

35. The method of any one of claims 30 to 34, wherein the one or more hydrogels are adapted for supporting growth and/or proliferation of living cells dispersed therein.

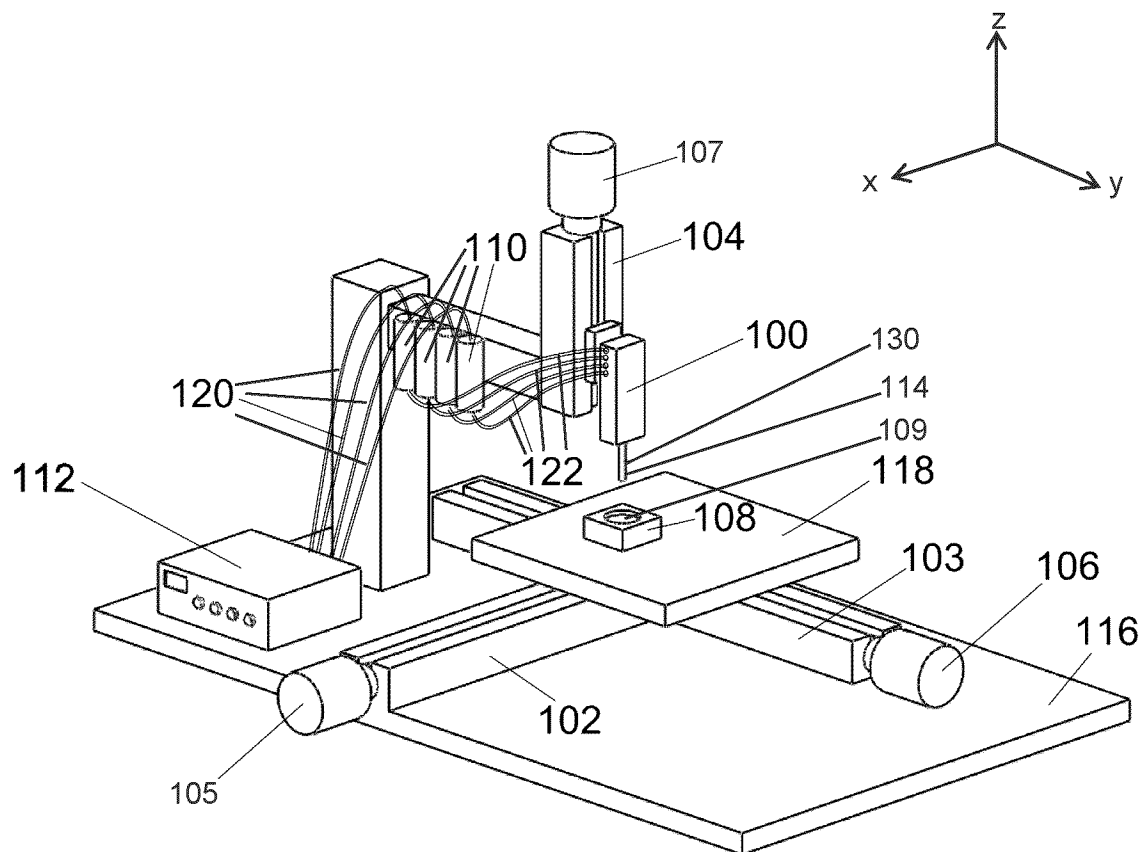


FIG. 1



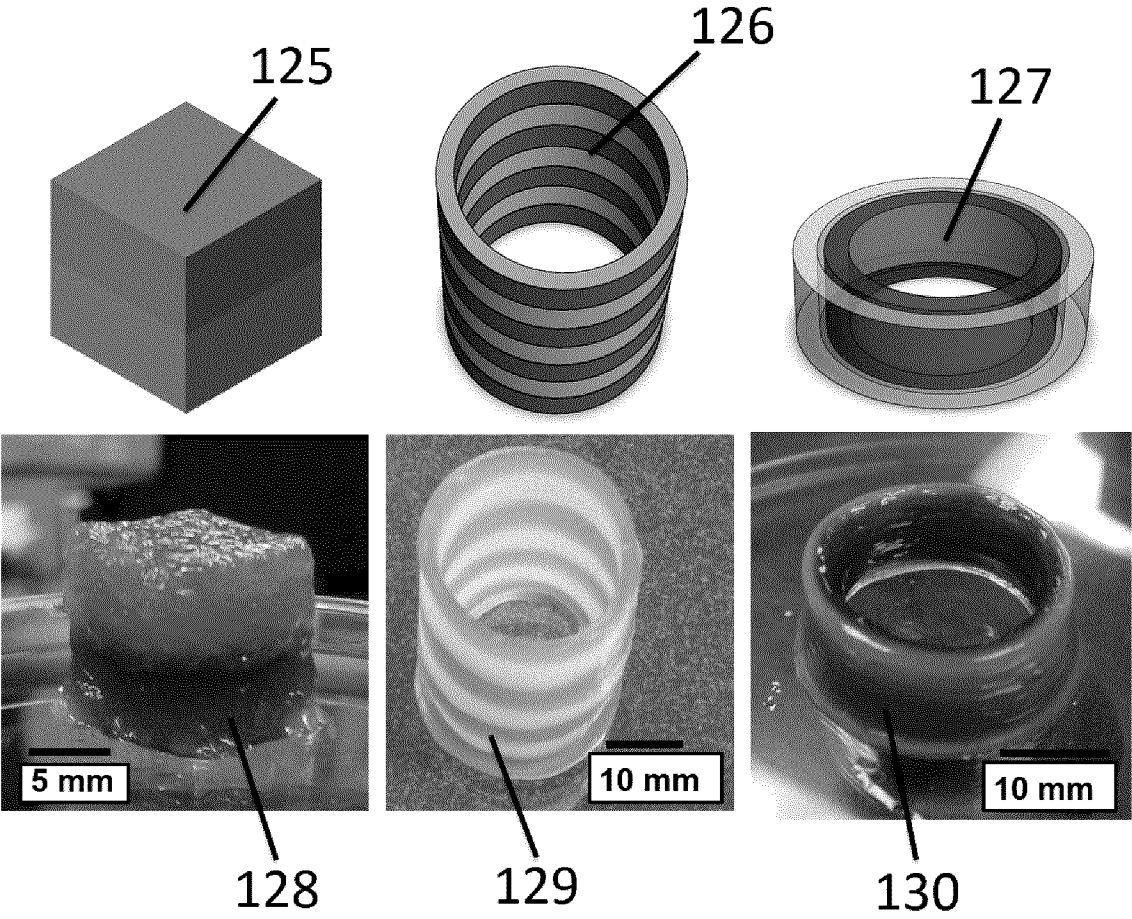


FIG. 2

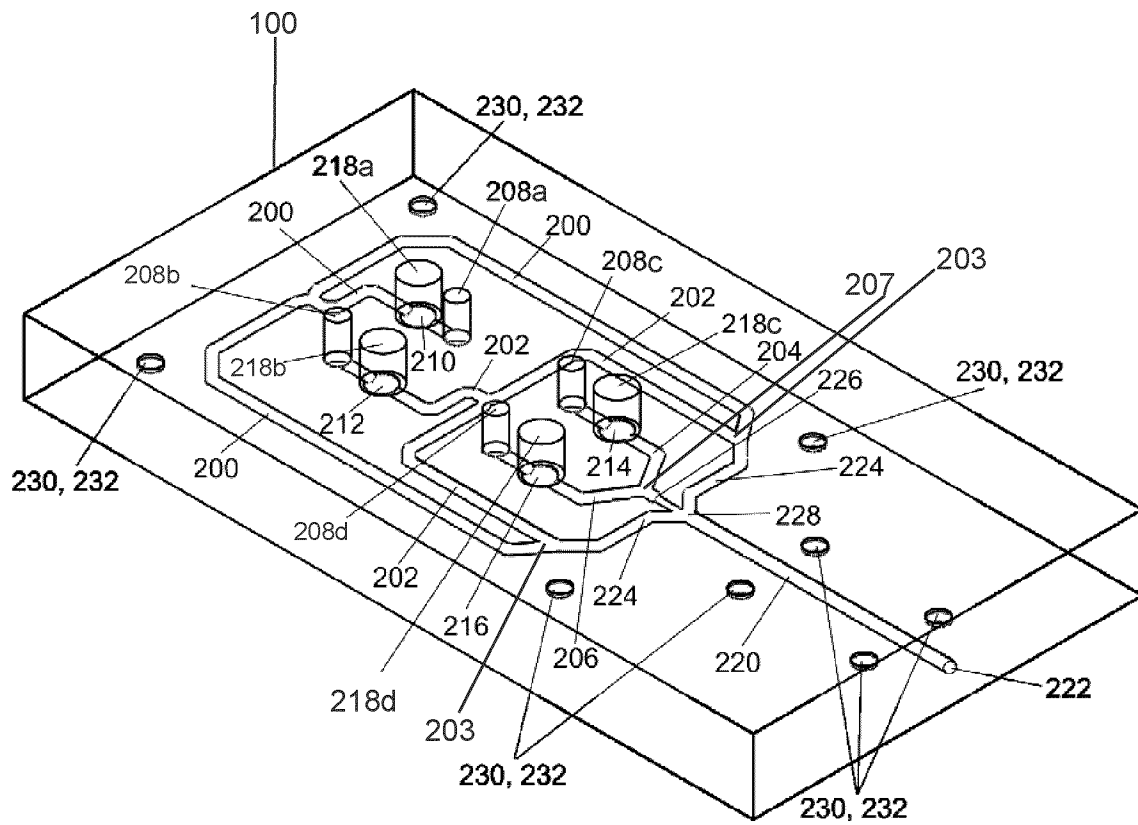


FIG. 3

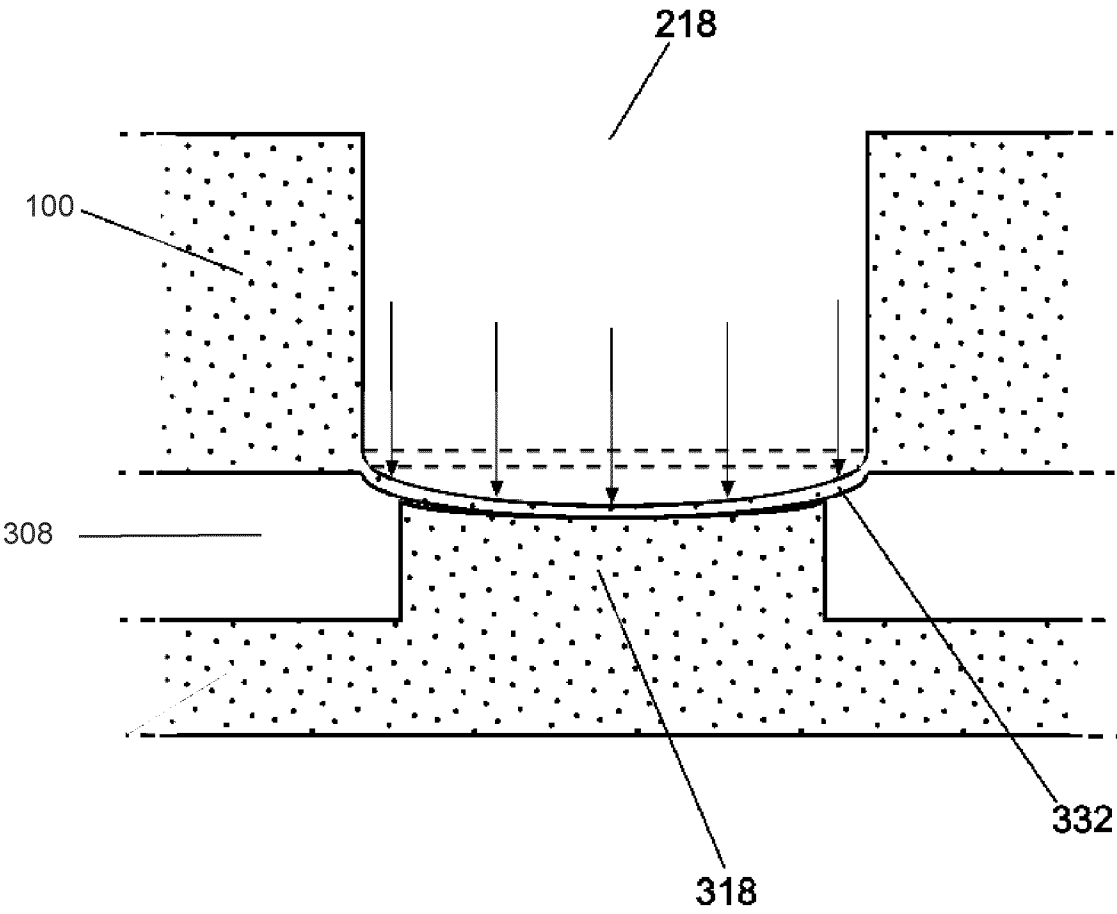


FIG. 4

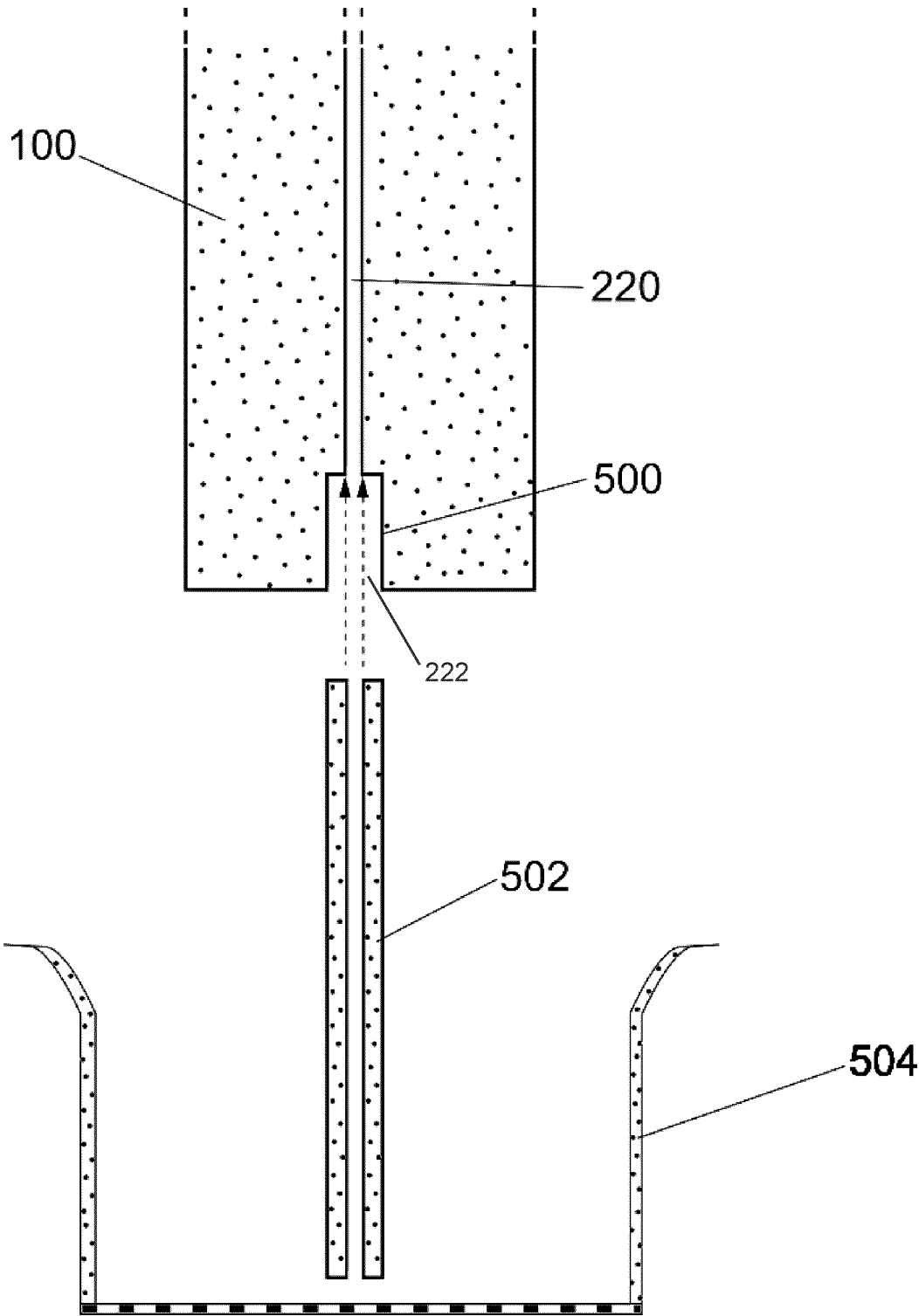


FIG. 5

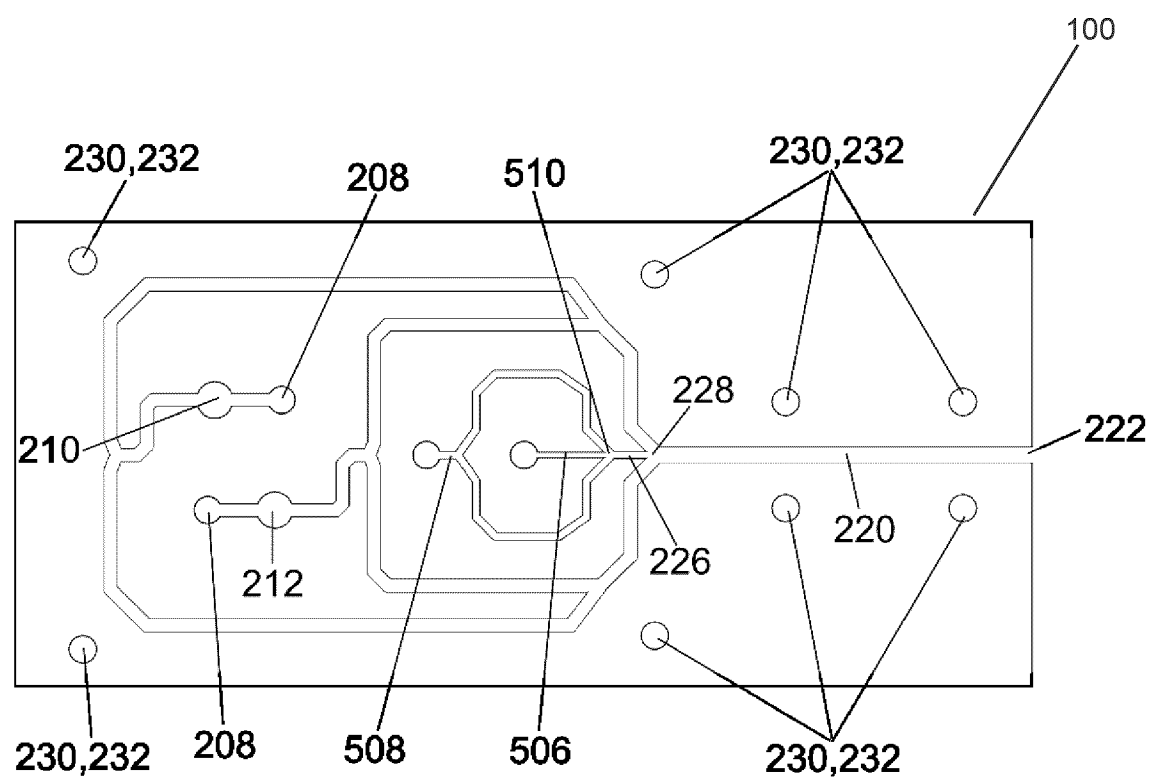


FIG. 6

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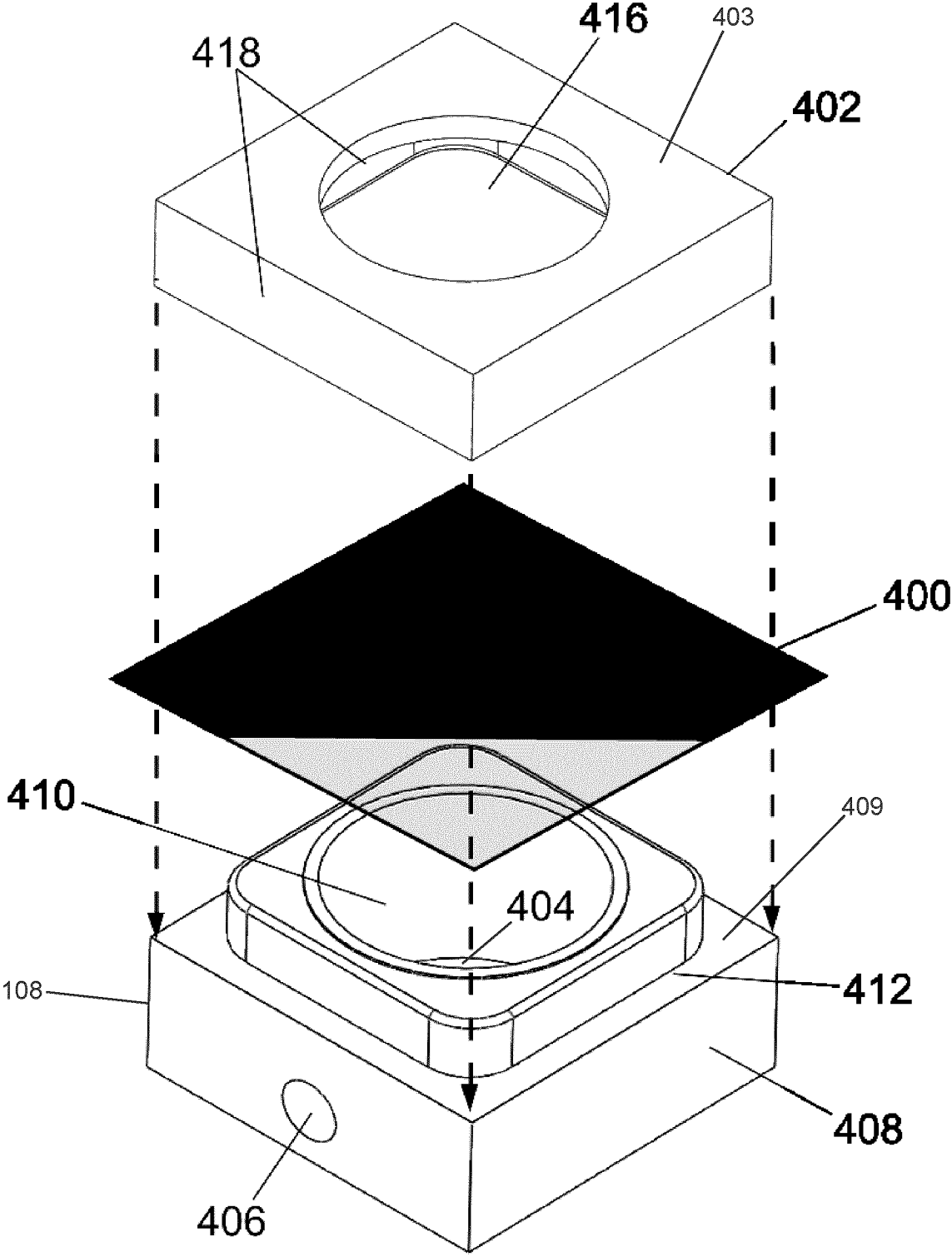


FIG. 7

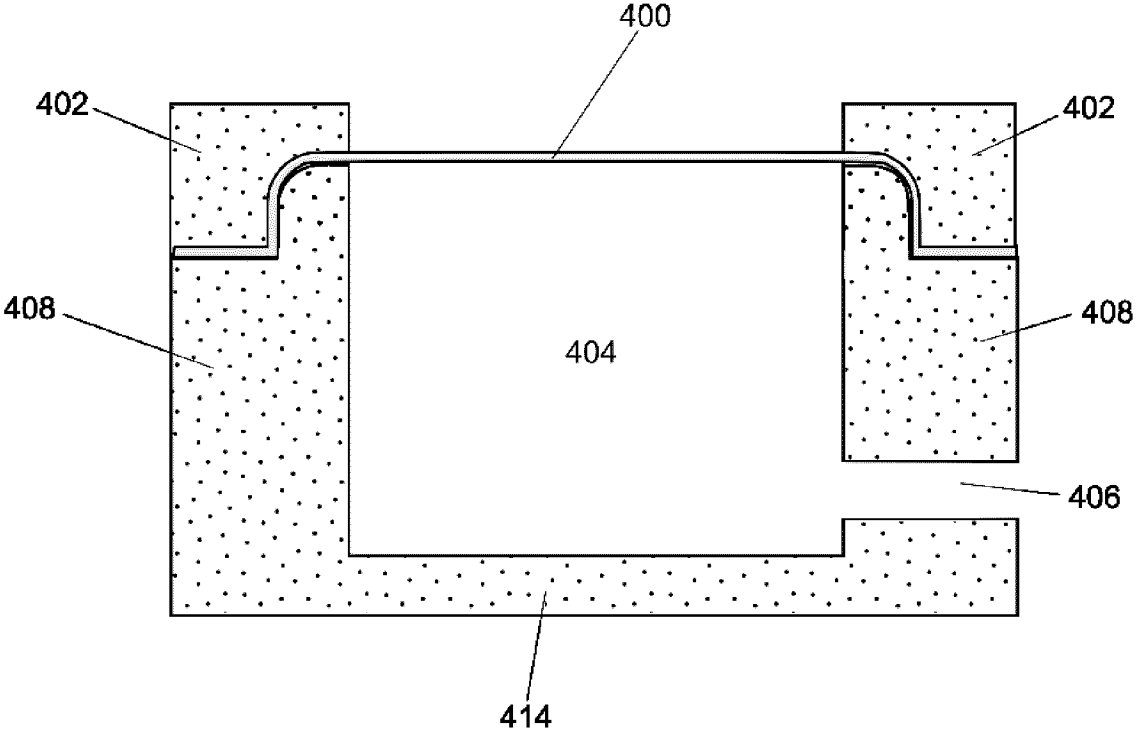


FIG. 8

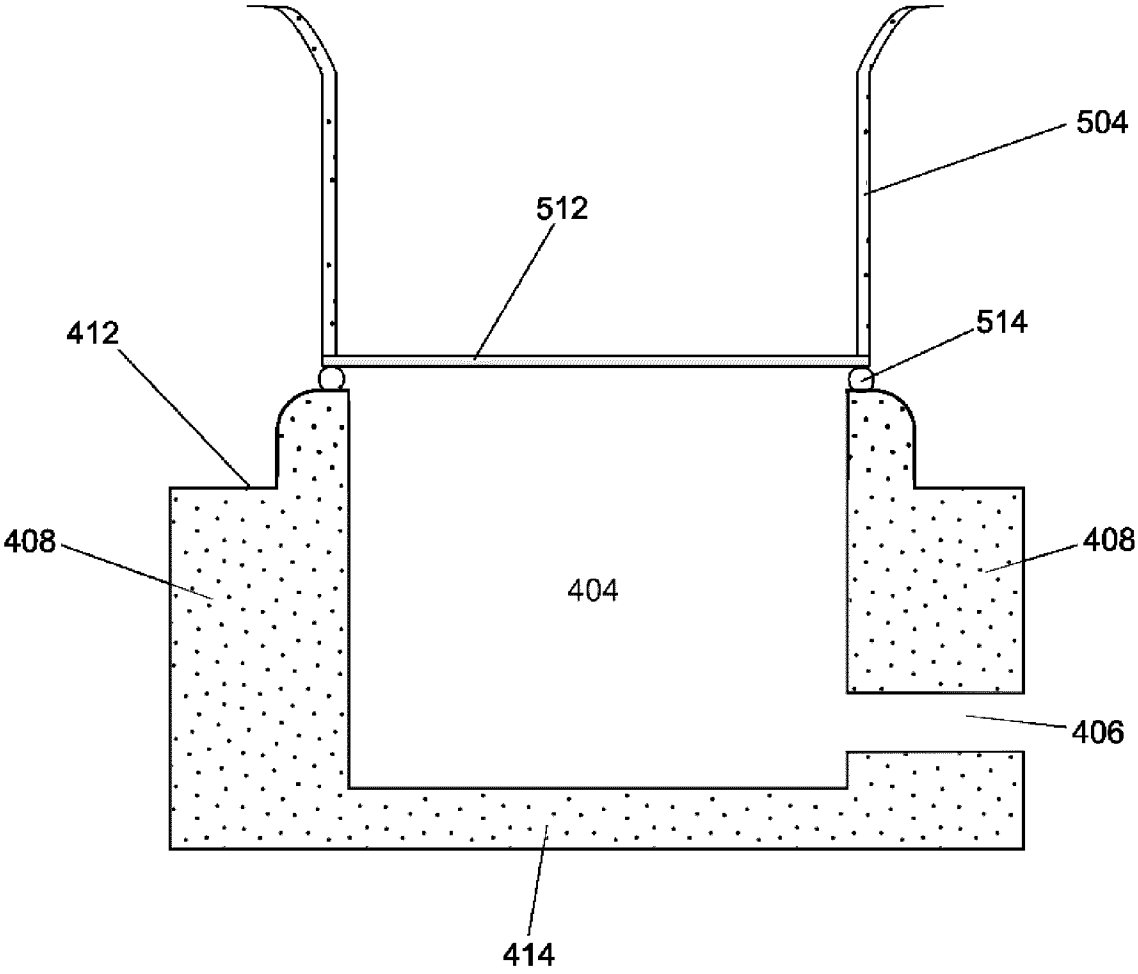


FIG. 9



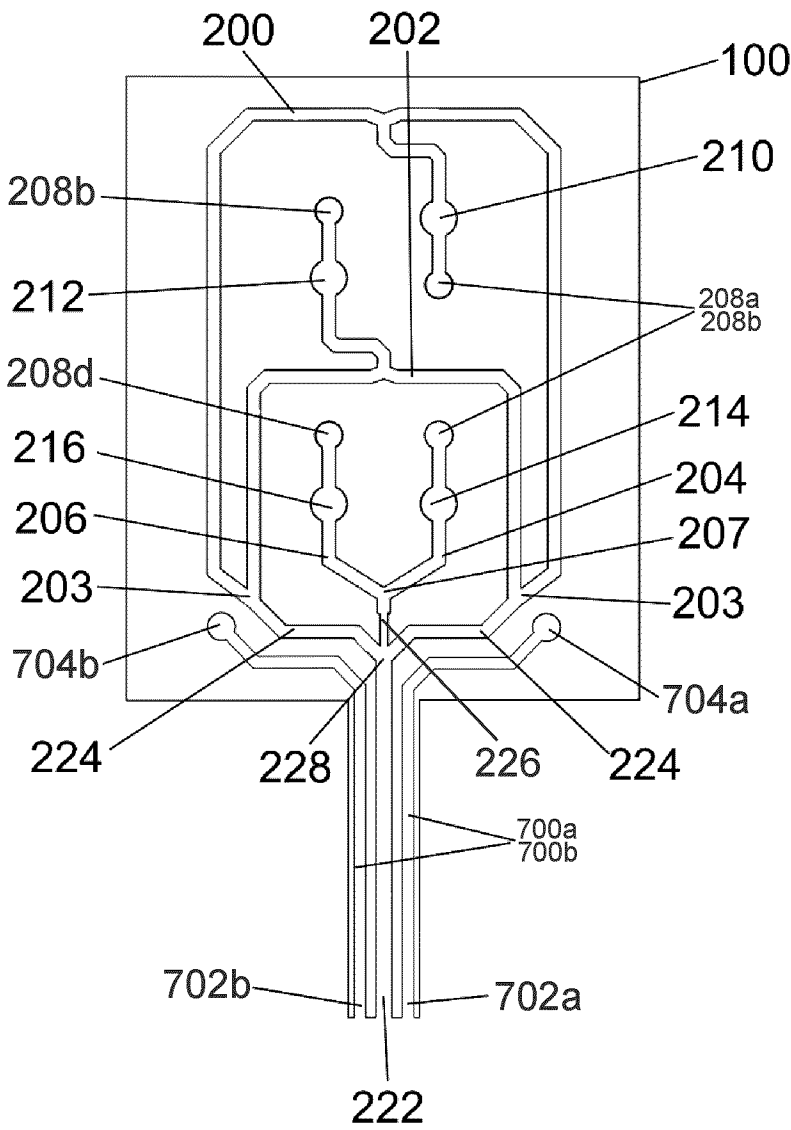


FIG. 10

## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2014/050556**

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC: <b>B29C 67/00</b> (2006.01), <b>B41J 2/015</b> (2006.01), <b>B41J 2/14</b> (2006.01), <b>B41J 3/00</b> (2006.01), <b>C08J 3/075</b> (2006.01), <b>C12M 3/00</b> (2006.01), <b>C12N 11/00</b> (2006.01)		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) <b>B29C 67/00</b> (2006.01), <b>B41J 2/015</b> (2006.01), <b>B41J 2/14</b> (2006.01), <b>B41J 3/00</b> (2006.01), <b>C08J 3/075</b> (2006.01), <b>C12M 3/00</b> (2006.01), <b>C12M 11/00</b> (2006.01), Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Orbit Questel: IPC(s) AND keywords hydrogel, print, 3 dimensional, microfluidic and similar terms Scopus: keywords, hydrogel, 3D printing, microfluidic, 3D structures and similar terms		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/054195 A2 (Murphy et al.) 26 April 2012 (26-04-2012) *cited by applicant* *whole document*	30, 31, 33, 34, 35
X, P	Transducers: 3D Alginate Constructs for Tissue Engineering Printed Using A Coaxial Flow Focusing Microfluidic Device, 16-20 June 2013 [retrieved on 15-09-2014]. Retrieved from the Internet: <URL: <a href="http://ieeexplore.ieee.org/xpls/icp.jsp?arnumber=6626990">http://ieeexplore.ieee.org/xpls/icp.jsp?arnumber=6626990</a> .	1-35
A	Nishiyama, Y. Journal of Biomedical Engineering: Development of a Three-Dimensional Bioprinter: Construction of Cell Supporting Structures Using Hydrogel and State-Of-The-Art Inkjet Technology, March 2009, Vol. 131, Issue 3, pages 0156-0161	
A	Moon et al., Tissue Engineering Part C: Methods: Layer by Layer Three-dimensional Tissue Epitaxy by Cell-laden Hydrogel Droplets, 2010, Vol. 16, Issue 1, pages 157-166	
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 17 September 2014 (17-09-2014)		Date of mailing of the international search report 08 October 2014 (08-10-2014)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476		Authorized officer  Melissa Bowles (819) 994-7546

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.

**PCT/CA2014/050556**

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2012054195A2	26 April 2012 (26-04-2012)	AU2011318437A1 CA2812766A1 CN103249567A EP2629975A2 IL225392D0 JP2013542728A KR20140009188A US2012116568A1 US2014012407A1	11 April 2013 (11-04-2013) 26 April 2012 (26-04-2012) 14 August 2013 (14-08-2013) 28 August 2013 (28-08-2013) 27 June 2013 (27-06-2013) 28 November 2013 (28-11-2013) 22 January 2014 (22-01-2014) 10 May 2012 (10-05-2012) 09 January 2014 (09-01-2014)