# Methods and devices for preparing and continuously printing multicellular cylinders onto biocompatible substrates

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## ABSTRACT

Methods and devices that provide scalable extrusion of cultured cells for use in forming three-dimensional tissue structures. In particular, described herein are methods for preparing and continuously extruding a compacted multicellular body material that may be used to form bioengineered tissue structures. Also described are extrusion tip assemblies that are adapted to receive a suspension of cellular material, and can be used both to prepare the cellular material (e.g. compaction) and to extrude the cellular material in an efficient manner for forming tissue structures.


## DESCRIPTION

METHODS AND DEVICES FOR PREPARING AND CONTINUOUSLY PRINTING

MULTICELLULAR CYLINDERS ONTO BIOCOMPATIBLE SUBSTRATES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This provisional patent application claims priority to U.S. provisional patent application no. 61/750,733, titled "DEVICE AND METHOD FOR FORMING

MULTICELLULAR CYLINDERS AND CONTINUOUSLY PRINTING MULTICELLULAR CYLINDERS ONTO BIOCOMPATIBLE SUBSTRATES" and filed on January 9, 2013; this provisional patent application is herein incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

FIELD

[0003] The devices and methods described herein relate generally to the fields of tissue formation, regenerative medicine, biomaterial and tissue engineering, pharmacology (e.g., drug testing), and academic research, including in particular the formation of tissue for consumption (engineered meat).

BACKGROUND

[0004] Tissue engineering by constructing three-dimensional tissue structures, which may be configured to mimic natural tissue structures, has been proposed using a variety of different techniques, including "organ printing". For example, U.S. Patent no. 8,241,905 (serial no. 10/590446) and U.S. Application no. 2012/01 16568 (serial no. 13/246,428) each describe methods and devices of organ (tissue) printing. Similarly, U.S. patent application no. US 2013/0029008 (application no. 13/558,928) describes the formation of comestible meat,

## CLAIMS (1)

**1.** What is claimed is:

1. A method of preparing and continuously extruding multicellular cylinders using an extrusion tip assembly having a body including an internal cavity, a nozzle at the proximal end of the body, and a venting piston at a distal end region of the body within the internal cavity, the method comprising:

blocking the nozzle of the extrusion tip assembly;

filling the internal cavity of the extrusion tip assembly with a suspension of cellular material;

securing the venting piston so that it cannot move within the internal cavity;

centrifuging the extrusion tip assembly to form a pellet of cellular material;

moving the venting piston until it contacts the pellet;

removing supernatant from the internal cavity of the extrusion tip assembly;

closing an opening through the venting piston;

unblocking the nozzle of the extrusion tip assembly; and

extruding cellular material from the nozzle.

2. The method of claim 1 , wherein blocking comprises inserting a plug into a capillary holder assembly forming the nozzle of the extrusion tip assembly.

3. The method of claim 1, wherein filling comprises filling through an opening in the venting piston.

4. The method of claim 1, wherein securing comprises engaging a piston retainer with the piston.

including by printing. Such methods may include the use of a cartridge or device holding a "bio ink" consisting of cultured cells. Unfortunately, such cartridges are not well adapted for scaling to high-volume operation, as they are inefficient, requiring larger volumes of materials (e.g., culture medium), and multiple steps to prepare and use.

[0005] Described herein are apparatuses (including devices and systems) and



devices may be used for preparing multicellular cylinders of uniform diameter, and depositing such cylinders onto biocompatible substrates continuously. The methods described allow the continuous extrusion of multicellular bodies

("continuous printing"). In general the multicellular bodies are multicellular tubes (e.g., cylinders). Any of the apparatuses described herein may be used to perform these methods.

SUMMARY OF THE DISCLOSURE

[0006] The present invention relates to methods and apparatuses (systems and devices) for preparing and continuously printing multicellular cylinders (e.g., multicellular tubes), including preparing and continuously extruding elongate multicellular bodies onto biocompatible substrates, where they may be grown into tissues. For example, described herein are methods and apparatuses that provide scalable extrusion of cultured cells for use in forming three- dimensional tissue structures. Described herein are extrusion tip assemblies that are adapted to receive a suspension of cultured cells and/or multicellular aggregates, and can be used to prepare the cells or multicellular aggregates for continuous extrusion in an efficient manner. These extrusion tip assemblies, by combining the preparation and extrusion of cellular material to form a multicellular body of sufficient size/amount, may improve the production of engineered tissues. Previously continuous extrusion of material (e.g., using a bio-printer) was limited to discrete lengths (including pellets) of material, as handling of larger amounts of material often resulted in poor yield and poor outcomes for the multicellular bodies.

[0007] For example, described herein are methods of preparing and/or continuously extruding cellular material. For example, described herein are methods of preparing and continuously extruding multicellular cylinders using an extrusion tip assembly having a body including an internal cavity, a nozzle at the proximal end of the body, and a venting piston at a distal end region of the body within the internal cavity. Such a method may include: blocking (e.g., plugging) the nozzle of the extrusion tip assembly; filling the internal cavity of the extrusion tip assembly with a suspension of cellular material; securing the venting piston so that it cannot move within the internal cavity; centrifuging the extrusion tip assembly to form a pellet of cellular material; moving the venting piston until it contacts the pellet; closing an opening through the venting piston; unplugging the nozzle of the extrusion tip assembly; and extruding cellular material from the nozzle. In some variations supernatant may be removed from the internal cavity of the extrusion tip assembly (either before or after advancing the piston within the cavity).

[0008] Blocking may comprise inserting a plug into a capillary holder assembly forming the nozzle of the extrusion tip assembly. Filling may comprise filling through an opening in the venting piston. Securing may comprise engaging a piston retainer with the piston. [0009] The method may also include releasing the venting piston after centrifuging so that it can slide within the internal cavity. The supernatant may be removed after moving the venting piston.

[00010] In some variations, closing the opening through the venting piston comprises inserting a piston plug into the opening. For example, closing the opening through the venting piston may comprise screwing a piston plug into a threaded region of the opening.

5. The method of claim 1 , further comprising releasing the venting piston after centrifuging so that it can slide within the internal cavity. 6. The method of claim 1, wherein the supernatant is removed after moving the venting piston.

7. The method of claim 1 , wherein closing the opening through the venting piston comprises inserting a piston plug

8. The method of claim 1, wherein closing the opening through the venting piston comprises screwing a piston plug into a threaded region of the opening.

9. The method of claim I, wherein unblocking the nozzle of the extrusion tip assembly comprises inserting a capillary lube into a capillary holder assembly forming the nozzle of the extrusion tip assembly.

10. The method of claim 1 , further comprising mounting the extrusion tip assembly into a bio-printer, extruder or bio-ptinlcr and extruder.

11. A method of preparing and continuously extruding multicellular cylinders using an extrusion tip assembly having a body including an internal cavity, a nozzle at the proximal end of the body, and a venting piston at a distal end region of the body within the internal cavity, the method comprising: filling the internal cavity of the extrusion tip assembly with a suspension of cellular material;

securing the venting piston so that it cannot move within the internal cavity;

centrifuging the extrusion tip assembly to form a pellet of cellular material;

moving the venting piston until it contacts the pellet;

closing an opening through the venting piston;

unblocking the nozzle of the extrusion tip assembly;

attaching an extrusio capillary to the nozzle of the extrusion tip assembly; and attaching the extrusion tip assembly to a bio-printer configured to extrude cellular

material from the extrusion capillary.

12. The method of claim 11, further comprising blocking the nozzle of the extrusion tip assembly.

13. The method of claim 1 1 , wherein filling comprises filling through an opening in the venting piston.

14. The method of claim 1 1 , wherein securing comprises engaging a piston retainer with the piston.

1 . The method of claim 11, further comprising releasing the venting piston ailcr ccntriiuging so that it can slide within the internal cavity.

16. The method of claim 11, wherein the supernatant is removed after moving the venting piston.

17. The method of claim 11 , wherein closing the opening through the venting piston comprises inserting a piston plug into the opening,

[00011] Unblocking the nozzle of the extrusion tip assembly may comprise inserting a capillary tube into a capillary holder assembly forming the nozzle of the extrusion tip assembly.

[00012] The method may further include mounting the extrusion tip assembly into a bio- printer, extruder or bio-printer and extruder.

[00013] In any of the variations of method of preparing and continuously



continuously extruding multicellular cylinders of material may use an extrusion tip assembly having a body including an internal cavity, a nozzle at the proximal end of the body, and a venting piston at a distal end region of the body within the internal cavity. Any of these methods may include the steps of: filling the internal cavity of the extrusion tip assembly with a suspension of cellular material; securing the venting piston so that it cannot move within the internal cavity; centrifuging the extrusion tip assembly to form a pellet of cellular material; moving the venting piston until it contacts the pellet; removing supernatant from the internal cavity of the extrusion tip assembly; closing an opening through the venting piston; unblocking the nozzle of the extrusion tip assembly; attaching an extrusion capillary to the nozzle of the extrusion tip assembly; and attaching the extrusion tip assembly to a bio-printer configured to extrude cellular material from the extrusion capillary.

[00014] Also described herein are apparatuses for preparing and continuously extruding multicellular cylinders (e.g., tubes, of any appropriate cross-section) are also described. For example, described herein are extrusion tip assembly devices for preparing and continuously extruding multicellular bodies that include: a body comprising an internal cavity for holding a suspension of cellular material; a nozzle region at a proximal end of the body; a venting piston at a distal end region of the body within, the venting piston configured to move within the internal cavity; a sealable opening through the venting piston; a piston plug configured to seal the sealable opening of the venting piston; and a piston retainer configured to prevent the piston plug from moving within the internal cavity when engaged.

[00015] The internal cavity of the body may comprise a glass outer surface, and may be configured as a glass tube. In some variations, the body is configured as an elongate body having a cylindrical internal cavity. [00016] In general, the internal cavity may be any appropriate shape or size. In particular, the internal cavity may be sized to allow continuous extrusion of extremely long cylinders of multicellular bodies. The extruded multicellular bodies may have a length of many cm (e.g., 10 cm, 20 cm, 30 cm, 40 cm, 50 cm, 60 cm, 70cm, 80 cm, 90 cm, 100 cm, 200 cm, 300 cm, 400 cm, 500 cm, etc.), and the internal cavity may include sufficient volume so that they may hold sufficient suspension of cellular material to continuously extrude (as opposed to discretely extrude) such a length of material. The cylinders may have any appropriate cross-section (e.g., round, oval, rectangular, square, crescent-shaped, bi-lobed, tri-lobed, etc.). Generally, the devices described herein may be referred to as devices for continuous printing, i.e. the nozzle continuously dispenses the cylindrical cellular material without interruption. Depending on the desired configuration of the multicellular body to be formed (e.g., sheets, etc.) the cylindrical material may be laid down continuously so that regions of the cylinder are adjacent or they can be separated by a certain predefined distance. In general, a shape may formed by a single elongate cylinder that is laid down in a serpentine or sigmoidal pattern so it doubles back so that each region is adjacent to itself; the regions of the cylinder forming the structure being laid down are parallel and in the same plane, but the printing can conform to printing on custom surfaces (e.g. one can even print on a curved surface). As discussed, the length of the continuous cylinder may depend on the volume enclosed in the syringe, and the area and shape of the printed construct can be varied and controlled by the printer. Thus, printing a structure such as a disk, square, rectangle, etc., depends on the layout provided by the printer. One benefit of the devices described herein is that they may allow a relatively large structure (e.g., sheet)

18. The method of claim 1 1, wherein closing the opening through the venting piston comprises screwing a piston plug into a threaded region of the opening. 1 . An extrusion Up assembly device for preparing and continuously extruding multicellular cylinders, the device comprising:

a body comprising an internal cavity for holding a

a nozzle region at a proximal end of the body;

a venting piston at a distal end region of (he body within, the venting piston configured to move within the internal cavity;

a scalable opening through the venting piston;

a piston plug configured to seal the scalable opening of the venting piston; and

a piston retainer configured to prevent the piston plug from moving within the internal cavity when engaged. 20. The device of claim 19, wherein the internal cavity of the body comprises an outer surface that is transparent and sterilizable.

2 . The device of claim 19, wherein the body is an elongate body having a cylindrical internal cavity.

22. The device of claim 19, wherein the nozzle region comprises a capillary holder attachment configured to hold a capillary tube from which cellular material can be extruded.

23. The device of claim 19, wherein the scalable opening through the venting piston comprises a threaded opening through the venting piston.

24. The device of claim 1 , wherein the piston plug comprises a threaded stein configured to male with threading within the scalable opening through the venting piston. 25. The device of claim 19, wherein the piston retainer comprises a threaded stem configured to mate with threading within tbe sealable opening through the venting piston.

26. An extrusion lip assembly device for preparing and continuously extruding multicellular cylindrical cylinders, the device comprising: an elongate body comprising an internal cavity for holding a suspension of cellular material;

a capillary holder attachment at a proximal end of the body configured to hold a capillary tube from which cellular material can be extruded;

a venting piston at a distal end region of the body, the venting piston configured to move within the internal cavity;

a sealable opening through the venting piston;

a piston plug configured to seal the sealable opening of the venting piston; and a piston retainer configured to prevent the piston plug from moving within the internal cavity when engaged.

of material to be printed from a single continuous extrusion.

[00017] In general, the nozzle region may comprise a capillary holder attachment configured to hold a capillary tube from which cellular material can be extruded. The nozzle can also be adapted to accommodate standard Luer-lock fitted needles.

[00018] The sealable opening through the venting piston may comprise a threaded opening through the venting piston.



configured to mate with threading within the sealable opening through the venting piston.

[00020] Also described herein are extrusion tip assembly devices for preparing and continuously extruding multicellular cylindrical bodies that include: an elongate body comprising an internal cavity for holding a suspension of cellular material; a capillary holder attachment at a proximal end of the body configured to hold a capillary tube from which cellular material can be extruded; a venting piston at a distal end region of the body, the venting piston configured to move within the internal cavity; a sealable opening through the venting piston; a piston plug configured to seal the sealable opening of the venting piston; and a piston retainer configured to prevent the piston plug from moving within the internal cavity when engaged.

BRIEF DESCRIPTION OF THE DRAWINGS

[00021] FIG. 1 illustrates one variation of a syringe (extrusion tip assembly), illustrating the capillary holder, body, and venting piston. The device is shown connected to a positive displacement extruder, which may form part of a bio-printer/extruder that may use any of the extrusion tip assemblies described herein.

[00022] FIG. 2 shows an expanded view of one variation of an extrusion tip assembly similar to the one shown in FIG. 1.

[00023] FIG. 3 shows an expanded view of the proximal (capillary) end of one variation of an extrusion tip assembly, including particularly the capillary holder portion of the extrusion tip assembly, and also illustrates the insertion of a capillary into the capillary holder.

[00024] FIGS. 4 A and 4B show expanded views of the distal (piston) end of the one variation of an extrusion tip assembly and illustrate interaction with a plug/engagement member (FIG. 4A) and a piston retainer member (FIG. 4B).

[00025] FIGS. 5A-5F illustrate operation of one variation of an extrusion tip assembly, in which the extrusion tip assembly is used to prepare and pellet the cells as well as for printing cellular material.

[00026] FIGS. 6A and 6B illustrate top and side perspective views, respectively of one pattern continuously extruded by an extrusion tip assembly as described herein.

DETAILED DESCRIPTION

[00027] Described herein are methods for continuously printing (e.g., using a bio-printer) prepared cellular material (e.g., multicellular bodies) that may be used to form bio-printed tissue structures, and apparatuses for performing these methods. These methods typically allow bio- printing of very long and continuous shaped having multiple turns, bends, and the like.

Continuous printing includes the extrusion of unbroken lengths of prepared cellular material that are long (e.g., >10 cm, >20 cm, >30 cm, >40 cm, >50 cm, >60 cm, >70cm, >80 cm, >90 cm, >100 cm, >200 cm, >300 cm, >400 cm, >500 cm, etc.). The methods described herein may include parallel continuous printing, in which either multiple extrusion tip assemblies are coupled adjacently and used to extrude, and/or one or more extrusions tip assembly includes multiple nozzles (or tips) for extruding parallel "lines" of cellular material. The methods and apparatuses described herein may be configured to print on flat, curved, or 3D surfaces.

Typically, the methods and extrusion tip assemblies for performing them described herein may be used with a bio-printer that may control the extrusion and formation of shapes from the material; the bio-printer may be adapted for continuous printing using one or more extrusion tip assembly.

[00028] In general, an extrusion tip assembly as described herein may be used to form multicellular cylinders of uniform diameter, and continuously deposit such cylinders onto biocompatible substrates. Any shaped tip ("capillary tube") may be used, including tips having circular cross-section, oval cross-section, rectangular cross-section, irregular (e.g., crescent-shaped, lobed, etc.) cross-sections, or the like. An extrusion tip assembly may be referred to as system or device (or part of a system or device), and may include alternative components (attachments) as described below, which may be used at different stages in the preparation and extrusion of the cellular material. An exemplary extrusion tip assembly is described below, and illustrated in the figures. It should be understood that the features and elements of the extrusion tip assembly

and methods of using them that are described herein may be adapted for use with a variety of configurations. Some, but not all, variations are also described herein.

[00029] The extrusion tip assemblies described herein, which may be referred to for convenience as syringes (though they may operate differently than traditional syringes), typically include a tip holder at the proximal end. The tip holder may be referred to as a capillary holder and the end it attaches to may be referred to as the capillary end. The tip holder may alternatively be configured as a nozzle and/or dedicated tip. The extrusion tip assembly may also include a body region



cells or multicellular aggregates of cells in suspension (e.g., dense cell suspension), process them to form a dense cell pellet (e.g., by centrifugation and removal of excess supernatant), and used with a bio- printer/extruder to extrude cells from the body through a tip (e.g., capillary) by applying force to the piston. Thus, these devices may be adapted for holding, concentrating (e.g., centrifugation) and extrusion. Further, the device may be adapted for use with a variety of tips (capillary tubes) and bio-printers/extruders.

[00030] The diameter of the cylinder extruded by an extrusion tip assembly may be predefined by the geometry of the tip of the device (e.g., the diameter of the nozzle opening) and may be limited by physiological requirements, imposed by the minimal thickness of the tissue where cells maintain their biological functions. [00031] The extrusion tip assemblies are typically configured to attach to a 3D bio-printer equipped with a positive-displacement extruder that is capable of precise positioning and movement of the device in three dimensions, as well as displacing the piston. A bio-printer equipped with such device may be used to create an initial configuration of cellular material that, upon post-printing self-assembly (i.e. fusion), may form multicellular structures, include, e.g., thick sheets of tissues. Thus, in some variations, the devices described herein enable rapid prototyping of extended configurations (or precursors) of biological tissue, therefore supplying complex 3D tissues consisting of one or multiple cell types with predefined structure. These tissues can be used in basic scientific research, pharmaceutical industry, tissue engineering and regenerative medicine. These devices and the methods of operating them are particularly valuable because they allow scaling up the production of biological material for applications where large quantities of biological tissues are needed (e.g., for the formation of extended-size constructs, as in the manufacturing of meat and leather engineering).

[00032] In general, the cellular material that may be used for printing may be prepared by culturing adherent cells according to standard protocols. Upon enzymatic removal from the culture flasks a single cell solution is obtained that is gently pelleted by centrifugation and re- suspended in a small amount of cell culture medium to facilitate transfer into the extrusion tip assembly (e.g., syringe).

[00033] As mentioned, the described devices and methods make the production of large quantities of biological tissues considerably more efficient, in terms of cost, time and accuracy, as they may speed up the process of assembly of 3D tissues, thus economizing on expensive components of tissue culturing, such as culture medium.

[00034] FIG. 1 illustrates one variation of an extrusion tip assembly. In the example shown in FIG. 1, the device includes a tip holder (e.g., capillary holder) (A), a body region (B), and piston (C). FIG. 1 also illustrates a positive displacement extruder (D), an attachment of a bio-printer that may be used with the extrusion tip assembly. For example, the piston and/or body may be configured to couple with a bio-printer including a positive displacement extruder (D). For example, the piston and/or body may be threaded or otherwise configured to engage (releasably engage) with the bio-printer including a positive displacement extruder.

[00035] In FIG. 1, the extrusion tip assembly includes tip holder configured as a capillary holder attachment (A) at the proximal end that serves to mount a capillary tube; the capillary tube may form the nozzle or "exit" from the apparatus. Any appropriate tip, including a capillary tube, may be used, and the tip holder may be adapted specifically to one or a range of tip (e.g., capillary tube) sizes, as described below. The opening of the tip is the nozzle from which the cellular material leaves the device. In some variations the capillary holder is optional; the proximal end may be a dedicated tip (rather than a holder) or a holder for a non-capillary tip.

[00036] The body of the device, syringe body (B), has biocompatible walls to which the cells/multicellular aggregates will not readily adhere. In some examples, the walls may be glass. Thus, in FIG. 1, the device includes a glass tube forming the inner walls of the internal cavity of the body for contacting the cellular material. The device also includes a piston (C), which is adapted as a venting piston by including an opening or aperture that may be closed off as described below. The assembly may also include a lock or retainer for the piston to hold the piston secure when centrifuging the device. The piston may include one or more openings extending the length (e.g., as a channel) though the piston body, allowing venting of the inner body chamber. Material within the body chamber may be added and/or removed using the opening through the piston, which may be referred to as the piston inner channel(s) or venting channel(s).

[00037] Thus, in general, the extrusion tip assembly may be used to both process (e.g., compact) cellular materials from an already dense suspension into a multicellular body for extrusion, and to extrude it during printing.

[00038] The extrusion tip assembly may be formed of a relatively stiff and sterilizable material, such as stainless steel, and fitted with high temperature rubber or Teflon O-rings and polyacrylic glass tube. In general the extrusion tip assembly is sterilizable without negatively impacting the function of the device. Thus, in this example, all of the components may be sterilized in a regular autoclave and assembled under sterile environment, such as a biosafety cabinet.

[00039] FIG. 2 depicts an exploded view of a portion of one example of an extrusion tip assembly, such as the variation shown in FIG. 1. In FIG. 2, the exemplary device is shown longitudinally expanded, to illustrate how the device may be



[00040] In this exemplary device, the extrusion tip assembly includes a rubber 0-ring (1), a capillary holder base (2), a syringe body (3), a polyacrylic (Lexan) tube (4), a tube retainer (5), a venting piston (6), and three piston O-rings (7). As shown in FIG. 2, a rubber O-ring (1) is fitted over the capillary holder base (2), and the capillary holder base slides in the syringe body (3), followed by the polyacrylic glass tube (4). The glass tube is secured by a tube retainer (5), which in this example is a hollow cylinder threaded on the outside that mates with the thread cut onto the inner surface of the syringe body. The tube retainer presses the glass tube against the O-ring, creating a seal; upon assembly its outer surface is flush with the syringe body. The tube retainer and glass tube have the same inside diameter. The piston (6) seals to the tube retainer and glass tube with three O-rings (7).

[00041] In general, the extrusion tip assembly may be configured differently for preparation of the cellular material (e.g., filling and compacting) and for extrusion. For example, the venting piston may be locked in position (e.g., using a piston retainer, not shown in FIG. 2) during centrifugation, and the venting piston may be plugged for extrusion. For the printing (extrusion) the nozzle, e.g., capillary holder base, may house a capillary tube, whereas for the cell compaction phase it may be blocked (e.g., plugged, such as with a short wire plug).

[00042] In some variations these different configurations are achieved by attaching one or more additional elements to the assembly. In some variations the different configurations may be achieved by actuating an element (e.g., lock, plug, seal, etc.) that is integral with the device. Thus, the configuration of the device may depend on the function the device has to fulfill in the workflow, described in more detail below.

[00043] FIG. 3 illustrates one variation of the proximal end of an extrusion tip assembly in which the tip (nozzle) is configured as a capillary holder assembly. In this example, the apparatus includes a rubber 0-ring (1), a threaded capillary holder base (2), a syringe body (3), the proximal end of a polyacrylic (Lexan) tube body region (4) is also illustrated, as is an example of a capillary tube (8), silicone gasket (9), capillary retainer elements (10), and a capillary fastener cap (1 1). As with any of the examples described herein the material chosen (e.g., silicone gasket, polyacrylic tube, etc.) may be modified or substituted without deviating from the structure (e.g., gasket, tube, etc.) and its related function.

[00044] As mentioned above, in this example, the nozzle of the device used in the printing step is a capillary tube coupled to the capillary holder attachment. In assembling the device, the capillary holder base (2) is integral with the syringe body (3), with the glass tube (4) and O-ring (1), assembled into the body of the apparatus. The capillary holder may be configured to accept any appropriately sized capillary tube. For example, in FIG. 3, the capillary holder base accepts capillary micropipettes of 1.5 mm outer and 0.3-0.5 mm inner diameters; these dimensions are customizable depending on the desired volume, shape and flow rate of the cellular material. The capillary micropipette (8) may be introduced into and moved through the silicone gasket (9) (OD of the former is slightly larger than the ID of the latter) until its end exits the gasket by about 1-2 mm. The capillary retainer elements (10) (shown in this example as two half solid cylinders with square shaped hole cut to fit the pipette's surface) wrap around the capillary and come in contact with the end surface of the silicone gasket. The capillary retainer elements (10) could be omitted, e.g., if larger diameter gaskets and capillaries are used. The capillary fastener cap (1 1), through its thread on its inner surface, mates with the capillary holder base (2) through threads on the latter's outer surface. The capillary fastener cap compresses the capillary retainer elements against the silicone gasket thus the latter is forced in its housing hole, where the axial deformation translates into radial pressure thus securing the capillary micropipette. In the absence of the capillary retainer elements (10), the capillary fastener cap (1 1) may press against a larger silicone gasket.

[00045] FIGS. 4 A and 4B depict different configurations of the assembly, particularly the attachments to the piston (6). In FIG. 4A, one variation of the distal (piston) end of the device is shown being assembled for extrusion, while in FIG. 4B, the piston end of the device is shown configured for compaction (centrifugation). In both FIGS. 4A and 4B, the distal end of the device shows a portion of the extrusion tip assembly body (3), a portion of the inner wall (e.g., polyacrylic, Lexan, tube) (4), and tube retainer (5), piston (6) and piston O-rings (7). FIG. 4A also shows a piston plug (12), while FIG. 4B shows a piston retainer (13). The piston plug (12) and piston retainer (13) may be used in separate phases of operation, and are illustrated in this example as separate components that may be attached (screwed on) to the main assembly during different phases of use. As mentioned above, in some configurations the piston plug and piston retainer may be integral with the rest of the device, e.g., as a valve and/or lock.

[00046] In this example, the two types of attachments for the piston, the piston retainer and the piston plug, may be attached into the opening (vent) through the piston. This opening may operate as a vent (e.g., when filling and/or removing

material from the internal cavity), but may be sealed during extrusion and/or during compaction. The capillary holder base may be plugged and the piston retainer (13) may be mounted into the piston (6) during compaction. When printing (extruding) with the device, a capillary micropipette may be fastened into the capillary holder base and a piston plug (12) may be mounted into the piston. As illustrated both the piston plug (12) and the piston retainer (13) may mount into the piston via threads. METHOD OF CONTINUOUS EXTRUSTION OF MULTICELLULAR BODIES

[00047] The extrusion tip assembly described above is one example of an apparatus that may be used to prepare and



extrusion, a blockable/openable tip region for extruding material, and a vented piston that can be locked in position during centrifugation and can be unlocked (and the vent/opening blocked) to allow sliding within the reservoir to extrude multicellular bodies from the tip. In operation, an extrusion tip assembly may efficiently both compact (e.g., pellet) cellular material, and to extrude it. [00048] FIGS. 5A-5F illustrate one method of operating an exemplary extrusion tip assembly, showing partially cut-away views of one variation of an extrusion tip assembly at different stages in of use. In this example, as shown in FIG. 5A, the extrusion tip assembly is configured so that it can be filled with cellular material by securing a wire plug (14) into the capillary holder assembly, with the venting piston (6) inserted into the glass tube/tube retainer near the end (proximal end) of the body of the extrusion tip assembly. The volume enclosed by the glass tube in the syringe holder is then filled through the opening through the piston (in this example a vent hole is located in the center of the piston) with a dense cellular suspension (15).

[00049] In FIG. 5B, a piston retainer (13) is mounted to the piston to prevent sliding the venting piston into the glass tube forming the internal cavity of the body during centrifugation.

The piston retainer locks the piston in position. The device is transferred into a centrifuge and gently spun to pellet the cells.

[00050] FIG. 5C shows the device after centrifugation. The dense cell suspension is separated into a supernatant (16) (essentially cell-free tissue culture medium) and a dense cell pellet (17).

[00051] In FIG. 5D, the piston retainer (13) is removed and the piston (6) is pushed down until it comes in contact with the cell pellet. The piston may be pushed down with any appropriate device. For example, a vented pushing handle may be attached to the piston; the vented pushing handle may allow the device to be driven manually or automatically. The venting in the pushing handle may be coupled to a vacuum or channel to remove the supernatant (16) as it is pushed down, or it may be pushed down, and then removed, as illustrated in FIG. 5D-5E. In some variations the piston level relative to any pellet/supernatant may be monitored; for example, a window though the extrusion tip assembly may allow the operator (or automatic device) to see the pellet position relative to the piston (6).

[00052] In FIG. 5D, the supernatant is displaced to the top of the piston through the threaded hole and may be disposed (e.g., via suction). As mentioned, the piston may be manually displaced, e.g., using a tool to push it down until it contacts (of just before it contacts) the pellet, or it may be automatically displaced.

[00053] In FIG. 5E, the piston plug (12) is attached to close off the vent of the piston, enclosing the cellular material into the internal cavity (e.g., glass tube) of the device. A piston plug (as shown in FIG. 4A) may be connected to the piston by a threading or other attachment, by a friction fit (e.g., one or more o-rings or the like), etc. In some variations the piston plug may be integral with the pushing handle.

[00054] As shown in FIG. 5F, the nozzle may be opened (e.g.., removing the wire plug) and a tip (e.g., capillary micropipette (8)) fastened into the tip holder assembly to form the nozzle. The device can then be transferred into a 3D bio-printer or extruder equipped with positive displacement control. Moving the printing head in 3D and advancing the piston with the positive displacement extruder may allow the cellular material to be extruded onto or into a suitable biocompatible substrate.

[00055] Once coupled to the bio-printer, the apparatus may push against (and/or pull) the piston to continuously extrude multicellular body material from the tip. A continuous length of multicellular bodies may be extruded in any appropriate length, including in particular lengths of greater than 10 cm (e.g., >20 cm, >30 cm, >40 cm, >50 cm, >60 cm, >70cm, >80 cm, >90 cm, >100 cm, >200 cm, >300 cm, >400 cm, >500 cm, etc.). The bio-printer may adjust the pattern that is printed, laying down curves, shapes, etc. The bio-printer may also be configured to form 3D shapes by continuously printing a line of multicellular bodies back on top of the earlier- extruded end of the line. The bio-printer may stop extruding and may automatically "cut" or end one line and/or begin another one. In some variations parallel lines of multicellular bodies are extruded from one or more extrusion tip assembly.

[00056] FIGS. 6A and 6B schematically illustrate the use of an exemplary extrusion tip assembly to continuously print multicellular material that can fuse to form a sheet. The extrusion tip assemblies described herein allow this structure (in this example, a rectangular sheet) to be formed from a single extruded cylinder laid down in the continuous serpentine path shown. The continuous length of multicellular body is >25 cm long in this example. In some variations, the method

may include continuously extruding long lengths (e.g., >10 cm, >20 cm, etc.) of multicellular material that can then be cultured and allowed to form sheets or other 3D structures; additional continuous extrusion of multicellular bodies may then be performed on the formed structures. As mentioned above, the continuous preparation and extrusion methods described above may be used as a part of (and may therefore include additional steps for) the formation of tissue such as comestible tissue, engineered organs, etc.

[00057] As used herein in the specification and claims, including as used in the examples and unless otherwise expressly



indicate that the value and/or position described is within a reasonable expected range of values and/or positions. For example, a numeric value may have a value that is +/- 0.1% of the stated value (or range of values), +/- 1% of the stated value (or range of values), +/- 2% of the stated value (or range of values), +/- 5% of the stated value (or range of values), +/- 10% of the stated value (or range of values), etc. Any numerical range recited herein is intended to include all sub-ranges subsumed therein. [00058] Although various illustrative embodiments are described above, any of a number of changes may be made to various embodiments without departing from the scope of the invention as described by the claims. For example, the order in which various described method steps are performed may often be changed in alternative embodiments, and in other alternative embodiments one or more method steps may be skipped altogether. Optional features of various device and system embodiments may be included in some embodiments and not in others.

Therefore, the foregoing description is provided primarily for exemplary purposes and should not be interpreted to limit the scope of the invention as it is set forth in the claims.

[00059] The examples and illustrations included herein show, by way of illustration and not of limitation, specific embodiments in which the subject matter may be practiced. As mentioned, other embodiments may be utilized and derived there from, such that structural and logical substitutions and changes may be made without departing from the scope of this disclosure. Such embodiments of the inventive subject matter may be referred to herein individually or collectively by the term "invention" merely for convenience and without intending to voluntarily limit the scope of this application to any single invention or inventive concept, if more than one is, in fact, disclosed. Thus, although specific embodiments have been illustrated and described herein, any arrangement calculated to achieve the same purpose may be substituted for the specific embodiments shown. This disclosure is intended to cover any and all adaptations or variations of various embodiments. Combinations of the above embodiments, and other embodiments not specifically described herein, will be apparent to those of skill in the art upon reviewing the above description.

## PATENT CITATIONS

| Cited Patent | Filing date | Publication date | Applicant | Title |
|---|---|---|---|---|
| KR100716015B1 * | | | | *Title not available* |
| US4465472 * | Nov 22, 1982 | Aug 14, 1984 | American Hospital Supply Corp. | Syringe cartridge and method |
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* Cited by examiner

## CLASSIFICATIONS

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|---|---|
| International Classification | C12M1/00 |
| Cooperative Classification | C12M45/02, B29L2031/7532, B29C67/0055, B29K2995/0056, B29C47/0004, B29C47/122, B29C47/0023, B29C47/765, B30B11/221, C12M21/08 |

## LEGAL EVENTS

| Date | Code | Event | Description |
|---|---|---|---|
| Sep 3, 2014 | 121 | Ep: the epo has been informed by wipo that ep was designated in this application | **Ref document number:** 14738125<br>**Country of ref document:** EP<br>**Kind code of ref document:** A1 |
| Jul 9, 2015 | NENP | Non-entry into the national phase in: | **Ref country code:** DE |
| Feb 3, 2016 | 122 | Ep: pct app. not ent. europ. phase | **Ref document number:** 14738125<br>**Country of ref document:** EP<br>**Kind code of ref document:** A1 |