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(54) Title: CAPILLARY-EMBEDDED RAPID ADAPTABLE MULTISCALE BIOPRINTING FOR ENGINEERING LARGE VASCULARIZED TISSUES

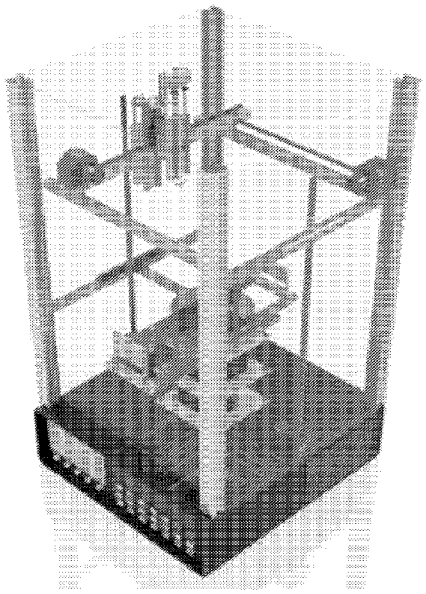

FIG. 4

(57) Abstract: A capillary-incorporated bioprinting system for rapid engineering of vascularized tissues, the system comprising a three-dimensional printing head, wherein the three-dimensional printing head is configured on a three-axis linear stage; a spinneret, wherein the spinneret is configured on a horizontal linear stage and the spinneret is affixed above the three-dimensional printing head; a UV light source, wherein the UV light source is operatively coupled to the three-dimensional printing head and the spinneret, and adjacent to the spinneret; and a pair of parallel bars, wherein the pair of parallel bars are configured to collect a plurality of electrospun aligned microtubes.

WO 2024/254588 A2

TITLE: Capillary-Embedded Rapid Adaptable Multiscale Bioprinting for Engineering Large Vascularized Tissues

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority to U.S. Appl. Serial No. 63/507,065, filed June 8, 2023, entitled "Capillary-Embedded Rapid Adaptable Multiscale Bioprinting for Engineering Large Vascularized Tissues," which patent application is commonly owned by the owner of the present invention. This patent application is incorporated herein in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to the field of three-dimensional (3D) bioprinting and electrospinning. In particular, the present disclosure relates to the combination of 3D bioprinting and electrospinning for the fabrication of multi-scale scaffolds integrated with artificial capillary vessels.

STATEMENT OF FEDERALLY FUNDED RESEARCH

[0003] This disclosure is related to federally sponsored research and development under the government funding from NSF Grant No. CMMI-2145108.

[0004] The invention was made with United States government support. The United States government has certain rights in the invention.

BACKGROUND

[0005] A major problem facing the healthcare industry is the shortage of transplant organs. In the United States, hundreds-of-thousands of healthcare patients are already waiting for organ transplants and each day the number of patients rises by dozens.

[0006] Advanced manufacturing has enabled the fabrication of 3D biological constructs through 3D bioprinting, which can allow for the repair of damaged tissues or organs. For most engineered tissues and organs, forming the microvascular structure is essential because the viability of tissues relies on the exchange of nutrients and waste substances, which is maintained by capillary vessels in native tissues.

[0007] For a scaffold to have long-term cell viability, the cells need to be within approximately 200 microns (μm) from the engineered tissue surface. However, it is extremely challenging and time-consuming to create a centimeter-sized microchannel diffusion system within a 3D bioprinted tissue.

[0008] Accordingly, there is extreme difficulty establishing the rapid fabrication of large 3D cell-laden constructs with effective capillary vasculatures, which have diameters ranging from 5 to 10 μm. This problem has stifled the creation of a centimeter-sized microchannel diffusion system within a 3D cell-laden scaffold.

[0009] Decellularized allogenic or xenogenic grafts may provide naturally and intact vascular conduits. But, nonetheless, these grafts have associated difficulties, such as limits in obtaining personalized organ scaffolds, limited types of organs, and potential disruption to the extracellular matrix (ECM).

[0010] Further, the current methods for promoting a complex vascular network formation are unable to accomplish the spatiotemporal scaling-up of the process that would be necessary for a rapid fabrication system for tissue constructs of a clinically-relevant size.

[0011] Therefore, there is a need for a solution that can provide a hybrid bioprinting technology that can rapidly incorporate thousands of porous microtubes per one square centimeter area, which function like capillary vessels, to a centimeter sized 3D printed scaffold.

SUMMARY OF THE DISCLOSURE

[0012] The present disclosure is directed to systems and a method for capillary-incorporated bioprinting that combines electrospinning and 3D bioprinting for fabricating multi-scale scaffolds integrated with biomimetic porous microtubes that function as capillary vessels.

[0013] To address the need disclosed above, the present disclosure provides a hybrid bioprinting technology that can incorporate thousands of porous microtubes to a centimeter sized 3D printed scaffold within several minutes. The present disclosure allows for a high resolution with a rapid speed of printing. The present disclosure provides a multi-scale biofabrication process for optimal tissue regeneration. In certain embodiments, the subsystem of porous microtube electrospinning can be incorporated into a wide range of additive manufacturing processes, including stereolithography, inkjet bioprinting, and fused deposition modeling.

[0014] In general, in one embodiment, the disclosure features a capillary-incorporated bioprinting system for rapid engineering of vascularized tissues. The system can include a three-dimensional printing head. The three-dimensional printing head can be configured on a three-axis linear stage. The system can also include a spinneret. The spinneret can be configured on a horizontal linear stage. The spinneret attached to the three-dimensional printing head can be retracted above or below the printing head. The system can also include a UV light source. The UV light source can be operatively coupled to the three-dimensional printing head and the spinneret. The UV light source can be adjacent to the spinneret. The system can also include a pair of distance adjustable parallel bars. The pair of parallel bars can be configured to collect a plurality of electrospun aligned microtubes.

[0015] In general, in another embodiment, the disclosure features a method for engineering vascularized tissues using a capillary-incorporated bioprinting system. The method can include printing a first layer of a hydrogel onto a printing area using a three-dimensional printing head. The method can include returning the three-dimensional printing head to a home position. The method can include electrospinning microfibers onto a pair of parallel bars. The electrospinning can align the microfibers on top of the first layer of the hydrogel. The parallel bars can rotate and align the fiber in any angle from 0 to 360 degrees on the printed layers. The method can include submerging, due to gravitational forces, the microfibers in the first layer of the hydrogel. The method can include, resultant from the submerging, forming porous microtubes in the first layer of hydrogel. The method can include cross-linking the first layer of the hydrogel by exposing the first layer of the hydrogel to a UV light source. The cross-linking can create a cross-linked base of the hydrogel.

[0016] In general, in another embodiment, the disclosure features a bioprinting electrospinning hybrid printer including a parallel plate collector. The parallel plate collector can include one or more plates with an adjustable distance and an adjustable angle. The bioprinting electrospinning hybrid printer can also include an electronic control module. The electronic control module can be configured to control one or more major stage-motion servos and one or more flow-control servos. The bioprinting electrospinning hybrid printer can also include a hybrid electrospinning bioprinting nozzle including one or more electrospinning syringes and one or more bioprinting syringes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Other advantages of the present disclosure will be apparent from the following detailed description of the disclosure in conjunction with embodiments as illustrated in the accompanying drawings, in which:

[0018] FIG. 1 depicts a prior capillary-incorporated system in a hydrogel scaffold.

[0019] FIG. 2A depicts sequential bioprinting-electrospinning-bioprinting operations, in accordance with certain embodiments of the present disclosure.

[0020] FIG. 2B depicts a capillary-incorporated bioprinting system, in accordance with certain embodiments of the present disclosure.

[0021] FIG. 3 depicts the capillary-incorporated bioprinting process for a scaffold with embedded fibrous networks and cells, in accordance with certain embodiments of the present disclosure.

[0022] FIG. 4 depicts a bioprinting electrospinning hybrid printer, in accordance with certain embodiments of the present disclosure.

[0023] FIGS. 5A-5E depict components in the bioprinting electrospinning hybrid printer, in accordance with certain embodiments of the present disclosure. **FIG. 5A** depicts a perspective view of the complete bioprinter electrospinning hybrid printer. **FIG. 5B** depicts an isometric view of a parallel plate collector. **FIG. 5C** depicts a side view of a parallel plate collector. **FIG. 5D** depicts an isometric view of a hybrid printhead. **FIG. 5E** depicts a side view of a hybrid printhead.

[0024] FIG. 6 depicts a variable angle parallel plate collector mechanism, in accordance with certain embodiments of the present disclosure.

[0025] FIG. 7A depicts a hybrid electrospinning bioprinting nozzle system, in accordance with certain embodiments of the present disclosure.

[0026] FIG. 7B depicts a hybrid electrospinning bioprinting nozzle system, with each component of the system depicted separately, which when configured together are in accordance with certain embodiments of the present disclosure.

[0027] FIG. 8 depicts a hybrid printhead three-axis motion system, in accordance with certain embodiments of the present disclosure.

[0028] FIG. 9 depicts a servo control connection layout, in accordance with certain embodiments of the present disclosure.

NOTATION AND NOMENCLATURE

[0029] Various terms are used to refer to particular system components. Different companies may refer to a component by different names – this document does not intend to distinguish between components that differ in name but not function. In the following discussion and in the claims, the terms "including" and "comprising" are used in an open-ended fashion, and thus should be interpreted to mean "including, but not limited to . . . ." Also, the term "couple" or "couples" is intended to mean either an indirect or a direct connection. Thus, if a first device couples to a second device, that connection may be through a direct connection or through an indirect connection via other devices and connections.

[0030] The terminology used herein is for the purpose of describing particular example embodiments only, and is not intended to be limiting. Following long-standing patent law convention, the terms "a" and "an" mean "one or more" when used in this application, including the claims.

[0031] As used herein, the singular forms "a," "an," and "the" may be intended to include the plural forms as well, unless the context clearly indicates otherwise. The method steps, processes,

and operations described herein are not to be construed as necessarily requiring their performance in the particular order discussed or illustrated, unless specifically identified as an order of performance. It is also to be understood that additional or alternative steps may be employed.

[0032] The terms first, second, third, etc. may be used herein to describe various elements, components, regions, layers and/or sections; however, these elements, components, regions, layers and/or sections should not be limited by these terms. These terms may be only used to distinguish one element, component, region, layer, or section from another region, layer, or section. Terms such as "first," "second," and other numerical terms, when used herein, do not imply a sequence or order unless clearly indicated by the context. Thus, a first element, component, region, layer, or section discussed below could be termed a second element, component, region, layer, or section without departing from the teachings of the example embodiments. The phrase "at least one of," when used with a list of items, means that different combinations of one or more of the listed items may be used, and only one item in the list may be needed. As used herein, the term "and/or" when used in the context of a listing of entities, refers to the entities being present singly or in combination. Thus, for example, the phrase "A, B, C, and/or D" includes A, B, C, and D individually, but also includes any and all combinations and subcombinations of A, B, C, and D. Accordingly, as an example, "at least one of: A, B, and C" includes any of the following combinations: A, B, C, A and B, A and C, B and C, and A and B and C. In another example, the phrase "one or more" when used with a list of items means there may be one item or any suitable number of items exceeding one.

[0033] Spatially relative terms, such as "inner," "outer," "beneath," "below," "lower," "above," "upper," "top," "bottom," and the like, may be used herein. These spatially relative terms can be used for ease of description to describe one element's or feature's relationship to another

element(s) or feature(s) as illustrated in the figures. The spatially relative terms may also be intended to encompass different orientations of the device in use, or operation, in addition to the orientation depicted in the figures. For example, if the device in the figures is turned over, elements described as "below" or "beneath" other elements or features would then be oriented "above" the other elements or features. Thus, the example term "below" can encompass both an orientation of above and below. The device may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptions used herein interpreted accordingly.

[0034] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0035] The present disclosure provides systems and methods for capillary-incorporated bioprinting combineing electrospinning and 3D bioprinting for fabricating multi-scale scaffolds integrated with biomimetic porous microtubes that function as capillary vessels.

[0036] The capillary-incorporated bioprinting system can include a scalable 3D bioprinted molecular scaffold that incorporates the blood vascular architecture to support tissue regeneration and organ development. Specifically, the system may allow for the incorporation of thousands of porous microtubes over an area of 1 cm^2 that function as capillary vessels and maintain a requisite diameter of approximately 5 to 10 μm in length.

[0037] The incorporation of the microtubes can generate a centimeter-sized 3D-printed scaffold a rate approximately ten times faster and with approximately 10-fold higher resolution than the present 3D printing technologies in the field. As such, the 3D bioprinting process may rapidly incorporate thousands of porous microtubes that function as capillary vessels to a centimeter-sized 3D printed scaffold within several minutes. From this, the system for porous microtube electrospinning can be incorporated into a wide range of additive manufacturing processes, including for example but not limited to stereolithography, inkjet bioprinting, and fused deposition modeling.

[0038] The adaptable 3D bioprinting process may, in some embodiments, allow for engineering large, vascularized tissues and capillaries. In such an embodiment, the process for capillary-incorporated bioprinting can combine electrospinning and 3D bioprinting. The 3D bioprinting may be extrusion-based in some embodiments. In other embodiments, the 3D bioprinting may be inkjet-based.

[0039] In some embodiments, the capillary-incorporated bioprinting system may be used to fabricate multiscale scaffolds integrated with biomimetic porous microtubes. In such an embodiment, the biomimetic porous microtubes may function as capillary vessels.

[0040] FIG. 1 depicts a prior capillary-incorporated system in a hydrogel scaffold. As shown in the embodiment of FIG. 1, the capillary-incorporated system can be generated on a 3D printed microtube-embedded hydrogel scaffold. The system depicted in FIG. 1 may be enhanced through the use of a three-axis linear stage, as depicted in FIG. 2.

[0041] FIG. 2A depicts sequential bioprinting-electrospinning-bioprinting operations, in accordance with certain embodiments of the present disclosure.

[0042] In some embodiments, a layer of co-axially electrospun core-sheath microtube can be sandwiched between alternate layers of 3D bio-compatible extrusion-printed hydrogels. In such an embodiment, automated alternate hydrogel-microtube-hydrogel fabrication can be accomplished without any manual handling that would potentially damage the fine electrospun microtube structure. Further, in such embodiment, the operations may include switching between random and highly aligned microtube orientations as per the design requirements.

[0043] In some embodiments, through the use of the sequential bioprinting-electrospinning-bioprinting operations depicted in FIG. 2, the system may have full control over microtube morphology (diameter, wall thickness, orientation) and the option to change fiber orientation direction.

[0044] FIG. 2B depicts a capillary-incorporated bioprinting system, in accordance with certain embodiments of the present disclosure.

[0045] In some embodiments, the capillary-incorporated bioprinting system can include a 3D printing head. For example, the 3D printing head may be a mechanical-driven or a pneumatic-driven extrusion-based 3D printing head. The 3D printing head may be located on a 3-axis, 4-axis, or 5-axis motorized stage.

[0046] In some embodiments, the capillary-incorporated bioprinting system can include a concentric dual-channel spinneret on a horizontal linear stage. For example, the concentric dual-channel spinneret may be set above the 3D printing head.

[0047] In some embodiments, the capillary-incorporated bioprinting system may also include a UV light source. The UV light source, in certain embodiments, may be adjacent to the spinneret. The UV light source may also be a 395nm UV light.

[0048] In the capillary-incorporated bioprinting system, there can also be a pair of parallel aluminum or stainless steel bars, which may be used as the collector for electrospinning aligned microtubes. The bars can be placed on a rotary stage that can adjust the orientation of the microtube alignment.

[0049] In certain embodiments, the capillary-incorporated bioprinting system provides enhanced capabilities based on the use of a three-axis linear stage and concentric dual-channel spinneret. In such an embodiment, the capillary-incorporated bioprinting system may also include working cycle for UV-curable hydrogels to support the polyethylene glycol diacrylate (PEGDA) or gelatin methacryloyl tissue scaffolds.

[0050] In some embodiments, a height-adjustable table may be included in the capillary-incorporated bioprinting system.

[0051] FIG. 3 depicts the capillary-incorporated bioprinting process for a scaffold with embedded fibrous networks and cells, in accordance with certain embodiments of the present disclosure.

[0052] As shown in FIG. 3, the working cycle for UV-curable hydrogels can begin by one layer of hydrogel being 3D printed. Following this, in such an embodiment as shown in FIG. 3 through use of the system in FIG. 2, the printing head can return to the home position. Before cross-linking, in such an embodiment, core-sheath microfibers will be electrospun onto two parallel collector bars. In this embodiment, the electrospinning of the core-sheath microfibers allows for forming aligned microfibers on top of the hydrogel. Due to gravity, microfibers, in the capillary-incorporated bioprinting system of FIG. 2 for use in the process of FIG. 3, can immediately submerge in the hydrogel and turn into porous microtubes. Accordingly, in such an embodiment, the hydrogel can then be cross-linked by UV exposure or other crosslinking mechanisms.

[0053] In such an embodiment, after the cross-linking, the printing table can be lowered, and the second layer of hydrogel can be printed on the cross-linked base. After the printing, in some embodiments where necessary, the excess microtubes outside the hydrogel can be trimmed.

[0054] Accordingly, the process shown in FIG. 3, for use with the system of FIG. 2, combines bioprinting methods with co-axial electrospinning methods.

[0055] FIG. 4 depicts a bioprinting electrospinning hybrid printer, in accordance with certain embodiments of the present disclosure. The hybrid printer of FIG. 4 can be designed to allow for tissue engineered production of perfused scaffold structures.

[0056] In certain embodiments, the motion system may be enclosed within a closed chamber to ensure sterile environment for cell encapsulated bioprinting. At the bottom of the bioprinting electrospinning hybrid printer, a control module may be present. The control module may provide high voltage potential difference for electrospinning, control for all the servos in the system, USB connection to control the stage motions and flowrates using standard G-codes, and combinations thereof. The bioprinting electrospinning hybrid printer of FIG. 4 can allow simultaneous bioprinting and electrospinning operations for biofabrication of microtube encapsulated 3D hydrogel structures. Further, the bioprinting electrospinning hybrid printer can include a parallel plate collection system with retractable distance and variable angle adjustment. The parallel plate collection system can allow for deposition of aligned microtubes along any desired angle along the XY-axis.

[0057] FIGS. 5A-5E depict components in the bioprinting electrospinning hybrid printer, in accordance with certain embodiments of the present disclosure. FIG. 5A depicts a perspective view of the complete bioprinter electrospinning hybrid printer. FIG. 5B depicts an isometric view of a

parallel plate collector. FIG. 5C depicts a side view of a parallel plate collector. FIG. 5D depicts an isometric view of a hybrid printhead. FIG. 5E depicts a side view of a hybrid printhead.

[0058] In some embodiments, the bioprinting electrospinning hybrid printer can be sterile and fully servo-controlled. For example, the bioprinting electrospinning hybrid printer may be designed to provide electronic servo-controlled flow systems for the bioprinting and electrospinning solution. In such an embodiment, the design would eliminate the need for auxiliary components, such as for example syringe pumps. In this embodiment, a single electronic control module can control the major stage-motion servos and the flow-control servos. By eliminating the need for any hydraulic or pneumatic pipe routings into the fabrication chamber, in such an embodiment, the system can be a sterile environment for biofabrication operations with cell incorporated bio-inks.

[0059] In some embodiments, the bioprinting electrospinning hybrid printer can include an adjustable distance and angle parallel plate collector. The parallel plate collectors, in such an embodiment, may collect highly aligned electrospun microfibers or microtubes. The overall fabrication process of aligned fiber and microtubes can depend on the distance of the plates from the electrospinning nozzle and the distance between the plates. For tissue engineering applications, repeated opening and closing of the enclosure could introduce risks of contaminations. Furthermore, in tissue engineering applications, manual tweaking of the plates introduces more process variability. Accordingly, in some embodiments, the bioprinting electrospinning hybrid printer may include a computer controlled parallel plate collection system that can control the inter-plate distance. Furthermore, in some embodiments, a secondary servo attached to the system allows changing the angle of the plates with respect to the bio-print bed, thus adding more design variability in the fabrication of the microtube incorporated hydrogel structures. The plates may

also have a resistive heating option to melt off the collected fibers and microtubes and deposit them directly on the hydrogel layer without any manual handling.

[0060] In some embodiments, the bioprinting electrospinning hybrid printer can include a hybrid electrospinning bioprinting nozzle. In the bioprinting electrospinning hybrid printer, in certain embodiments, the electrospinning syringes and bioprinting syringes may be combined into a single and common hybrid nozzle system. Accordingly, all the nozzles may be controlled using mini-scale, high precision servos instead of the traditional pneumatic or hydraulic systems. In such an embodiment, the bioprinting nozzle can be extended or retracted along the z-axis using a separate bioprinthead control servo, which may reduce the interference of the bioprinting nozzle with the electrospinning nozzle during each of their individual operation procedures. Also, each of the servos may be individually controlled, triggered, stopped, and initiated using servo control systems running on G-codes. In some embodiments, for example, the bioprinter and the electrospinning syringes can be switched off individually when the other nozzles are operational, thus reducing the impact of material dripping.

[0061] FIG. 6 depicts a variable angle parallel plate collector mechanism, in accordance with certain embodiments of the present disclosure.

[0062] The parallel plate collection system, in certain embodiments, allows for the combined flexibility of handling-free microtube deposition with precision and control over fiber morphology and alignment angle. As shown in FIG. 6, in some embodiments, a rack-pinion gear system coupled with a servo motor at the bottom of the system can control the gap between the parallel plates. The precise gap control can be a fabrication parameter that varies with different electrospinning polymer solutions, and other process parameters, such as collector-nozzle distance and operating voltage. The gap between the plates can be controlled using the rotational angle

position of the parallel plate retraction servo using a standard G-code program, which can ensure repeatability of the same gap position down to the mm-scale and thus a more precise microtube fabrication method.

[0063] In some embodiments, a secondary servo may function as a parallel plate rotation servo. The parallel plate rotation servo may be positioned directly beneath the housing for the retraction servo. The parallel plate rotation servo can, in certain embodiments, rotate all the attachments, including but not limited to the parallel plate retraction servo, rack/pinion gear system, parallel plates, above the parallel plate rotation servo along the XY-plane. By doing so, in such an embodiment, the parallel plate rotation servo can allow the collection of the fibers and microtubes in any angular position with respect to the X or Y-axis on the build-plate. Therefore, the parallel plate rotation servo can provide more design options to generate complex fiber and microtube infused hydrogel structure designs for research applications.

[0064] In some embodiments, the print plate may be mounted on a servo system that can move the print plate along the Z-axis. In such an embodiment, once a first layer of hydrogel is printed on the print plate, the aligned microtubes can be collected between the build plates keeping a certain gap from the hydrogel layer. Through the process, once the first layer of hydrogel and aligned fibers are fabricated, the print-bed Z-axis servo may lift the print-bed up so that the hydrogel layer just touches the aligned fibers. The precise position of this Z-travel can be controlled using G-codes to ensure that the fibers are positioned precisely at the interface of the hydrogel.

[0065] In some embodiments, the parallel plates can have two operation modes. The first operation mode, for example, may allow the parallel plate to be connected in the negative/ground terminal and thus act as collector surfaces for the electrospinning operation. The second operation mode,

for example, may switch the connection to the plates from a single potential to a series-connected system, turning the parallel plates into resistive heating sources. The second operation mode, in some embodiments, may melt the fibers from the ridges of the plates and softly allow them to land on the hydrogel surface under gravitational forces without any other external forces, which may ensure that the fibers are not deformed from handling.

[0066] In some embodiments, a separate precision spray nozzle may be added to the system. The separate precision spray nozzle may allow chemical crosslinking without manual-handling.

[0067] FIG. 7A depicts a hybrid electrospinning bioprinting nozzle system, in accordance with certain embodiments of the present disclosure. FIG. 7B depicts a hybrid electrospinning bioprinting nozzle system, with each component of the system depicted separately, which when configured together are in accordance with certain embodiments of the present disclosure.

[0068] The hybrid nozzle system can eliminate the problem of mounting the two different fabrication nozzles onto separate motion-axis systems and combines them under the same three-axis motion system, thus allowing the easy operation of both the nozzles using the same control system platform.

[0069] In some embodiments, a core-sheath syringe holder system can consist of two syringe holders. In certain embodiments, the core-sheath syringe holder system can also consist of two mechanical servo systems to individually control the flowrates of the two solutions. In some embodiments, the polymer solution holder system can also consist of a single-syringe holder that can be mechanically controlled using a servo mounted at the back of the syringe holder. Positioned in the back of the bioprinting syringe holder, in some embodiments, a sliding channel can exist for attachment with the bioprint head and electrospinning head junction body. For example, the junction body can be fixed to the coaxial syringe system on one side. In such an embodiment, on

the other side, the junction body can have sliding channels that attach to the bioprinting syringe holder system. In certain embodiments, as shown in FIGS. 7A and 7B, a servo mounted on the top of the junction body can controls the Z-axis motion of the bioprinting nozzle. Using this servo mechanism, in such an embodiment, the bioprint head can be moved up and down during the toggling operation between electrospinning and bioprinting mode. In some embodiments, as shown in FIG. 7B, the bioprinting nozzle system can include a heating pad operatively connected to the bioprinting extruder.

[0070] FIG. 8 depicts a hybrid printhead three-axis motion system, in accordance with certain embodiments of the present disclosure.

[0071] As shown in FIG. 8, the z-axis slider arm can be mounted between the aluminum housing frames to support the weight of the system during the motion. Two y-axis slider arms can be bolted or welded to the z-axis slider arm. Both of the slider arms can have channel grooves to support the sliding of the x-axis slider arm over each slider arm. In some embodiments, one of the y-axis slider arms can host the y-axis control servo system. In such an embodiment, the x-axis slider arm can rest between the two y-axis slider arms. As shown in FIG. 8, the hybrid printhead can be mounted directly on the x-axis slider arm and slide along the slider arm when the x-axis servos are engaged. Accordingly, in some embodiments, the x-axis servo screw-rod can run directly through the junction body in the hybrid printhead system and control the motion via screw-driven motion.

[0072] FIG. 9 depicts a servo control connection layout, in accordance with certain embodiments of the present disclosure. Specifically, FIG. 9 depicts the connection layout for the logical servo control systems. All of the servo connections can originate from the servo control module system, which can be located at the base of the printer housing. In some embodiments, the same module system controls the high-voltage for the electrospinning operation and acts as a USB interface for

computer G-codes and software connection. The six red connection lines, as shown in FIG. 9, can indicate logical control connection of the major power-driven servos in the assembly. For example, in some embodiments, there can be a three-axis servo for the printhead, a one z-axis servo for the print bed, and two rotational servos for the parallel plate fiber collection system. In some embodiments, there can be servo connections that lead from the servo control unit to the printhead. These connections, in such an embodiment, may control the two flowrate servos of the coaxial electrospinning system, one flowrate servo for the bioprinting system, and one z-axis motion servo for retracting the bio printhead during the electrospinning operation.

[0073] The foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the described embodiments. However, it should be apparent to one skilled in the art that the specific details are not required in order to practice the described embodiments. Thus, the foregoing descriptions of specific embodiments are presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the described embodiments to the precise forms disclosed. It should be apparent to one of ordinary skill in the art that many modifications and variations are possible in view of the above teachings.

[0074] While embodiments of the disclosure have been shown and described, modifications thereof can be made by one skilled in the art without departing from the spirit and teachings of the disclosure. The embodiments described and the examples provided herein are exemplary only, and are not intended to be limiting. Many variations and modifications of the disclosure disclosed herein are possible and are within the scope of the disclosure. The scope of protection is not limited by the description set out above, but is only limited by the claims which follow, that scope including all equivalents of the subject matter of the claims.

[0075] Embodiments can include a system, a method, and/or a computer program product. The computer program product may include a computer readable storage medium (or media) having computer readable program instructions thereon for causing a processor to carry out aspects of the present disclosure.

[0076] Those skilled in the art will appreciate that the steps described herein may be carried out in a variety ways and that no particular ordering is required. It will be further understood from the foregoing description that modifications and changes may be made in various embodiments of the present disclosure without departing from its true spirit. The descriptions in this specification are for purposes of illustration only and are not to be construed in a limiting sense.

[0077] Consistent with the above disclosure, the examples of systems and methods enumerated in the following clauses are specifically contemplated and are intended as a non-limiting set of examples.

[0078] Clause 1. A capillary-incorporated bioprinting system for rapid engineering of vascularized tissues, the system including a three-dimensional printing head, where the three-dimensional printing head is configured on a three-axis linear stage; a spinneret, where the spinneret is configured on a horizontal linear stage and the spinneret is affixed above the three-dimensional printing head; a UV light source, where the UV light source is operatively coupled to the three-dimensional printing head and the spinneret, and adjacent to the spinneret; and a pair of parallel bars, where the pair of parallel bars are configured to collect a plurality of electrospun aligned microtubes.

[0079] Clause 2. The system of any foregoing clause, where the three-dimensional printing head is a pneumatic-driven extrusion-based three-dimensional printing head.

[0080] Clause 3. The system of any foregoing clause, where the spinneret is a concentric dual-channel spinneret.

[0081] Clause 4. The system of any foregoing clause, where the UV light source is 395 nm UV light.

[0082] Clause 5. The system of any foregoing clause, where the pair of parallel bars comprise aluminum.

[0083] Clause 6. The system of any foregoing clause further including a rotary stage, where the rotary stage is operatively coupled to the pair of parallel bars to adjust the orientation of the electrospun aligned microtubes.

[0084] Clause 7. The system of any foregoing clause further including one or more hydrogels, where the hydrogels are operatively configured to support a tissue scaffold.

[0085] Clause 8. The system of any foregoing clause further including a printing area, where the printing area is configured to support a bioprinted vascularized tissue.

[0086] Clause 9. The system of any foregoing clause, where the printing area is a glass table.

[0087] Clause 10. The system of any foregoing clause, where the printing area is adjustable to one or more heights.

[0088] Clause 11. A method for engineering vascularized tissues using a capillary-incorporated bioprinting system, the method including printing a first layer of a hydrogel onto a printing area using a three-dimensional printing head; returning the three-dimensional printing head to a home position; electrospinning microfibers onto a pair of parallel bars, where the electrospinning aligns the microfibers on top of the first layer of the hydrogel; submerging, due to gravitational forces, the microfibers in the first layer of the hydrogel; resultant from the submerging, forming porous microtubes in the first layer of hydrogel; and cross-linking the first layer of the hydrogel by

exposing the first layer of the hydrogel to a UV light source, where the cross-linking creates a cross-linked base of the hydrogel.

[0089] Clause 12. The method of any foregoing clause, where the three-dimensional printing head is configured on a three-axis linear stage.

[0090] Clause 13. The method of any foregoing clause, where the microfibers are core-sheath microfibers.

[0091] Clause 14. The method of any foregoing clause, where the printing area is configured to support a bioprinted vascularized tissue.

[0092] Clause 15. The method of any foregoing clause, where the printing area is a glass table.

[0093] Clause 16. The method of any foregoing clause, where the printing area is adjustable to one or more heights.

[0094] Clause 17. The method of any foregoing clause further including lowering the printing area.

[0095] Clause 18. The method of any foregoing clause further including printing a second layer of the hydrogel on the cross-linked base of the hydrogel.

[0096] Clause 19. The method of any foregoing clause further including trimming one or more excess microtubes, where the excess microtubes are situated in the printing area outside the hydrogel.

[0097] Clause 20. The method of any foregoing clause further including adjusting an orientation of the porous microtubes using a rotary stage, where the rotary stage is coupled to the pair of parallel bars.

[0098] Clause 21. A bioprinting electrospinning hybrid printer including a parallel plate collector, where the parallel plate collector including one or more plates with an adjustable distance and an

adjustable angle; an electronic control module, where the electronic control module is configured to control one or more major stage-motion servos and one or more flow-control servos; and a hybrid electrospinning bioprinting nozzle including one or more electrospinning syringes and one or more bioprinting syringes.

[0099] Clause 22. The bioprinting electrospinning hybrid printer of any foregoing clause, where the one or more major stage-motion motors and the one or more flow-control motors comprise servos.

[0100] Clause 23. The bioprinting electrospinning hybrid printer of any foregoing clause, where the one or more major stage-motion motors and the one or more flow-control motors comprise DC motors.

[0101] Clause 24. The bioprinting electrospinning hybrid printer of any foregoing clause further including a computer controlled parallel plate collection system, where the computer controlled parallel plate collection system is operatively connected to the parallel plate collector to adjust the adjustable distance and the adjustable angle.

[0102] Clause 25. The bioprinting electrospinning hybrid printer of any foregoing clause, where the plates are configured to heat collected microtubes.

REFERENCES

[0103] Zhang, N., Qavi, I., Halder, S., & Tan, G. (2023). Biomimetic hydrogel scaffolds embedded with porous microtubes as perfusion channels. Manufacturing Letters, 35, 184-193.

CLAIMS

WHAT IS CLAIMED IS:

1. A capillary-incorporated bioprinting system for rapid engineering of vascularized tissues, the system comprising:

(a) a three-dimensional printing head, wherein the three-dimensional printing head is configured on a three-axis linear stage;

(b) a spinneret, wherein the spinneret is configured on a horizontal linear stage and the spinneret is affixed above the three-dimensional printing head;

(c) a UV light source, wherein the UV light source is

(i) operatively coupled to the three-dimensional printing head and the spinneret, and

(ii) adjacent to the spinneret; and

(d) a pair of parallel bars, wherein the pair of parallel bars are configured to collect a plurality of electrospun aligned microtubes.

2. The system of Claim 1, wherein the three-dimensional printing head is a pneumatic-driven extrusion-based three-dimensional printing head.

3. The system of Claim 1, wherein the spinneret is a concentric dual-channel spinneret.

4. The system of Claim 1, wherein the UV light source is 395 nm UV light.

5. The system of Claim 1, wherein the pair of parallel bars comprise aluminum.

6. The system of Claim 1 further comprising a rotary stage, wherein the rotary stage is operatively coupled to the pair of parallel bars to adjust the orientation of the electrospun aligned microtubes.

7. The system of Claim 1 further comprising one or more hydrogels, wherein the hydrogels are operatively configured to support a tissue scaffold.

8. The system of Claim 1 further comprising a printing area, wherein the printing area is configured to support a bioprinted vascularized tissue.

9. The system of Claim 8, wherein the printing area is a glass table.

10. The system of Claim 8, wherein the printing area is adjustable to one or more heights.

11. A method for engineering vascularized tissues using a capillary-incorporated bioprinting system, the method comprising:

(a) printing a first layer of a hydrogel onto a printing area using a three-dimensional printing head;

(b) returning the three-dimensional printing head to a home position;

(c) electrospinning microfibers onto a pair of parallel bars, wherein the electrospinning aligns the microfibers on top of the first layer of the hydrogel;

(d) submerging, due to gravitational forces, the microfibers in the first layer of the hydrogel;

(e) resultant from the submerging, forming porous microtubes in the first layer of hydrogel; and

(f) cross-linking the first layer of the hydrogel by exposing the first layer of the hydrogel to a UV light source, wherein the cross-linking creates a cross-linked base of the hydrogel.

12. The method of Claim 11, wherein the three-dimensional printing head is configured on a three-axis linear stage.

13. The method of Claim 11, wherein the microfibers are core-sheath microfibers.

14. The method of Claim 11, wherein the printing area is configured to support a bioprinted vascularized tissue.

15. The method of Claim 14, wherein the printing area is a glass table.

16. The method of Claim 14, wherein the printing area is adjustable to one or more heights.

17. The method of Claim 11 further comprising lowering the printing area.

18. The method of Claim 11 further comprising printing a second layer of the hydrogel on the cross-linked base of the hydrogel.

19. The method of Claim 11 further comprising trimming one or more excess microtubes, wherein the excess microtubes are situated in the printing area outside the hydrogel.

20. The method of Claim 11 further comprising adjusting an orientation of the porous microtubes using a rotary stage, wherein the rotary stage is coupled to the pair of parallel bars.

21. A bioprinting electrospinning hybrid printer comprising:

(a) a parallel plate collector, wherein the parallel plate collector comprising one or more plates with an adjustable distance and an adjustable angle;

(b) an electronic control module, wherein the electronic control module is configured to control one or more major stage-motion motors and one or more flow-control motors; and

(c) a hybrid electrospinning bioprinting nozzle comprising one or more electrospinning syringes and one or more bioprinting syringes.

22. The bioprinting electrospinning hybrid printer of Claim 21, wherein the one or more major stage-motion motors and one or more flow-control motors comprise servos.

23. The bioprinting electrospinning hybrid printer of Claim 21, wherein the one or more major stage-motion motors and one or more flow-control motors comprise DC motors.

24. The bioprinting electrospinning hybrid printer of Claim 21 further comprising a computer controlled parallel plate collection system, wherein the computer controlled parallel plate collection system is operatively connected to the parallel plate collector to adjust the adjustable distance and the adjustable angle.

25. The bioprinting electrospinning hybrid printer of Claim 21, wherein the plates are configured to heat collected microtubes.

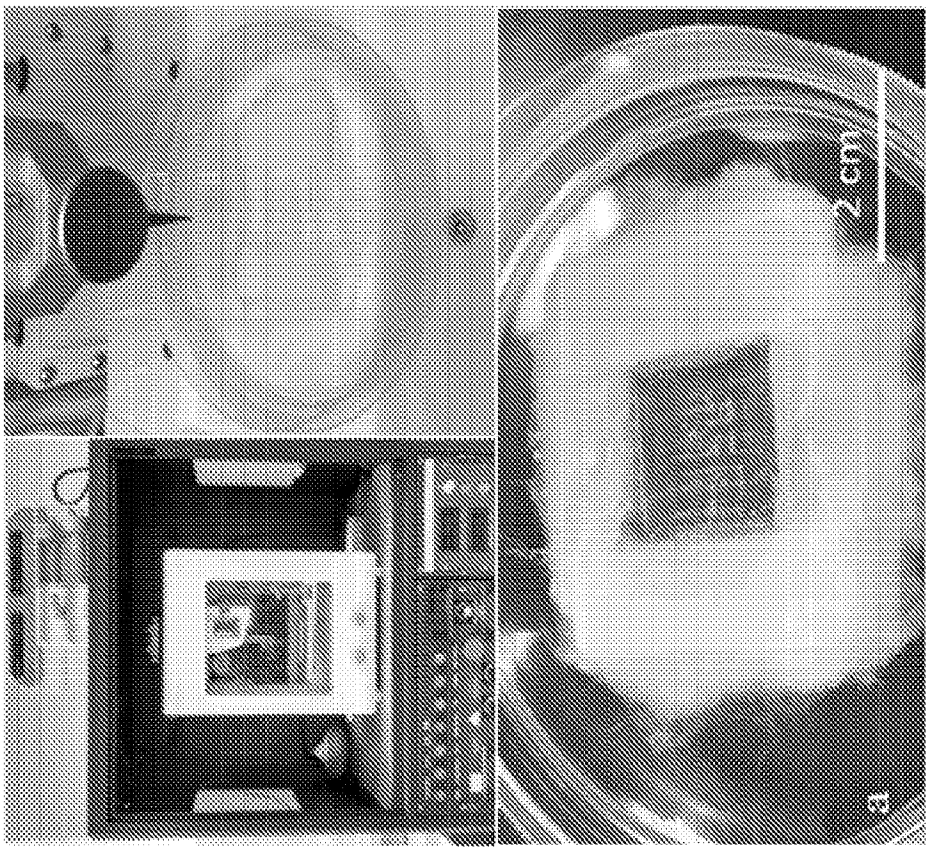

FIG. 1

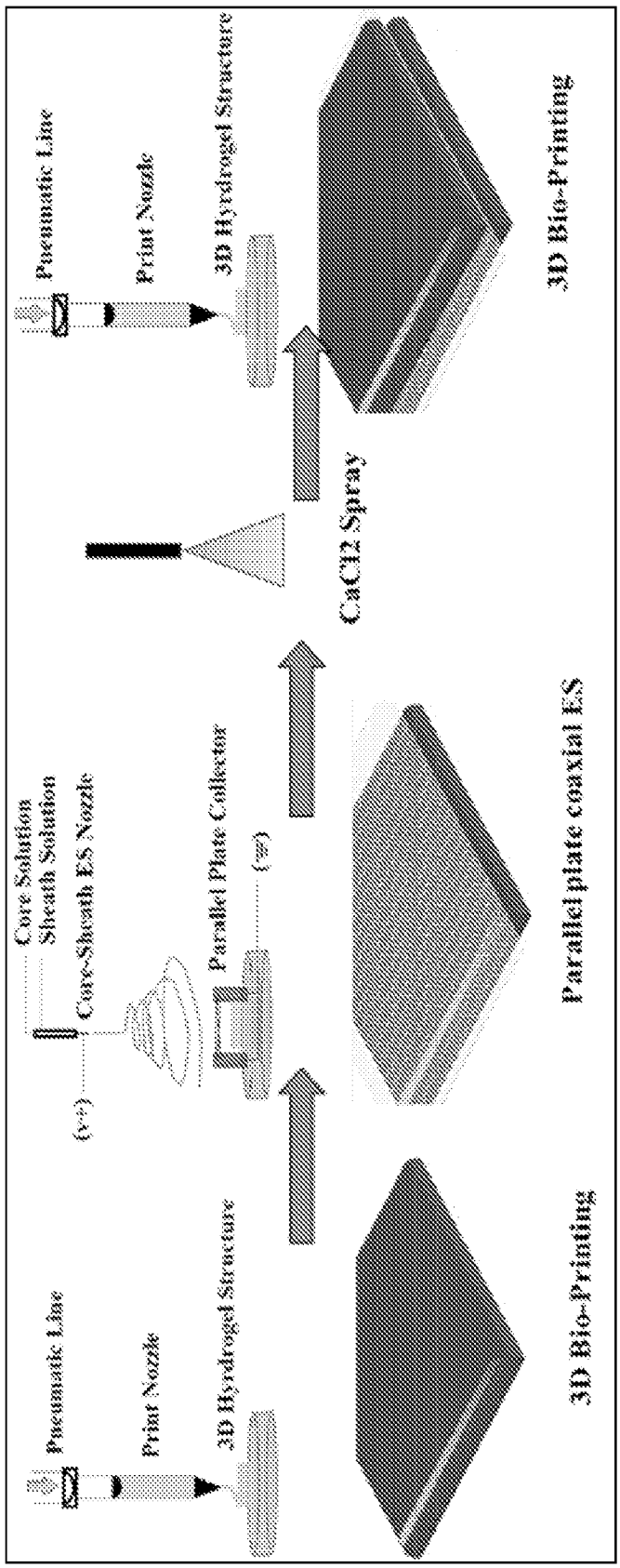
Pneumatic Line
Print Nozzle
3D Hydrogel Structure
3D Bio-Printing
CaCl2 Spray
Core Solution
Sheath Solution
Core-Sheath ES Nozzle
Parallel Plate Collector
Parallel plate coaxial ES
3D Bio-Printing

FIG. 2A

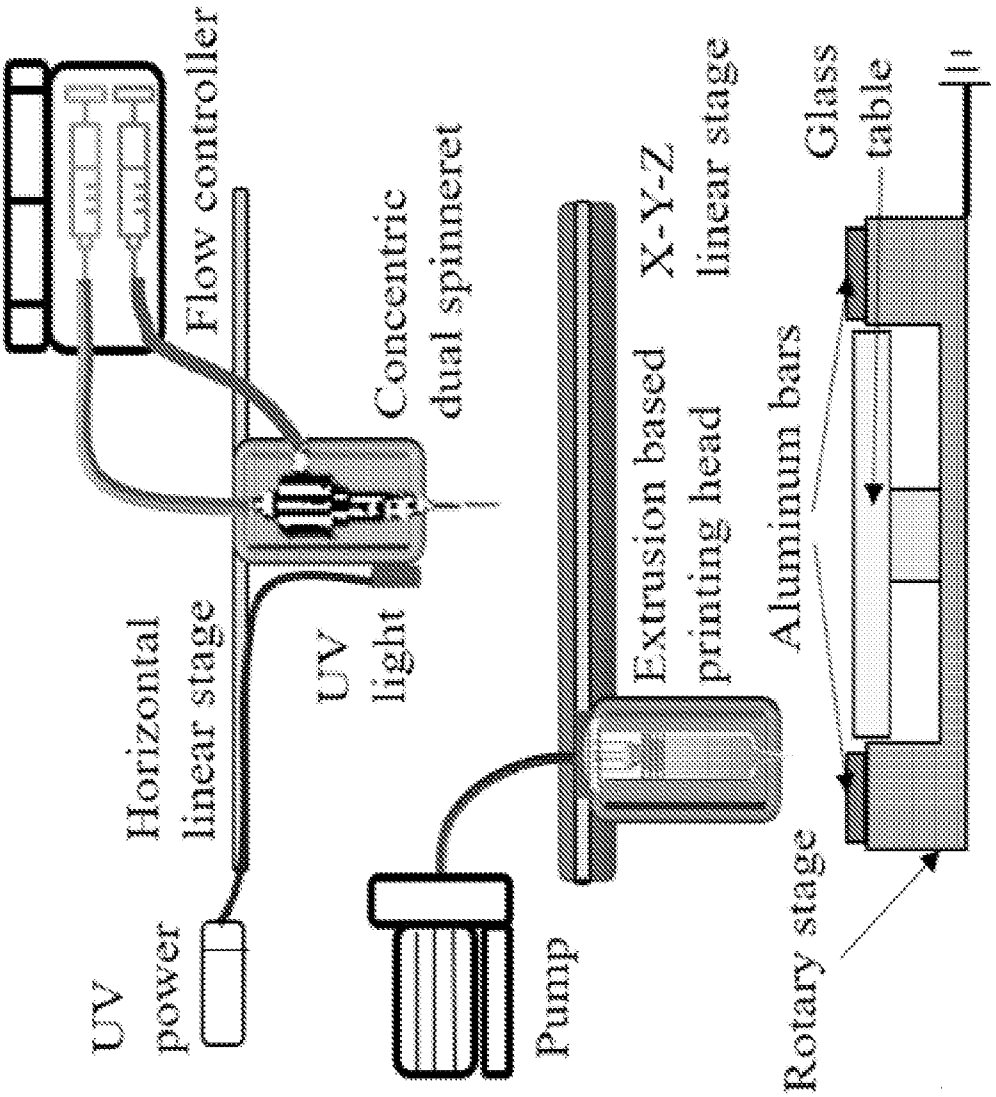
UV power
Horizontal linear stage
Flow controller
UV light
Concentric dual spinneret
Pump
Extrusion based printing head
X-Y-Z linear stage
Aluminum bars
Glass table
Rotary stage

FIG. 2B

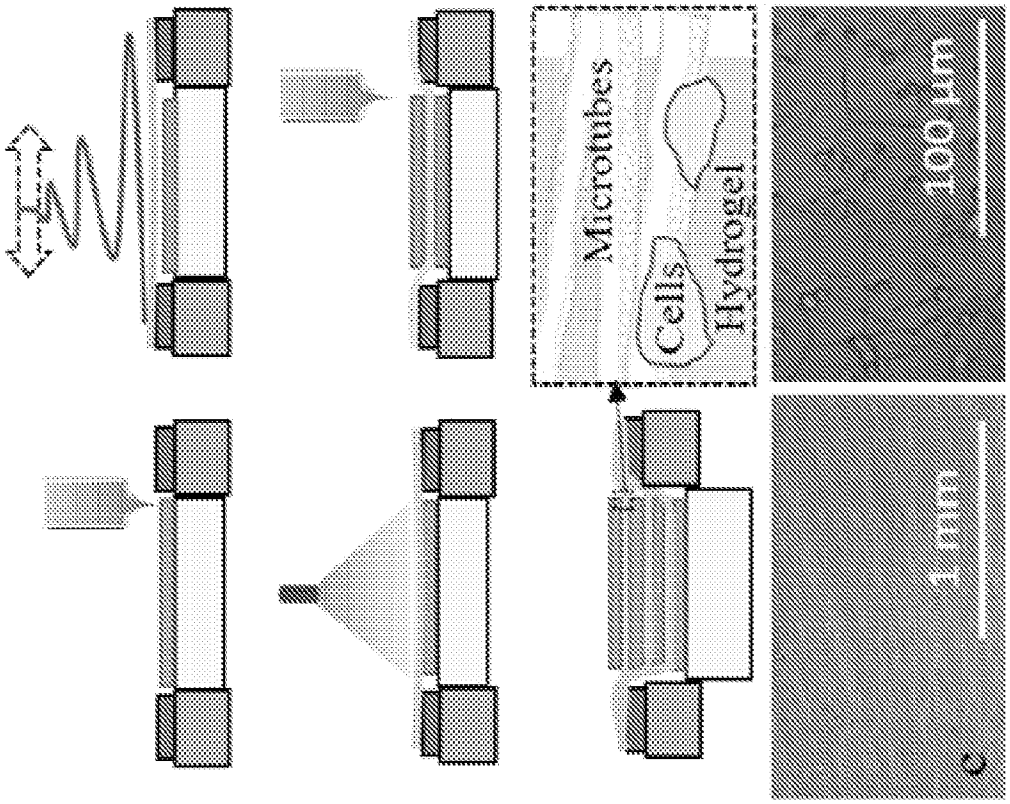

FIG. 3

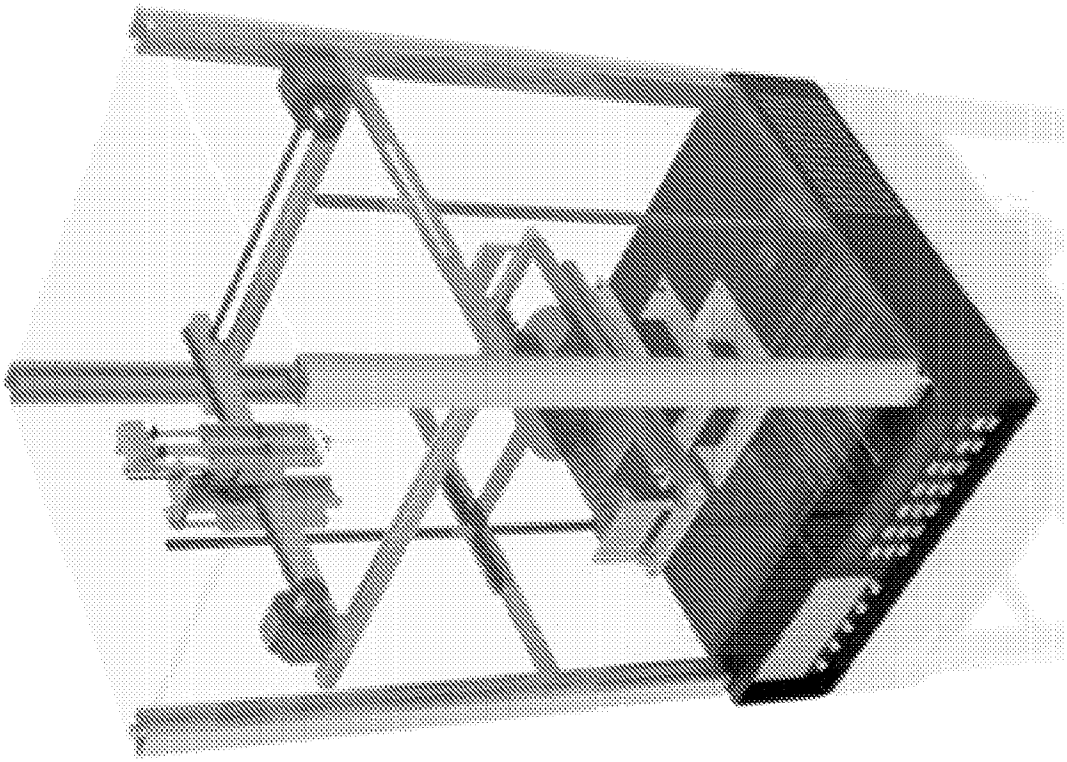

FIG. 4

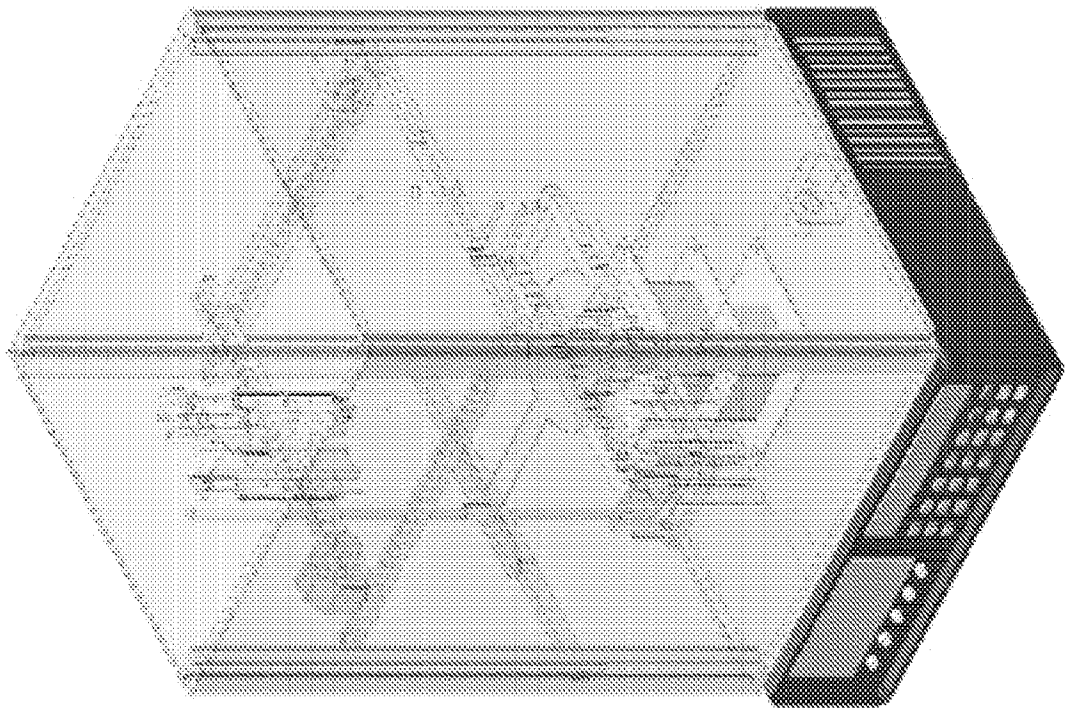

FIG. 5A

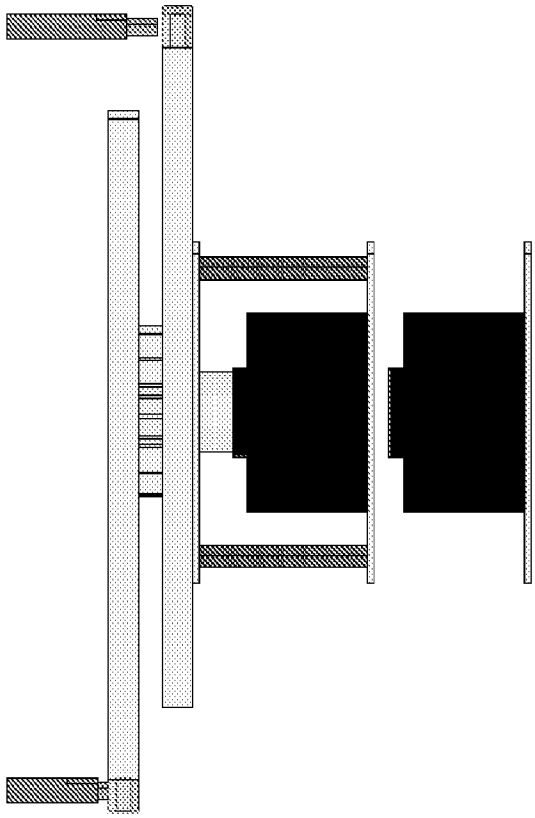

FIG. 5C

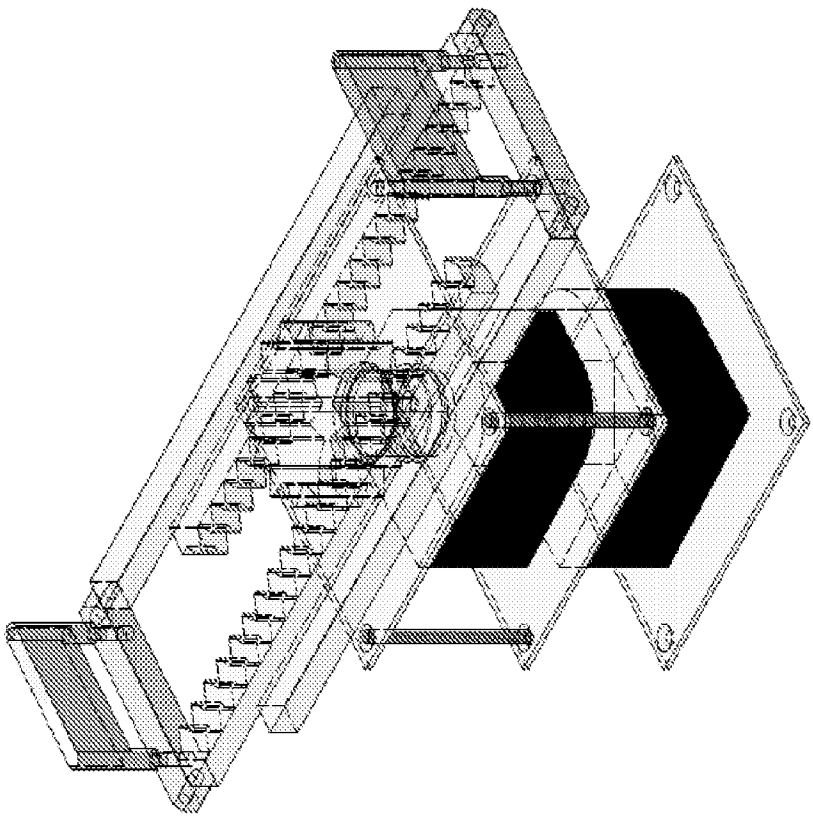

FIG. 5B

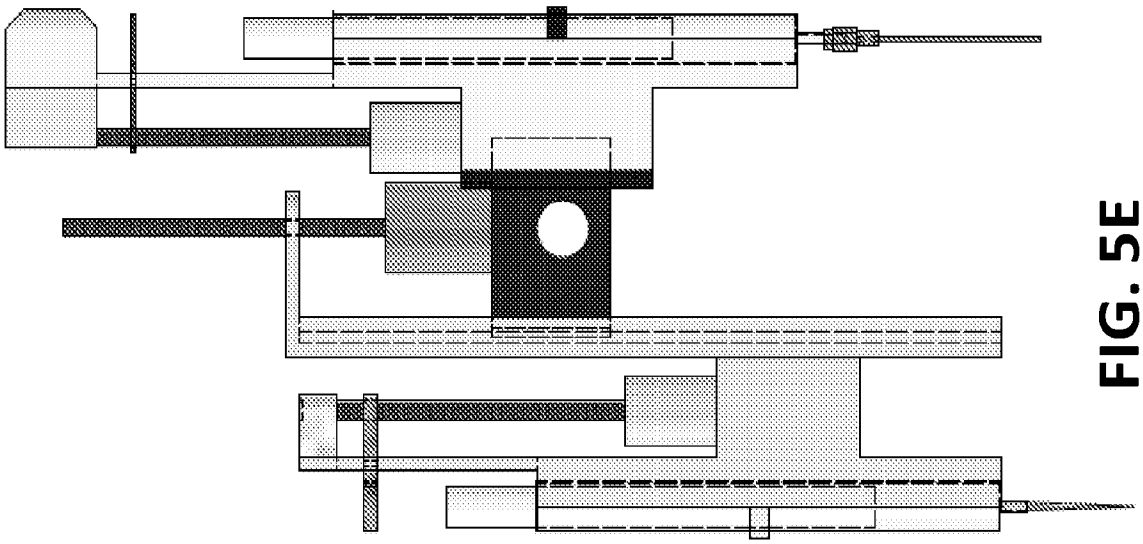

FIG. 5E

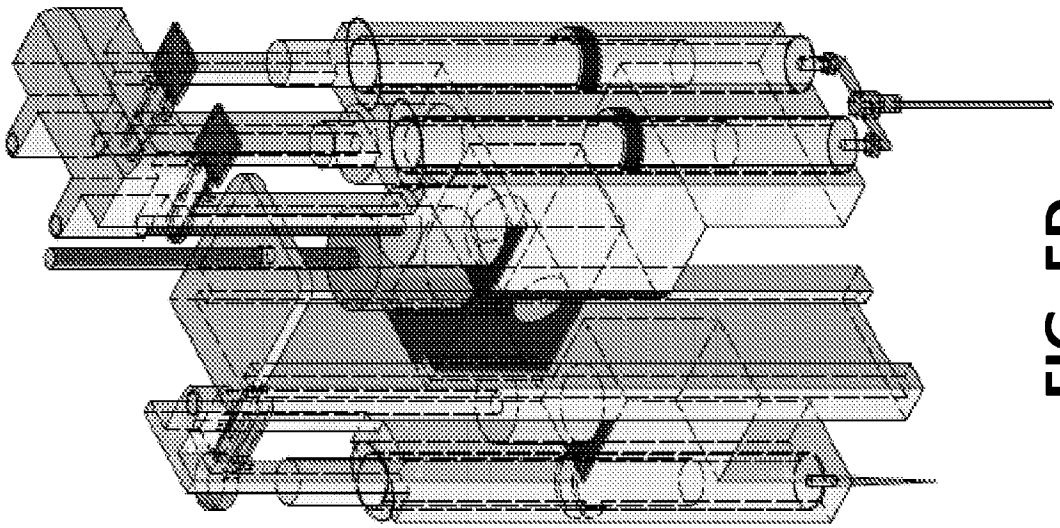

FIG. 5D

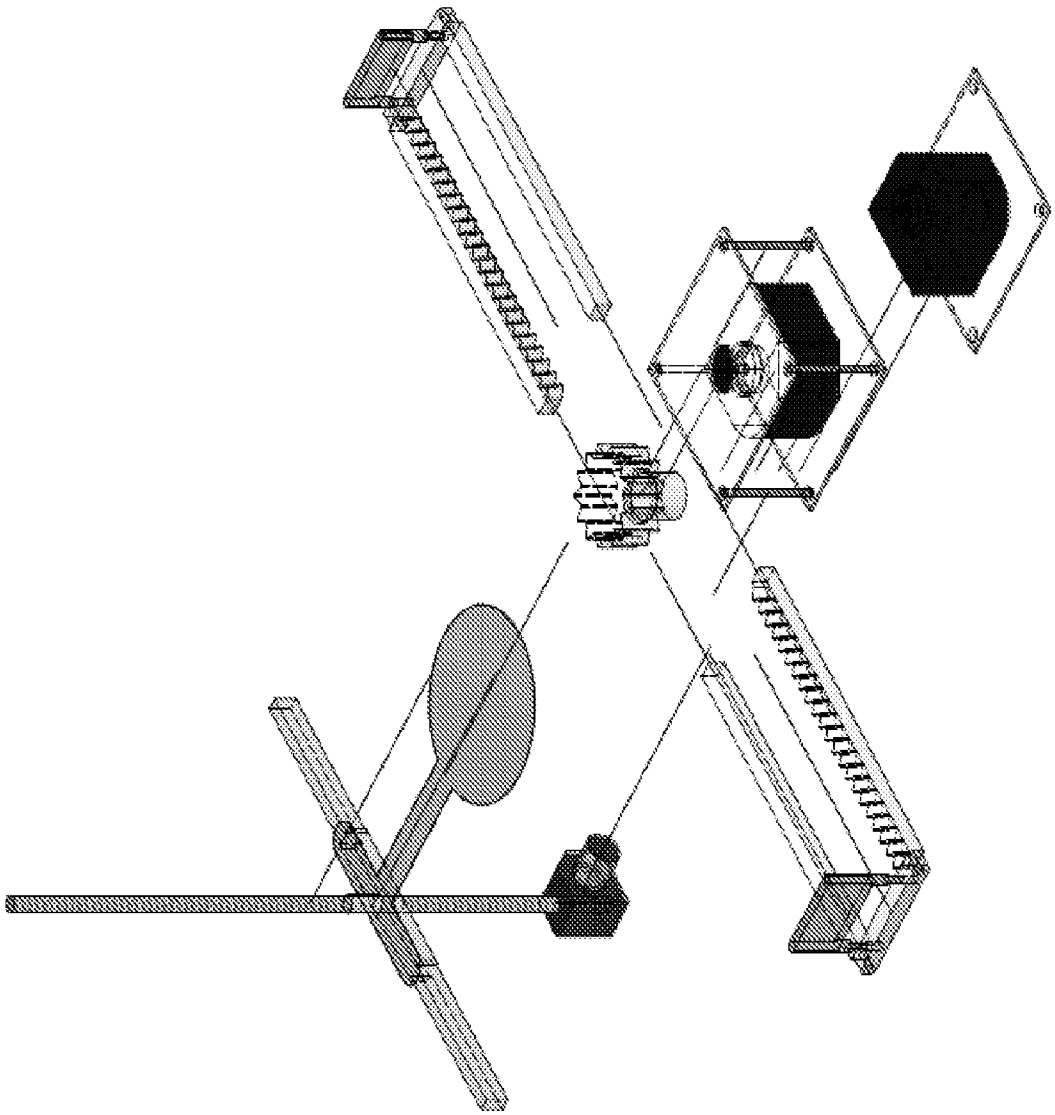

FIG. 6

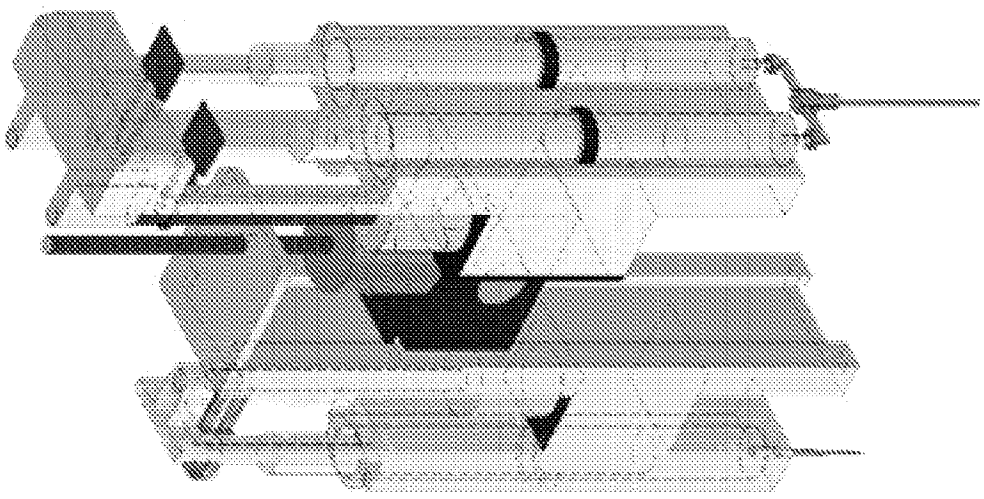

FIG. 7A

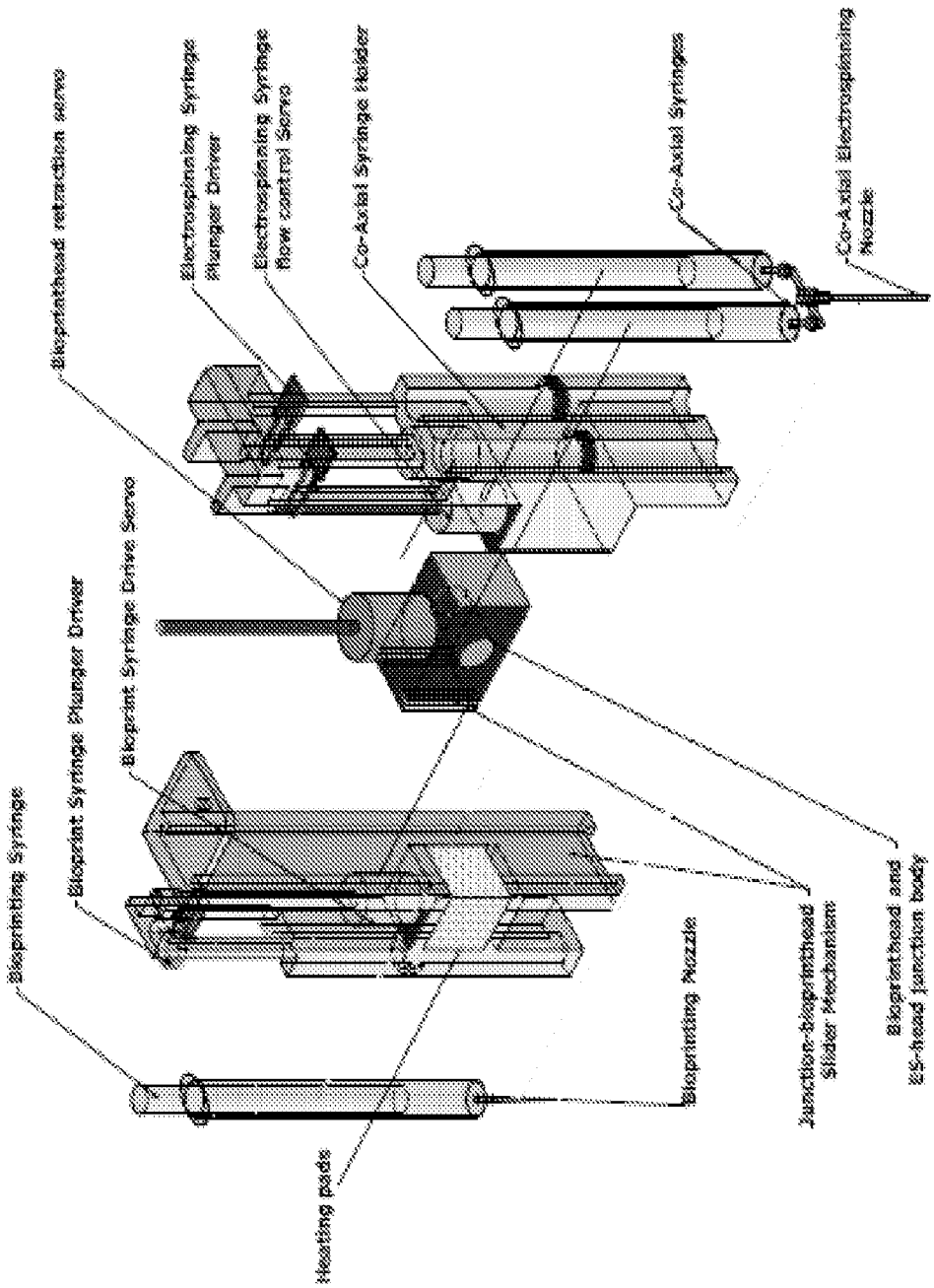
Bioprinthead retraction servo
Electrospinning Syringe Plunger Driver
Electrospinning Syringe flow control Servo
Co-Axial Syringe Holder
Co-Axial Syringes
Co-Axial Electrospinning Nozzle
Bioprinting Syringes
Bioprint Syringe Plunger Driver
Bioprint Syringe Drive Servo
Heating pads
Bioprinting Nozzle
Junction-bioprinthead Slider Mechanism
Bioprinthead and ES-head junction body

FIG. 7B

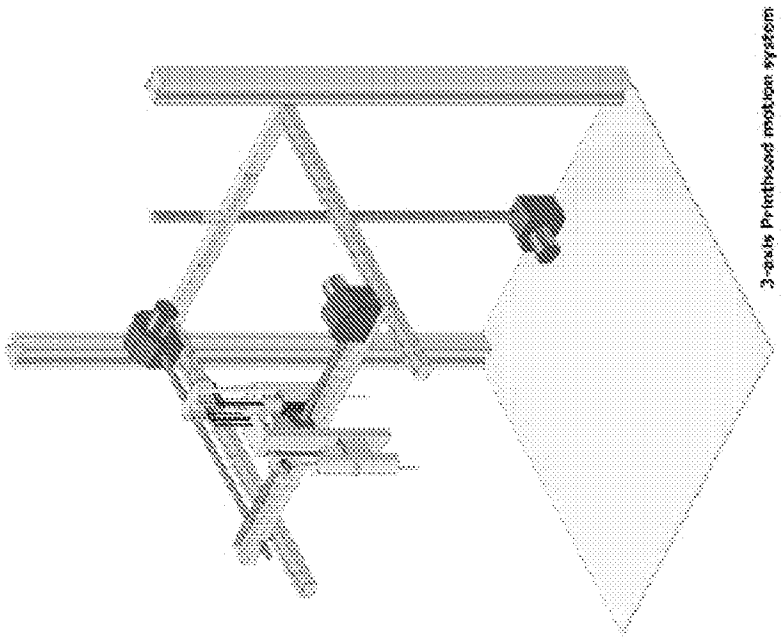

FIG. 8

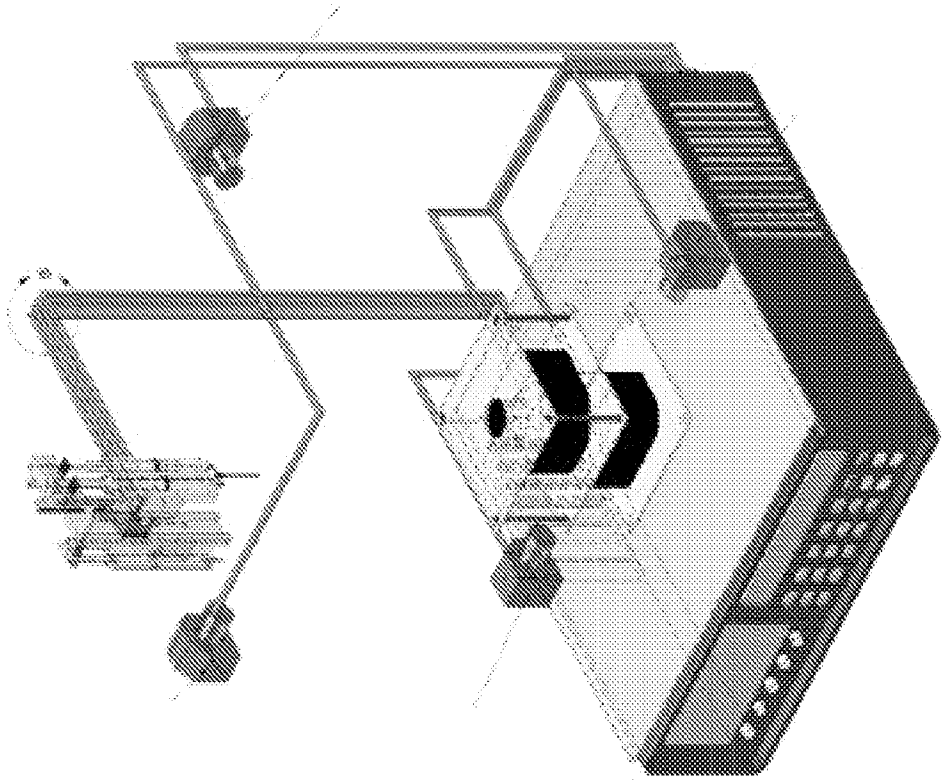

FIG. 9