



(51) International Patent Classification:

B29C 64/112 (2017.01) *B33Y 30/00* (2015.01)
B29C 64/20 (2017.01)

(21) International Application Number:

PCT/CN2019/072991

(22) International Filing Date:

24 January 2019 (24.01.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicants: **INSTITUTE OF GENETICS AND DEVELOPMENTAL BIOLOGY, CHINESE ACADEMY SCIENCES** [CN/CN]; No.1 Beichen West Road, Chaoyang District, Beijing 100101 (CN). **THE CHINESE UNIVERSITY OF HONGKONG** [CN/CN]; Shatin, NT, Hong Kong (CN). **TSINGHUA UNIVERSITY** [CN/CN]; No.1, Tsinghua Yuan, Haidian District, Beijing 100084 (CN).

(72) Inventors: **WANG, Xiujie**; No.1 Beichen West Road, Chaoyang District, Beijing 100101 (CN). **WANG, Changling Charlie**; The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (CN). **LIU, Yongjin**; No.1 Tsinghua University, Haidian District, Beijing 100084 (CN). **ZHANG, Zeyu**; No.1 Beichen West Road, Chaoyang District, Beijing 100101 (CN). **SHI, Qingqing**; No.1 Beichen West Road, Chaoyang District, Beijing 100101 (CN).

(74) Agent: **METIS IP (CHENGDU) LLC**; Room 808, 8th Floor, Building B7, Block D, Tianfu Jingrong Center, No. 99 West Section Of Hupan Road, Tianfu New District, Chengdu, Sichuan 610213 (CN).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

(54) Title: SYSTEMS AND METHODS FOR THREE-DIMENSIONAL BIOPRINTING

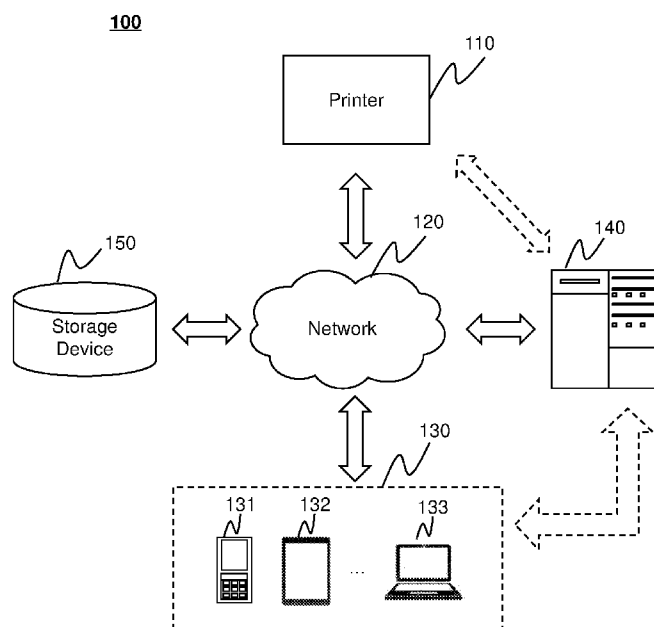


FIG. 1

(57) Abstract: Systems and methods for three-dimensional bioprinting are provided. The method may include depositing, using a printer head, a droplet of bio-ink on a printing surface of a substrate. The printing surface may be immersed in a liquid. An attachment of the droplet of bio-ink to the printing surface may be prompted by a first interaction between the droplet of bio-ink and the printing surface, and the attachment of the droplet of bio-ink to the printing surface may be further prompted by a second interaction between the droplet of bio-ink and the liquid.

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

SYSTEMS AND METHODS FOR THREE-DIMENSIONAL BIOPRINTING

TECHNICAL FIELD

[0001] The present disclosure generally relates to three-dimensional (3D) printing, and more particularly, to systems and methods for 3D printing in a liquid environment, printing an object including a tubular structure, and printing a 3D object with bio-materials.

BACKGROUND

[0002] 3D printing usually refers to a technology that creates a 3D object by depositing materials or solidify the materials on a printing surface of a substrate. 3D bioprinting has been utilized to print biological components including tissue or organs. A solidification process is often employed in the existing 3D bioprinting technology to solidify the printed biological components, which likely causes damages to cells and decreases cell survival rates. Besides, the biological functions of the printed tissue or organs are often limited at least partly due to the difficulty of generating a vascular network. Thus, it is desirable to provide systems and methods for printing 3D tissue or organs with improved cell survival rate and biological functions.

SUMMARY

[0003] According to an aspect of the present disclosure, a method for three-dimensional (3D) printing in a liquid environment is provided. The method may include depositing, using a printer head, a droplet of bio-ink on a printing surface of a substrate. The printing surface may be immersed in a liquid. An attachment of the droplet of bio-ink to the printing surface may be prompted by a first interaction between the droplet of bio-ink and the printing surface, and the attachment of the droplet of bio-ink to the printing surface may be further prompted by a second interaction between the droplet of bio-ink and the liquid.

[0004] According to another aspect of the present disclosure, a method for printing

an object including a tubular structure is provided. The method may include depositing, using a printer head, a first material on a substrate that includes a scaffold having the tubular structure; and providing a second material, which is a fluid, within the tubular structure while depositing the first material on the substrate. The scaffold may be permeable to the second material and may be configured to allow the second material to reach the first material. The second material may be configured to enhance or maintain an activity of the first material.

[0005] According to a further aspect of the present disclosure, a method for printing a three-dimensional (3D) object is provided. The method may include (a) depositing first bio-ink including first cells on a printing surface of a substrate; (b) culturing the first cells deposited on the printing surface in a first cell culture medium for a first time period; (c) depositing second bio-ink including second cells on the deposited printing surface; (d) culturing the second cells deposited on the printing surface in a second cell culture medium for a second time period; and (e) repeating (a)~(d) and allowing the deposited first cells and second cells to cohere to form a 3D object.

[0006] According to a still further aspect of the present disclosure, a system for three-dimensional (3D) printing is provided. The system may include one or more printer heads configured to deposit one or more types of bio-ink; a positioning device that is connected to the one or more printer heads and is configured to drive the one or more printer heads to move relative to a printing surface of a substrate; a control module that is configured to control the positioning device so that the one or more printer heads are in place to deposit a droplet of bio-ink on one or more target positions on the printing surface immersed in a liquid. The liquid may include a material such that an attachment of the droplet of bio-ink on the printing surface is prompted by a first interaction between the droplet of bio-ink and the printing surface, and the attachment of the droplet of bio-ink on the printing surface is further prompted by a second interaction between the droplet of bio-ink and the liquid.

[0007] Additional features will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the

following and the accompanying drawings or may be learned by production or operation of the examples. The features of the present disclosure may be realized and attained by practice or use of various aspects of the methodologies, instrumentalities, and combinations set forth in the detailed examples discussed below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The present disclosure is further described in terms of exemplary embodiments. These exemplary embodiments are described in detail with reference to the drawings. The drawings are not to scale. These embodiments are non-limiting exemplary embodiments, in which like reference numerals represent similar structures throughout the several views of the drawings, and wherein:

[0009] FIG. 1 is a schematic diagram illustrating an exemplary printing system according to some embodiments of the present disclosure;

[0010] FIG. 2 is a schematic diagram illustrating exemplary hardware and/or software components of an exemplary computing device according to some embodiments of the present disclosure;

[0011] FIG. 3 is a schematic diagram illustrating exemplary hardware and/or software components of an exemplary mobile device according to some embodiments of the present disclosure;

[0012] FIG. 4 is a block diagram illustrating an exemplary printer according to some embodiments of the present disclosure;

[0013] FIG. 5 is a block diagram illustrating an exemplary processing device according to some embodiments of the present disclosure;

[0014] FIG. 6A is a schematic diagram illustrating an exemplary printer according to some embodiments of the present disclosure;

[0015] FIGs. 6B-6D are schematic diagrams illustrating exemplary tilted printer heads according to some embodiments of the present disclosure;

[0016] FIG. 7 is a flowchart illustrating a general process of 3D printing according to some embodiments of the present disclosure;

[0017] FIG. 8 is a schematic diagram illustrating an exemplary printing process according to some embodiments of the present disclosure;

[0018] FIGs. 9A-9B are schematic diagrams of exemplary substrates having a complex structure according to some embodiments of the present disclosure;

[0019] FIG. 10 is a flowchart illustrating an exemplary printing process in a liquid environment according to some embodiments of the present disclosure;

[0020] FIG. 11 is a schematic diagram illustrating an exemplary front view of printing in a liquid environment according to some embodiments of the present disclosure;

[0021] FIG. 12 is a schematic diagram illustrating an interaction between biological components deposited on a printing surface and a liquid according to some embodiments of the present disclosure;

[0022] FIG. 13 is a flowchart illustrating an exemplary process of printing an object including a tubular structure according to some embodiments of the present disclosure;

[0023] FIG. 14 is a schematic diagram of providing a second material within a tubular structure of a scaffold according to some embodiments of the present disclosure;

[0024] FIG. 15 is a flowchart illustrating an exemplary cyclic 3D printing process according to some embodiments of the present disclosure;

[0025] FIG. 16 is a schematic diagram of an exemplary cyclic 3D printing process according to some embodiments of the present disclosure;

[0026] FIGs. 17A-17B are photos of exemplary droplets of bio-ink deposited by the printer head according to some embodiments of the present disclosure;

[0027] FIG. 17C is a schematic diagram illustrating exemplary positions of droplets of bio-ink deposited by the printer head according to some embodiments of the present disclosure;

[0028] FIG. 18A is a fluorescence microscopy image of a droplet of bio-ink according to some embodiments of the present disclosure;

[0029] FIG. 18B is a fluorescence microscopy image of the vascular scaffold after 12 h of culturing post printing according to some embodiments of the present

disclosure;

[0030] FIG. 18C is a fluorescence microscopy image of the vascular scaffold after 24 h of culturing post printing according to some embodiments of the present disclosure;

[0031] FIG. 18D is an image showing the printer head, the scaffold, and the liquid environment in a printing process according to some embodiments of the present disclosure;

[0032] FIG. 19 is a diagram illustrating the degree of DNA break for the robot printed epidermal cells and the manually handled epidermal cells according to some embodiments of the present disclosure;

[0033] FIG. 20 is a scanning electron microscopy image of the manually seeded endothelial cells and the robot printed endothelial cells on the vascular scaffold after 12 h and 72 h of culturing post printing according to some embodiments of the present disclosure;

[0034] FIG. 21 is a schematic diagram illustrating an exemplary cyclic 3D printing process according to some embodiments of the present disclosure; and

[0035] FIG. 22 is a fluorescence microscopy image of the vascular scaffolds after 1st, 2nd, 4th, and 6th round of the cyclic 3D printing process according to some embodiments of the present disclosure.

DETAILED DESCRIPTION

[0036] In the following detailed description, numerous specific details are set forth by way of examples in order to provide a thorough understanding of the relevant disclosure. However, it should be apparent to those skilled in the art that the present disclosure may be practiced without such details. In other instances, well-known methods, procedures, systems, components, and/or circuitry have been described at a relatively high-level, without detail, in order to avoid unnecessarily obscuring aspects of the present disclosure. Various modifications to the disclosed embodiments will be readily apparent to those skilled in the art, and the general

principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the present disclosure. Thus, the present disclosure is not limited to the embodiments shown, but to be accorded the widest scope consistent with the claims.

[0037] The terminology used herein is for the purpose of describing particular example embodiments only and is not intended to be limiting. 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. It will be further understood that the terms “comprise,” “comprises,” and/or “comprising,” “include,” “includes,” and/or “including,” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0038] It will be understood that the term “system,” “engine,” “unit,” “module,” and/or “block” used herein are one method to distinguish different components, elements, parts, section or assembly of different level in ascending order. However, the terms may be displaced by another expression if they achieve the same purpose.

[0039] Generally, the word “module,” “unit,” or “block,” as used herein, refers to logic embodied in hardware or firmware, or to a collection of software instructions. A module, a unit, or a block described herein may be implemented as software and/or hardware and may be stored in any type of non-transitory computer-readable medium or another storage device. In some embodiments, a software module/unit/block may be compiled and linked into an executable program. It will be appreciated that software modules can be callable from other modules/units/blocks or from themselves, and/or may be invoked in response to detected events or interrupts. Software modules/units/blocks configured for execution on computing devices (e.g., processor 230 as illustrated in FIG. 2) may be provided on a computer-readable medium, such as a compact disc, a digital video disc, a flash drive, a magnetic disc, or any other tangible medium, or as a digital download (and can be originally stored in a compressed or installable format that

needs installation, decompression, or decryption prior to execution). Such software code may be stored, partially or fully, on a storage device of the executing computing device, for execution by the computing device. Software instructions may be embedded in firmware, such as an EPROM. It will be further appreciated that hardware modules/units/blocks may be included in connected logic components, such as gates and flip-flops, and/or can be included of programmable units, such as programmable gate arrays or processors. The modules/units/blocks or computing device functionality described herein may be implemented as software modules/units/blocks, but may be represented in hardware or firmware. In general, the modules/units/blocks described herein refer to logical modules/units/blocks that may be combined with other modules/units/blocks or divided into sub-modules/sub-units/sub-blocks despite their physical organization or storage. The description may be applicable to a system, an engine, or a portion thereof.

[0040] It will be understood that when a unit, engine, module or block is referred to as being “on,” “connected to,” or “coupled to,” another unit, engine, module, or block, it may be directly on, connected or coupled to, or communicate with the other unit, engine, module, or block, or an intervening unit, engine, module, or block may be present, unless the context clearly indicates otherwise. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0041] These and other features, and characteristics of the present disclosure, as well as the methods of operation and functions of the related elements of structure and the combination of parts and economies of manufacture, may become more apparent upon consideration of the following description with reference to the accompanying drawings, all of which form a part of this disclosure. It is to be expressly understood, however, that the drawings are for the purpose of illustration and description only and are not intended to limit the scope of the present disclosure. It is understood that the drawings are not to scale.

[0042] As used herein, “bio-ink” refers to a bio-material (often a mixture) having a generally a fluidic form that can be deposited on a surface to generate an object

based on 3D bioprinting techniques. In some embodiments, the bio-ink may include cellular components and/or non-cellular components. The cellular components may include free cells, cell aggregates, multicellular bodies, encapsulated cells, or the like, or any combination thereof. The multicellular bodies may include, for example, multicellular spheroids formed by different types of cells such as endothelial cells and/or cardiac cells. The non-cellular components may include one or more nutrients for cells, encapsulation materials, pH buffer agents, or the like, or any combination thereof. In some embodiments, the bio-ink may also include supportive components. The supportive components may form supportive structures before or after the bio-ink is deposited, providing structural support and/or mechanical protection for the printed biological components. The supportive components may or may not be removed from the generated object.

[0043] As used herein, a “substrate” refers to a material that the bio-ink is deposited on. In some embodiments, the substrate may become a part of the object to be generated by printing. The substrate may include a scaffold of various shapes and/or sizes. Cells from the bio-ink may attach to the substrate for further proliferation and/or differentiation. The substrate may be artificially synthesized or derived from natural organisms. Additionally or alternatively, the substrate may include biological components formed by living cells, and the bio-ink may be deposited on the biological components to form a 3D object. The substrate may be biodegradable or non-biodegradable. In some embodiments, the substrate may not become a part of the object to be generated. For example, the substrate may be a mold, a support, etc. In some embodiments, the substrate may be of any shape, such as a sphere, a semi-sphere, a cylinder, a cubic, an irregular shape, etc. In some embodiments, the substrate may have a tubular structure. In some embodiments, the substrate may include one or more branches and/or one or more bending structures. In some embodiments, the substrate may be coated with a hydrophilic layer (e.g., hydrogel). For instance, the hydrophilic layer may include cross-linked or non-cross-linked hydrophilic polymers that are non-toxic to biological components such as cells. The hydrophilic polymers may include, for example,

polylactic acid, polyvinyl alcohol, polyglycolic acid, collagen, gelatin, chitosan, or the like, or a combination thereof. In some embodiments, hydrophilic or polar functional groups may be grafted onto the printing surface, including hydroxyl groups, carboxyl groups, amino groups, phosphate groups, or the like, or a combination thereof. In some embodiments, the printing surface of the substrate may have been deposited with one or more biological components.

[0044] The present disclosure provides mechanisms (which can include methods, systems, materials, computer-readable medium, products, etc.) for printing an object using a printer. In some embodiments, the object may be printed in a liquid environment. For example, the printer may deposit droplets of bio-ink on a printing surface of a substrate when the printing surface is immersed in a liquid. An attachment of the droplets of bio-ink to the printing surface may be prompted by a first interaction between the droplets of bio-ink and the printing surface, and further prompted by a second interaction between the droplets of bio-ink and the liquid. For example, the liquid may be hydrophobic and the second interaction may be a hydrophobic interaction, while the first interaction may be a hydrophilic interaction. In some embodiments, the object may include a tubular structure. A fluid material may be provided within a tubular structure of a substrate (e.g., a scaffold). The scaffold may be permeable to the fluid material. The fluid material may support the survival of living cells deposited on the scaffold. In some embodiments, a cyclic printing strategy may be applied to generate the object. In each cycle, the bio-ink including biological components may be deposited on the printing surface of the substrate, and then the printed biological components may be cultured to cohere. In some embodiments, the composition of the bio-ink used in different cycles may vary. The object with a 3D structure may be generated after a plurality of cycles. The object may include a part of or an entire organ, tissue, etc.

[0045] FIG. 1 is a schematic diagram illustrating an exemplary printing system according to some embodiments of the present disclosure. The printing system 100 shown in FIG. 1 may be configured to print an object according to a predetermined printing strategy. The printing system 100 may include a printer 110, a network 120,

one or more terminals 130, a processing device 140, and/or a storage device 150.

[0046] In some embodiments, the printer 110 may be configured to print the object according to the predetermined printing strategy. In certain embodiments, the printing strategy may include one or more pathways of one or more printer heads of the printer 110, the volume of each droplet of bio-ink to be deposited, and/or other parameters related to a printing process for printing the object. In certain embodiments, the printing strategy may also include the make-up and components of the bio-ink, which may or may not be the same as the components of the printed object. The object may include non-biological components and/or biological components. For example, the non-biological components may include materials such as but not limited to plastics, metal, alloy, gypsum, or the like, or any combination thereof. The biological components may be printed using a type of ink that is referred to as "bio-ink". The object to be printed using bio-ink may include an organ (e.g., a heart, a liver, a kidney) or a portion thereof, tissue (e.g., blood vessels), or the like, or any combination thereof. The printer 110 may be any type of printer, such as an inkjet printer, a laser-assisted printer, an extrusion printer, etc. More descriptions regarding the printer 110 may be found elsewhere in the present disclosure (e.g., FIGs. 4 and 6A and relevant descriptions thereof). In some embodiments, the printer 110 may communicate with one or more components of the printing system 100 (e.g., the terminal(s) 130, the processing device 140, the storage device 150). For example, the printer 110 may obtain a predetermined printing strategy from the storage device 150 via the network 120.

[0047] The components of the printing system 100 may be connected in various ways. Merely by way of example, the printer 110 may be connected to the processing device 140 through the network 120. As another example, the printer 110 may be connected to the processing device 140 directly as indicated by the bi-directional arrow in dotted lines linking the printer 110 and the processing device 140. As a further example, the storage device 150 may be connected to the processing device 140 directly or through the network 120. As still a further example, the terminal 130 may be connected to the processing device 140 directly

(as indicated by the bi-directional arrow in dotted lines linking the terminal 130 and the processing device 140) or through the network 120.

[0048] The network 120 may include any suitable network that can facilitate the exchange of information and/or data for the printing system 100. In some embodiments, one or more components of the printing system 100 (e.g., the printer 110, the terminal(s) 130, the processing device 140, and the storage device 150) may communicate information and/or data with one or more other components of the printing system 100 via the network 120. For example, the processing device 140 may obtain image data from the printer 110 via the network 120. As another example, the processing device 140 may obtain user instruction(s) from the terminal(s) 130 via the network 120. The network 120 may be or include a public network (e.g., the Internet), a private network (e.g., a local area network (LAN)), a wired network, a wireless network (e.g., an 802.11 network, a Wi-Fi network), a frame relay network, a virtual private network (VPN), a satellite network, a telephone network, routers, hubs, switches, server computers, and/or any combination thereof. For example, the network 120 may include a cable network, a wireline network, a fiber-optic network, a telecommunications network, an intranet, a wireless local area network (WLAN), a metropolitan area network (MAN), a public telephone switched network (PSTN), a Bluetooth™ network, a ZigBee™ network, a near field communication (NFC) network, or the like, or any combination thereof. In some embodiments, the network 120 may include one or more network access points. For example, the network 120 may include wired and/or wireless network access points such as base stations and/or internet exchange points through which one or more components of the printing system 100 may be connected to the network 120 to exchange data and/or information.

[0049] The terminal(s) 130 may be connected to and/or communicate with the printer 110, the processing device 140, and/or the storage device 150. For example, the terminal(s) 130 may receive an instruction inputted by a user and transmit the instruction to the printer 110 and/or the processing device 140. As another example, the terminal(s) 130 may obtain a 3D model of the object to be

printed from the storage device 150 so that the user may view and/or modify the 3D model of the object via the terminal(s) 130. In some embodiments, the terminal(s) 130 may include a mobile device 131, a tablet computer 132, a laptop computer 133, or the like, or any combination thereof. For example, the mobile device 131 may include a mobile phone, a personal digital assistant (PDA), a gaming device, a navigation device, a point of sale (POS) device, a laptop, a tablet computer, a desktop, or the like, or any combination thereof. In some embodiments, the terminal(s) 130 may include an input device, an output device, etc. The input device may include alphanumeric and other keys that may be input via a keyboard, a touchscreen (for example, with haptics or tactile feedback), a speech input, an eye tracking input, a brain monitoring system, or any other comparable input mechanism. The input information received through the input device may be transmitted to the processing device 140 via, for example, a bus, for further processing. Other types of the input device may include a cursor control device, such as a mouse, a trackball, or cursor direction keys, etc. The output device may include a display, a speaker, a printer, or the like, or a combination thereof. In some embodiments, the terminal(s) 130 may be part of the processing device 140.

[0050] The processing device 140 may process data and/or information obtained from the printer 110, the storage device 150, the terminal(s) 130, or other components of the printing system 100. For example, the processing device 140 may obtain the 3D model of the object from the storage device 150 and determine a printing strategy for printing the object. In some embodiments, the processing device 140 may be a single server or a server group. The server group may be centralized or distributed. In some embodiments, the processing device 140 may be local to or remote from the printing system 100. For example, the processing device 140 may access information and/or data from the printer 110, the storage device 150, and/or the terminal(s) 130 via the network 120. As another example, the processing device 140 may be directly connected to the printer 110, the terminal(s) 130, and/or the storage device 150 to access information and/or data. In some embodiments, the processing device 140 may be implemented on a cloud

platform. For example, the cloud platform may include a private cloud, a public cloud, a hybrid cloud, a community cloud, a distributed cloud, an inter-cloud, a multi-cloud, or the like, or a combination thereof. In some embodiments, the processing device 140 may be implemented by a computing device 200 having one or more components as described in connection with FIG. 2.

[0051] The storage device 150 may store data, instructions, and/or any other information. In some embodiments, the storage device 150 may store data obtained from the processing device 140, the terminal(s) 130, and/or the storage device 150. In some embodiments, the storage device 150 may store data and/or instructions that the processing device 140 may execute or use to perform exemplary methods described in the present disclosure. In some embodiments, the storage device 150 may include a mass storage, removable storage, a volatile read-and-write memory, a read-only memory (ROM), or the like, or any combination thereof. Exemplary mass storage may include a magnetic disk, an optical disk, a solid-state drive, etc. Exemplary removable storage may include a flash drive, a floppy disk, an optical disk, a memory card, a zip disk, a magnetic tape, etc. Exemplary volatile read-and-write memory may include a random access memory (RAM). Exemplary RAM may include a dynamic RAM (DRAM), a double data rate synchronous dynamic RAM (DDR SDRAM), a static RAM (SRAM), a thyristor RAM (T-RAM), and a zero-capacitor RAM (Z-RAM), etc. Exemplary ROM may include a mask ROM (MROM), a programmable ROM (PROM), an erasable programmable ROM (EPROM), an electrically erasable programmable ROM (EEPROM), a compact disk ROM (CD-ROM), and a digital versatile disk ROM, etc. In some embodiments, the storage device 150 may be implemented on a cloud platform as described elsewhere in the disclosure.

[0052] In some embodiments, the storage device 150 may be connected to the network 120 to communicate with one or more other components of the printing system 100 (e.g., the processing device 140, the terminal(s) 130). One or more components of the printing system 100 may access the data or instructions stored in the storage device 150 via the network 120. In some embodiments, the storage

device 150 may be part of the processing device 140.

[0053] This description is intended to be illustrative, and not to limit the scope of the present disclosure. Many alternatives, modifications, and variations will be apparent to those skilled in the art. The features, structures, methods, and other characteristics of the exemplary embodiments described herein may be combined in various ways to obtain additional and/or alternative exemplary embodiments. For example, the storage device 150 may be a data storage including cloud computing platforms, such as public cloud, private cloud, community, and hybrid clouds, etc. As another example, the printer 110, the processing device 140, and the storage device 150 may be integrated into a single device. However, those variations and modifications do not depart the scope of the present disclosure.

[0054] FIG. 2 is a schematic diagram illustrating exemplary hardware and/or software components of an exemplary computing device according to some embodiments of the present disclosure. In some embodiments, the processing device 140 may be implemented on the computing device. The computing device 200 shown in FIG. 2 may include a processor 210, a storage 220, an input/output (I/O) 230 and a communication port 240.

[0055] The processor 210 may execute computer instructions (e.g., program code) and perform functions of the processing device 140 in accordance with techniques described herein. The computer instructions may include, for example, routines, programs, objects, components, data structures, procedures, modules, and functions, which perform particular functions described herein. For example, the processor 210 may obtain a 3D model of an object to be printed. As another example, the processor 210 may determine a printing strategy based on the 3D model. In some embodiments, the processor 210 may include one or more hardware processors, such as a microcontroller, a microprocessor, a reduced instruction set computer (RISC), an application specific integrated circuits (ASICs), an application-specific instruction-set processor (ASIP), a central processing unit (CPU), a graphics processing unit (GPU), a physics processing unit (PPU), a microcontroller unit, a digital signal processor (DSP), a field programmable gate

array (FPGA), an advanced RISC machine (ARM), a programmable logic device (PLD), any circuit or processor capable of executing one or more functions, or the like, or any combination thereof.

[0056] Merely for illustration, only one processor is described in the computing device 200. However, it should be noted that the computing device 200 in the present disclosure may also include multiple processors. Thus operations and/or method steps that are performed by one processor as described in the present disclosure may also be jointly or separately performed by the multiple processors. For example, if in the present disclosure the processor of the computing device 200 executes both process A and process B, it should be understood that process A and process B may also be performed by two or more different processors jointly or separately in the computing device 200 (e.g., a first processor executes process A and a second processor executes process B, or the first and second processors jointly execute processes A and B).

[0057] The storage 220 may store data/information obtained from the printer 110, the terminal(s) 130, the storage device 150, and/or any other component of the X-ray imaging system 100. The storage 220 may be similar to the storage device 150 described in connection with FIG. 1, and the detailed descriptions are not repeated here.

[0058] The I/O 230 may input and/or output signals, data, information, etc. In some embodiments, the I/O 230 may enable a user interaction with the processing device 140. In some embodiments, the I/O 230 may include an input device and an output device. Examples of the input device may include a keyboard, a mouse, a touchscreen, a microphone, a sound recording device, or the like, or a combination thereof. Examples of the output device may include a display device, a loudspeaker, a printer, a projector, or the like, or a combination thereof. Examples of the display device may include a liquid crystal display (LCD), a light-emitting diode (LED)-based display, a flat panel display, a curved screen, a television device, a cathode ray tube (CRT), a touchscreen, or the like, or a combination thereof.

[0059] The communication port 240 may be connected to a network (e.g., the

network 120) to facilitate data communications. The communication port 240 may establish connections between the processing device 140 and the printer 110, the terminal(s) 130, and/or the storage device 150. The connection may be a wired connection, a wireless connection, any other communication connection that can enable data transmission and/or reception, and/or any combination of these connections. The wired connection may include, for example, an electrical cable, an optical cable, a telephone wire, or the like, or any combination thereof. The wireless connection may include, for example, a Bluetooth™ link, a Wi-Fi™ link, a WiMax™ link, a WLAN link, a ZigBee link, a mobile network link (e.g., 3G, 4G, 5G), or the like, or any combination thereof. In some embodiments, the communication port 240 may be and/or include a standardized communication port, such as RS232, RS485. In some embodiments, the communication port 240 may be a specially designed communication port. For example, the communication port 240 may be designed in accordance with the digital imaging and communications in medicine (DICOM) protocol.

[0060] FIG. 3 is a schematic diagram illustrating exemplary hardware and/or software components of an exemplary mobile device according to some embodiments of the present disclosure. In some embodiments, the terminal (s) 130 may be implemented on the mobile device. The mobile device 300 shown in FIG. 3 may include a communication platform 310, a display 320, a graphics processing unit (GPU) 330, a central processing unit (CPU) 340, an I/O 350, a memory 360, and a storage 390. In some embodiments, any other suitable component, including but not limited to a system bus or a controller (not shown), may also be included in the mobile device 300. In some embodiments, a mobile operating system 370 (e.g., iOS™, Android™, Windows Phone™) and one or more applications 380 may be loaded into the memory 360 from the storage 390 in order to be executed by the CPU 340. The applications 380 may include a browser or any other suitable mobile apps for receiving and rendering information relating to image processing or other information from the processing device 140. User interactions with the information stream may be achieved via the I/O 350 and provided to the processing device 140

and/or other components of the printing system 100 via the network 120. For example, the user interactions may include an instruction for the printer 110 to start, suspend, continue, or end a printing process. As another example, the user interactions may include a setting and/or an adjustment of parameters related to a printing process, such as a time interval for calibration, a moving velocity of one or more printer heads, a volume of each droplet of bio-ink to be deposited, or the like, or any combination thereof.

[0061] To implement various modules, units, and their functionalities described in the present disclosure, computer hardware platforms may be used as the hardware platform(s) for one or more of the elements described herein. A computer with user interface elements may be used to implement a personal computer (PC) or any other type of work station or terminal device. A computer may also act as a server if appropriately programmed.

[0062] FIG. 4 is a block diagram illustrating an exemplary printer according to some embodiments of the present disclosure. In some embodiments, the printer 110 may include one or more printer heads 402, one or more positioning devices 404, a container 406, one or more substrate fixing devices 408, one or more fluid systems 410, one or more controllers 412, and one or more calibration assemblies 414.

[0063] The printer head(s) 402 may be configured to deposit one or more droplets of ink on a printing surface of a substrate. In some embodiments, the ink may be inorganic. In some embodiments, the ink may be bio-ink, which is herein used as examples for the description of the printer and/or the printing process. In some embodiments, the one or more printer heads may be loaded with the same or different bio-inks. In some embodiments, the printer head(s) 402 may be driven by the positioning device(s) 404 to move to one or more target positions on the printing surface of the substrate based on a predetermined printing strategy. In some embodiments, the printer head(s) 402 may deposit one or more droplets of bio-ink on the target positions. In some embodiments, if a printer head 402 is moved to a target position on the printing surface of the substrate, there may be a certain distance (also referred to as a first safe distance) (e.g., 10 μm , 20 μm , 25 μm , 50 μm ,

75 μm , 100 μm , 150 μm , 200 μm , 300 μm , 400 μm , 500 μm , 600 μm , 700 μm , 800 μm , 900 μm , 1000 μm , etc.) between a front end of the printer head 402 and the target position. In some embodiments, the first safe distance between the front end of the printer head 402 and the target position may be configured to prevent the front end of the printer head 402 to collide or damage the substrate. In some embodiments, the first safe distance between the front end of the printer head 402 and the target position may be configured to provide a space for the droplet(s) of bio-ink. In some embodiments, the first safe distance may be determined according to the size of a droplet of bio-ink.

[0064] In some embodiments, the printer head 402 may include a pipette, a nozzle, a bio-ink container, a temperature adjuster (e.g., heater or cooler), or the like, or a combination thereof. In some embodiments, the pipette may include a plunger. In some embodiments, the pipette may connect to the nozzle. In some embodiments, the pipette may load bio-ink from the bio-ink container to the nozzle and/or dispense the loaded bio-ink to the printing surface through the movement of the plunger. In some embodiments, the movement of the plunger may be controlled by the controllers 412. In some embodiments, the pipette may include a pump to load bio-ink from the bio-ink container to the nozzle and/or dispense the loaded bio-ink to the printing surface. In some embodiments, the pump may be controlled by the controllers 412. In some embodiments, the nozzle may be straight or bended, which may result in varying (e.g., increased) degrees of freedom for the printer head 402 to deposit the bio-ink at the target positions and/or from various angles. In some embodiments, the nozzle may be made of a material that is non-toxic to biological components (e.g., cells), including non-toxic glass, plastics, ceramics, metal, fiber, or the like, or a combination thereof. In some embodiments, the bio-ink container may be filled with bio-ink and provide the bio-ink for the pipette and/or the nozzle. In some embodiments, the bio-ink container may be made of a material that is non-toxic to biological components (e.g., cells) including non-toxic glass, plastics, ceramics, metal, fiber, or the like, or a combination thereof. In some embodiments, the bio-ink container may be integrated in the printer 110. In some

embodiments, the bio-ink container may not be a portion of the printer 110. For example, the bio-ink container may be mounted on the printer 110 and may have a certain distance from the printer head 402, and accordingly, the bio-ink container may be in fluidic communication with the pipette or the nozzle of the printer head 402. As another example, the bio-ink container may be separated from the printer head 402, and accordingly, the printer head 402 may be moved to approach the bio-ink container and load bio-ink from the bio-ink container. In some embodiments, temperature adjuster may be used to change the temperature of components of the printing system 100. For example, the temperature adjuster can adjust the temperature of the bio-ink. Using the heater as an example, it may heat the bio-ink and/or keep the bio-ink under an appropriate temperature range, such as but not limited 36-37 °C, so that the activity of biological components in the bio-ink may be maintained. In some embodiments, the heater may be controlled by the controllers 412. In some embodiments, the heater may include one or more resistors connected to a power source and a thermometer. The power source may provide a current flowing through the one or more resistors and generate heat. In some embodiments, the heater may be mounted inside a heating chamber surrounding an outer surface of the nozzle, the pipette, and/or the bio-ink container. For instance, the heating chamber may be filled with a heating fluid (e.g., water) so that the heat can be evenly transferred to the bio-ink and keep the bio-ink under the appropriate temperature range based on the temperature measured by the thermometer. In some embodiments, the bio-ink in the nozzle, the pipette, and/or the bio-ink container may be heated using a same heater mounted in a same heating chamber, or using separate heaters mounted in separate heating chambers. In some embodiments, the printing head 402 may include a heating fluid system instead of a heater so as to heat the bio-ink. For example, the heating fluid system may provide the heating fluid under the appropriate temperature range to the heating chamber so that the bio-ink may be heated.

[0065] The positioning device(s) 404 may be configured to drive the printer head(s) 402 to move. The positioning device(s) 404 may be connected with the printer

head(s) 402. In some embodiments, if the bio-ink container is separated from the printer head(s) 402, and the positioning device(s) 404 may drive the printer head(s) 402 to move to the bio-ink container. In some embodiments, during a printing process, the positioning device(s) 404 may drive the printer head(s) 402 to move relative to the printing surface of the substrate. In some embodiments, the positioning device(s) 404 may include one or more extendable poles, one or more air cylinders, a carousel, etc. In this case, the positioning device(s) 404 may drive the printer head(s) 402 to move along an X-axis direction, a Y-axis direction, and/or a Z-axis direction. The X-axis, Y-axis, and Z-axis may refer to the three axes in a 3D Cartesian coordinate system. In some embodiments, the positioning device(s) 404 may include one or more robotic arms connected to the one or more printer head(s) 402. A robotic arm may include a plurality of arm units that are connected one by one. Each of the plurality of arm units may rotate around an axis, which enables the printer head(s) 402 to move freely in various directions. In some embodiments, the robotic arm may be connected to one or more components of the calibration assembly 414 for calibrating the location of the printer head(s) 402.

[0066] The container 406 may be configured to accommodate materials such as but limited to a liquid. In some embodiments, the container 406 may also be configured to provide support for the substrate. In some embodiments, the container 406 may be filled with a liquid. In some embodiments, the liquid may provide a liquid environment for the substrate during a printing process. In some embodiments, the liquid may be a hydrophobic liquid and may immerse the printing surface of the substrate. In some embodiments, the hydrophobic liquid may prompt the attachment of deposited biological components (e.g., cells) in the bio-ink to the substrate. In some embodiments, the liquid may be a hydrophilic liquid (e.g., a cell culture medium) that can support the survival of deposited biological components (e.g., cells) and/or promote proliferation (and/or differentiation) of the deposited biological components. In some embodiments, the container 406 may be made of a material that is non-toxic to biological components (e.g., cells). For instance, the container 406 may be made of non-toxic glass, plastics, ceramics, metal, fiber, or the

like, or a combination thereof. Merely by way of example, the container 406 may be made of titanium. The external surface and/or the internal surface of the container 406 may have various shapes including, for example, a cylinder shape, a cubic shape, a hemisphere shape, a trapezoid shape, a semi-ellipsoidal shape, a straight prism shape, an inclined prism shape, a truncated cone shape, a truncated pyramid shape, a turbinate shape, a truncated tetrahedron shape, an irregular shape, or the like, or a combination thereof.

[0067] In some embodiments, the container 406 may be equipped with a temperature adjuster (e.g., heater or cooler). The temperature adjuster may be used to change the temperature of components of the printing system 100. For example, the temperature adjuster can adjust the temperature of the liquid in the container 406. Using the heater as an example, it may heat the liquid and/or keep the liquid under an appropriate temperature range, such as but not limited 36-37 °C, so that the activity of biological components in the bio-ink may be maintained during a printing process. In some embodiments, the heater may be controlled by the controllers 412. In some embodiments, the heater may include one or more resistors connected to a power source and a thermometer. The power source may provide a current flowing through the one or more resistors and generate heat. In some embodiments, the heater may be mounted inside the container 406. In some embodiments, the heater may be mounted on the side wall and/or the bottom wall of the container 406.

[0068] The substrate fixing device 408 may be configured to fix the substrate. In some embodiments, at least a portion of the substrate fixing device 408 may be mounted on the container 406, fixing the substrate in the container 406. In some embodiments, the substrate fixing device 408 may include one or more fixing poles, one or more fasteners, one or more rotary components, or the like, or a combination thereof. In some embodiments, the fixing poles may be configured to fix the substrate. In some embodiments, the fasteners may be configured to fix the fixing poles on the container 406. In some embodiments, the fixing poles may be made of a material including but not limited to glass, plastics, ceramics, metal, fiber, etc. In

some embodiments, the fasteners may be fixed to the internal wall and/or external wall of the container 406 through, for example, a threaded connection, glue joint, bonding, bolted connection, or the like, or a combination thereof. In some embodiments, the fasteners may be made of a material including glass, plastics, ceramics, metal, fiber, etc. In some embodiments, a rotary component may include a motor or a connection piece connected to the motor. In some embodiments, at least a portion of a fixing pole may pass through a through-hole of a fastener, and an end of the fixing pole may connect to a rotary component. In some embodiments, the rotary components may be configured to drive the fixing poles to rotate, and may further drive the substrate to rotate via the rotation of the fixing poles. In some embodiments, the rotary components may drive the substrate to rotate during a printing process and/or a culturing process. In some embodiments, the rotary components may rotate the fixing poles synchronously in a coordinated direction (clockwise or anti-clockwise) so that the substrate may rotate as a whole.

[0069] Merely by way of example, as shown in FIG. 6A, the substrate fixing device 408 may include a pair of fixing poles 608 (e.g., a first fixing pole and a second fixing pole), a pair of fasteners 607 (e.g., a first fastener and a second fastener), and a pair of rotary components 609 (e.g., a first motor and a second motor). Specifically, a first end of the first fixing pole may be connected with a first end of the substrate, a second end of the first fixing pole may be connected with the first motor, and the first fixing pole may pass through a through-hole of the first fastener. Accordingly, a first end of the second fixing pole may be connected with a second end of the substrate, a second end of the second fixing pole may be connected with the second motor, and the second fixing pole may pass through a through-hole of the second fastener. The first motor may drive the first fixing pole to move and may further drive the first end of the substrate to move via the rotation of the first fixing pole. The second motor may drive the second fixing pole to move and may further drive the second end of the substrate to move via the rotation of the second fixing pole. The first motor and the second motor may rotate the fixing poles 608 synchronously in a coordinated direction so that the substrate may rotate as a whole.

[0070] In some embodiments, the substrate may include a tubular structure, and the fixing poles may be hollow. In some embodiments, the hollow fixing poles may be in fluidic communication with the tubular structure of the substrate. In some embodiments, the fluid system 410 may input a fluid (e.g., a cell culture medium) into the tubular structure of the substrate via a hollow fixing pole and output the fluid via another hollow fixing pole.

[0071] The fluid system(s) 410 may be configured to provide one or more fluids to one or more components of the printer 110. In some embodiments, a fluid system may include a pump, an inlet of a fluid, an outlet of a fluid, and one or more fluid passages configured between the inlet and outlet of the fluid. In some embodiments, the fluid system(s) 410 may include a first fluid system and a second fluid system. In some embodiments, as shown in FIG. 6A, the first fluid system may be configured to provide a first material into the printer head(s) 402. In some embodiments, the first material may refer to the bio-ink used for printing an object. For example, the first fluid system may provide the first material to the bio-ink container of the printer head(s) 402. As another example, the first fluid system may provide bio-ink to the printer head(s) 402 via, for example, a tube connected to the nozzle of the printer head(s) 402. The first material may be loaded to the printer head(s) 402 automatically or semi-automatically. In some embodiments, a plurality of first fluid systems may be configured to provide the first material with different compositions to the printer head(s) 402. In some embodiments, an operator may manually load the first material to the printer head(s) 402. In some embodiments, as shown in FIG. 6A, the second fluid system may be configured to provide a second material to a tubular structure of a substrate (e.g., a scaffold) during a printing process or a culturing process. In some embodiments, the substrate may be permeable to the second material. In some embodiments, the second material may include a cell culture medium, real blood, artificial blood, or the like, or a combination thereof. In some embodiments, the second material does not include any cells.

[0072] The controller(s) 412 may be configured to control the operations of one or more components of the printer 110 (e.g., the printer head(s) 402, the positioning

device(s) 404, the substrate fixing device 408, the fluid system 410, and/or the calibration assembly 414). For example, the controller(s) 412 may adjust the position of the printer head(s) 402 by controlling the movement of the positioning device(s) 404 connected with the printer head(s) 402. As another example, the controller(s) 412 may transmit a signal to the fluid system(s) 412 to start, pause, continue, or stop loading of the first material into the printer head(s) 402 and/or flowing of the second material in the substrate. As a further example, the controller(s) 412 may transmit a signal to the rotary components of the substrate fixing device 408 to control the rotation of the substrate. As yet a further example, the controller(s) 412 may transmit a signal to the calibration assembly 414 to control the calibration of the position of the printer head(s) 402.

[0073] In some embodiments, the controller(s) 412 may include one or more hardware modules. In some embodiments, the hardware modules may include connected logic units (e.g., gates, flip-flops, or the like), and/or programmable units (e.g., programmable gate arrays or processors). In some embodiments, the controller(s) 412 may include signal processing circuitry, memory circuitry, one or more processors, one or more single chip microcomputers, or the like, or a combination thereof. In some embodiments, at least a portion of the controller(s) 412 may be integrated in one or more printed circuit boards of the printer 110.

[0074] The calibration assembly 414 may be configured to calibrate the position of the printer head(s) 402. In some embodiments, the calibration assembly 414 may reduce or eliminate an error between an actual position and an identified position of the printer head(s) 402 (or the nozzle of the printer head(s) 402) so that the actual position of the printer head(s) 402 can be consistent with the identified position of the printer head(s) 402. The identified position of the printer head(s) 402 may refer to the position of the printer head(s) 402 that is recognized, recorded or detected by one or more components of the printing system 100 (e.g., the processing device 140 of the printing system 100). In some embodiments, one or more first calibration processes may be implemented before a printing process. In certain embodiments, there is only one first calibration process. In some embodiments, one or more

second calibration processes may be performed during a printing process. In some embodiments, the first calibration process and the second calibration processes may be performed based on a same calibration unit or different calibration units.

[0075] In some embodiments, the calibration assembly 414 may include a first calibration unit and a second calibration unit. The first calibration unit may be configured to calibrate the position of the printer head(s) 402 before the printing process. The second calibration unit may be configured to calibrate one or more positions of the printer head(s) 402 during the printing process. In some embodiments, the first calibration unit may include a calibrator located at a preset calibration position. The controller(s) 412 may control the nozzle of the printer head(s) 402 to move to the calibrator by adjusting one or more arm units of the robotic arm(s). The processing device 140 may recognize the identified position of the printer head(s) 402 and calibrate the identified position of the printer head(s) 402 based on the actual position (i.e., the preset calibration position) of the printer head(s) 402. Each arm unit of the robotic arm may be calibrated in this way or other ways.

[0076] The second calibration unit may include one or more sensors configured to detect real-time positions of the printer head(s) 402 during the printing process. Exemplary sensors may include a camera, a position sensor, or the like, or any combination thereof. In some embodiments, the position sensor may include an optical position sensor, a magnetic position sensor, an ultrasonic position sensor, a radar sensor, or the like, or any combination thereof. Merely by way of example, the position sensor may include one or more transmitters and one or more receptors. The one or more transmitters may transmit electromagnetic waves (e.g., infrared rays, laser rays), sound waves (e.g., ultrasonic waves) or other signals toward the printer head(s) 402. The receptor may receive the electromagnetic waves and/or sound waves reflected by the printer head(s) 402, and thus the real-time positions of the printer head(s) 402 may be determined based on the locations of the transmitter(s) and the receptor(s). In some embodiments, one or more reflectors may be mounted on the printer head(s) for reflecting the electromagnetic waves,

sound waves or other signals. In some embodiments, the second calibration unit may perform the calibration process before the printing process instead of the first calibration unit.

[0077] It should be noted that the above description of the printer 110 is merely provided for the purposes of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations or modifications may be made under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the printer 110 may include one or more additional components. For example, the printer 110 may further include a communication assembly configured to transmit or receive data from the one or more terminals 130 and/or the processing device 140. In some embodiments, the fluid system(s) 410 may be omitted or not included. In essence, the printer 110 may include one or more components as illustrated in FIG. 4. For example, the printer 110 may include only the printer head(s) 402, or the positioning device(s) 404, or the substrate fixing device 408, or the controller 412, or the calibration assembly 414, or a combination of any of these components. In some embodiments, the container 406 and/or the fluid system 410 may also be considered part of the printer 110. In some embodiments, the calibration assembly 414 may include a commercial calibration tool or device.

[0078] FIG. 5 is a block diagram illustrating an exemplary processing device according to some embodiments of the present disclosure. The processing device 140 may include an acquisition module 502, a target position determination module 504, a pathway determination module 506, and a control module 508. In some embodiments, the processing device 140 may be implemented on various components of the printing system 100 (e.g., the processor 210 of the computing device 200 illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 illustrated in FIG. 3).

[0079] The acquisition module 502 may be configured to acquire information related to the printing system 100. In some embodiments, the acquisition module 502 may

acquire data from one or more components of the printing system 100. For example, the acquisition module 502 may acquire information associated with the actual position of the printer head(s) 402 from the calibration assembly 414. As another example, the acquisition module 502 may acquire a geometric model relating to an object to be printed (and/or a substrate on which printing may be performed) from the storage device 150. In some embodiments, the geometric model may be a 3D model. In some embodiments, the 3D model may be obtained or reconstructed based on one or more imaging technologies including, for example, a computed tomography (CT) imaging, a magnetic resonance (MR) imaging, a structured light stereoscopic imaging, etc. In some embodiments, the 3D model may be obtained by measuring the object to be printed (and/or a substrate on which printing may be performed).

[0080] In some embodiments, the acquisition module 502 may acquire or receive one or more target positions on a printing surface of the substrate from, for example, the storage device 150, the terminal(s) 130, or an external data source. In some embodiments, the acquisition module 502 may acquire or receive one or more pathways for the printer head(s) 402 from, for example, the storage device 150, the terminal(s) 130, or an external data source. In some embodiments, the acquisition module 502 may acquire or receive one or more instructions provided by a user (or an operator) via, for example, the terminal(s) 130.

[0081] The target position determination module 504 may be configured to determine one or more target positions on a printing surface of the substrate. The printer head(s) 402 may deposit the bio-ink on the one or more target positions. In some embodiments, the one or more target positions may be determined based on the geometric model. In some embodiments, the target positions may be uniformly distributed on the surface of the geometric model. In some embodiments, the target position determination module 504 may recognize one or more target regions on the surface of the geometric model based on for example, image segmentation of the surface of the geometric model, a user input, a default setting of the printing system 100, or the like. In some embodiments, the target position determination module

504 may determine the target positions in the target regions. For example, the positions may be uniformly distributed in the target regions. In some embodiments, the target position determination module 504 may determine one or more sets of target positions. In some embodiments, the printer head(s) 402 may deposit different bio-inks on different sets of target positions based on the determinations of the target position determination module 504. In some embodiments, there may be a distance between two adjacent positions. In some embodiments, the target position determination module 504 may determine the distance between two adjacent target positions based on a dimension of the printing surface (e.g., a dimension of a target region) and/or a dimension of the droplet of bio-ink. For example, in some embodiments, the distance between two adjacent target positions may not be less than a diameter of a droplet of bio-ink, so as to avoid the fusion of two or more droplets of bio-ink. The distance may be, for example, 500 μm , 600 μm , 700 μm , 800 μm , 900 μm , 1000 μm , 1100 μm , 1200 μm , 1300 μm , 1400 μm , 1500 μm , etc. In some embodiments, the fusion of two or more droplets of bio-ink may make it more difficult for deposited biological components (e.g., cells) to attach to the substrate.

[0082] The pathway determination module 506 may be configured to determine one or more pathways of the printer head(s) 402. In some embodiments, a pathway may include a route of the printer head from an initial position to a first target position of the printing surface, or from a first target position to a second target position of the printing surface, or a route of the printer head from an initial position to a series of target positions in a sequential manner. In some embodiments, the pathway of the printer head(s) 402 may be a space curve. In some embodiments, the pathway(s) may be determined based on the geometric model, the one or more target positions, a current position of the printer head(s) 402, and/or the degrees of freedom for the printer head(s) 402. For example, the pathway determination module 506 may determine a pathway by geometric computation based on a current position of a printer head, a target position on the printing surface, and the degrees of freedom for the printer head. In some embodiments, if two or more printer heads 402 are

utilized to concurrently deposit bio-ink at different target positions, the pathway determination module 506 may also perform a collision check on the pathways of the two or more printer heads 402. If a possible collision is predicted, the pathway determination module 506 may modify one or more of the pathways of the printer head(s) 402.

[0083] The control module 508 may be configured to control one or more operations of the processing device 140. In some embodiments, the control module 508 may cause the acquisition module 502 to acquire information related to the printing system 100 upon an instruction provided by a user (or an operator) or an instruction stored in the storage device 150. In some embodiments, the control module 508 may cause the target position determination module 504 to determine the target position(s) on the printing surface upon an instruction provided by a user or an instruction stored in the storage device 150. In some embodiments, the control module 508 may cause the pathway determination module 506 to determine the pathway(s) of the printer head(s) 402 upon an instruction provided by a user or an instruction stored in the storage device 150. In some embodiments, the control module 508 may communicate with (or cooperate with) the controller 412 to control one or more components of the printer 110 to perform an operation.

[0084] Merely by way of example, if the acquisition module 502 receives an instruction from the terminal(s) 130 to print on the substrate, the control module 508 may cause the target position determination module 504 to determine the target position(s) on the printing surface, cause the pathway determination module 506 to determine the pathway(s) of the printer head(s) 402, communicate with the controller 412 to generate a control signal for the printer head(s) 402, and cause the printer head(s) 402 to move based on the determined pathway(s) and deposit bio-ink on the target position(s).

[0085] In some embodiments, the control module 508 may be implemented as software modules and may be stored in any type of non-transitory computer-readable medium or other storage devices. For example, the control module 508 may be stored in the processing device 140. In some embodiments, a software

module may be compiled and linked into an executable program. Software modules configured for execution on computing devices (e.g., a processor of computing device 120) can be provided on a computer-readable medium, such as a compact disc, a digital video disc, a flash drive, a magnetic disc, or any other tangible medium, or as a digital download (and can be originally stored in a compressed or installable format that requires installation, decompression, or decryption prior to execution). Such software code can be stored, partially or fully, on a memory device of the executing computing device, for execution by the computing device. Software instructions can be embedded in a firmware, such as an EPROM. In some embodiments, the control module 508 of the processing device 140 may be integrated with the controller 412 of the printer 110.

[0086] It should be noted that the above description of the processing device 140 is merely provided for the purposes of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations or modifications may be made under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the processing device 140 may include one or more additional modules. For example, the processing device 140 may further include a storage module configured to store data generated by the above-mentioned modules in the processing device 140. In some embodiments, the target position determination module 504 and/or the pathway determination module 506 may be omitted or implemented on an external processing device.

[0087] FIG. 6A is a schematic diagram illustrating an exemplary printer according to some embodiments of the present disclosure. The printer 600 shown in FIG. 6A may be configured to print an object. The printer 600 may include a printer head 602, a robotic arm 604, a container 606, a substrate fixing device, a first fluid system 610, a second fluid system 611, a controller 612.

[0088] Merely for illustration, only one printer head 602 and one robotic arm 604 are shown in FIG. 6A. It should be noted, however, that the printer 600 may have more than one printer head 602, wherein each of the printer head(s) 602 may be

connected to a robotic arm 604. In some embodiments, the robotic arm 604 may include a plurality of arm units (e.g., 2 arm units, 3 arm units, 4, arm units, 5 arm units, 6 arm units, 7 arm units, 8 arm units, 9 arm units, 10 arm units, etc.). Each of the plurality of arm units may rotate around at least one axis. In some embodiments, the robotic arm 604 may include a plurality of axes corresponding to the plurality of arm units (e.g., 2 axes, 3 axes, 4 axes, 5 axes, 6 axes, 7 axes, 8 axes, 9 axes, 10 axes, etc.). For example, the robotic arm 604 may include three arm units and three corresponding axes. The robotic arm 604 shown in FIG. 6A is a 6-axis robotic arm. The controller 612 may control the robotic arm 604 to drive (and/or guide) the printer head 602 to move relative to a printing surface of a substrate 605. The printer head 602 may approach one or more target positions on the printing surface under the guidance of the robotic arm 604. In some embodiments, the controller 612 may control the printer head 602 to deposit one or more droplets of bio-ink through a nozzle 603 of the printer head 602. In some embodiments, the controller 612 may control the first fluid system 611 to provide a first material into the printer head 602. The first material may refer to the bio-ink to be printed on the substrate 605. Specifically, the first material may include cell culture medium and a plurality of biological components (e.g., cells). The first fluid system 611 may provide bio-ink to the printer head 602 via a tube (not shown) connected to the nozzle 603 of the printer head 602. The first material may be loaded to the printer head 602 automatically. In some embodiments, the controller 612 may control a calibration assembly (e.g., the calibration assembly 414 shown in FIG. 4) to calibrate the position of the printer head 602 before and/or during a printing process. More descriptions of the printer head 602 may be found elsewhere in the present disclosure (e.g., FIG. 4 and the descriptions thereof).

[0089] The substrate 605 may be fixed in the container 606 by the substrate fixing device. The substrate 605 shown in FIG. 6A may include a scaffold having a tubular structure. In some embodiments, the substrate 605 may have various shapes and/or sizes. For example, the substrate 605 may be a cuboid, a cylinder, a sphere, any polyhedron, or any irregular shape appearing in an organism, or the like,

or a combination thereof. In some embodiments, the substrate 605 may have a complex structure. For example, the substrate 605 may include one or more branching structures (see FIG. 9A), one or more bending structures (see FIG. 9B), or the like, or a combination thereof. As another example, the substrate 605 may include a plurality of tubular structures that form a vascular network.

[0090] As shown in FIG. 6A, the substrate fixing device may include a pair of fixing poles 608 (e.g., a first fixing pole and a second fixing pole), a pair of fasteners 607 (e.g., a first fastener and a second fastener), and a pair of rotary components 609 (e.g., a first motor and a second motor). Specifically, a first end of the first fixing pole may be connected with a first end of the substrate, a second end of the first fixing pole may be connected with the first motor, and the first fixing pole may pass through a through-hole of the first fastener. Accordingly, a first end of the second fixing pole may be connected with a second end of the substrate, a second end of the second fixing pole may be connected with the second motor, and the second fixing pole may pass through a through-hole of the second fastener. The controller 612 may control the first motor to drive the first fixing pole to move and further drive the first end of the substrate to move via the rotation of the first fixing pole. The controller 612 may control the second motor to drive the second fixing pole to move and further drive the second end of the substrate to move via the rotation of the second fixing pole. The controller 612 may control the first motor and the second motor to rotate the fixing poles 608 synchronously in a coordinated direction so that the substrate may rotate as a whole.

[0091] The substrate 605 may include a tubular structure, and the fixing poles may be hollow. In some embodiments, the hollow fixing poles may be in fluidic communication with the tubular structure of the substrate 605. As shown in FIG. 6A, the controller 612 may control the second fluid system 610 to provide a second material to the tubular structure of the substrate 605 during a printing process or a culturing process. As shown in FIG. 6A, the second material may be provided in the tubular structure of the substrate 605 from an inlet port 613 and may be output from the tubular structure to the outlet port 614. In some embodiments, the

substrate 605 may be permeable to the second material. In some embodiments, the second material may include a cell culture medium, real blood, artificial blood, or the like, or a combination thereof. In some embodiments, the second material does not include any cells. In some embodiments, the second material may reach the deposited biological components on the substrate 605 and/or support the survival of the biological components.

[0092] In some embodiments, a liquid may be loaded into the container 606. In some embodiments, the liquid may provide a liquid environment for the substrate 605 during a printing process. In some embodiments, the liquid may be a hydrophobic liquid. The liquid may immerse the printing surface of the substrate 605 before a printing process. As shown in FIG. 6A, an internal surface of the container 606 may have a semi ellipsoid shape, a hemisphere shape, a trapezoid shape, or the like (i.e., the bottom sides of the internal surface may be shorter than the upper sides of the internal surface), so as to save the liquid consumption. Other shapes and sizes of the container 606 may also be applied in the present disclosure.

[0093] FIGs. 6B-6D are schematic diagrams illustrating exemplary tilted printer heads according to some embodiments of the present disclosure. As shown in FIG. 6B, the printer head 602 may be tilted relative to the printing surface of the substrate 605 and may approach a target position on the printing surface of the substrate 605 from various angles. Therefore, droplets of bio-ink may be deposited on the printing surface of the substrate 605 from various angles. In some embodiments, the printer head 602 may be tilted relative to an axial direction of the substrate 605 (see FIG. 6C). In some embodiments, the printer head 602 may be tilted relative to a transversal direction of the substrate 605 (see FIG. 6D). As shown in FIGs. 6C and 6D, the tilt angle α of the printer head 602 may refer to an angle between the nozzle 603 and a vertical direction (shown by a vertical dash-dot line). In some embodiments, the controller 612 may change the tilt angle α of the printer head 602 by adjust the robotic arm 604. In some embodiments, the substrate 605 may include an inclined plane or a curved surface (as shown in FIG. 6D).

[0094] It should be noted that the above description in FIGs. 6A-6D is merely

provided for the purposes of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations or modifications may be made under the teachings of the present disclosure.

However, those variations and modifications do not depart from the scope of the present disclosure. For example, a bended nozzle may be applied to deposit the bio-ink from the tilt angle α . As another example, the robotic arm 604 may be coupled with a calibration assembly, such as a camera or a position sensor.

[0095] FIG. 7 is a flowchart illustrating a general process of 3D printing according to some embodiments of the present disclosure. In some embodiments, one or more operations of process 700 illustrated in FIG. 7 may be implemented in the printing system 100 illustrated in FIG. 1. For example, at least a part of the process 700 may be stored in the storage device 150 in the form of instructions, and invoked and/or executed by the processing device 140 (e.g., the processor 210 of the computing device 200 as illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 as illustrated in FIG. 3). As another example, at least a part of the process 700 may be executed by the printer 110.

[0096] In 701, the processing device 140 (e.g., the acquisition module 502) may obtain a model relating to an object. In some embodiments, the model relating to the object may be a geometric model (e.g., a reconstructed 3D model) obtained or reconstructed based on one or more imaging techniques including, for example, a CT imaging, an MR imaging, a single photon emission computed tomography (SPET), a structured light stereoscopic imaging, etc. In some embodiments, the model relating to the object may be obtained by measuring the object (and/or a substrate on which printing may be performed). In some embodiments, the object may be a biological object to be printed. For example, the object may include a heart, a liver, a kidney, a lung, a stomach, an intestine, a pancreas, a urinary bladder, a pharynx, a larynx, a gallbladder, a lymph node, a spleen, a nerve, a bone, a skin, a tumor, or the like, or a portion thereof. In some embodiments, the reconstructed 3D model may be segmented to a plurality of layers for determining the pathway of the printer head(s) 402. In some embodiments, the object may be a substrate on which

printing may be performed. For example, the object may include a scaffold (e.g., a vascular scaffold, a liver scaffold, a heart scaffold, a nerve scaffold, or the like).

[0097] In 703, the processing device 140 may determine a printing strategy. In some embodiments, the printing strategy may include a strategy related to the types of one or more bio-inks, an amount of each type of bio-ink, a printing sequence of the bio-inks, or the like. In some embodiments, the printing strategy may include a strategy related to the printer head(s) 402. For example, the printing strategy may include the number of printer heads 402 to be utilized. If a plurality of printer heads 402 will be utilized, the printing strategy may include whether the plurality of printer heads 402 deposit the bio-ink(s) concurrently or alternately, or an execution sequence of the plurality of printer heads 402 to deposit the bio-ink(s). In some embodiments, the printing strategy may include one or more target positions on the printing surface of the substrate, one or more pathways of the printer head(s) 402, and/or one or more parameters related to a printing process. The parameters related to the printing process may include but not be limited to the positioning of the printer head(s) 402 relative to the printing surface, the volume of each droplet of bio-ink to be deposited, a moving velocity of the printer head(s) 402 from a target position to a next target position, a time interval for calibration, a time point to stop or suspend the printing process, or the like, or any combination thereof.

[0098] In 705, bio-ink may be prepared. In some embodiments, the bio-ink may be prepared automatically, semi-automatically, or manually. The composition of the bio-ink may be determined according to the object to be printed. The bio-ink may include cellular components and non-cellular components as described elsewhere in the present disclosure. In some embodiments, the bio-ink may include living cells. In some embodiments, the cells may include undifferentiated stem cells, intermediately differentiated stem cells, terminally differentiated cells, or a combination thereof. In some embodiments, the cells may include cardiac cells, renal cells, hepatic cells, lung cells, gastric cells, pancreatic cells, gallbladder cells, bladder cells, spleen cells, tracheal cells, nerve cells, bone cells, intestinal cells, epithelial cells, muscle cells, fibroblasts, secretory cells, ciliated cells, fat cells, blood

cells, immune cells, cancer cells, or the like, or a combination thereof. In some embodiments, the endothelial cells may include vascular endothelial cells, lymphatic endothelial cells, or the like, or a combination thereof. In some embodiments, the non-cellular components may include cell culture medium, real blood, artificial blood, or the like, or a combination thereof. Merely for illustration, for printing a blood vessel, the bio-ink may include cell culture medium (or real blood, artificial blood) endothelial cells, and/or smooth muscle cells, or the like, or a combination thereof.

[0099] In 707, a 3D printer may perform printing on a substrate. In some embodiments, the 3D printer may be the printer 110 shown in FIG. 4 and/or the printer 600 shown in FIG. 6A. In some embodiments, the bio-ink may be provided to the printer head(s) 402 via the first fluid system 611. In some embodiments, the 3D printer may control the printer head(s) 402 to deposit one or more droplets of the bio-ink on one or more target positions on a printing surface of the substrate according to the printing strategy. In some embodiments, the controller 412 and/or the control module 508 may control the movement of the printer head(s) 402 by controlling the movement of the robotic arm connected to the printer head(s) 402. In some embodiments, the positions of the printer head(s) 402 may be calibrated before and/or during the printing process by the calibration assembly 414.

[0100] In 709, a post-printing process may be performed. In some embodiments, the post-printing process may allow the deposited cells to attach to the substrate or allow the deposited cells to proliferate and cohere to form a tissue or tissues, an organ or organs, or a portion or combination thereof. For example, in the post-printing process, the substrate including the deposited cells may be kept in a stationary status for a certain period of time (e.g., 20 min, 30 min, 40 min, 1 h, 2 h). During the period of time, the deposited cells, especially living cells, may attach firmly onto the substrate and will not easily be detached from the substrate. As another example, in the post-printing process, the substrate including the deposited cells may be transferred into a bioreactor for incubation, wherein a cell culture medium may be provided to support the proliferation and/or the differentiation of the deposited cells.

[0101] FIG. 8 is a schematic diagram illustrating an exemplary printing process according to some embodiments of the present disclosure. In some embodiments, one or more operations of process 800 illustrated in FIG. 8 may be implemented in the printing system 100 illustrated in FIG. 1. For example, at least a part of the process 800 may be stored in the storage device 150 in the form of instructions, and invoked and/or executed by the processing device 140 (e.g., the processor 210 of the computing device 200 as illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 as illustrated in FIG. 3). As another example, at least a part of the process 800 may be executed by the printer 110.

[0102] In 801, the processing device 140 (e.g., the acquisition module 502) may receive information relating to one or more target positions on a printing surface. The printing surface may be a surface of a substrate. In some embodiments, the acquisition module 502 may receive the information relating to the target positions on the printing surface from, for example, the storage device 150, the terminal(s) 130, or an external data source. In some embodiments, the target position determination module 504 may determine the information relating to the target positions on the printing surface based on a geometric model relating to the substrate. The geometric model relating to the substrate may include information such as a dimension of the substrate, surface morphology of the substrate, etc. In some embodiments, the acquisition module 502 may acquire the information relating to the target positions from the target position determination module 504. In some embodiments, a distance between adjacent target positions may be determined based on a dimension of the printing surface, and/or a dimension of a droplet of bio-ink. In some embodiments, the distance between adjacent target positions may be greater than a threshold, so as to avoid aggregation of one or more droplets of bio-ink. In some embodiments, the threshold may be determined based on the volume of a droplet. For example, the distance between adjacent target positions may be greater than a diameter of a droplet.

[0103] In some embodiments, the printing surface may be a surface of a scaffold, such as a vascular scaffold, a liver scaffold, a heart scaffold, a nerve scaffold, etc.

The scaffold may become a part of the object to be printed. In some embodiments, the printing surface may be a surface of a mold, a support, etc. The mold and/or the support may be used to collect the deposited bio-ink. In some embodiments, the deposited biological components may coalesce to form the object to be printed through a self-assembly effect. In some embodiments, the printing surface may include biological components. The biological components may include cells growing on the substrate and/or cells deposited on the substrate. In some embodiments, the printing surface may be a horizontal surface or an incline surface. In some embodiments, the printing surface may be a flat plane and/or a curved surface.

[0104] In 803, the processing device 140 (e.g., the pathway determination module 506) may determine a pathway of a printer head 402 to the one or more target positions on the printing surface based on the information. In some embodiments, the pathway of the printer head 402 may include a plurality of motion parameters related to the movement of the printer head 402. In some embodiments, the motion parameters may include a moving direction and a velocity of the printer head 402 from an initial position to a target position, and/or from the target position to a next target position. In some embodiments, the pathway determination module 506 may determine the pathway based on a geometric model of the substrate, the one or more target positions, a current position of the printer head(s) 402, and/or the degrees of freedom for the printer head(s) 402. In some embodiments, the movement of the printer head 402 may be controlled by the controller 412. In some embodiments, the controlling of the printer head 402 may be realized by adjusting the movement of the positioning device(s) 404. In some embodiments, the positioning device(s) 404 may include a robotic arm connected to the printer head 402. In some embodiments, the robotic arm may include a plurality of arm units. In some embodiments, the pathway of the printer head 402 may further include a moving direction and a velocity of each arm units.

[0105] In some embodiments, a plurality of printer heads 402 may be connected to a plurality of robotic arms. The plurality of printer heads 402 may move

independently and deposit the bio-ink concurrently and/or alternately. The processing device 140 may determine a plurality of pathways of the plurality of printer heads 402. In some embodiments, the processing device 140 (e.g., the pathway determination module 506) may further perform a collision check on the plurality of pathways. If there is a possible collision, the processing device 140 may adjust one or more pathways of the plurality of pathways.

[0106] In 805, the calibration assembly 414 of the printer 110 may calibrate a position of the printer head 402 relative to the printer 110. In some embodiments, a calibration process may be implemented before a printing process to eliminate or reduce an error between an actual position and an identified position (also referred to as detected position) of the printer head(s) 402. The detected position of the printer head(s) 402 may be adjusted based on the actual position of the printer head(s) 402. In some embodiments, the calibration assembly 414 may include a first calibration unit. In some embodiments, the first calibration unit may include a calibrator located at a preset calibration position. In some embodiments, before a printing process, the controller(s) 412 may control the nozzle of the printer head 402 to move to the calibrator by adjusting the positioning device (e.g., one or more arm units of the robotic arm(s)), so that the actual position of the printer head(s) 402 may be the same as the preset calibration position. In some embodiments, the calibration assembly 414 may include a second calibration unit. In some embodiments, the second calibration unit may include one or more sensors. In some embodiments, during the printing process, the one or more sensors may detect the real-time positions of the printer head 402. Exemplary sensors may include a camera, a position sensor, or the like, or any combination thereof.

[0107] In 807, the bio-ink may be loaded to the printer 110. In some embodiments, the bio-ink may be loaded to the printer 110 via a first fluid system (e.g., the first fluid system 611 shown in FIG. 6). The first fluid system may continuously or discontinuously provide the bio-ink for the printer head(s) 402. For example, the first fluid system 611 may continuously provide the bio-ink to the printer 110 so that the printer 110 does not have to suspend the printing process to reload the bio-ink.

As another example, the first fluid system 611 may reload the bio-ink after the printer head 402 runs out of bio-ink. In some embodiments, an operator may manually load the bio-ink to the printer 110. In some embodiments, the controller 412 may suspend a printing process if the printer head 110 runs out of bio-ink, and may resume the printing process after the bio-ink is reloaded to the printer 110.

[0108] In 809, the controller 412 may control the printer head 402 to approach the one or more target positions sequentially based on the determined pathway. In some embodiments, the controller 412 may control the positioning device(s) 404 to drive the printer head 402 to approach the one or more target positions according to the determined pathway.

[0109] In 811, the controller 412 may change an axial direction of the printer head 402. In some embodiments, the substrate may have a complex structure. The printer head 402 may deposit the bio-ink from various angles (as described in FIGs. 9A-9B). For example, the axial direction of the printer head 402 may be changed from a vertical direction to an inclined direction before or during the printing process. In some embodiments, the controller 412 may change the axial direction of the printer head 402 (e.g., tilt the printer head 402) by adjusting the positioning device(s) 404 (e.g., the robotic arm 604 shown in FIG. 6A) connected to the printer head 402. In some embodiments, the controller 412 may change the axial direction of the printer head 402 for one or more times during the printing process to deposit the bio-ink on different target positions of the printing surface. In some embodiments, operation 811 may be omitted. In some embodiments, the axial direction of the printer head 402 may remain unchanged before and/or during the printing process. In some embodiments, the nozzle of the printer head 402 (e.g., the nozzle 603 shown in FIG. 6) may be replaced by another nozzle to change the printing angle (e.g., the tilt angle α shown in FIGs. 6C and 6D). For example, a straight nozzle may be replaced with a bended nozzle.

[0110] In 813, the printer head 402 may deposit one or more droplets of bio-ink on the one or more target positions. In some embodiments, the deposition of the droplets of bio-ink may be controlled by the controller 412. In some embodiments,

two or more printer heads may be used in a printing process. In some embodiments, the printer heads may be loaded with the same or different bio-inks. In some embodiments, the printer heads may deposit the bio-ink on the printing surface of the substrate concurrently or alternately. In some embodiments, a droplet of bio-ink may have a predetermined volume (e.g., 1 μ l, 1.5 μ l, 2 μ l, 5 μ l, 10 μ l, etc.). In some embodiments, a user (e.g., an operator) may view and/or adjust the volume of the droplets of bio-ink via the terminal 130. In some embodiments, the volume of each droplet of bio-ink may be the same or different. For example, the volume of a droplet of bio-ink including multicellular bodies may be set greater than a droplet of bio-ink including free cells. In some embodiments, the controller 412 may control the movement of the printer heads 402 by controlling the movement of the positioning device(s) 404 connected to the printer heads 402.

[0111] In 815, the biological components deposited on the printing surface may be cultured. In some embodiments, the deposited biological components may include cells, such as but limited to living cells. In some embodiments, the deposited cells may be cultured to allow the cells to proliferate and/or differentiate. For example, the cells may cohere to form 3D-structured tissue. As another example, the deposited biological components may include undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. The undifferentiated stem cells and/or the intermediately differentiated stem cells may differentiate into other types of cells under an appropriate condition. For instance, an agent that can induce differentiation of the undifferentiated stem cells and/or the intermediately differentiated stem cells may be added in a culture medium for culturing the deposited biological components. In some embodiments, the object to be printed may have a tubular structure (e.g., a vessel or a vascular network). For example, the object to be printed may be a heart including a vascular network. In some embodiments, the deposited biological components may be cultured for angiogenesis. In some embodiments, operation 815 may be omitted. In some embodiments, the object to be printed may include no cell. For example, the object may be a 3D-structured scaffold used for

transplantation, such as an artificial skin, an artificial endocranium, an artificial pericardium, etc.

[0112] It should be noted that the above description of the process 800 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made to the process 800 under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, operations 801 through 811 may be performed in any order. In some embodiments, one or more operations of the process 800 may be added or omitted. For example, operations 811 and/or 815 may be omitted.

[0113] FIGs. 9A-9B are schematic diagrams of exemplary substrates having a complex structure according to some embodiments of the present disclosure. In some embodiments, the substrate 605 may have a tubular structure. In some embodiments, the substrate 605 may include a vessel or a vascular network. As illustrated in FIG. 9A, the substrate 605 may have one or more branches. In some embodiments, the object to be printed may have a vessel or a vascular network that is connected to the tubular structure of the substrate 605. For example, the vascular network may include a plurality of capillary vessels that are interconnected. At least a portion of the vascular network (e.g., one or more capillary vessels) may be connected to the tubular structure of the substrate 605. Merely by way of example, the printer head 402 may deposit the bio-ink on the one or more branches to obtain the one or more capillary vessels that are connected to the tubular structure of the substrate 605. In some embodiments, the printer head 402 may deposit the bio-ink on one or more target positions on the surface of the branches from one or more printing angles. As shown in FIG. 9A, the tilt angle α may refer to an angle between the nozzle 603 and a vertical direction (shown by a vertical dash-dot line). In some embodiments, the substrate 605 may have a varying diameter and may be bended (as shown in FIG. 9B). In some embodiments, the printer head 402 may deposit the bio-ink on one or more target positions on a curved surface of the

substrate 605 without touching other parts of the substrate 605. In this situation, the processing device 140 may determine pathway(s) for the printer head 402 based on the geometric model of the substrate 605 before the printing process and/or check the position(s) of the printer head 402 on the pathway(s) so that the printer head 402 would not touch other parts of the substrate 605. In some embodiments, the printing surface of the substrate 605 may be irregular. For example, the substrate 605 may include one or more branches as illustrated in FIG. 9A and/or one or more bending structures as illustrated in FIG. 9B.

[0114] In some embodiments, during a printing process, the controller 412 may change the tilt angle α of the printer head 402 for different target positions (e.g., on different branches or bending structures) of the substrate 605, which may provide more degrees of freedom for printing on the substrate 605 having a complex structure. In some embodiments, if the tilt angle α of the printer head 402 needs to be changed during a printing process, the controller 412 may suspend the printing process, move the printer head 402 from a current target position to a certain position that has a second safe distance (e.g., 10 cm, 20 cm, 30 cm, 40 cm, 50 cm, etc.) away from the substrate 605, change the tilt angle α of the printer head 402, and/or move the printer head 402 to a next target position. In some embodiments, the processing device 140 may consider the change of the tilt angle α of the printer head 402 in the determination of the pathway(s) of the printer head 402 before the printing process.

[0115] It should be noted that the above description of FIGs. 9A-9B is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. For example, the substrate 605 may be of any shape, such as a cuboid, a sphere, a semi-sphere, a cylinder, any irregular shape, etc.

[0116] FIG. 10 is a flowchart illustrating an exemplary printing process in a liquid environment according to some embodiments of the present disclosure. In some

embodiments, one or more operations of process 1000 illustrated in FIG. 10 may be implemented in the printing system 100 illustrated in FIG. 1. For example, at least a part of the process 1000 may be stored in the storage device 150 in the form of instructions, and invoked and/or executed by the processing device 140 (e.g., the processor 210 of the computing device 200 as illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 as illustrated in FIG. 3). As another example, at least a part of the process 1000 may be executed by the printer 110.

[0117] In 1001, a substrate may be fixed. In some embodiments, the substrate may be fixed by a substrate fixing device (e.g., the substrate fixing device 608 shown in FIG. 6A). In some embodiments, at least a portion of the substrate fixing device 608 may be mounted on a container and fix the substrate in the container. The substrate may be fixed by the substrate fixing device 608 in various ways. For example, each end of the substrate may be fixed by a fixing pole of the substrate fixing device 608. More descriptions of the substrate fixing device 608 and the fixation of the substrate may be found elsewhere in the present disclosure (e.g., FIGs. 4 and 6 and the descriptions thereof).

[0118] In 1003, a liquid may be loaded in a container (e.g., the container 606 shown in FIG. 6A). In some embodiments, the liquid may provide a liquid environment for the substrate during a printing process (see FIG. 11). In some embodiments, the liquid may be a hydrophobic liquid and may immerse the printing surface of the substrate. In some embodiments, the liquid may be a conducting liquid, such as an electrolyte solution with a proper concentration (e.g., a normal saline solution with a concentration of 0.9 g/100 ml) that can maintain the osmotic balance for the biological components deposited on the substrate. In some embodiments, the liquid may be loaded in the container by an operator or by a fluid system (not shown) before the printing process.

[0119] In 1005, the printer head 402 may deposit one or more droplets of bio-ink on a printing surface of the substrate. In some embodiments, the printing surface may be immersed in the liquid during the printing process. In some embodiments, at least a portion of a nozzle of the printer head 402 may also be immersed in the liquid

loaded in the container 606. In some embodiments, one or more printer heads 402 may concurrently or alternately deposit the same or different bio-inks on the printing surface according to one or more predetermined pathways. In some embodiments, the controller 412 may control the movement of the one or more printer heads 402 by adjusting the one or more positioning devices 404 connected to the one or more printer heads 402.

[0120] In some embodiments, an attachment of a droplet of bio-ink to the printing surface of the substrate may be prompted by a first interaction and/or a second interaction (see FIG. 11). Herein a droplet of bio-ink is considered attached to the printing surface if a significant portion (e.g., more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) of the drop of bio-ink, especially the solid component of the droplet of bio-ink (e.g., biological components in the bio-ink), is attached to the printing surface. In certain embodiments, the attachment of the droplet to the printing surface is prompted solely or mainly by the first interaction. In certain embodiments, the attachment of the droplet to the printing surface is prompted solely or mainly by the second interaction. In certain embodiments, the attachment of the droplet to the printing surface is prompted by the first interaction and the second interaction, as joint forces. The first interaction may be an interaction between the droplet of bio-ink and the printing surface of the substrate. The second interaction may be an interaction between the droplet of bio-ink and the liquid. In some embodiments, the first interaction may be an attracting force between the droplet of bio-ink and the printing surface of the substrate. In some embodiments, the second interaction may be a repelling force between the droplet of bio-ink and the liquid. In some embodiments, printing in a liquid environment may avoid the introduction of a solidification process, which may cause damage to biological components and greatly decrease the survival rate of the biological components.

[0121] In some embodiments, the first interaction may be a hydrophilic interaction, a polar interaction, an electric interaction, a magnetic interaction, or the like, or any combination thereof. In some embodiments, the second interaction may be a hydrophobic interaction, a buoyance force, or the like, or any combination thereof.

In some embodiments, the liquid may be a hydrophobic liquid. In some embodiments, the hydrophobic liquid may include mineral oil, and any other type of hydrophobic liquid that is not harmful to biological components (e.g., cells). In some embodiments, the printing surface of the substrate may be hydrophilic. In some embodiments, the first interaction between the droplet of bio-ink and the printing surface may be a hydrophilic interaction. In some embodiments, the second interaction between the droplet of bio-ink and the liquid may be a hydrophobic interaction. In some embodiments, the deposited biological components (e.g., cells) may include polar functional groups. In some embodiments, the printing surface of the substrate may be grafted with polar functional groups to increase the polar interaction between the droplet of bio-ink and the printing surface. In some embodiments, the deposited biological components (e.g., cells) may have been marked with charged or magnetized nanoparticles. In some embodiments, an electric or magnetic field may be applied to the substrate to facilitate the droplet of bio-ink to attach to the printing surface of the substrate.

[0122] In 1007, the liquid may be removed. After a printing process is finished, the liquid may be removed, for example, by using a liquid pumping device. It should be noted that the liquid may be removed at an appropriate removal rate, so as to prevent or reduce mechanical damage to the deposited biological components. For example, if the liquid flows too fast during the removal of the liquid, a relatively large shear force may cause mechanical damage to the deposited biological components.

[0123] In 1009, the deposited printing surface may be washed. In some embodiments, after the liquid is removed, there may be some residual liquid on the internal wall of the container 606 and/or the deposited printing surface. In some embodiments, the container 606 and/or the deposited printing surface may be washed by one or more washing liquids. In some embodiments, the container 606 and the deposited printing surface may be washed by different liquids. In some embodiments, the container 606 and/or the deposited printing surface may be washed for a plurality of times. In some embodiments, the washing liquid may include a hydrophilic liquid, a hydrophobic liquid, or a cell culture medium. For

example, the hydrophilic liquid may include ethanol, a phosphate buffer saline (PBS) solution, or a normal saline (NS) solution, or the like. In some embodiments, the washing liquid may be added in the container 606 and may immerse the deposited printing surface. In some embodiments, the washing liquid may be added in the container 606 and may not immerse the deposited printing surface. In some embodiments, the residual liquid may be a hydrophobic liquid (e.g., mineral oil) and may float on top of the washing liquid after the washing liquid is loaded in the container. In some embodiments, the washing liquid and the residual liquid may be removed, for example, using the liquid pumping device.

[0124] Merely by way of example, in a first time of washing, ethanol may be loaded in the container 606 to remove at least a portion of residual mineral oil on the internal wall of the container 606. Specifically, a relatively small amount of ethanol may be loaded to the container 606 along the internal wall of the container 606 but may not reach the deposited printing surface. The ethanol and at least a portion of the residual mineral oil may be removed. In a second time of washing, a PBS solution may be loaded to the container 606 and may immerse the deposited printing surface. The residual mineral oil (if any) may float on top of the PBS solution. The PBS solution and at least a portion of the residual mineral oil (if any) may be removed. In a third time of washing, a cell culture medium may be loaded to the container 606 and may immerse the deposited printing surface. The residual mineral oil (if any) may float on top of the cell culture medium. The cell culture medium and at least a portion of the residual mineral oil (if any) may be removed. In some embodiments, the first time of washing may be repeated for one or more times. In some embodiments, the second time of washing may be repeated for one or more times. In some embodiments, the third time of washing may be repeated for one or more times.

[0125] In 1011, the biological components deposited on the printing surface may be cultured. As described in connection with operation 815 of FIG. 8, the biological components deposited on the printing surface may be cultured after a printing process for cell proliferation, cell differentiation, cell cohesion, cell migration, or the

like, or any combination thereof. In some embodiments, the substrate fixing device 608 may be removed from the container 606. In some embodiments, the substrate along with the at least a portion of the substrate fixing device 608 (e.g., the fixing pole(s)) may be transferred to a culture container loaded with a cell culture medium. In some embodiments, the substrate may be removed from the substrate fixing device 608 and placed in a culture container loaded with a cell culture medium. In some embodiments, a cell culture medium may be loaded into the container 606 and the deposited biological components may be immersed in the culture medium. In some embodiments, the cell culture medium may include one or more nutrients and/or one or more factors (or inhibitors) that may support the survival of the cells in the deposited biological components. In some embodiments, the factors (or inhibitors) may include adrenomedullin, an autocrine motility factor, a ciliary neurotrophic factor, an epidermal growth factor (EGF), apoptosis inhibitors, or the like. In some embodiments, the cell culture medium may include one or more angiogenic factors that may induce one or more cells to coalesce to form one or more capillary vessels. For example, the angiogenic factors may include a vascular endothelial growth factor (VEGF), a fibroblast growth factor (FGF), angiopoietins, matrix metalloproteinase (MMP), delta-like ligand 4 (DII4), class 3 semaphorins (SEMA3s), or the like, or any combination thereof.

[0126] In some embodiments, the culture container or the container 606 may be placed in a bioreactor and the deposited biological components may be cultured at an appropriate temperature for a time period. For example, the deposited biological components may be cultured at 37 °C. In some embodiments, the deposited biological components may be cultured for 2h, 4h, 8h, 24h, 48h, 3 days, 5 days, etc. In some embodiments, the cell culture medium may be replaced with fresh cell culture medium every 8h, 16h, 24h, etc.

[0127] It should be noted that the above description of the process 1000 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made to the process 1000 under the teachings of the

present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the substrate fixing device 608 may include one or more rotary components (e.g., motors). In some embodiments, the rotary component(s) may cause the substrate to rotate in a printing process (e.g., in operation 1005) or a culturing process (e.g., in operation 1011). For example, after a first region on the printing surface of the substrate are deposited with biological components, the rotary component(s) may cause the substrate to rotate so that a second region on the printing surface (e.g., a second region opposite to the first region) may be exposed to the printer head(s) 402. As another example, the substrate may be rotated during the culturing process to facilitate the material exchange between the deposited biological components and the cell culture medium. In some embodiments, the substrate may be rotated manually at a time interval during the culturing process. In some embodiments, one or more of the operations 801 through 811 may be added in the process 1000 and may be performed before the operation 1005.

[0128] FIG. 11 is a schematic diagram illustrating an exemplary front view of printing in a liquid environment according to some embodiments of the present disclosure. As shown in FIG. 11, a robotic arm 1101 may be connected with a printer head 1102 and may drive the printer head 1102 to approach a substrate 1104. A liquid 1103 may be loaded in a container 1109. The liquid 1103 may provide a liquid environment for the printing process. The substrate 1104 and at least a portion of the nozzle of the printer head 1102 may be immersed in the liquid 1103. One or more droplets of ink (e.g., bio-ink) may be deposited in the liquid environment and attach to the printing surface of the substrate 1104. The attachment of the droplet of bio-ink may be prompted by a first interaction between the droplet of bio-ink and the substrate 1104, and may be further prompted by a second interaction between the droplet of bio-ink and the liquid. In some embodiments, the liquid 1103 may be a hydrophobic liquid, and accordingly, the first interaction may be a hydrophilic interaction, and the second interaction may be a hydrophobic interaction.

[0129] In some embodiments, the substrate 1104 may be fixed by a substrate fixing

device. Merely by way of example, the substrate fixing device may include two fixing poles 1106 that are connected to two ends of the substrate 1104 via one or more connecting pieces 1105. In some embodiments, the substrate fixing device may include one or more fasteners 1110, and/or one or more rotary components 1107. In some embodiments, the fasteners 1110 may be sealed with the container 1109 to prevent liquid leakage. More descriptions of the substrate fixing device may be found elsewhere in the present disclosure (e.g., FIGs. 4 and 6 and the descriptions thereof).

[0130] FIG. 12 is a schematic diagram illustrating an interaction between biological components deposited on a printing surface and a liquid according to some embodiments of the present disclosure. As illustrated in FIG. 12, one or more biological components 1202 may be deposited on a printing surface 1201 by the printer head 402. In some embodiments, the printing surface 1201 may be a curved surface or an inclined plane. Before the biological components 1202 can properly attach to the printing surface 1201, the deposited biological components 1202 may tend to fall from the printing surface 1201 due to gravity (or it may be difficult for the deposited biological components 1202 to attach to the printing surface 1201 under gravity) or other forces (e.g., buoyancy). In some embodiments, the biological components 1202 and the printing surface 1201 may be immersed in a liquid. The liquid may include a plurality of liquid molecules 1203. In some embodiments, the biological components 1202 may be hydrophilic, and the liquid may be hydrophobic. In some embodiments, liquid molecules 1203 surrounding the biological components 1202 may provide a hydrophobic interaction (as shown by a plurality of arrows in FIG. 12) to prompt the deposited biological components to attach to the printing surface 1201 (e.g., with a repelling force). In some embodiments, the printing surface of the substrate may be hydrophilic. In some embodiments, the attachment of the biological components 1202 to the printing surface 1201 may also be prompted by a hydrophilic interaction between the biological components 1203 and the printing surface 1201 (e.g., with an attracting force). For example, the substrate may be synthesized using a hydrophilic polymer,

such as polylactic acid, polyvinyl alcohol, polyglycolic acid, collagen, gelatin, chitosan, or the like, or any combination thereof. As another example, the substrate may be coated with a hydrophilic layer (e.g., hydrogel). For instance, the hydrophilic layer may include cross-linked or non-cross-linked hydrophilic polymers that are non-toxic to biological components such as cells. The hydrophilic polymers may include polylactic acid, polyvinyl alcohol, polyglycolic acid, collagen, gelatin, chitosan, or the like, or a combination thereof. As another example, hydrophilic or polar functional groups (e.g., hydroxyl groups, carboxyl groups, amino groups, phosphate groups, or the like) may be grafted onto the printing surface 1201. In some embodiments, the printing surface 1201 may have been deposited with one or more biological components. The hydrophilic and hydrophobic interactions herein described serve merely as examples, and it would be possible that the printing surface is hydrophobic and the liquid surrounding the deposited material is hydrophilic. As long as one or both of the interactions facilitate the attachment of deposited materials, the first and second interactions can be any type of forces. In addition, it would be entirely possible that there are more than two types of interactions that are playing a role in the 3-D printing process, where these interactions can promote the attachment of the deposited material.

[0131] It should be noted that the above description of FIG. 12 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made to FIG. 12 under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. For example, the interaction between the biological components 1202 and the liquid may include a buoyance force, which is not shown in FIG. 12.

[0132] FIG. 13 is a flowchart illustrating an exemplary process of printing an object including a tubular structure according to some embodiments of the present disclosure. In some embodiments, one or more operations of process 1300 illustrated in FIG. 13 may be implemented in the printing system 100 illustrated in

FIG. 1. For example, at least a part of the process 1300 may be stored in the storage device 150 in the form of instructions, and invoked and/or executed by the processing device 140 (e.g., the processor 210 of the computing device 200 as illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 as illustrated in FIG. 3). As another example, at least a part of the process 1300 may be executed by the printer 110. In some embodiments, the object to be printed may be an artificial blood vessel, a urethra, a lymph vessel, or a portion or combination thereof, or the like. In some embodiments, the object to be printed may have a vascular network. For example, the object may be a part of a tissue of an organ, such as a heart, a liver, a kidney, a lung, a stomach, an intestine, a pancreas, a urinary bladder, a pharynx, a larynx, a gallbladder, a lymph node, a spleen, etc. In some embodiments, the vascular network may include a plurality of tubular structures varying in length and/or diameter. In some embodiments, the tubular structure of the object may provide a fluid material to provide nutrients, oxygen and/or other components to at least a part of the object, so as to keep the activity and/or functions of the object.

[0133] In 1301, the printer head 402 may deposit a first material on a substrate that includes a scaffold having a tubular structure. In some embodiments, the first material may refer to bio-ink. In some embodiments, the substrate may include a scaffold. In some embodiments, the scaffold may include a tubular structure. In some embodiments, the first material may include one or more biological components and/or a cell culture medium that may support the survival of the biological components. In some embodiments, the biological components may include somatic cells, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. In some embodiments, the biological components may include cardiac cells, renal cells, hepatic cells, lung cells, gastric cells, pancreatic cells, gallbladder cells, bladder cells, spleen cells, tracheal cells, nerve cells, bone cells, cancer cells, intestinal cells, epithelial cells, muscle cells, fibroblasts, secretory cells, ciliated cells, fat cells, blood cells, immune cells, or the like, or any combination thereof. In some embodiments,

the biological components may include free cells. In some embodiments, the biological components may include multicellular bodies. For example, the multicellular bodies may include multicellular spheroids formed by endothelial cells and cardiac cells.

[0134] In some embodiments, one or more printer heads 402 may deposit the same or different first materials on one or more target positions of the printing surface of the substrate according to one or more predetermined pathways. In some embodiments, a fluid system (e.g., the first fluid system 611 shown in FIG. 6A) may provide the first material to the printer heads 402. More descriptions regarding the deposition of the first material may be found elsewhere in the present disclosure (e.g., operation 707 in FIG. 7, operation 813 in FIG. 8 and descriptions thereof).

[0135] In 1303, a fluid system (e.g., the second fluid system 610 shown in FIG. 6A) may provide a second material within the tubular structure while the printer head 402 is depositing the first material on the substrate. In some embodiments, the scaffold may be permeable to the second material and may allow the second material to reach the first material. For example, the tubular structure may be porous. The second material may reach the first material through holes in the tubular structure of the scaffold. In some embodiments, the second material may be configured to enhance or maintain an activity of the first material. As used herein, an “activity” may refer to a biological activity, a chemical activity, etc. In some embodiments, the biological activity may include cell survival, cell attachment, cell integration, cell migration, cell proliferation, cell differentiation, or the like, or any combination thereof. In some embodiments, the second material may include a cell culture medium, real blood, artificial blood, or the like, or a combination thereof.

[0136] In 1305, the biological components deposited on the printing surface may be cultured. In some embodiments, during a culturing process, a cell culture medium may be provided to immerse the biological components and/or support the survival of the biological components. In some embodiments, the printing surface may have been deposited with one or more layers of biological components. In some embodiments, the cell culture medium may permeate and/or diffuse from peripheral

biological components to inner biological components. More descriptions regarding a culturing process after the biological components are deposited on the printing surface may be found elsewhere in the present disclosure (e.g., operation 709 in FIG. 7, operation 815 in FIG. 8, operation 1011 in FIG. 10, and descriptions thereof). In some embodiments, operation 1305 may be omitted.

[0137] In 1307, the second material may be provided within the tubular structure while the biological components deposited on the printing surface are cultured. In some embodiments, the second material provided within the tubular structure of the scaffold may permeate the tubular structure and reach a portion of the deposited biological components (e.g., from inner biological components to peripheral biological components, see FIG. 14). This may facilitate the deposited biological components to maintain an activity to proliferate and/or differentiate. In some embodiments, in the culturing process, the substrate may be rotated (e.g., while the second material is provided within the tubular structure). In some embodiments, operation 1307 may be omitted.

[0138] It should be noted that the above description of the process 1300 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made to the process 1300 under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the process 1000 of printing an object in a liquid environment may be combined with the process 1300, for example, to print an object having a tubular structure in a liquid environment. In some embodiments, one or more of operations 801 through 811 may be performed before operation 1301.

[0139] FIG. 14 is a schematic diagram of providing a second material within a tubular structure of a scaffold according to some embodiments of the present disclosure. As shown in FIG. 14, a first material 1401 may have been deposited on the scaffold 1403 that includes a scaffold having a tubular structure. In some embodiments, the first material 1401 may be bio-ink including biological components

(e.g., cells). In some embodiments, a second material 1402 may be provided within the tubular structure during a printing process and/or a culturing process. For example, the second material 1402 may flow from a side of the tubular structure to another side of the tubular structure as illustrated by the arrows in FIG. 14. The second material 1402 may be configured to maintain and/or enhance the activity of the first material 1401. In some embodiments, the scaffold 1403 may be permeable to the second material 1402, and the second material 1402 may reach the first material 1401 deposited on the printing surface of the scaffold 1403. In some embodiments, there may be a vascular network including a plurality of capillary vessels associated with the first material 1401. In some embodiments, at least a portion of the capillary vessels may be connected to the tubular scaffold. In some embodiments, the second material 1402 may be capable of passing through the vascular network to the at least a portion of the capillary vessels to maintain and/or enhance the activity of the first material and/or other biological components. For example, the second material 1402 may be a culture medium can be passed through the vascular network as an average flow rate of, e.g., 1 ml/min.

[0140] It should be noted that the above description of FIG. 14 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made under the teachings of the present disclosure.

However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the tubular structure may be a complex structure. For example, the diameter of the tubular structure may vary. As another example, a part of the tubular structure may be straight and a part of the tubular structure may be curved.

[0141] FIG. 15 is a flowchart illustrating an exemplary cyclic 3D printing process according to some embodiments of the present disclosure. In some embodiments, one or more operations of process 1500 illustrated in FIG. 15 may be implemented in the printing system 100 illustrated in FIG. 1. For example, at least a part of the process 1500 may be stored in the storage device 150 in the form of instructions,

and invoked and/or executed by the processing device 140 (e.g., the processor 210 of the computing device 200 as illustrated in FIG. 2, the GPU 330 or CPU 340 of the mobile device 300 as illustrated in FIG. 3). As another example, at least a part of the process 1500 may be executed by the printer 110.

[0142] In 1501, the printer head 402 may deposit first bio-ink including first cells on a printing surface of a substrate. In some embodiments, the first cells may include somatic cells, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. In some embodiments, the somatic cells may include cardiac cells, renal cells, hepatic cells, lung cells, gastric cells, pancreatic cells, gallbladder cells, bladder cells, spleen cells, tracheal cells, nerve cells, bone cells, cancer cells, intestinal cells, epithelial cells, muscle cells, fibroblasts, secretory cells, ciliated cells, fat cells, blood cells, immune cells, or the like, or any combination thereof. In some embodiments, the first cells may include one or more types of somatic cells. For example, the first cells may include a first type of somatic cells and a second type of somatic cells. In some embodiments, the first type of somatic cells may include endothelial cells. In some embodiments, the second type of somatic cells may include cardiomyocytes, hepatocytes, pneumonocytes, nephrocytes, splenocytes, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. In some embodiments, the somatic cells may include free cells. In some embodiments, the somatic cells may include multicellular bodies. For example, the multicellular bodies may include multicellular spheroids formed by endothelial cells and cardiac cells. In some embodiments, the printer head 402 may deposit one or more droplets of first bio-ink on the printing surface of the substrate immersed in a liquid environment as described in connection with FIG. 10. In some embodiments, the liquid environment may include a hydrophobic liquid and may prompt the deposited droplets of first bio-ink to attach to the printing surface. In some embodiments, the substrate may have a scaffold including a tubular structure. In some embodiments, a material, which is a fluid (e.g., a cell culture medium, real blood, artificial blood, or a combination thereof), may be provided

within the tubular structure to maintain or enhance an activity of the deposited first cells as described in connection with FIG. 13. In some embodiments, the substrate may be rotated during a printing process.

[0143] In 1503, first cells deposited on the printing surface may be cultured in a first cell culture medium for a first time period. In some embodiments, the deposited first cells may be cultured in a bioreactor. For example, the deposited first cells may be cultured at 37 °C. In some embodiments, the deposited first cells may be cultured for 2h, 4h, 8h, 24h, 48h, 3 days, 5 days, etc. In some embodiments, the first cell culture medium may be replaced with fresh cell culture medium every 8h, 16h, 24h, etc. In some embodiments, one or more angiogenic factors may be introduced into the first cell culture medium to induce the deposited first cells to coalesce to form a plurality of capillary vessels. In some embodiments, after the first cells are deposited on the printing surface in a liquid environment including a first liquid, the first liquid in the container may be replaced with the first cell culture medium. For example, the first liquid may be removed from the container and the first cells deposited on the printing surface, the first cells and the container may be washed, and the first cell culture medium may be loaded to the container. More descriptions regarding washing the deposited first cells and the container may be found elsewhere in the present disclosure (e.g., FIG. 10 and the description thereof). In some embodiments, the substrate may be rotated during a culturing process. In some embodiments, a material, which is a fluid (e.g., a cell culture medium, real blood, artificial blood, or a combination thereof), may be provided within the tubular structure when the first cells are cultured to maintain an activity of the deposited first cells.

[0144] In 1505, the printer head 402 may deposit second bio-ink including second cells on the deposited printing surface. In some embodiments, the deposited printing surface may include the deposited first cells. In some embodiments, the second cells may include somatic cells, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. In some embodiments, the somatic cells may include cardiac cells, renal cells,

hepatic cells, lung cells, gastric cells, pancreatic cells, gallbladder cells, bladder cells, spleen cells, tracheal cells, nerve cells, bone cells, cancer cells, intestinal cells, epithelial cells, muscle cells, fibroblasts, secretory cells, ciliated cells, fat cells, blood cells, immune cells, or the like, or any combination thereof. In some embodiments, the second cells may include one or more types of somatic cells. For example, the second cells may include a first type of somatic cells and a second type of somatic cells. In some embodiments, the first type of somatic cells may include endothelial cells. In some embodiments, the second type of somatic cells may include cardiomyocytes, hepatocytes, pneumonocytes, nephrocytes, splenocytes, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof. In some embodiments, the somatic cells may include free cells. In some embodiments, the somatic cells may include multicellular bodies. For example, the multicellular bodies may include multicellular spheroids formed by endothelial cells and cardiac cells. In some embodiments, the printer head 402 may deposit one or more droplets of second bio-ink on the deposited printing surface of the substrate immersed in a liquid environment as described in connection with FIG. 10. In some embodiments, the liquid environment may include a hydrophobic liquid and may prompt the deposited droplets of second bio-ink to attach to the printing surface. In some embodiments, the substrate may have a scaffold including a tubular structure. In some embodiments, a material, which is a fluid (e.g., a cell culture medium, real blood, artificial blood, or a combination thereof), may be provided within the tubular structure to maintain or enhance an activity of the deposited first cells and/or the deposited second cells as described in connection with FIG. 13. In some embodiments, the substrate may be rotated during a printing process.

[0145] In some embodiments, the first bio-ink and the second bio-ink may have the same or different compositions. In some embodiments, the first bio-ink and the second bio-ink may include the same or different types of cell culture medium. In some embodiments, the first cells and the second cells may include one or more same types of cells. In some embodiments, a percentage of each type of cells for

the first cells is the same as or different from a percentage of a corresponding type of cells for the second cells. For example, the first cells may include 90% endothelial cells and 10% cardiomyocytes, while the second cells may include 20% endothelial cells and 80% cardiomyocytes. As another example, the first cells and the second cells may include multicellular bodies, and the multicellular bodies for the first cells and the second cells may both include 90% cardiomyocytes and 10% endothelial cells. In some embodiments, the first cells and the second cells may include one or more different types of cells. For example, the first cells may include free endothelial cells while the second bio-ink may include cardiomyocytes.

[0146] In 1507, the second cells deposited on the printing surface may be cultured in a second cell culture medium for a second time period. In some embodiments, the deposited second cells may be cultured in a bioreactor. For example, the deposited second cells may be cultured at 37 °C. In some embodiments, the deposited second cells may be cultured for 2h, 4h, 8h, 24h, 48h, 3 days, 5 days, etc. In some embodiments, the second cell culture medium may be replaced with fresh cell culture medium every 8h, 16h, 24h, etc. In some embodiments, one or more angiogenic factors may be introduced into the second cell culture medium to induce the deposited second cells to coalesce to form a plurality of capillary vessels. In some embodiments, after the second cells are deposited on the printing surface in a liquid environment including a second liquid, the second liquid in the container may be replaced with the second cell culture medium. For example, the second liquid may be removed from the container and the second cells deposited on the printing surface, the second cells and the container may be washed, and the second cell culture medium may be loaded to the container. In some embodiments, the first liquid and the second liquid may be the same or different. More descriptions regarding washing the deposited second cells and the container may be found elsewhere in the present disclosure (e.g., FIG. 10 and the description thereof). In some embodiments, the substrate may be rotated during a culturing process. In some embodiments, a material, which is a fluid (e.g., a cell culture medium, real blood, artificial blood, or a combination thereof), may be provided within the tubular

structure when the second cells are cultured to maintain an activity of the deposited second cells and/or the deposited first cells.

[0147] In some embodiments, the first cell culture medium and the second cell culture medium may include the same or different compositions. In some embodiment, the first time period for culturing the first cells and the second time period for culturing the second cells may be the same or different.

[0148] In 1509, a user and/or the processing device 140 may determine whether a condition is satisfied. In response to a determination that the condition is not satisfied, the process 1500 may return to operation 1501 and repeating operations 1501 through 1507. In some embodiments, the operations 1501 through 1507 may be repeated for a plurality of times for allowing the deposited first cells and the second cells to cohere to form a 3D object. In some embodiments, the substrate may include a tubular structure (e.g., a vessel or a vascular network), and at least a portion of the plurality of capillary vessels may connect with the tubular structure, so that the first cell culture medium or the second cell culture medium may be capable of passing through the tubular structure to the at least a portion of the plurality of capillary vessels. In response to a determination that the condition is satisfied, the process 1500 may proceed to 1511. In 1511, the printing process may be ended. In some embodiments, the bio-ink used for printing may be different from that used in a previous printing cycle. For example, in a first printing cycle, the first bio-ink and the second bio-ink may be deposited, while in a second printing cycle, a third bio-ink and a fourth bio-ink may be deposited on the printing surface, respectively.

[0149] In some embodiments, the condition may be related to the volume and/or the thickness of the printed biological components. For example, if the operator observes that the volume of the printed biological components is greater than a volume threshold (e.g., 1 cm^3 , 5 cm^3), the operator may determine that the condition is satisfied and determine to end the printing process. As another example, if the operator observes that the thickness of the printed biological components is greater than a thickness threshold (e.g., 3 mm, 5 mm, 10 mm), the operator may determine that the condition is satisfied. As another example, a camera (e.g., the camera of

the calibration assembly 414) may obtain an image of the printed biological components on the substrate. The processing device 140 may determine whether the volume and/or the thickness of the printed biological components satisfy the condition.

[0150] In some embodiments, the condition may include whether the capillary vessels and/or a vascular network are generated. For example, the operator may obtain a sample of the printed biological components and perform a histological staining on the sample of the printed biological components. If a plurality of capillary vessels and/or a vascular network is observed, the operator may determine that the condition is satisfied. In some embodiments, the condition may include whether the first cells and the second cells are cohered. If the first cells and the second cells cohere to each other in the sample of the printed biological components, the condition may be satisfied. In some embodiments, the condition may relate to an integral function of the printed biological components. In some embodiments, the operator may test the integral function of the printed biological components. For example, if the object to be printed is a heart, the operator may test the blood pressure when the heart pumps. If the blood pressure is greater than a threshold, the condition may be satisfied. As another example, if the object to be printed is a kidney or a liver, the cell culture medium that has been used for culturing the printed biological components may be utilized to analyze the concentration of one or more components that are related to the function(s) of the kidney or the liver. If the concentration of the component(s) falls in a normal range, the condition may be satisfied and the printing process may be ended.

[0151] It should be noted that the above description of FIG. 15 is merely provided for the purpose of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations and modifications may be made under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, only one of the first bio-ink and the second bio-ink may be used in the printing cycle. For example, the printing cycle

may only include operations 1501, 1503 and 1509. That is, after the first cells are cultured, the user or the processing device 140 may determine whether the condition is satisfied (i.e., operation 1507 may be omitted). In response to a determination that the condition is satisfied, the printing process may be ended in 1511. In some embodiments, operation 1503 may be omitted. That is, after the second bio-ink is deposited in 1505, the first cells and the second cells may be cultured in 1507. In some embodiments, a printing process may include a plurality of printing cycles, in one or more printing cycles of the plurality of printing cycles, operations 1505 and/or 1507 may be performed, while in the other printing cycles of the plurality of printing cycles, operations 1505 and/or 1507 may be skipped or omitted. In some embodiments, after the first cells and/or the second cells are deposited on the printing surface in one or more printing cycles, one or more layers of adhesive materials (e.g., hydrogel) may be deposited on the printed cells (first cells and/or second cells) to facilitate the immobilization and/or proliferation of the printed cells.

[0152] FIG. 16 is a schematic diagram of an exemplary cyclic 3D printing process according to some embodiments of the present disclosure. As shown in FIG. 16, a nozzle 1601 of the printer head 402 may deposit first bio-ink 1602 on one or more target positions of a substrate 1604. The first bio-ink 1602 may include first cells 1605. One or more layers of first cells 1605 may attach to the substrate 1604. Merely by way of example, only one layer of the first cells 1605 are shown in FIG. 16. The deposited first cells 1605 may then be cultured for a first time period for certain cell activities such as but not limited to cell proliferation and/differentiation. As used herein, a layer of cells may refer to a contiguous, substantially contiguous or non-contiguous sheet of cells. The nozzle 1601 may deposit second bio-ink 1603 on one or more target positions on the substrate 1604. The second bio-ink 1603 may be deposited on the surface of the deposited first cells 1605. One or more layers of the second cells 1606 may attach to the first cells 1605. Merely by way of example, only one layer of the second cells 1606 are shown in FIG. 16. The second cells 1606 may be cultured for a second time period for certain cell activities such as but not limited to cell proliferation and/differentiation. In a next printing

cycle, the first bio-ink 1602 and the second bio-ink 1603 may be sequentially deposited on the surface of the deposited second cells 1606 and/or the deposited first cells 1605. After a plurality of printing cycles and culturing processes, the deposited first cells 1605 and the deposited second cells 1606 may coalesce to form a 3D structure of an organ, a tissue, or a portion thereof. More descriptions of the first bio-ink 1602, the first cells 1605, the second bio-ink 1603, the second cells 1606 may be found elsewhere in the present disclosure (e.g., FIG. 15 and the description thereof).

[0153] In some embodiments, the first bio-ink 1602 and the second bio-ink 1603 may be deposited in a liquid environment. In some embodiments, the liquid environment may include a liquid (e.g., a hydrophobic liquid). In some embodiments, the liquid may be removed before each culturing process, and the first cells 1605 and the second cells 1606 may be washed before each culturing process (not shown in FIG. 16). In some embodiments, the first bio-ink 1602 and the second bio-ink 1603 may have the same or different compositions. In some embodiments, the first time period for culturing the first cells 1605 and the second time period for culturing the second cells 1606 may be the same or different.

[0154] It should be noted that the above description regarding FIG. 16 is merely provided for the purposes of illustration, and not intended to limit the scope of the present disclosure. For persons having ordinary skills in the art, multiple variations or modifications may be made under the teachings of the present disclosure. However, those variations and modifications do not depart from the scope of the present disclosure. In some embodiments, the second scanning direction may be determined by other means. For example, the nozzle 1601 may be curved instead of straight as shown in FIG. 16. As another example, one or more nozzles 1601 of the printer head 402 may concurrently or alternately deposit the first cells 1605 and/or the second cells 1606.

EXAMPLES

Example 1 Cells deposited in a liquid environment

[0155] This example shows that 3D printing in a hydrophobic liquid environment can reduce mechanical damage to cells during printing and prompt the attachment of the cells to a printing surface of a substrate.

[0156] Materials

[0157] Vascular endothelial cells, cell culture medium, phosphate buffer saline (PBS), hydrophobic liquid, hydrogel, polylactic acid solution, and bio-ink were used in this example. The hydrophobic liquid provides a hydrophobic liquid environment for cell printing. Mineral oil was used as the hydrophobic liquid in this example. The bio-ink was prepared according to the following operations: the primary and/or differentiated human cerebral microvascular endothelial cells were cultured in the cell culture medium under 37 °C for 24 h to obtain a vascular endothelial cell culture; 5% collagen, 10% Matrigel, 5 nM Y-27632, 5 ng/ml basic fibroblast growth factor (bFGF), and 10 ng/ml vascular endothelial growth factor (VEGF) were added to the vascular endothelial cell culture to obtain the bio-ink.

[0158] Scaffold preparation

[0159] A vascular scaffold was used as the substrate for printing. The vascular scaffold was prepared based on electrostatic spinning. A polylactic acid solution was used for the preparation of the scaffold. The surface of the vascular scaffold was coated with a 30% Matrigel solution and incubated at 37 °C to form a hydrogel layer covering the surface of the vascular scaffold before printing. The hydrogel layer is hydrophilic and beneficial to the immobilization and proliferation of the cells deposited on the vascular scaffold.

[0160] Robotic arm

[0161] The printer included a robotic arm. The robotic arm was mounted on the printer. The robotic arm of the printer was coupled to a printer head. The robotic arm was a 6-axis robotic arm, including six arm units that were connected one by one. Each arm unit could move around an axis by 360 degrees. Thus, the printer

head could move freely in any direction in or across any plane in a 3D space surrounding the printing surface of the substrate.

[0162] Printer head

[0163] The printer head of the printer was configured to deposit one or more droplets of bio-ink on one or more target positions of the printing surface of the substrate according to a determined pathway. The printer head included a pipette configured to receive and dispense the bio-ink. The printer head was operatively coupled to a control module, a temperature controller, and a volume control unit. The bio-ink was loaded into the pipette and stored temporarily in the pipette. The total volume of the bio-ink loaded into the pipette could be adjusted by adjusting a rotary knob of the volume control unit. The pipette was configured to dispense droplets of the bio-ink through a nozzle of the pipette. The temperature controller included a heating device that can keep the loaded bio-ink at 37 °C. The control module was configured to control the pipette to dispense a certain volume of a droplet on each target position of the printing surface. In this example, the volume of a droplet was set as 1 μ l. After the printer head dispensed all the bio-ink loaded in the nozzle, the printer head could reload bio-ink in the nozzle. The nozzle could be replaced after several times of loading/dispensing operation. The printer head was automatically reloaded with the bio-ink from a bio-ink loading device. The bio-ink loading device included a bio-ink container and a support for aligning the pipette and the bio-ink container.

[0164] Liquid environment in the printing process

[0165] The printing process was performed in the hydrophobic liquid environment. The hydrophobic liquid environment was provided by a hydrophobic liquid (mineral oil used as the hydrophobic liquid in this example) filled in a container. The substrate was fixed by a substrate fixing device that is fixed on the container. The printing surface of the substrate was immersed in the hydrophobic liquid during printing. At least a part of the nozzle of the printer head was also immersed in the hydrophobic liquid during printing. The hydrophobic interaction between the mineral oil and the deposited cells promoted the deposited cells to attach to the hydrophilic

surface of the substrate. The mineral oil did not have a negative effect on the survival, the proliferation and the functions of the deposited cells. The container was coupled to a temperature controller. The temperature controller was configured to maintain the temperature of mineral oil as 37 °C during the printing process.

[0166] Pathway determination

[0167] Prior to the printing process, the processor determined a pathway of the printer head based on a 3D model relating to the substrate to be printed. The model of the substrate was obtained by measuring the substrate. A 3D coordinate system was constructed for the 3D model. A control module of the printer acquired information relating to the target positions on the printing surface of the substrate. The control module determined the pathway of the printer head based on the acquired information, the coordinate of the 3D model of the substrate, and a position of the substrate relative to the printer. A distance between two adjacent target positions for depositing droplets of the bio-ink was set as 1 mm based on a dimension of the printing surface and a dimension of the droplet of the bio-ink. The distance was set to prevent the droplets from aggregating with each other, which may affect the attachment of cells to the printing surface of the substrate.

[0168] Calibration

[0169] A calibration was performed before printing to reduce possible error between the actual position and the identified position of the nozzle of the printer head. The nozzle of the printer head was moved to a preset calibration position by adjusting one or more arm units of the robotic arm. The identified position of the nozzle of the printer head was recognized by the control module. The identified position was calibrated based on the actual position (i.e., the preset calibration position). Each arm unit of the robotic arm was calibrated in this way. The operations mentioned above were repeated for at least five times for the calibration of the position of the printer head.

[0170] During printing process, the actual position of the nozzle of the printer head was recognized based on infrared rays detected by a position sensor mounted on the robotic arm, and the actual position of the nozzle of the printer head was

adjusted if a position drift was detected, so that the actual motion trajectory of the nozzle of the printer head was calibrated in real time, and the motion drift of the nozzle of the printer head was corrected.

[0171] Printing process

[0172] The temperature controller heated the mineral oil and kept the mineral oil at 37 °C. The printing surface of the substrate was immersed in the mineral oil. The robotic arm was driven by the control module and accordingly, the printer head was moved based on the predetermined pathway. The nozzle of the printer head approached a target position on the printing surface of the substrate. Both the nozzle of the printer head and the printing surface were immersed in the mineral oil. The control module controlled the pipette of the printer head to deposit a droplet of bio-ink on the target position of the printing surface. The hydrophilic interaction between the droplet of bio-ink and the printing surface prompted the attachment of the droplet of bio-ink to the printing surface. The hydrophobic interaction between the droplet of bio-ink and the mineral oil further prompted the attachment of the droplet of bio-ink to the printing surface. The cells in the bio-ink attached to the printing surface and further immobilized on the printing surface.

[0173] The nozzle of the printer head was sequentially moved and approached the next target position on the printing surface of the substrate according to the determined pathway. The control module controlled the pipette of the printer head to deposit another droplet of bio-ink on the next target position of the printing surface. A plurality of vascular endothelial cells were deposited on a plurality of target positions on the printing surface of the substrate in the similar way.

[0174] FIGs. 17A-17B are photos of exemplary droplets of bio-ink deposited by the printer head according to some embodiments of the present disclosure. FIG. 17C is a schematic diagram illustrating exemplary positions of droplets of bio-ink deposited by the printer head according to some embodiments of the present disclosure. As shown in FIGs. 17A-17C, the printer head deposited bio-ink droplets 1702, 1703, and 1704 on different target positions on the substrate 1701 in a petri dish when the printing surface of the substrate 1701 and the nozzle of the printer head (not shown)

were immersed in mineral oil. The distance between the front end of the nozzle and the target position on the printing surface was 1 mm. As shown in FIG. 17B, the bio-ink droplets 1702, 1703, and 1704 were attaching to the printing surface of the substrate 1701, probably due to the hydrophobic interaction between the bio-ink and the mineral oil, the buoyance force provided by the mineral oil, and the gravity force. The bio-ink droplets 1702, 1703, and 1704 had a globular shape in the mineral oil under homogeneous hydrophobic interaction between the bio-ink and the mineral oil. Bio-ink droplets 1705, 1706, and 1707 were deposited directly on the surface of the petri dish. As shown in FIG. 17A, the bio-ink droplets 1705, 1706, and 1707 also had a globular shape in the mineral oil under homogeneous hydrophobic interaction between the bio-ink and the mineral oil. In FIG. 17C, the positions of the bio-ink droplets 1702, 1703 and 1704 on the substrate 1701 were shown in the longitudinal section, the vertical view and the side view. A coordinate system was used to determine the target positions on the printing surface of the substrate 1701. The X axis was a horizontal axis, the Y axis was a vertical axis, and the Z axis was the longitudinal axis of the substrate 1701. These results indicates that the bio-ink droplets deposited in a hydrophobic liquid environment may approach the printing surface of the substrate 1701 for attachment.

[0175] The washing process

[0176] After the printing process, the mineral oil was removed from the container and the printing surface of the substrate. Ethanol was added in the container to remove some residual mineral oil on the surface of the container. PBS was added in the container to immerse the printed cells on the printing surface of the substrate to further wash some residual mineral oil on the surface of the container and the printing surface of the substrate. Other remaining mineral oil floating on the surface of the PBS was removed using a pump. The PBS in the container was then removed.

[0177] Culturing process of the printed cells

[0178] The container was filled with the cell culture medium, and the printed cells on the printing surface of the substrate were immersed in the cell culture medium. The

container and the printed cells were placed in a bioreactor. The printed cells were cultured under 37 °C for 48 h. The cell culture medium was replaced every 48 hours.

[0179] Attachment of cells to the printing surface

[0180] The attachment of deposited cells to the printing surface of the substrate was investigated. The human cerebral microvascular endothelial cells were labelled with green fluorescent protein (GFP) and were used to prepare the bio-ink. The bio-ink was deposited on the substrate in a liquid environment following the procedures described previously in this example. The printed biological components on the substrate were cultured as previously described in this example. A droplet of bio-ink before printing and the substrate (a vascular scaffold) after 12 h and 24 h of culturing post printing were observed using a fluorescence microscope (Leica fluorescence microscope (DMI 3000B)). FIG. 18A is a fluorescence microscopy image of a droplet of bio-ink according to some embodiments of the present disclosure. The green portion was the GFP synthesized by the cells and indicated the position of the cells. FIG. 18B is a fluorescence microscopy image of the vascular scaffold after 12 h of culturing post printing according to some embodiments of the present disclosure. FIG. 18C is a fluorescence microscopy image of the vascular scaffold after 24 h of culturing post printing according to some embodiments of the present disclosure. As shown in FIGs. 18B-18C, the deposited cells and cells proliferated from the deposited cells were attached to the printing surface of the vascular scaffold. In the enlarged view of a central region in FIG. 18B, each dotted box indicated a cell aggregate from a droplet of bio-ink, and the cell aggregates from different droplets of bio-ink coalesced and fused to form a 3D structure. The length of the scale bars in FIGs. 18A-18B was 50 μ m and the length of the scale bar in FIG. 18C was 2.5 mm. FIG. 18D is an image showing the printer head 1801, the scaffold 1802, and the liquid environment 1803 in the printing process according to some embodiments of the present disclosure. These results suggest that cells deposited on the printing surface in a liquid environment and cells proliferated from the deposited cells can successfully attach to the vascular scaffold.

Example 2 A printed object with a tubular structure

[0181] This example shows that providing a cell culture medium within the tubular structure of the object during printing can promote the proliferation of the printed cells and the formation of a vascular network.

[0182] Materials

[0183] The materials used in this example are similar as described in Example 1. The cells used for preparing the bio-ink were HeLa cells (i.e., human cerebral microvascular endothelial cells (EC)).

[0184] Scaffold preparation

[0185] A vascular scaffold including a tubular structure was used as the substrate for printing. The vascular scaffold was prepared based on electrostatic spinning. A polylactic acid solution was used for the preparation of the scaffold. The surface of the vascular scaffold was coated with a 30% Matrigel solution and incubated at 37 °C to form a hydrogel layer covering the surface of the vascular scaffold before printing. The hydrogel layer is hydrophilic and beneficial to the immobilization and proliferation of the cells deposited on the substrate.

[0186] Each end of the vascular scaffold was fixed with a fixing pole. Each of the fixing poles was coupled to a motor. The fixing poles on both sides of the vascular scaffold were hollow. The two motors can rotate synchronously. The two motors were controlled by a control module of the printer. A pipe was connected to the vascular scaffold via a fluid pathway inside each of the fixing poles.

[0187] Printer head

[0188] The materials were prepared similarly as Example 1.

[0189] Pathway determination

[0190] The pathway was determined similarly as Example 1.

[0191] Calibration

[0192] The calibration was performed similarly as Example 1.

[0193] Printing process

[0194] The robotic arm was driven by the control module and accordingly, the

printer head was moved based on the predetermined pathway. The nozzle of the printer head approached a target position on the printing surface of the substrate. The control module controlled the pipette of the printer head to deposit a droplet of bio-ink on the target position of the printing surface. A fluid system provided the cell culture medium within the tubular structure of the vascular scaffold while depositing the bio-ink. The fluid system includes a pump configured to drive the flow of the cell culture medium. The pump was coupled to the control module of the printer. The control module of the printer controlled the start and stop of the flow of the cell culture medium, and the flow rate of the cell culture medium flowing through the tubular structure. The flow rate of the cell culture medium was set as 1 ml/min during printing process in this example.

[0195] The two motors coupled to the rotary components driven the rotary components to rotate and induce the vascular scaffold to rotate. The nozzle of the printer head was sequentially moved and approached a next target position on the printing surface of the substrate according to the determined pathway. The control module controlled the pipette of the printer head to deposit another droplet of bio-ink on the next target position of the printing surface. A plurality of vascular endothelial cells were deposited on a plurality of target positions on the printing surface of the substrate in the similar way.

[0196] Culturing process of the printed cells

[0197] The printed cells and the vascular scaffold were placed in a bioreactor. During the culturing process, the fluid system provided the cell culture medium within the tubular structure of the vascular scaffold. The flow rate of the cell culture medium was set as 1 ml/min during culturing process in this example. During the culturing process, the vascular scaffold was rotated at a rotation rate of 0.2 r/min in this example.

[0198] Detection of cells after printing

[0199] The cell damage after manually depositing the cells on a vascular scaffold using a pipette and depositing the cells on the vascular scaffold using the printing system according to the procedures described previously in this example was

investigated. The EC deposited using the printing system according to the procedures described previously in this example were referred to as “robot printed EC”. The manually deposited EC were referred to as “manually handled EC” or “manually seeded EC”). The degrees of DNA break for the robot printed EC and the manually handled EC were tested using the TUNEL kit after printing, respectively. In the TUNEL test, a negative control group of EC were treated without labeling and a positive control group of EC were treated by DNase I. FIG. 19 is a diagram illustrating the degree of DNA break for the robot printed EC and the manually handled EC according to some embodiments of the present disclosure. As shown in FIG. 19, the degrees of DNA break of the three repeat groups of robot printed EC were 0.1%, 0.1%, and 0.2%, respectively, which were significantly lower than the degree of DNA break of the manually handled EC (0.3%). These results indicate that printing the EC using the printing system according to the procedures described previously in this example do not cause excessive apoptosis in EC.

[0200] The manually seeded EC and the robot printed EC on the vascular scaffold were cultured at 37 °C with 5% CO₂ for 72 h after printing. The morphology of the manually seeded EC and the robot printed EC on the vascular scaffold after 12 h and 72 h of culturing were investigated using a scanning electron microscope (SEM). The results were shown in FIG. 20. FIG. 20 is a scanning electron microscopy image of the manually seeded endothelial cells and the robot printed endothelial cells on the vascular scaffold after 12 h and 72 h of culturing post printing according to some embodiments of the present disclosure. The length of the scale bar in FIG. 20 was 100 μm. As shown in FIG. 20, the morphology and proliferation of the robot printed EC and the manually seeded EC are similar, which indicates that printing EC using the printing system according to the procedures described previously in this example do not negatively affect the cell morphology and proliferation. The results of the TUNEL test and the SEM images in this example suggest that printing EC using the printing system according to the procedures described previously in this example may achieve a lower extent of cell damage as compared to manually seeded EC.

Example 3 A 3D object generated by cyclic 3D printing

[0201] This example shows that a 3D object having a vascular network can be obtained by cyclic 3D printing.

[0202] Materials

[0203] Human cerebral microvascular endothelial cells, cardiomyocytes, a first cell culture medium, a second cell culture medium, phosphate buffer saline (PBS), hydrophobic liquid, hydrogel, polylactic acid solution, a first bio-ink, and a second bio-ink were used in this example. The hydrophobic liquid provides hydrophobic liquid environment for cell printing. Mineral oil is used as the hydrophobic liquid in this example. The first bio-ink was prepared according to the following operations: first cells (i.e., 10-20% primary and/or differentiated human cerebral microvascular endothelial cells and 80-90% primary and/or differentiated human cardiomyocytes) were cultured in the first cell culture medium under 37 degrees for 24 hours to obtain a first cell culture; 5% collagen, 10% Matrigel, 5 nM Y-27632, 5 ng/ml bFGF, and 10 ng/ml VEGF were added to the first cell culture to obtain the first bio-ink. The second bio-ink was prepared in a similar manner as the first bio-ink.

[0204] Scaffold preparation

[0205] The scaffold was prepared similarly as Example 2.

[0206] Robotic arm

[0207] The robotic arm was prepared similarly as Example 1.

[0208] Printer head

[0209] The printer head was prepared similarly as Example 1.

[0210] Liquid environment in the printing process

[0211] The liquid environment was prepared similarly as Example 1.

[0212] Pathway determination

[0213] The pathway was determined similarly as Example 1.

[0214] Calibration

[0215] The calibration was performed similarly as Example 1.

[0216] Cyclic 3D printing process

[0217] FIG. 21 is a schematic diagram illustrating an exemplary cyclic 3D printing process according to some embodiments of the present disclosure. As shown in FIG. 21, the printing environment for the first cells and the second cells was a liquid environment. The front end of the nozzle of the printer head and the printing surface of the substrate were immersed in mineral oil. In a 3D printing cycle, cells were deposited on the substrate using a printer. The printer was equipped with a 6-axis robotic arm used to drive a printer head to deposit the first cells or the second cells. After depositing the first cells or the second cells, the mineral oil was removed from the container, and the printing surface of the vascular scaffold and the container were washed. Then the first cell culture medium or the second cell culture medium were added to the container. The container was placed in a bioreactor to incubate the biological components deposited on the vascular scaffold at 37 °C with 5% CO₂ for 24 h. After culturing the biological components, the first cell culture medium or the second cell culture medium was removed, and the printing surface of the vascular scaffold and the container were washed. Mineral oil was added to the container to form a liquid environment for printing the first cells or the second cells. The 3D printing cycle was repeated for 6 rounds. Details of the cyclic 3D printing process were provided in the following descriptions.

[0218] The temperature controller heated the mineral oil and kept the mineral oil at 37 °C. The printing surface of the vascular scaffold was immersed in the mineral oil. The pipette of the printer head was loaded with the first bio-ink. The robotic arm was driven by the control module and accordingly, the printer head was moved based on the predetermined pathway. The nozzle of the printer head approached a target position on the printing surface of the vascular scaffold. Both the nozzle of the printer head and the printing surface were immersed in the mineral oil. The control module controlled the pipette of the printer head to deposit a droplet of the first bio-ink on the target position of the printing surface. The hydrophilic interaction between the droplet of the first bio-ink and the printing surface prompted the attachment of the droplet of the first bio-ink to the printing surface. The hydrophobic interaction between the droplet of the first bio-ink and the mineral oil further

prompted the attachment of the droplet of the first bio-ink to the printing surface. A fluid system provided the first cell culture medium within the tubular structure of the vascular scaffold while depositing the first bio-ink. The fluid system includes a pump configured to drive the flow of the first cell culture medium. The pump was coupled to the control module of the printer. The control module of the printer controlled the start and stop of the flow of the first cell culture medium, and the flow rate of the first cell culture medium flowing through the tubular structure. The flow rate of the first cell culture medium was set as 1 ml/min during printing process in this example. The cells in the first bio-ink attached to the printing surface and further immobilized on the printing surface. The two motors coupled to the rotary components driven the rotary components to rotate and induce the vascular scaffold to rotate for 90 degrees. The nozzle of the printer head was sequentially moved and approached a next target position on the printing surface of the vascular scaffold according to the determined pathway. The control module controlled the pipette of the printer head to deposit another droplet of the first bio-ink on the next target position of the printing surface. A layer of first cells were deposited on the printing surface of the vascular scaffold in the similar way.

[0219] After depositing the first cells, the mineral oil was removed from the container and the printing surface of the vascular scaffold. Ethanol was added in the container to remove some residual mineral oil on the surface of the container. PBS was added in the container to immerse the printed first cells on the printing surface of the vascular scaffold to further wash some residual mineral oil on the surface of the container and the printing surface of the vascular scaffold. Other remaining mineral oil floating on the surface of the PBS was removed using a pump. The PBS in the container was then removed.

[0220] The container was filled with the first cell culture medium, and the printed first cells on the printing surface of the vascular scaffold were immersed in the first cell culture medium. The container and the printed first cells were placed in a bioreactor. The printed first cells were cultured under 37 °C with 5% CO₂ for 24 hours. During the culturing process, the fluid system provided the first cell culture

medium within the tubular structure of the vascular scaffold. The flow rate of the first cell culture medium was set as 1 ml/min during culturing process in this example. During the culturing process, the vascular scaffold was rotated at a rotation rate of 0.2 r/min in this example.

[0221] After culturing process, the container and the printed first cells were taken out from the bioreactor. The first cell culture medium was removed from the container and the printing surface of the vascular scaffold. Mineral oil was reloaded into the container, and the printing surface of the vascular scaffold was immersed in the mineral oil. The pipette of the printer head was loaded with the second bio-ink. The robotic arm was driven by the control module and accordingly, the printer head was moved based on the predetermined pathway. The nozzle of the printer head approached a target position on the printing surface of the vascular scaffold. Both the nozzle of the printer head and the printing surface were immersed in the mineral oil. The control module controlled the pipette of the printer head to deposit a droplet of the second bio-ink on the target position of the printing surface. The hydrophilic interaction between the droplet of the second bio-ink and the printing surface prompted the attachment of the droplet of the second bio-ink to the printing surface. The hydrophobic interaction between the droplet of the second bio-ink and the mineral oil further prompted the attachment of the droplet of the second bio-ink to the printing surface. A fluid system provided the second cell culture medium within the tubular structure of the vascular scaffold while depositing the second bio-ink. The flow rate of the second cell culture medium was set as 1 ml/min during printing process in this example. The cells in the second bio-ink attached to the printing surface and further immobilized on the printing surface. The two motors coupled to the rotary components driven the rotary components to rotate and induce the vascular scaffold to rotate for 90 degrees. The nozzle of the printer head was sequentially moved and approached a next target position on the printing surface of the vascular scaffold according to the determined pathway. The control module controlled the pipette of the printer head to deposit another droplet of the second bio-ink on the next target position of the printing surface. A layer of second cells were

deposited on the printed first cells of the vascular scaffold in the similar way.

[0222] After depositing the second cells, the mineral oil was removed from the container and the printing surface of the vascular scaffold. Ethanol was added in the container to remove some residual mineral oil on the surface of the container. PBS was added in the container to immerse the printed second cells on the printing surface of the vascular scaffold to further wash some residual mineral oil on the surface of the container and the printing surface of the vascular scaffold. Other remaining mineral oil floating on the surface of the PBS was removed using a pump. The PBS in the container was then removed.

[0223] Then the container was filled with the second cell culture medium, and the printed second cells on the printing surface of the vascular scaffold were immersed in the second cell culture medium. The container and the printed second cells were placed in a bioreactor. The printed second cells were cultured under 37 °C for 24 hours. During the culturing process, the fluid system provided the second cell culture medium within the tubular structure of the vascular scaffold. The flow rate of the second cell culture medium was set as 1 ml/min during culturing process in this example. During the culturing process, the vascular scaffold was rotated at a rotation rate of 0.2 r/min in this example.

[0224] The process of the printing of the first cells, the culturing of the first cells, the printing of the second cells, and the culturing of the second cells were cycled for a plurality of times. The cultured first cells and second cells obtained after each 3D printing cycle were observed under a microscope. When the printed cells were not macroscopic or had a volume smaller than 1 cm³, operations of another 3D print cycle was performed based on the printed cells. When the printed cells had a volume greater than 1 cm³, the cyclic 3D printing was ended, and thus, a printed 3D object was obtained.

[0225] Analysis for the printed cells after different rounds of printing

[0226] The human cerebral microvascular endothelial cells were labelled with GFP and the human cardiomyocytes were labelled with red fluorescent protein (RFP). The labelled human cerebral microvascular endothelial cells and the human

cardiomyocytes were used to prepare the bio-ink. The bio-ink was deposited on the vascular scaffold in a liquid environment according to the cyclic 3D printing process following the procedures described previously in this example. The surface of the vascular scaffold were observed after the 1st, 2nd, 4th, and 6th round of the cyclic 3D printing process using a fluorescence microscope (Leica fluorescence microscope (DMI 3000B)). The results were shown in FIG. 22. FIG. 22 is a fluorescence microscopy image of the vascular scaffolds after 1st, 2nd, 4th, and 6th round of the cyclic 3D printing process according to some embodiments of the present disclosure. The length of the scale bar in FIG. 22 was 50 μ m. After 1st round of printing, human cerebral microvascular endothelial cells (green) and human cardiomyocytes (red) attached to the vascular scaffold were observed. After 2nd round and 4th round of printing, more attached human cerebral microvascular endothelial cells and human cardiomyocytes were observed on the vascular scaffold. After 6th round of printing, a 3D structure formed by the printed biological components was observed. These results suggest that the cyclic 3D printing method described in this example may be used to print a 3D object.

[0227] It will be apparent to those skilled in the art that various changes and modifications can be made in the present disclosure without departing from the spirit and scope of the disclosure. In this manner, the present disclosure may be intended to include such modifications and variations if the modifications and variations of the present disclosure are within the scope of the appended claims and the equivalents thereof.

[0228] Having thus described the basic concepts, it may be rather apparent to those skilled in the art after reading this detailed disclosure that the foregoing detailed disclosure is intended to be presented by way of example only and is not limiting. Various alterations, improvements, and modifications may occur and are intended to those skilled in the art, though not expressly stated herein. These alterations, improvements, and modifications are intended to be suggested by this disclosure, and are within the spirit and scope of the exemplary embodiments of this disclosure.

[0229] Moreover, certain terminology has been used to describe embodiments of the present disclosure. For example, the terms “one embodiment,” “an embodiment,” and “some embodiments” mean that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Therefore, it is emphasized and should be appreciated that two or more references to “an embodiment” or “one embodiment” or “an alternative embodiment” in various portions of this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures or characteristics may be combined as suitable in one or more embodiments of the present disclosure.

[0230] Further, it will be appreciated by one skilled in the art, aspects of the present disclosure may be illustrated and described herein in any of a number of patentable classes or context including any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof. Accordingly, aspects of the present disclosure may be implemented entirely hardware, entirely software (including firmware, resident software, micro-code, etc.) or combining software and hardware implementation that may all generally be referred to herein as a “module,” “unit,” “component,” “device,” or “system.” Furthermore, aspects of the present disclosure may take the form of a computer program product embodied in one or more computer-readable medium having computer readable program code embodied thereon.

[0231] A computer readable signal medium may include a propagated data signal with computer readable program code embodied therein, for example, in baseband or as part of a carrier wave. Such a propagated signal may take any of a variety of forms, including electromagnetic, optical, or the like, or any suitable combination thereof. A computer readable signal medium may be any computer readable medium that is not a computer readable storage medium and that may communicate, propagate, or transport a program for use by or in connection with an instruction execution system, apparatus, or device. Program code embodied on a computer readable signal medium may be transmitted using any appropriate

medium, including wireless, wireline, optical fiber cable, or the like, or any suitable combination of the foregoing.

[0232] Computer program code for carrying out operations for aspects of the present disclosure may be written in any combination of one or more programming languages, including an object-oriented programming language such as Java, Scala, Smalltalk, Eiffel, JADE, Emerald, C++, C#, VB. NET, Python or the like, conventional procedural programming languages, such as the "C" programming language, Visual Basic, Fortran 2003, Perl, COBOL 2002, PHP, ABAP, dynamic programming languages such as Python, Ruby, and Groovy, or other programming languages. The program code may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through any type of network, including a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider) or in a cloud computing environment or offered as a service such as a Software as a Service (SaaS).

[0233] Furthermore, the recited order of processing elements or sequences, or the use of numbers, letters, or other designations, therefore, is not intended to limit the claimed processes and methods to any order except as may be specified in the claims. Although the above disclosure discusses through various examples what is currently considered to be a variety of useful embodiments of the disclosure, it is to be understood that such detail is solely for that purpose and that the appended claims are not limited to the disclosed embodiments, but, on the contrary, are intended to cover modifications and equivalent arrangements that are within the spirit and scope of the disclosed embodiments. For example, although the implementation of various components described above may be embodied in a hardware device, it may also be implemented as a software-only solution, e.g., an installation on an existing server or mobile device.

[0234] Similarly, it should be appreciated that in the foregoing description of embodiments of the present disclosure, various features are sometimes grouped together in a single embodiment, figure, or description thereof to streamline the disclosure aiding in the understanding of one or more of the various embodiments. This method of disclosure, however, is not to be interpreted as reflecting an intention that the claimed subject matter requires more features than are expressly recited in each claim. Rather, claim subject matter lie in less than all features of a single foregoing disclosed embodiment.

WHAT IS CLAIMED IS

1. A method for three-dimensional (3D) printing in a liquid environment, comprising:
depositing, using a printer head, a droplet of bio-ink on a printing surface of a substrate, the printing surface being immersed in a liquid, wherein
an attachment of the droplet of bio-ink to the printing surface is prompted by a first interaction between the droplet of bio-ink and the printing surface; and
the attachment of the droplet of bio-ink to the printing surface is further prompted by a second interaction between the droplet of bio-ink and the liquid.
2. The method of claim 1, wherein the substrate includes a tissue scaffold.
3. The method of claim 1, wherein the droplet of bio-ink includes living biological components.
4. The method of claim 1, wherein the droplet of bio-ink includes a plurality of cells.
5. The method of claim 4, wherein the cells include endothelial cells.
6. The method of claim 5, wherein the endothelial cells include vascular endothelial cells, or lymphatic endothelial cells, or a combination thereof.
7. The method of claim 4, wherein the cells include cardiac cells, renal cells, hepatic cells, lung cells, gastric cells, pancreatic cells, gallbladder cells, bladder cells, spleen cells, tracheal cells, nerve cells, bone cells, or cancer cells, or a combination thereof.
8. The method of claim 4, wherein the cells include intestinal cells, epithelial cells, muscle cells, fibroblasts, secretory cells, ciliated cells, fat cells, blood cells, or immune cells, or a combination thereof.

9. The method of claim 4, wherein the cells include undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof.
10. The method of any one of claims 1-9, wherein the first interaction includes a hydrophilic interaction.
11. The method of any one of claims 1-9, wherein the first interaction includes a polar interaction.
12. The method of any one of claims 1-9, wherein the second interaction includes a hydrophobic interaction.
13. The method of any one of claims 1-9, wherein the second interaction includes a non-polar interaction.
14. The method of any one of claims 1-9, wherein the liquid includes a hydrophobic liquid.
15. The method of claim 14, wherein the hydrophobic liquid includes a mineral oil.
16. The method of any one of claims 1-15, wherein the substrate is configured to provide support for biological components or function as a portion of a biological organ.
17. The method of any one of claims 1-16, wherein the substrate is made of a biocompatible material.
18. The method of claim 17, wherein the biocompatible material is biodegradable.

19. The method of any one of claims 1-18, wherein the printing surface of the substrate includes non-biological components, or biological components, or a combination thereof.
20. The method of any one of claims 1-19, wherein the printing surface of the substrate includes one or more substances that are configured to facilitate an attachment of the droplet of bio-ink onto the printing surface of the substrate.
21. The method of claim 20, wherein the one or more substances include hydrogel.
22. The method of any one of claims 1-21, wherein the printing surface of the substrate includes a plurality of cells.
23. The method of any one of claims 1-22, further comprising:
- receiving information relating to one or more target positions on the printing surface;
 - determining a pathway of the printer head to the one or more target positions on the printing surface based on the information; and
 - controlling the printer head to approach the one or more target positions sequentially based on the determined pathway.
24. The method of claim 23, wherein the one or more target positions on the printing surface include:
- a first target position and a second target position, which are adjacent to each other for depositing of the bio-ink; wherein
 - a distance between the first target position and the second target position is determined based on a dimension of the printing surface, or a dimension of the droplet of bio-ink.
25. A method for printing an object including a tubular structure, comprising:

depositing, using a printer head, a first material on a substrate that includes a scaffold having the tubular structure; and

providing a second material, which is a fluid, within the tubular structure while depositing the first material on the substrate, wherein

the scaffold is permeable to the second material and is configured to allow the second material to reach the first material; and

the second material is configured to enhance or maintain an activity of the first material.

26. The method of claim 25, wherein the first material includes one or more biological components.

27. The method of claim 26, wherein the one or more biological components include somatic cells, undifferentiated stem cells, in differentiated stem cells, or terminally differentiated cells, or a combination thereof.

28. The method of claim 25, wherein the second material includes a cell culture medium, real blood, artificial blood, endothelial cells, or smooth muscle cells, or a combination thereof.

29. The method of claim 25, wherein the object includes part or entirety of an organ.

30. The method of claim 25, wherein the object includes an artificial blood vessel.

31. The method of claim 25, wherein the first material includes a plurality of living cells and the second material is configured to support the survival of the living cells, the second material including a cell culture medium, real blood, artificial blood, or endothelial cells, or a combination thereof.

32. The method of claim 25, wherein the object including a tubular structure includes

a vascular scaffold or a vascular network.

33. The method of any one of claims 25-32, wherein the substrate is configured to provide support for biological components or function as a portion of a biological organ.

34. The method of any one of claims 25-33, wherein the substrate is made of a biocompatible material.

35. The method of claim 34, wherein the biocompatible material is biodegradable.

36. The method of any one of claims 25-35, wherein a printing surface of the substrate includes non-biological components, or biological components, or a combination thereof.

37. The method of any one of claims 25-36, wherein a printing surface of the substrate includes one or more substances that are configured to facilitate an attachment of the first material onto the printing surface of the substrate.

38. The method of claim 37, wherein the one or more substances include hydrogel.

39. The method of any one of claims 25-38, wherein a printing surface of the substrate includes a plurality of cells.

40. The method of any one of claims 25-39, further comprising:

receiving information relating to one or more target positions on a printing surface of the substrate;

determining a pathway of the printer head to the one or more target positions on the printing surface based on the information; and

controlling the printer head to approach the one or more target positions

sequentially based on the determined pathway.

41. The method of claim 40, wherein the one or more target positions on the printing surface include:

a first target position and a second target position, which are adjacent to each other for depositing of the bio-ink; wherein

a distance between the first target position and the second target position is determined based on a dimension of the printing surface, or a dimension of the droplet of bio-ink.

42. A method for printing a three-dimensional (3D) object, comprising:

(a). depositing first bio-ink including first cells on a printing surface of a substrate;

(b). culturing the first cells deposited on the printing surface in a first cell culture medium for a first time period;

(c). depositing second bio-ink including second cells on the deposited printing surface;

(d). culturing the second cells deposited on the printing surface in a second cell culture medium for a second time period; and

(e). repeating (a)~(d) and allowing the deposited first cells and second cells to cohere to form a 3D object.

43. The method of claim 42, wherein depositing first bio-ink including first cells on a printing surface of a substrate comprises:

depositing the first bio-ink including first cells on the printing surface that is being immersed in a first liquid.

44. The method of claim 43, wherein depositing first bio-ink including first cells on a printing surface of a substrate further comprises:

removing the first liquid from the first cells deposited on the printing surface; and

washing the first cells deposited on the printing surface.

45. The method of claim 43, wherein the first liquid is loaded in a container, and the method further comprises:

replacing the first liquid with the first cell culture medium in the container.

46. The method of claim 45, wherein replacing the first liquid with the first cell culture medium in the container comprises:

removing the first liquid from the container and the first cells deposited on the printing surface;

washing the container and the first cells deposited on the printing surface; and

loading the first cell culture medium to the container.

47. The method of claim 42, wherein depositing second bio-ink including second cells on the deposited printing surface comprises:

depositing the second bio-ink including second cells on the deposited printing surface in a second liquid.

48. The method of claim 47, wherein depositing second bio-ink including second cells on the deposited printing surface comprises:

removing the second liquid from the second cells deposited on the printing surface; and

washing the second cells deposited on the printing surface.

49. The method of claim 47, wherein the second liquid is loaded in a container, and the method further comprises:

replacing the second liquid with the second cell culture medium in the container.

50. The method of claim 49, wherein replacing the second liquid with the second cell culture medium in the container comprises:

removing the second liquid from the container and the second cells deposited on the printing surface;

washing the container and the second cells deposited on the printing surface;
and

loading the second cell culture medium to the container.

51. The method of claim 42, wherein the first cells or the second cells include one or more types of somatic cells.

52. The method of claim 51, wherein the one or more types of somatic cells include a first type of somatic cells and a second type of somatic cells, the first type of somatic cells including endothelial cells, the second type of somatic cells including cardiomyocytes, hepatocytes, pneumonocytes, nephrocytes, splenocytes, undifferentiated stem cells, intermediately differentiated stem cells, or terminally differentiated cells, or a combination thereof.

53. The method of claim 42, wherein the first cells and the second cells include one or more same types of cells.

54. The method of claim 53, wherein a percentage of each type of cells for the first cells is the same as or different from a percentage of a corresponding type of cells for the second cells.

55. The method of claim 42, wherein the first cells and the second cells include one or more different types of cells.

56. The method of claim 42, wherein the first bio-ink or the second bio-ink further includes a cell culture medium.

57. The method of claim 42, wherein allowing the deposited first cells and second

cells to cohere to form a 3D object comprises:

inducing the deposited first cells or the to coalesce to form a plurality of capillary vessels by introducing one or more angiogenic factors into the first cell culture medium.

58. The method of claim 57, wherein the substrate includes a tubular scaffold, and at least a portion of the plurality of capillary vessels connect with the tubular scaffold, so that the first cell culture medium or the second cell culture medium is capable of passing through the tubular scaffold to the at least a portion of the plurality of capillary vessels.

59. The method of claim 42, wherein the substrate includes a vascular scaffold or a vascular network.

60. The method of claim 59, wherein depositing first bio-ink including first cells on a printing surface of a substrate comprises:

providing a fluid of a material within the vascular scaffold or the vascular network while depositing the first bio-ink including the first cells on the vascular scaffold or the vascular network, wherein

the vascular scaffold or the vascular network is permeable to the cell culture medium and is configured to allow the cell culture medium to reach the first cells.

61. The method of claim 59, wherein culturing the first cells deposited on the printing surface in a first cell culture medium for a first time period comprises:

providing a fluid of the first cell culture medium within the vascular scaffold or the vascular network while culturing the first cells, wherein

the vascular scaffold or the vascular network is permeable to the first cell culture medium and is configured to allow the first cell culture medium to reach the first cells.

62. The method of claim 59, wherein depositing second bio-ink including second cells on the deposited printing surface comprises:

providing a fluid of a material within the vascular scaffold or the vascular network while depositing the second bio-ink including the second cells on the vascular scaffold or the vascular network, wherein

the vascular scaffold or the vascular network is permeable to the cell culture medium and is configured to allow the cell culture medium to reach the second cells.

63. The method of claim 59, wherein culturing the second cells deposited on the printing surface in a second cell culture medium for a second time period comprises:

providing a fluid of the second cell culture medium within the vascular scaffold or the vascular network while culturing the second cells, wherein

the vascular scaffold or the vascular network is permeable to the second cell culture medium and is configured to allow the second cell culture medium to reach the second cells.

64. The method of claim 60 or claim 62, wherein the material includes a cell culture medium, real blood, or artificial blood, or a combination thereof.

65. The method of any one of claims 42-64, wherein the substrate is configured to provide support for biological components or function as a portion of a biological organ.

66. The method of any one of claims 42-65, wherein the substrate is made of a biocompatible material.

67. The method of claim 66, wherein the biocompatible material is biodegradable.

68. The method of any one of claims 42-67, wherein the printing surface of the

substrate includes non-biological components, or biological components, or a combination thereof.

69. The method of any one of claims 42-68, wherein the printing surface of the substrate includes one or more substances that are configured to facilitate an attachment of the first cells and/or the second cells onto the printing surface of the substrate.

70. The method of claim 69, wherein the one or more substances include hydrogel.

71. The method of any one of claims 42-70, wherein the printing surface of the substrate includes a plurality of cells.

72. The method of any one of claims 42-71, further comprising:

receiving information relating to one or more target positions on the printing surface;

determining a pathway of the printer head to the one or more target positions on the printing surface based on the information; and

controlling the printer head to approach the one or more target positions sequentially based on the determined pathway.

73. The method of claim 72, wherein the one or more target positions on the printing surface include:

a first target position and a second target position, which are adjacent to each other for depositing of the bio-ink; wherein

a distance between the first target position and the second target position is determined based on a dimension of the printing surface, or a dimension of the droplet of bio-ink.

74. A system for three-dimensional (3D) printing, comprising:

one or more printer heads configured to deposit one or more types of bio-ink;
a positioning device that is connected to the one or more printer heads and is configured to drive the one or more printer heads to move relative to a printing surface of a substrate;

a control module that is configured to:

control the positioning device so that the one or more printer heads are in place to deposit a droplet of bio-ink on one or more target positions on the printing surface immersed in a liquid;

wherein the liquid includes a material such that an attachment of the droplet of bio-ink on the printing surface is prompted by a first interaction between the droplet of bio-ink and the printing surface, and the attachment of the droplet of bio-ink on the printing surface is further prompted by a second interaction between the droplet of bio-ink and the liquid.

75. The system of claim 74, further comprising a container configured to accommodate the liquid to immerse the printing surface.

76. The system of claim 74, wherein the first interaction includes a hydrophilic interaction.

77. The system of claim 74, wherein the second interaction includes a hydrophobic interaction, and the liquid includes a hydrophobic liquid.

78. The system of claim 74, wherein the positioning device includes a robotic arm.

79. The system of claim 78, wherein the robotic arm is rotatable around at least two axes.

80. The system of claim 78, the robotic arm is a 6-axis robotic arm.

81. The system of any one of claims 78-80, wherein the control module is further configured to:

change an axial direction of the one or more printer heads by operating the positioning device so that the axial direction of the one or more printer heads is different from a vertical direction in a 3D space.

82. The system of any one of claims 74-80, wherein the control module is further configured to:

receive information relating to the one or more target positions on the printing surface;

determine a pathway of the one or more printer heads to the one or more target positions on the printing surface based on the information; and

control the positioning device to drive the one or more printer heads to approach the one or more target positions sequentially based on the determined pathway.

83. The system of claim 82, further comprising a calibration assembly that is configured to calibrate a position of the one or more printer heads.

84. The system of claim 83, wherein the calibration assembly includes a camera or a position sensor that is configured to detect the position of the one or more printer heads.

85. The system of claim 84, wherein the position sensor is configured to detect the position of the one or more printer heads based on one or more infrared rays.

86. The system of claim 83 or 84, wherein the control module is further configured to:

acquire information associated with the position of the one or more printer heads from the calibration assembly;

determine the position of the one or more printer heads based on the information; and

adjust the one or more printer heads to a target position based on the determined position or adjust the pathway of the one or more printer heads.

87. The system of claim 82, wherein the pathway of the one or more printer heads is a space curve.

88. The system of any one of claims 74-87, wherein the printing surface is a curved surface in the 3D space.

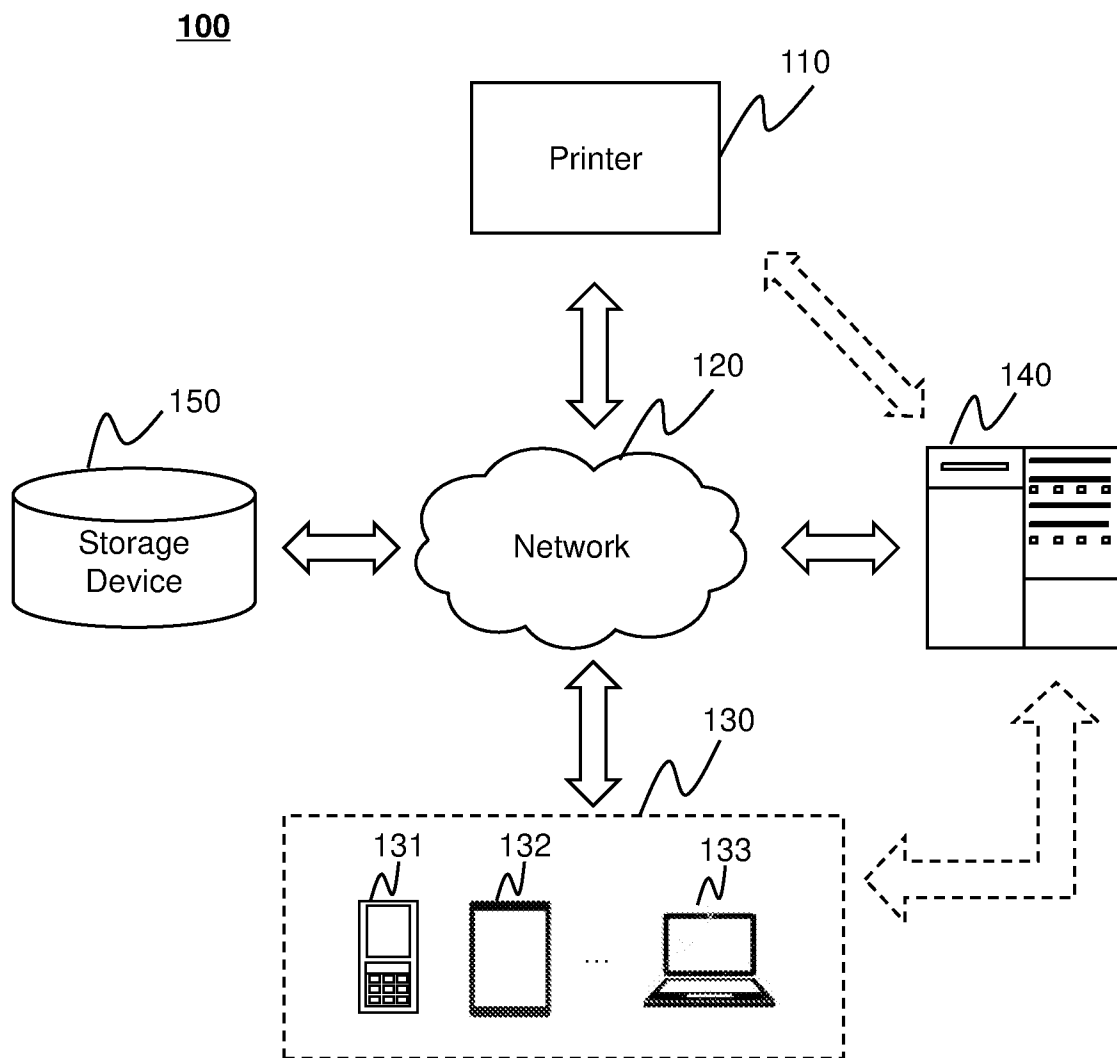


FIG. 1

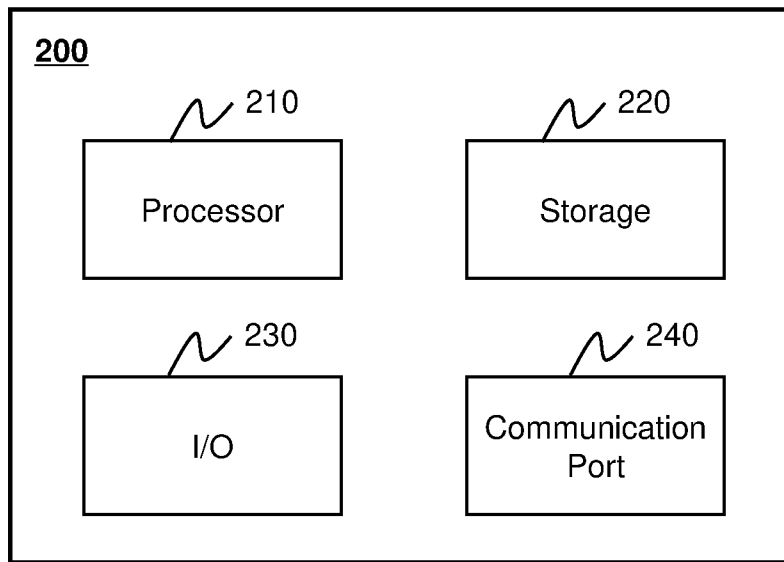
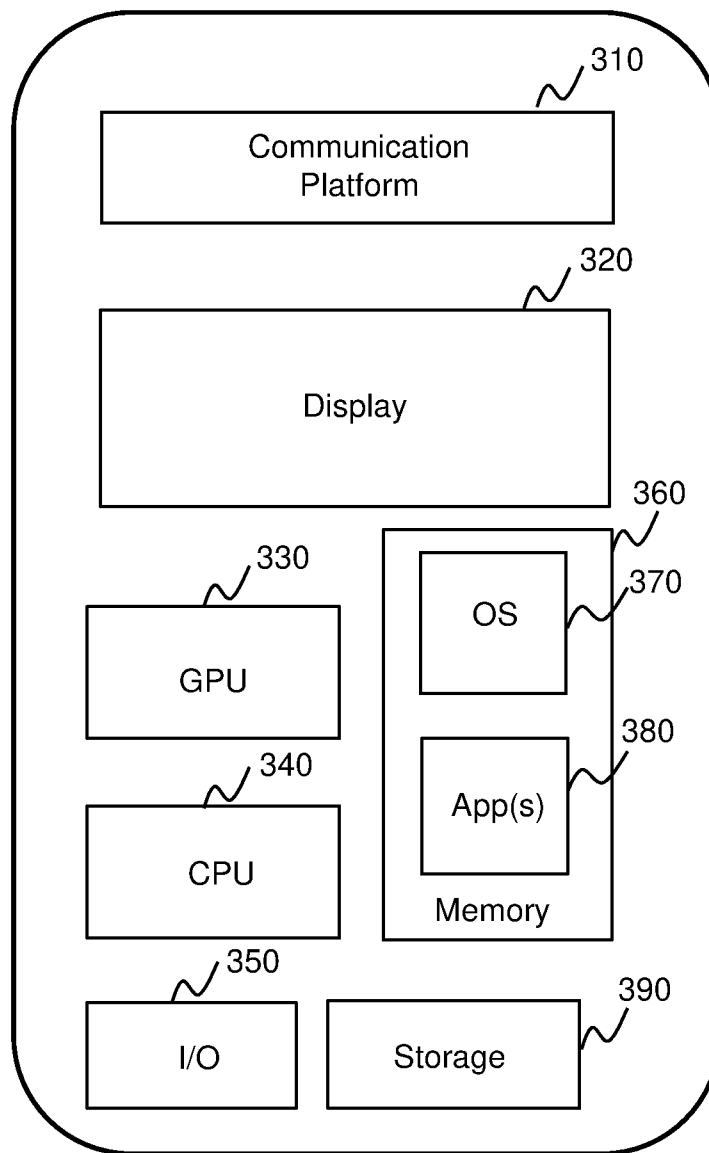
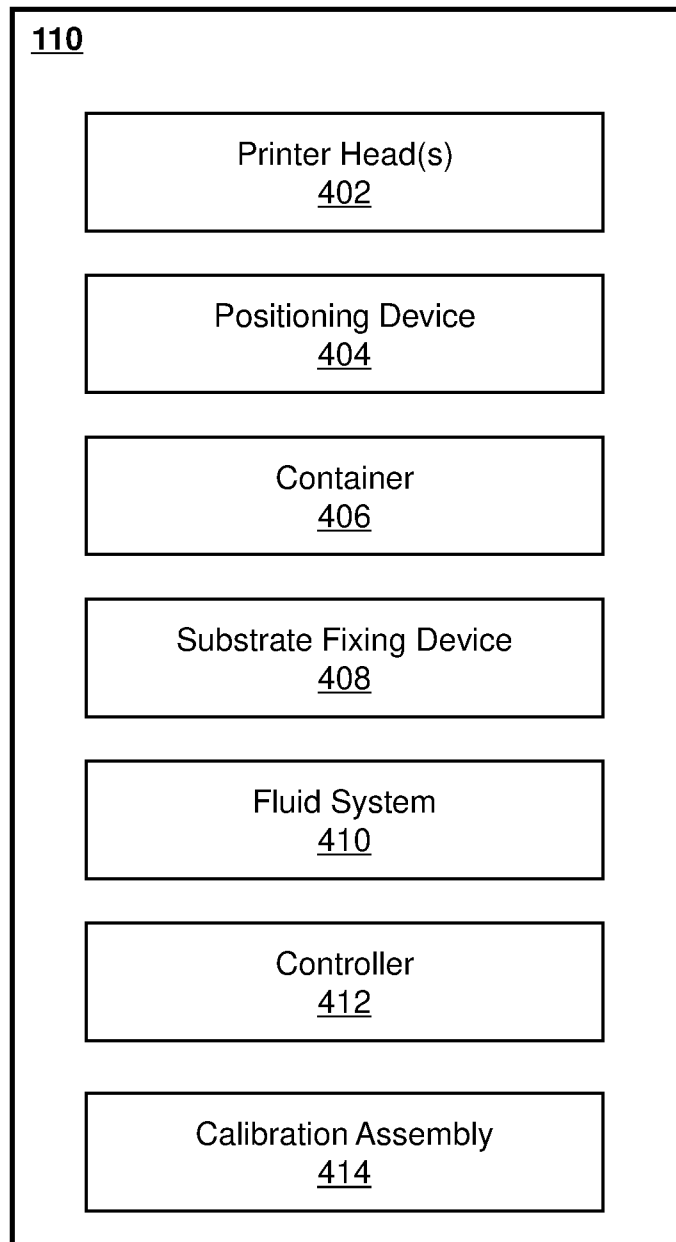
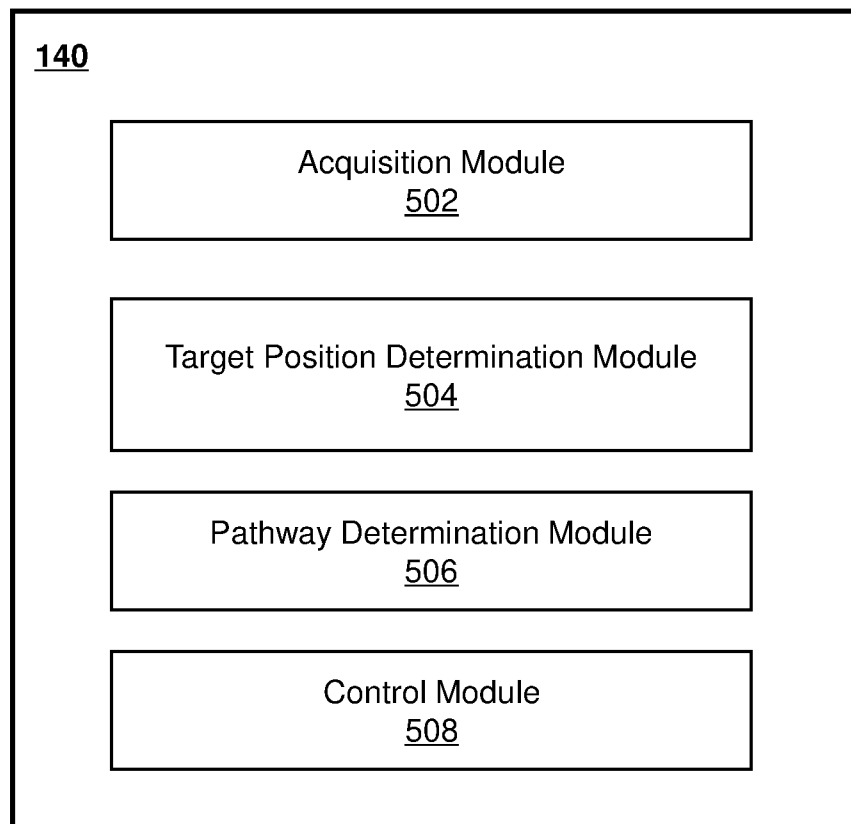


FIG. 2

300**FIG. 3**

**FIG. 4**

**FIG. 5**

600

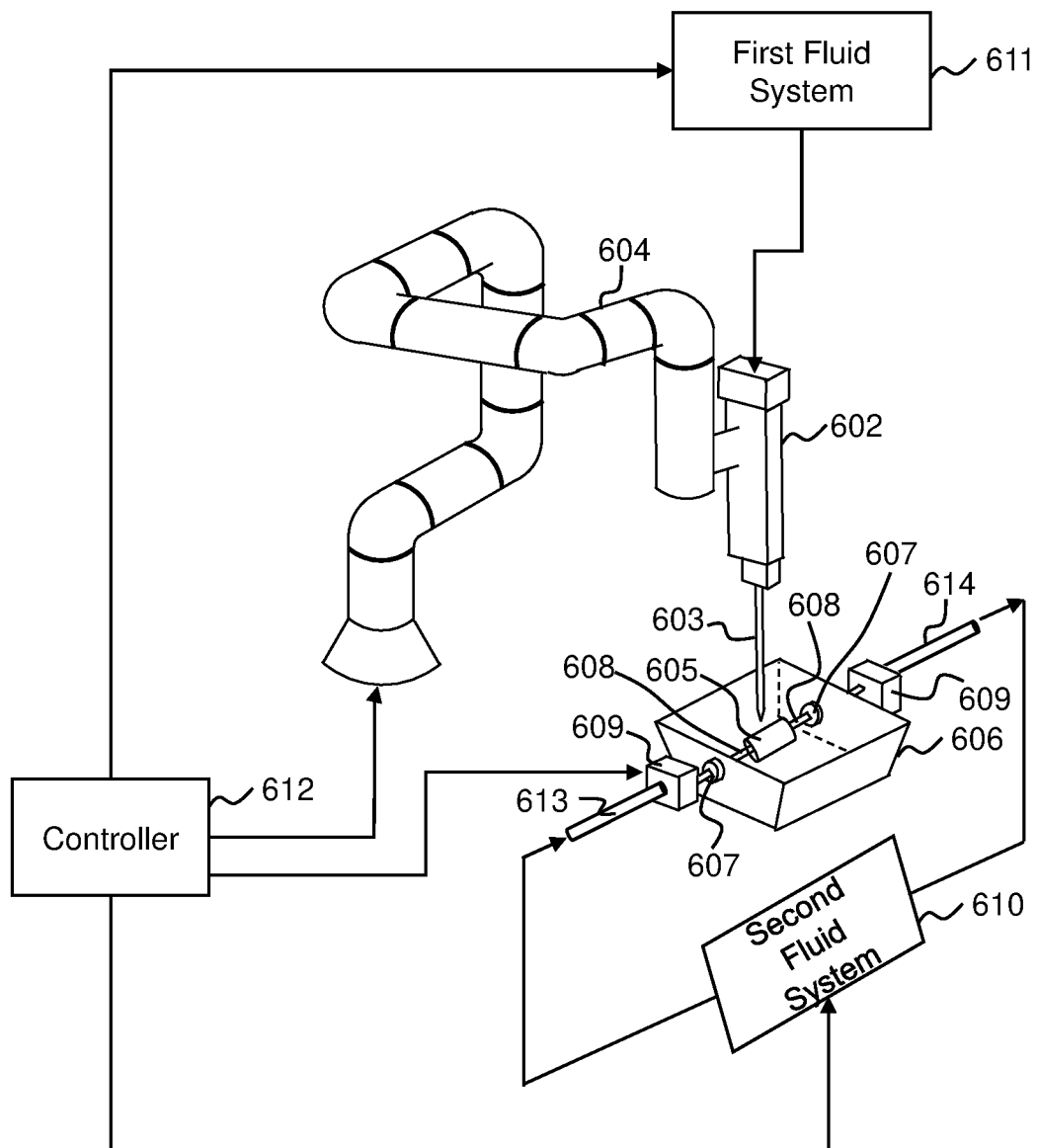


FIG. 6A

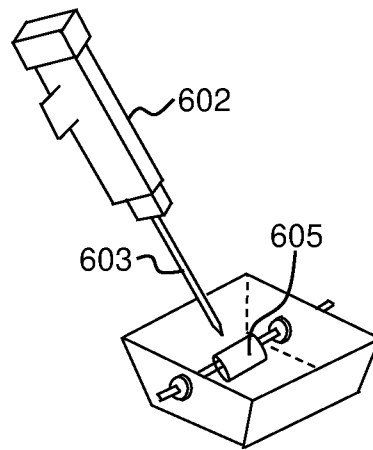


FIG. 6B

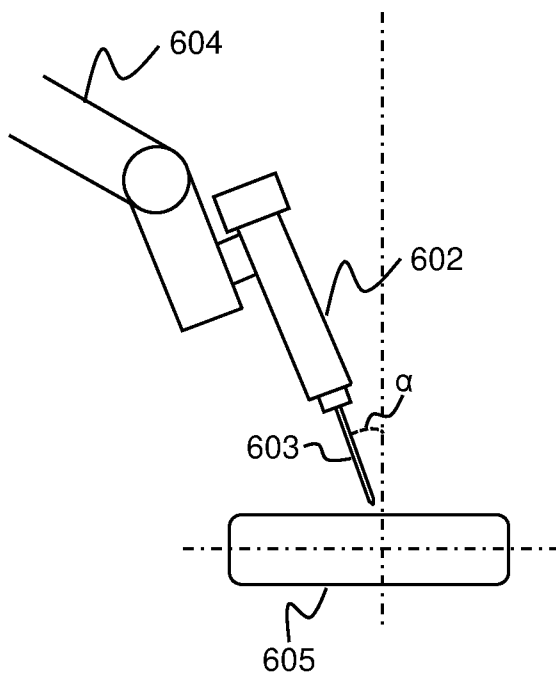


FIG. 6C

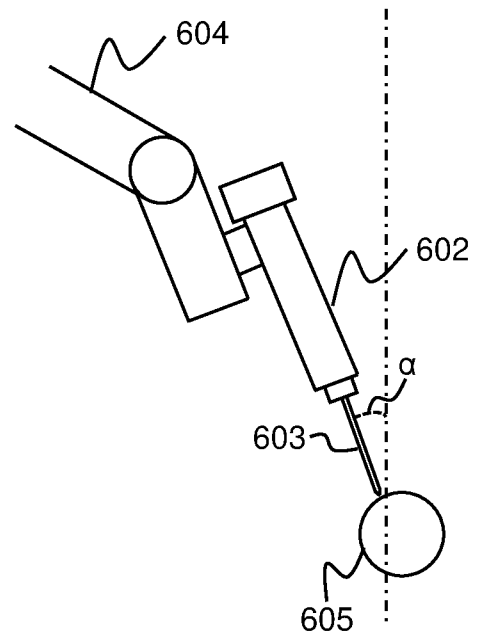
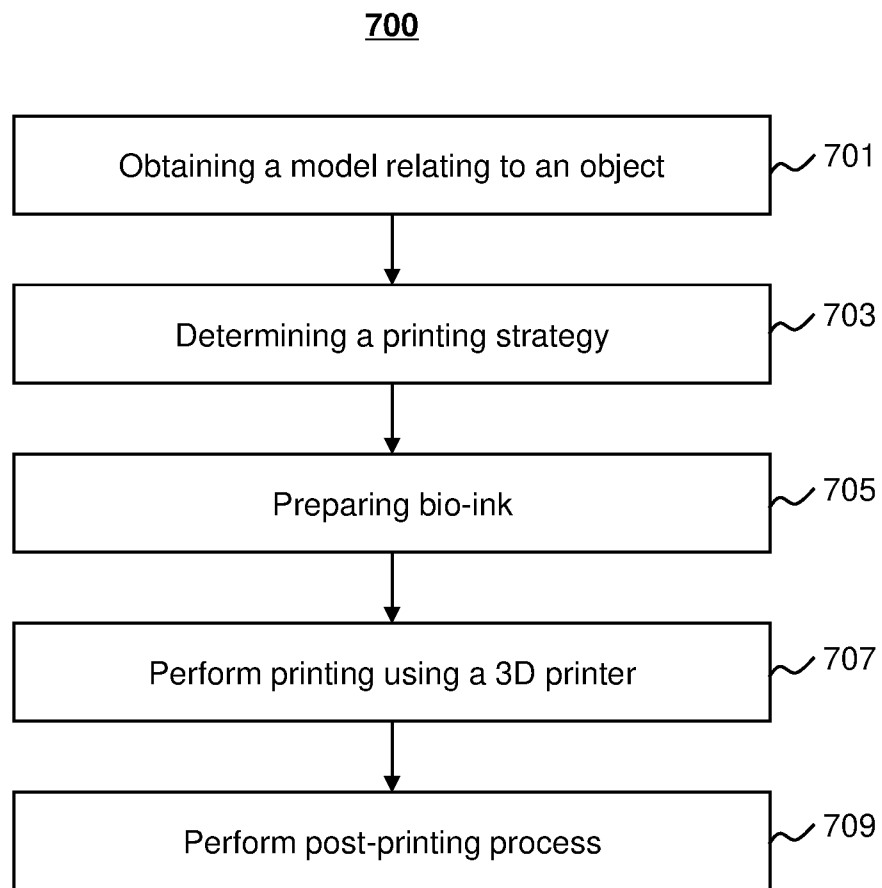
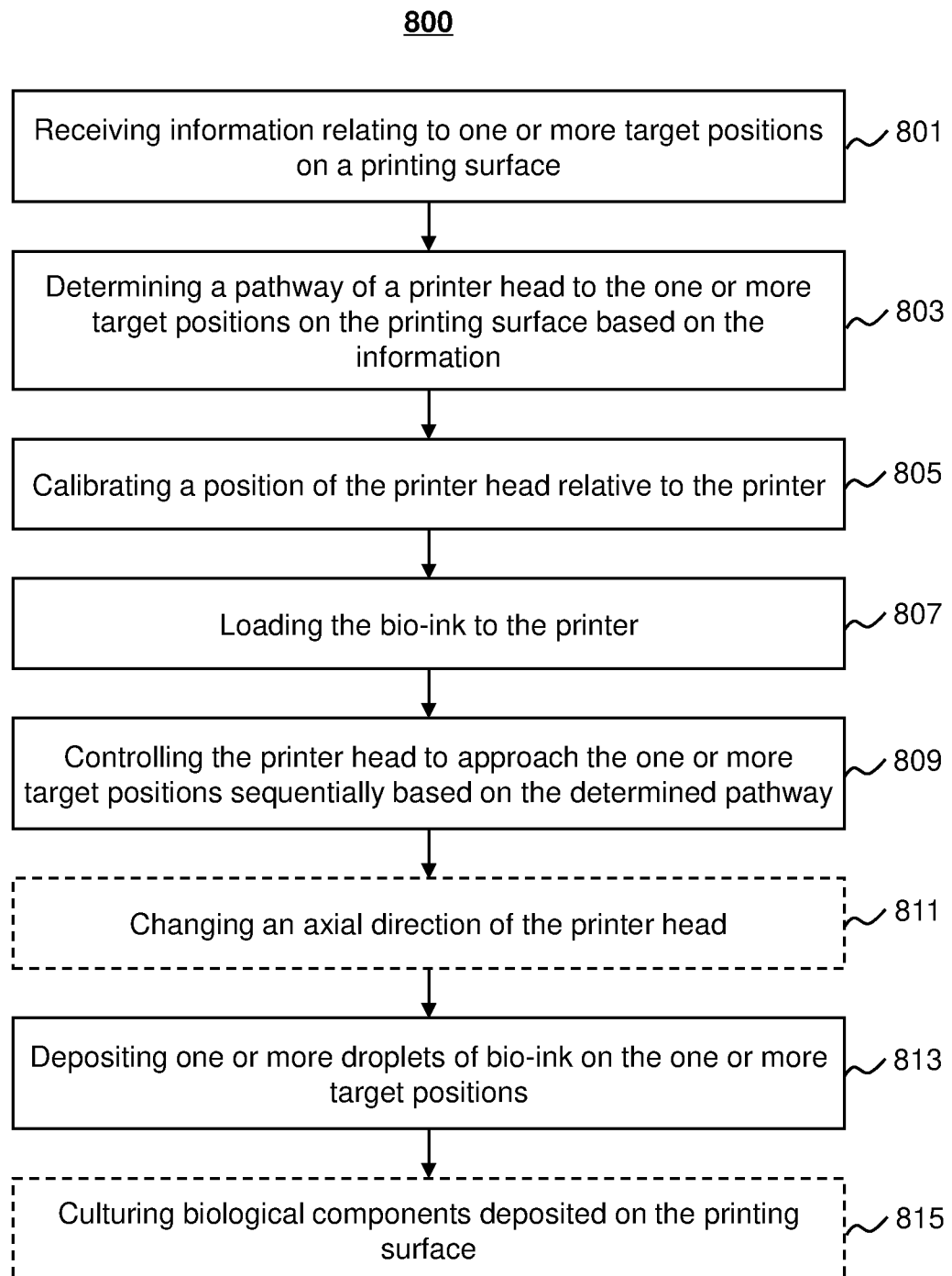


FIG. 6D

**FIG. 7**

**FIG. 8**

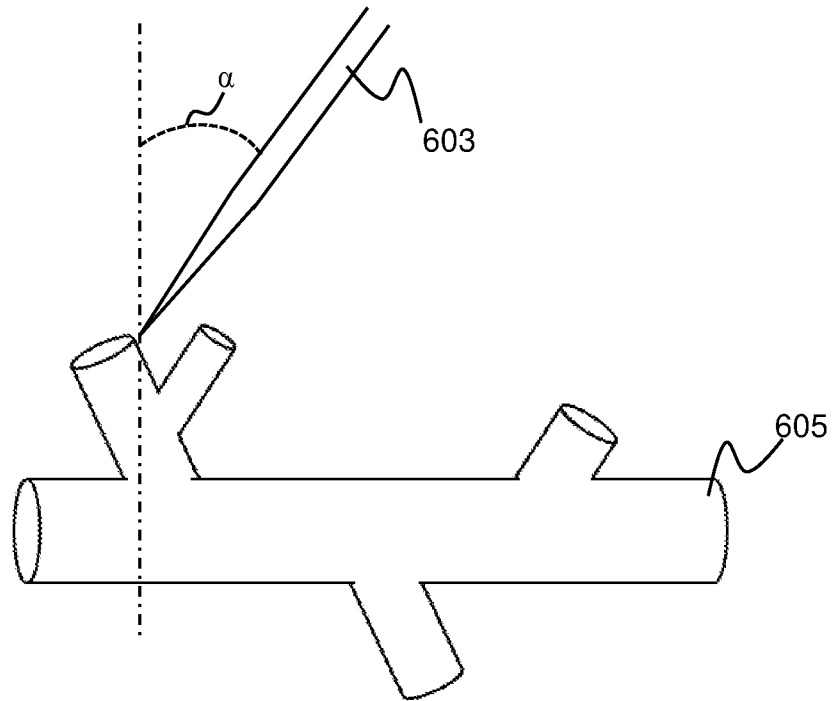


FIG. 9A

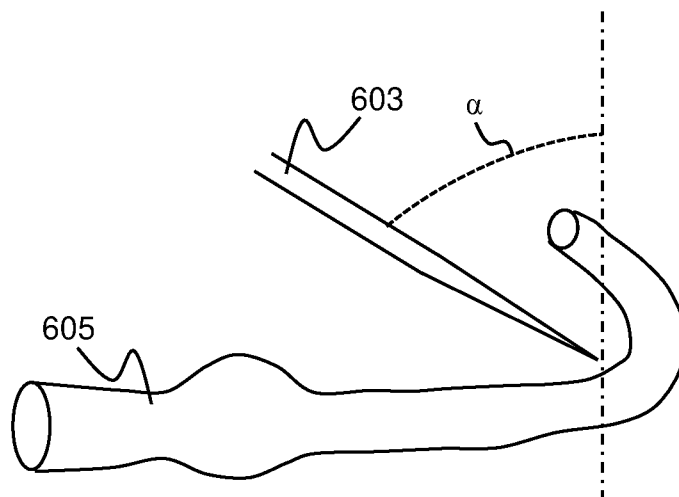
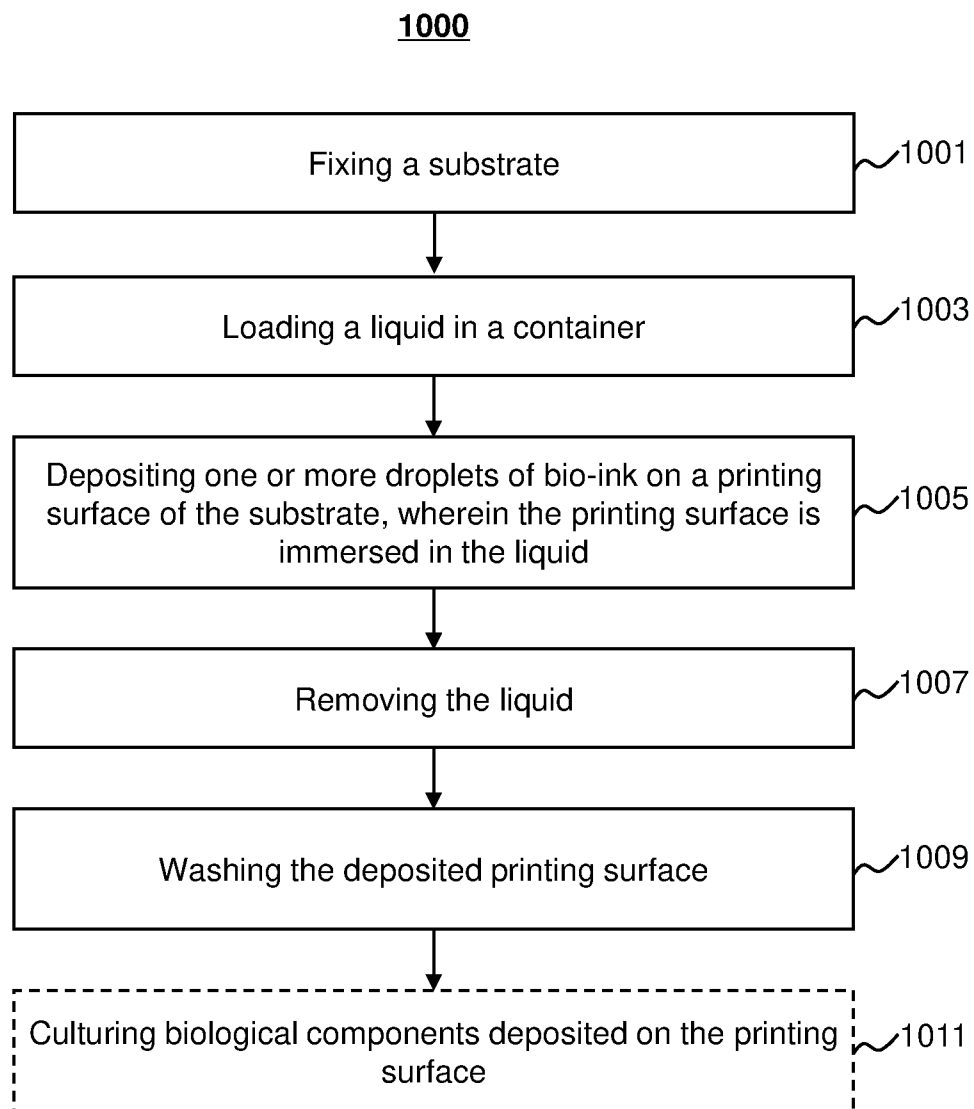


FIG. 9B

**FIG. 10**

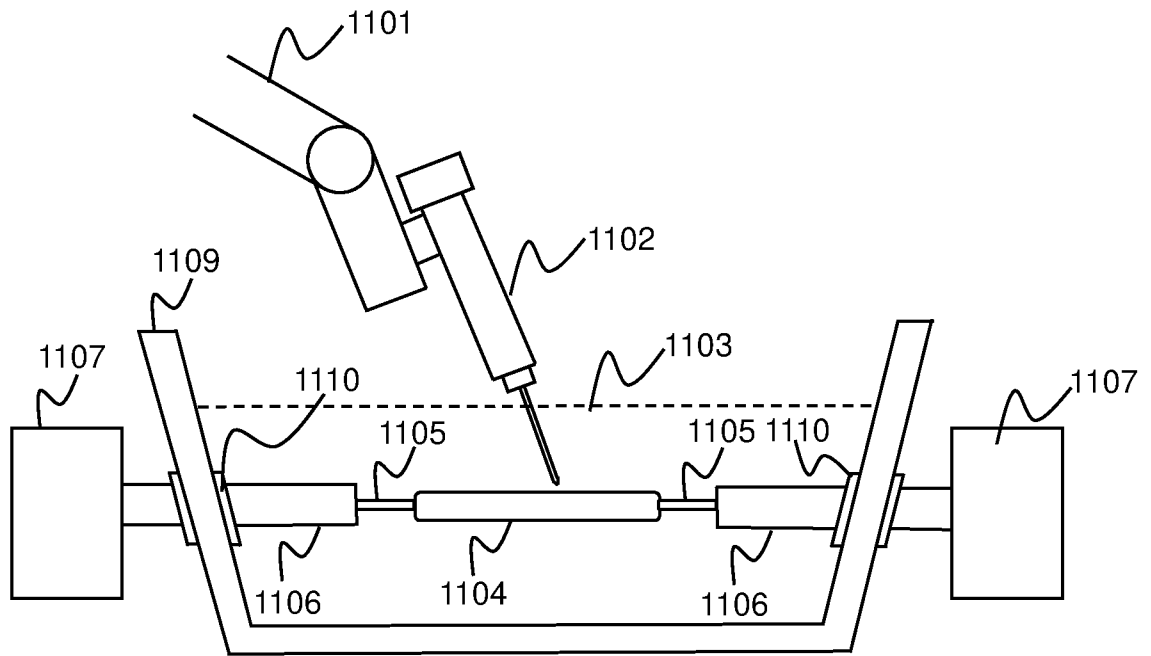


FIG. 11

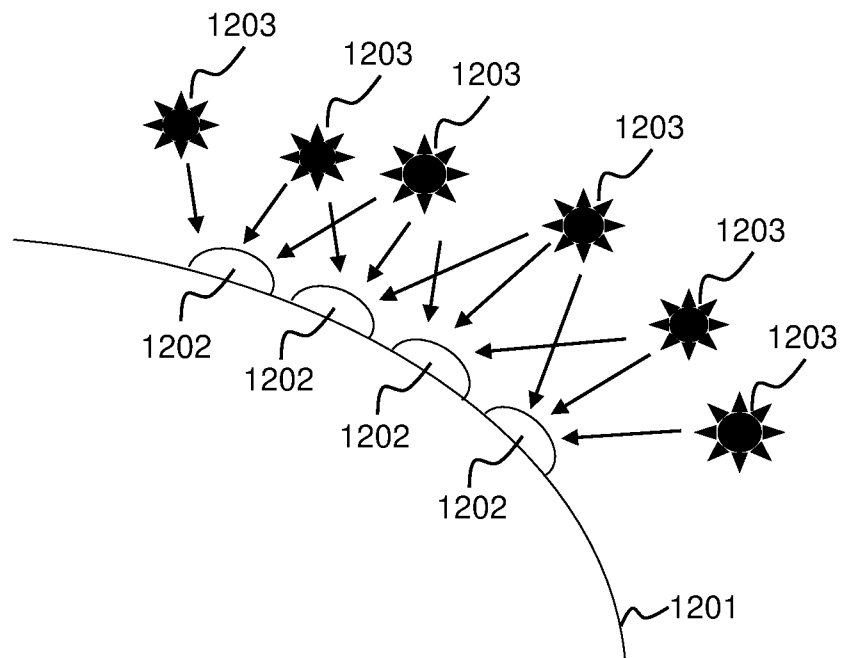
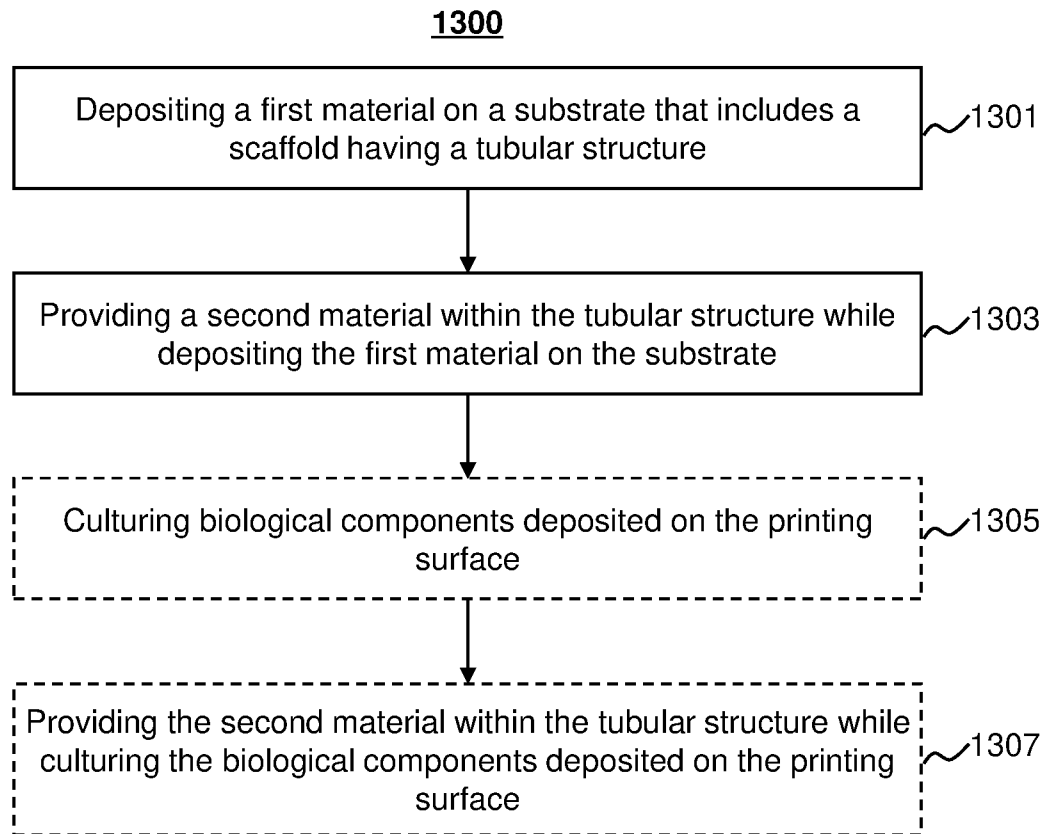
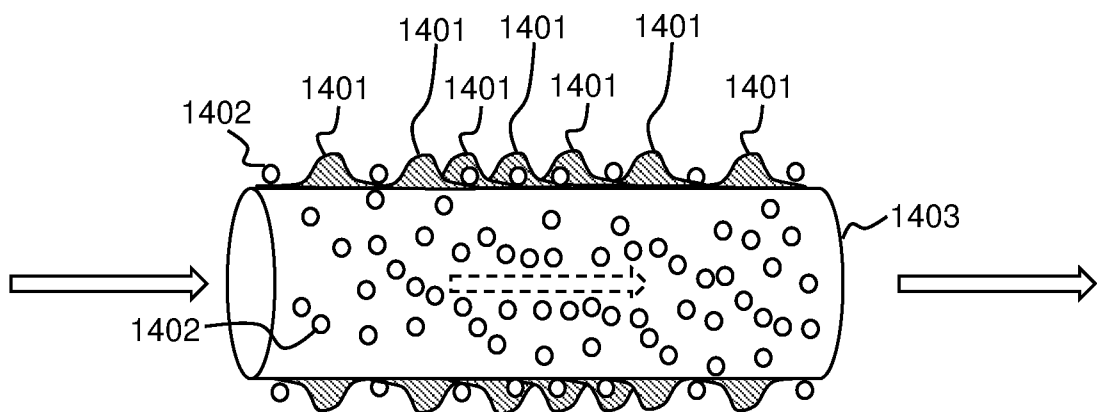
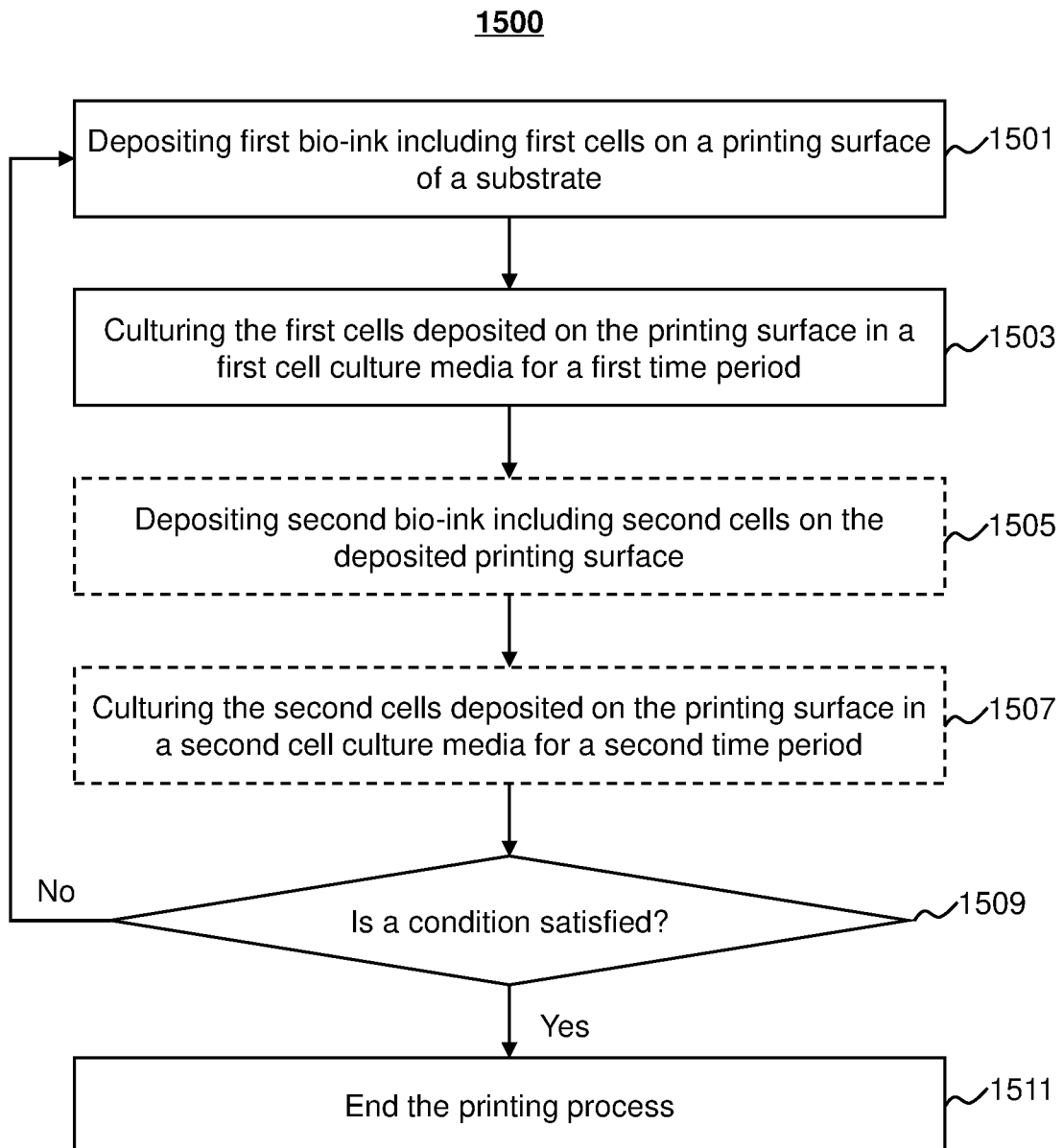


FIG. 12

**FIG. 13****FIG. 14**

**FIG. 15**

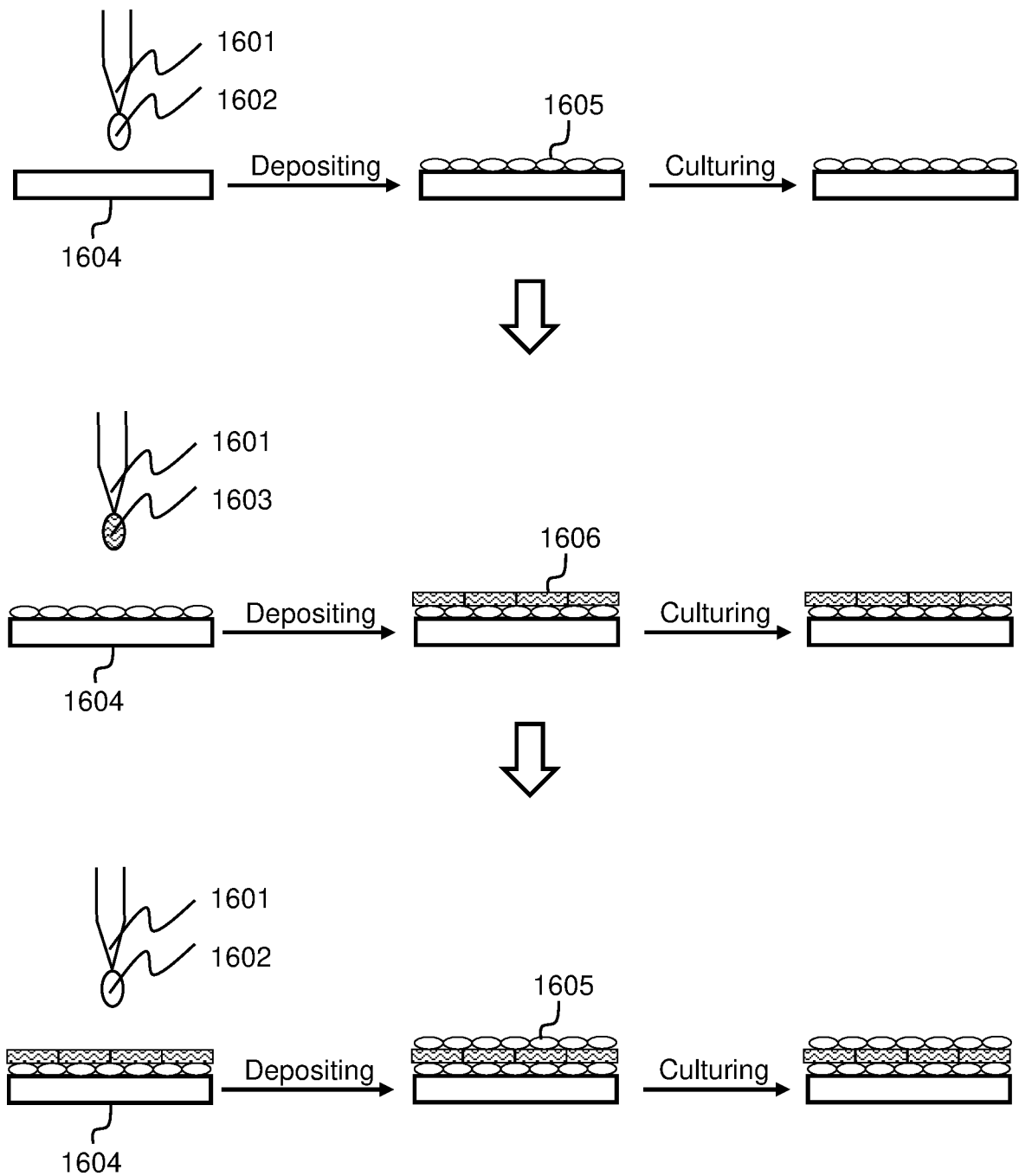


FIG. 16

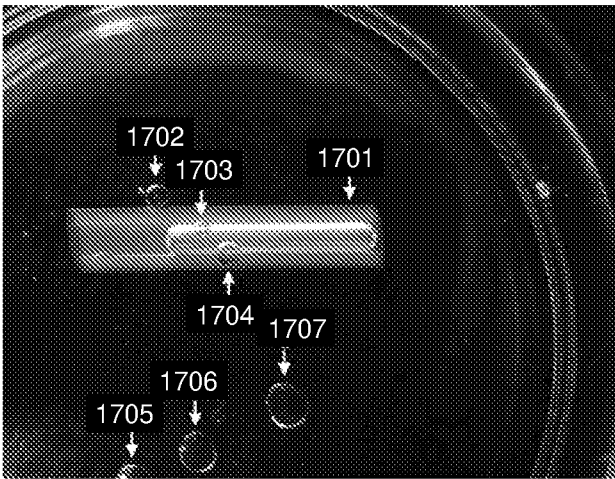


FIG. 17A

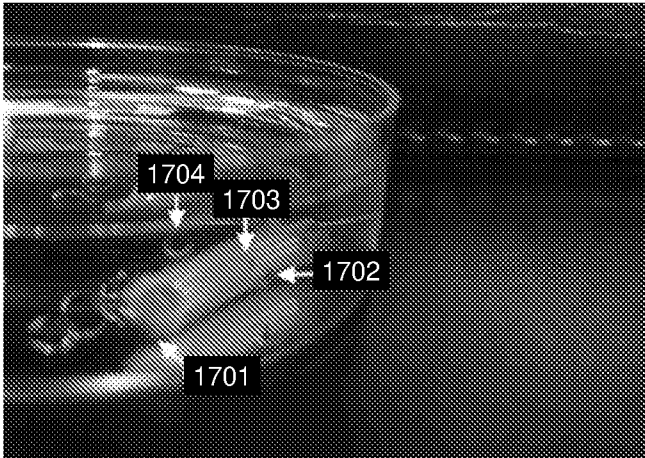


FIG. 17B

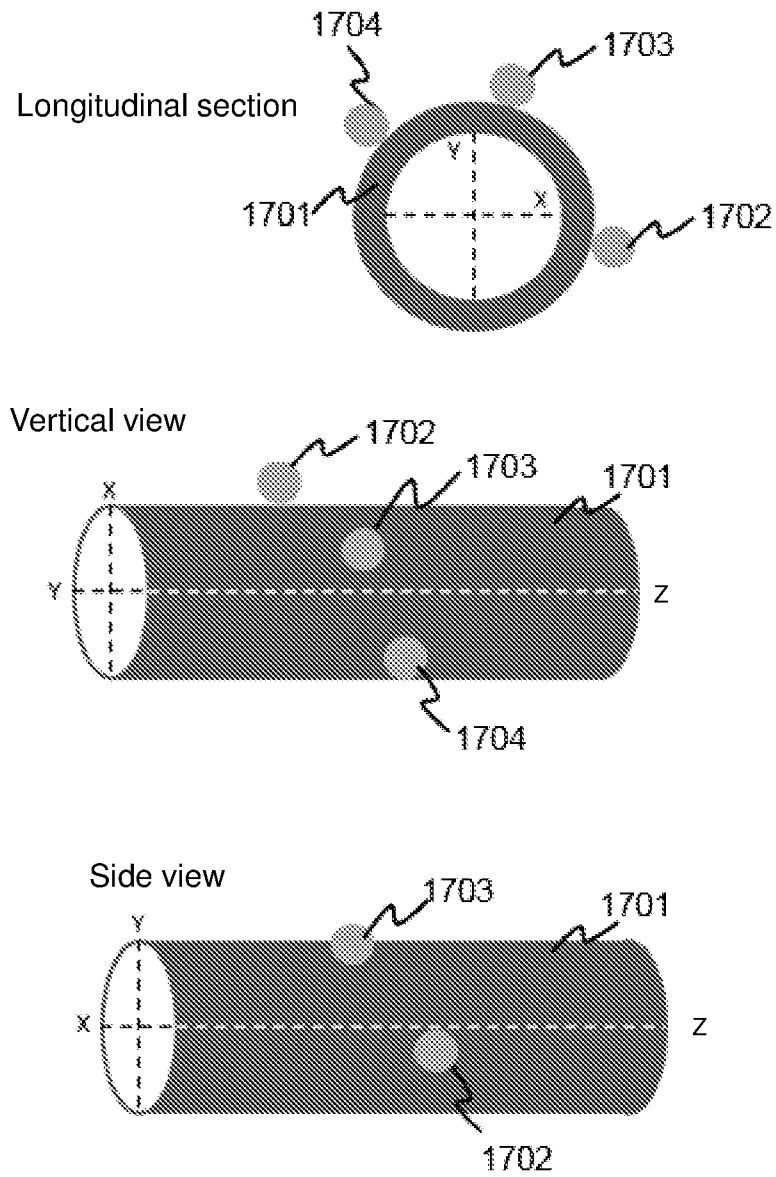


FIG. 17C

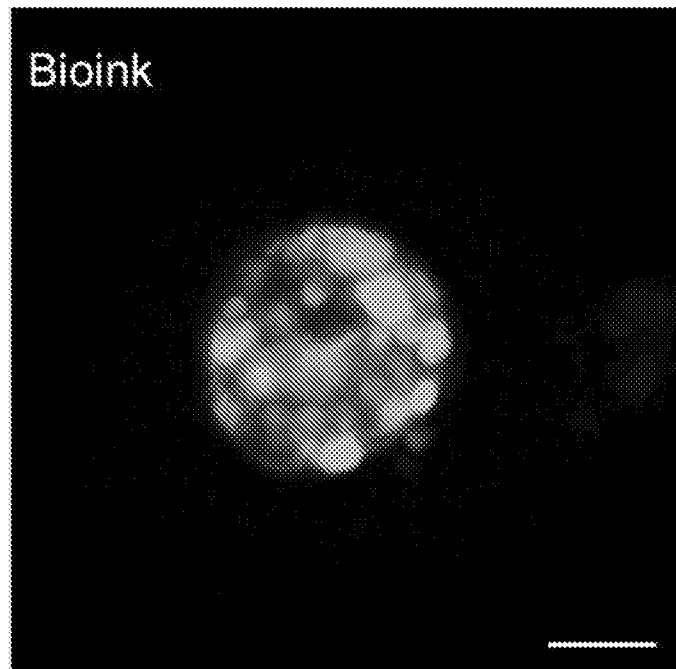


FIG. 18A

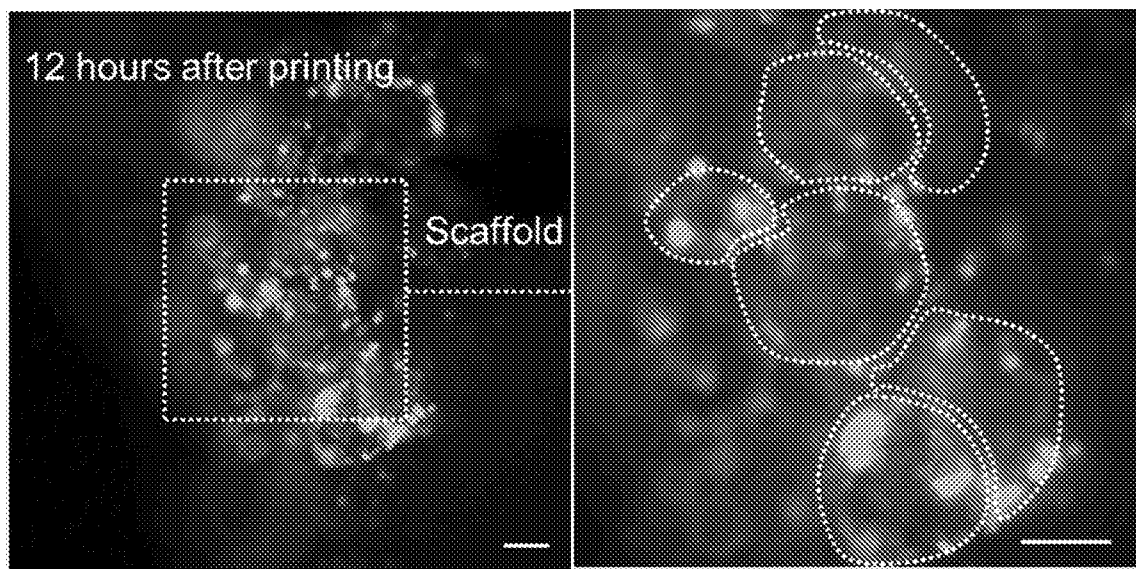


FIG. 18B

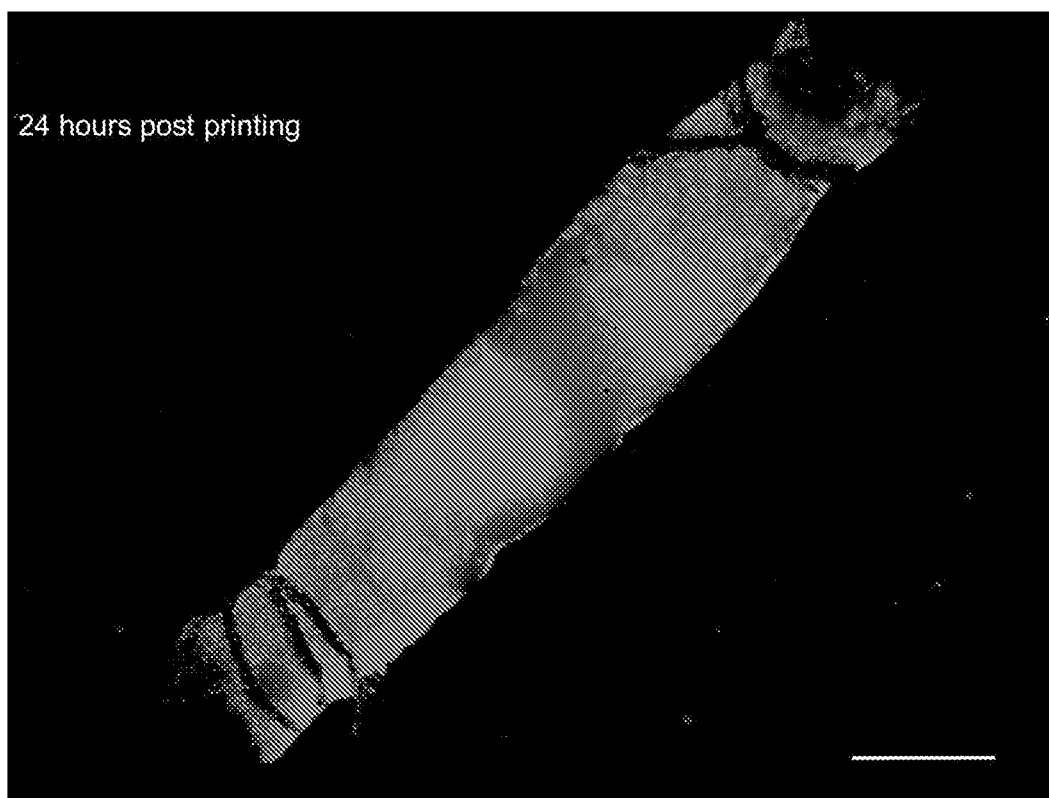


FIG. 18C

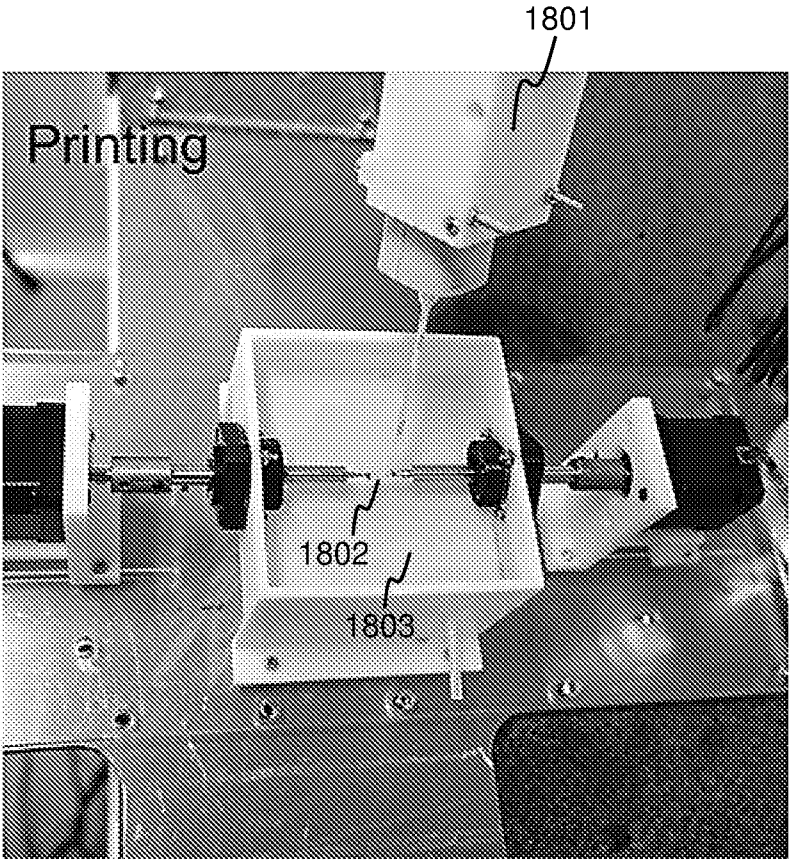


FIG. 18D

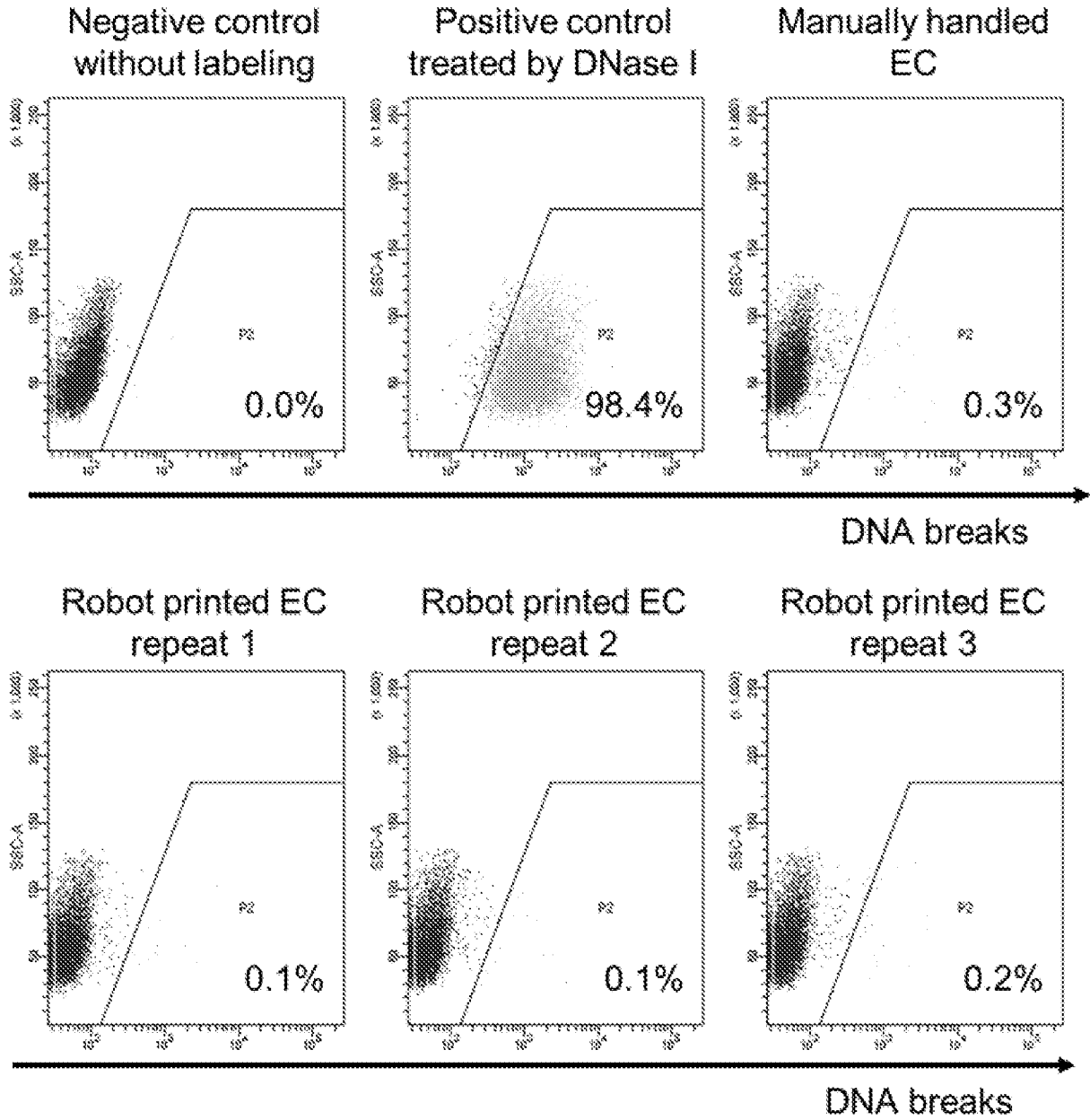


FIG. 19

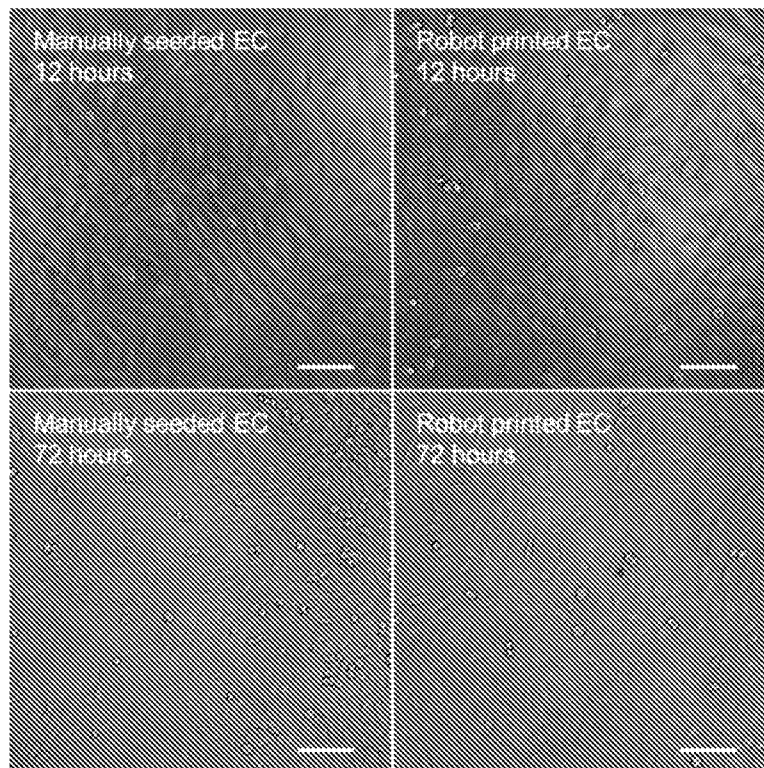


FIG. 20

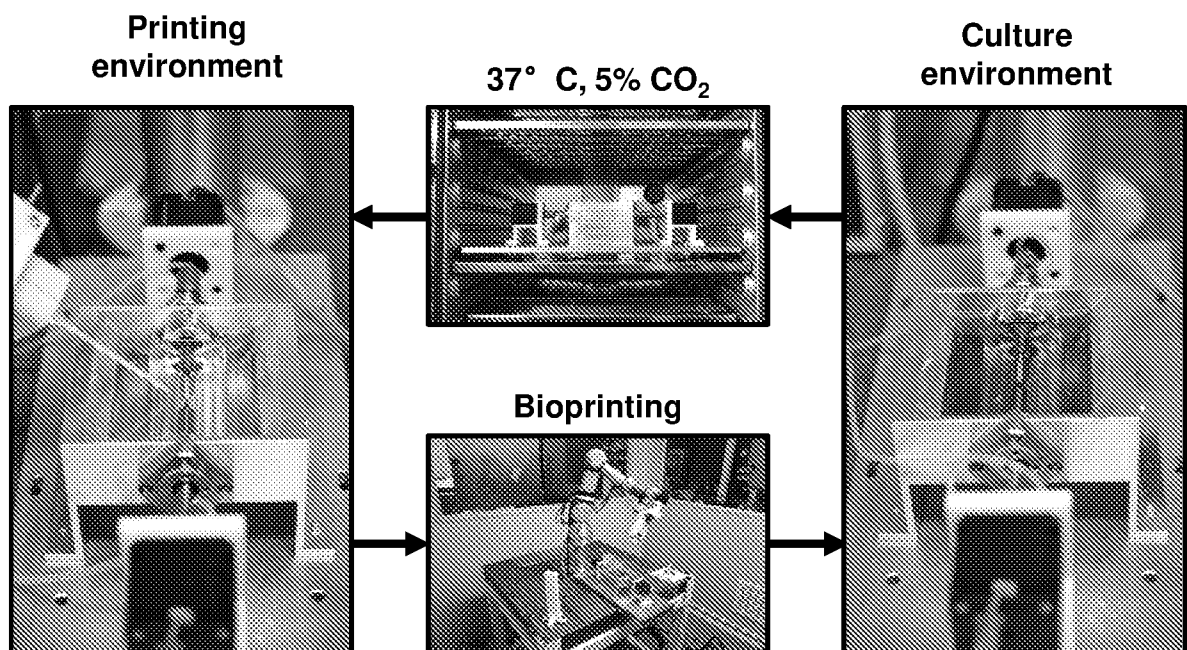


FIG. 21

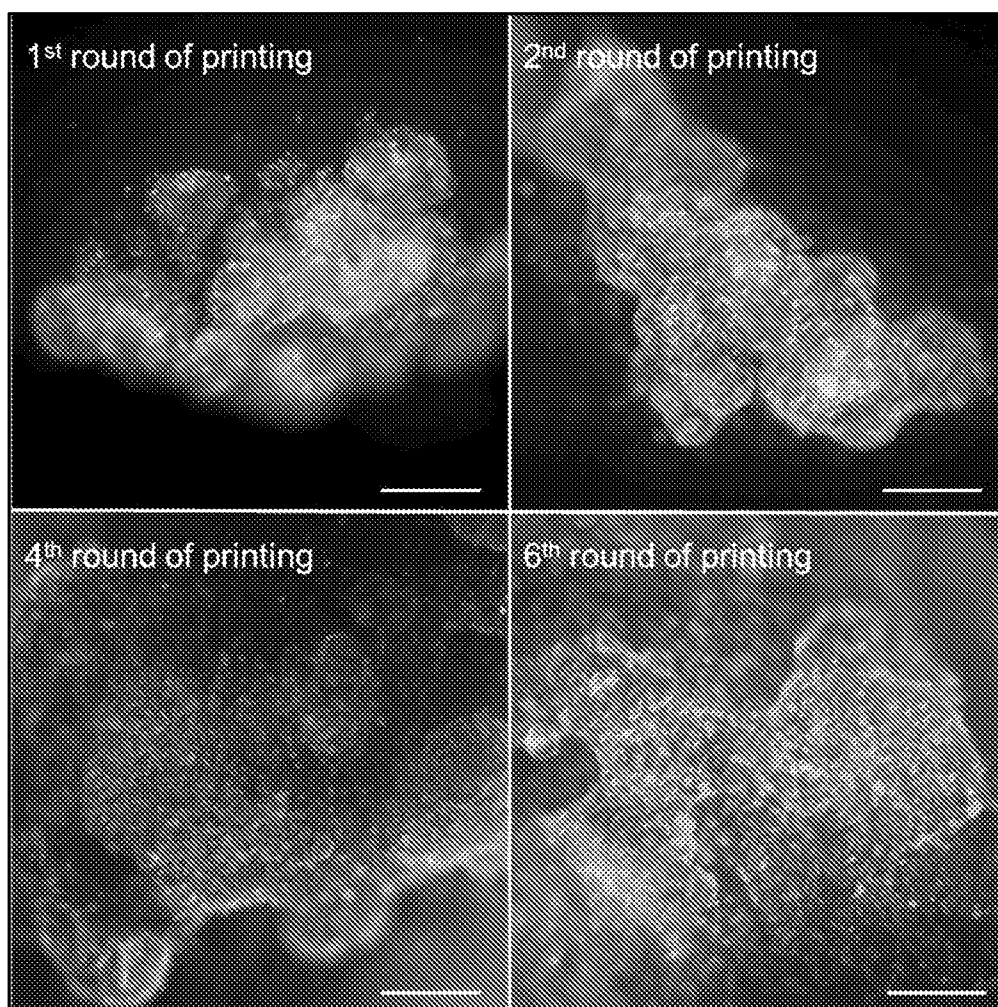


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/072991

A. CLASSIFICATION OF SUBJECT MATTER

B29C 64/112(2017.01)i; B29C 64/20(2017.01)i; B33Y 30/00(2015.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

B29C; B33Y

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNTXT, JPABS, USTXT, JPTXT, CNKI, ISI Web of Knowledge, Elsevier Science Direct; three-dimensional, WANG Xiuji, three dimensional, deposit+, bio-ink, ink, 3D, print+, liquid, cell?, biological, scaffold

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018130949 A1 (ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNEEPFL) 19 July 2018 (2018-07-19) Paragraphs 9-45, 92-106, 113, 122-125, Fig. 1-9	1-88
X	CN 105582571 A (UNIV. ZHEJIANG) 18 May 2016 (2016-05-18) Paragraphs 6-27	1-88
A	CN 106510898 A (UNIV. CHINA RENMIN) 22 March 2017 (2017-03-22) Claims 1-9	1-88
A	CN 108883572 A (3D SYSTEMS INC.) 23 November 2018 (2018-11-23) Claims 1-27	1-88
A	US 2017140848 A1 (TEXAS TECH UNIVERSITY SYSTEM) 18 May 2017 (2017-05-18) Claims 1-42	1-88

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

12 October 2019

Date of mailing of the international search report

22 October 2019

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Authorized officer

NIU, Peili

Facsimile No. (86-10)62019451

Telephone No. 86-(10)-53962685

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2019/072991

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2018130949	A1	19 July 2018	GB	201700368	D0	22 February 2017
				CN	110177584	A	27 August 2019
CN	105582571	A	18 May 2016	CN	105582571	B	09 November 2018
CN	106510898	A	22 March 2017	None			
CN	108883572	A	23 November 2018	JP	2019503290	A	07 February 2019
				EP	3408073	A1	05 December 2018
				US	2017217091	A1	03 August 2017
				WO	2017132464	A1	03 August 2017
US	2017140848	A1	18 May 2017	US	10311993	B2	04 June 2019