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(71) Applicant: **TUFTS UNIVERSITY** [US/US]; Ballou Hall, Medford, Massachusetts 02155 (US).

(72) Inventors: **KUO, Catherine K.**; 63 Appleton Street, Unit #2, Boston, Massachusetts 02116 (US). **BROWN, Jeffrey P.**; 46 East Albion Street, Somerville, Massachusetts 02145 (US).

(74) Agent: **OCCHIUTI, Frank R.**; Occhiuti & Rohlicek LLP, 321 Summer Street, Boston, Massachusetts 02210 (US).

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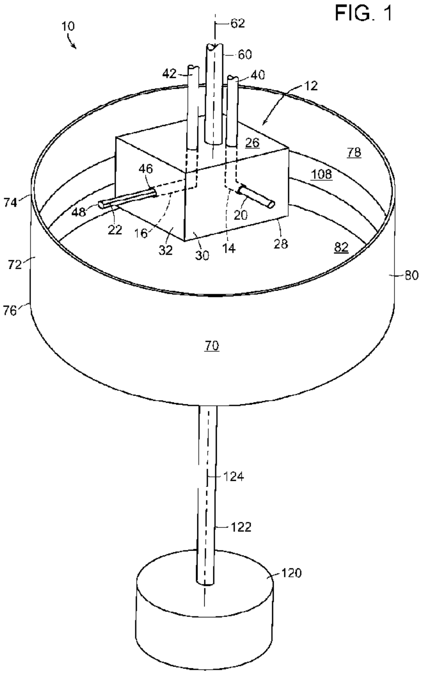
(54) **Title**: APPARATUS AND METHOD FOR FORMING A NANOFIBER HYDROGEL COMPOSITE

FIG. 1



(57) **Abstract**: A method for making an electrospun nano- or micro-fiber hydrogel composite includes a platform suspended within a housing and a motor connected to the housing or platform so as to cause rotation of the housing relative to the platform, or vice versa. The platform supports nozzles that are directed toward a collector formed on an interior surface of the housing. At least one nozzle is configured to discharge an electrospun fiber, and at least one other nozzle is configured to discharge a gellable solution. After multiple revolutions of the housing, the electrospun fiber forms a scaffold that is interspersed with hydrogel and the other discharged substances. This novel method of fabrication yields composites consisting of a variety of materials, structures, biological and chemical components, and mechanical properties that will have utility in a variety of fields including regenerative medicine, tissue engineering, drug delivery, wound healing and more.

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APPARATUS AND METHOD FOR FORMING A NANOFIBER HYDROGEL COMPOSITE

CROSS REFERENCE TO RELATED APPLICATION

[001] This application claims priority to U.S. Provisional Application No. 61/825,191, filed on May 20, 2013, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[002] One goal of tissue engineering is to generate replacement materials for transplantation into animals as a replacement for damaged or diseased tissues. In some cases, tissues are fabricated in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for a construct which permits loading of drugs, protein, chemicals, and cells into a semi-solid shape for implantation. Hydrogels have been developed to serve this purpose and have the advantage of being able to encapsulate cells and biologically active molecules in a spatially controlled manner, but many hydrogels are easily deformed and lack sufficient integrity, strength and rigidity to remain in place or withstand forces applied in vivo. Nano- and micro-fiber scaffolds for supporting cells and other substances are also favorable as scaffold structures, and can be fabricated with greater mechanical properties, but face the challenge of uniform cell incorporation and integration throughout the nano- or mirco-fiber scaffold either during fabrication or post-fabrication. The fabrication of a nanofiber-hydrogel composite in which each material is uniformly distributed throughout the composite scaffold is desirable to blend the best attributes of each, but is challenging to achieve. This invention offers a novel apparatus and method to achieving a nanofiber-hydrogel composite structure in which components of the composite structure are uniformly integrated amongst each other.

SUMMARY

[003] In some aspects, an apparatus for forming an electrospun nano- or micro-fiber hydrogel composite is provided. The apparatus includes a platform, a housing including

a sidewall that surrounds a periphery of the platform, and motor connected to one of the housing and the platform so as to rotate the one of the housing and the platform relative to the other of the housing and the platform about a rotational axis that extends parallel to the sidewall of the housing. The apparatus also includes a nozzle configured to discharge an electrospun fiber, the nozzle supported by the platform and including a discharge end that faces an interior surface of the housing, and an input end configured to receive a dischargeable substance.

[004] The apparatus may include one or more of the following features: The platform is stationary, and the housing rotates about the platform. The housing is stationary, and the platform rotates within the housing. The platform is suspended within the housing. The apparatus further includes a power supply connected to the nozzle, the power supply configured to provide the nozzle with an electrical charge. The nozzle and at least a portion of the interior surface of the housing are at different electrical potentials. The nozzle is at a higher electrical potential than the portion of the interior surface of the housing. The nozzle is a single-lumen nozzle. The nozzle is double-lumen nozzle in which the lumens are arranged coaxially. The nozzle includes a longitudinal axis that extends in a direction transverse to the rotational axis. The nozzle includes a first nozzle longitudinal axis, and the apparatus further includes a second nozzle supported by the platform, the second nozzle including a second nozzle longitudinal axis that lies in the same plane as the first nozzle longitudinal axis or in a plane that is parallel to a plane defined by the first nozzle longitudinal axis, and is non-parallel relative to the first nozzle longitudinal axis. The apparatus further includes a third nozzle supported by the platform, the third nozzle including a third nozzle longitudinal axis that lies in the same plane as the first nozzle longitudinal axis or in a plane that is parallel to a plane defined by the first nozzle longitudinal axis, and is non-parallel relative to one of the first nozzle longitudinal axis and the second nozzle longitudinal axis.

[005] The apparatus may also include one or more of the following additional features: The apparatus further includes a feed device connected to the nozzle using tubing, the feed device configured to feed a solution to the nozzle. The feed device includes a

syringe pump. The feed device includes a rotary pump. The feed device includes a compressor that is configured to apply air pressure within the tubing. The feed device includes a pressurized fluid storage tank that is configured to apply fluid pressure within the tubing. The housing sidewall includes an inner surface arranged to face the platform, the housing sidewall bounded by a first end and a second end that is spaced apart from the first end, the inner surface comprising a metal substrate coated with an insulation layer, wherein the insulation layer is discontinuous such that a region of the metal substrate is exposed, the region extending continuously about a circumference of the housing sidewall at a location spaced apart from each of the first end and the second end. The region is recessed. The nozzle is oriented relative to the region so as to direct a discharged substance toward the region. The region is located relative to the housing sidewall so as to include a line defined by the intersection of the housing sidewall with a plane that includes the nozzle. The housing sidewall includes an inner surface arranged to face the platform, the housing sidewall bounded by a first end and a second end that is spaced apart from the first end, the inner surface including a non-electrically conducting substrate and an electrically-conductive band disposed on the substrate, the band extending continuously about a circumference of the sidewall at a location spaced apart from each of the first end and the second end, and the band is at a different electric potential than the nozzle. The band is recessed relative to an interior-facing surface of the substrate. The nozzle is oriented relative to the region so as to direct a discharged substance toward the region.

[006] In some aspects, a method of forming a micro- or nano-fiber hydrogel composite that includes a micro- or nano-fiber scaffold that supports a hydrogel is provided. The method includes providing a strand of micro- or nano-fiber; depositing a first portion of the strand on a receiving surface; and applying the hydrogel to the first portion of the strand. The method further includes depositing another portion of the strand on the receiving surface in an orientation that is generally aligned with the first portion; applying the hydrogel to the another portion of the strand; repeating the depositing another portion step and the applying hydrogel to the another portion step until a three dimensional composite is achieved.

[007] The method may include one or more of the following additional steps and/or features: Applying second substance to the corresponding portion of the strand, the second substance including at least one of a bioactive fluid or chemoactive fluid. The step of applying second substance occurs after each application of the hydrogel. The step of applying second substance occurs before each application of the hydrogel. The fibers are formed of a natural polymer. The fibers are formed of a synthetic polymer. The fibers are formed of a blend of natural polymers and synthetic polymers. The hydrogel includes one or more of a gellable solution, biological cells, biological molecules, proteins, and chemical molecules. The second substance includes a bioactive fluid. The method further includes a step of exposing the scaffold to a chemoactive or gelling agent. The step of exposing the scaffold to a gelling agent occurs concurrently with the depositing and applying steps. The step of exposing the scaffold to a gelling agent occurs after completion of all the depositing and applying steps, whereby the scaffold as a whole is exposed to the gelling agent. The step of exposing the scaffold to a gelling agent occurs before the depositing and applying steps in a manner such that gelation is a timed process that occurs at some time after the application of the gelling agent. The step of exposing the scaffold to a gelling agent comprises exposure to ultra violet light. The step of exposing the scaffold to a gelling agent includes application of crosslinking chemicals to the scaffold.

[008] The method may also include one or more of the following additional steps and/or features: The method further includes providing a second strand of micro- or nano-fiber; depositing a first portion of the second strand on the receiving surface; applying the hydrogel to the first portion of the second strand, depositing another portion of the second strand on the receiving surface in an orientation that is generally aligned with the first portion of the second strand; applying the hydrogel to the another portion of the second strand; and repeating the depositing another portion step and the applying hydrogel to the another portion step. The step of providing a second strand occurs subsequent to the step of providing the first strand. The step of applying the hydrogel occurs continuously.

[009] Advantageously, a device and method are provided to create a fiber-reinforced hydrogel for supporting drugs, protein, chemicals, biological, other materials and cells in a semi-solid form for use as an implantable replacement tissue. By reinforcing the hydrogel with nano- or micro-fibers or a structural polymer, the resulting construct is able to withstand in vivo forces and maintain its shape and integrity after implantation. Similar to natural tissues in the body, the fiber-reinforced hydrogel has both fiber and gel-like components.

[0010] Further advantageously, the hydrogel is injected directly within a nano- or micro-fiber scaffold during its formation. In particular, tissue cells and their supporting substances are injected directly in the construct while it is being formed in such a way that the scaffold and hydrogel are mutually interspersed in a controllable and desirable ratio, and controlled (for example, uniform) distribution of cells throughout the construct is attained. The resulting composite is advantageous over some conventionally engineered tissues that include laminar arrangements of defined fiber layers and gel layers since the layers of the conventional tissues do not always bond well and tend to delaminate.

[0011] Building the cells and hydrogel directly in the construct during formation of the construct in such a way that the scaffold and hydrogel are mutually interspersed as described herein is further advantageous since there is no need to force the hydrogel (before or after gelation) or cells into the nanofiber scaffold secondarily after the nanofiber scaffold has first been formed. This can be compared to some conventionally engineered tissues in which a hydrogel and/or cells are applied to a previously-formed scaffold and in which the hydrogel (before or after gelation) or cells are forced into the interior portions of the scaffold (e.g., via vacuum or centrifugation), or tend to infiltrate poorly to interior portions of the scaffold, for example due to small pore sizes of the scaffold or other reasons.

[0012] Building the cells directly in the construct during formation of the construct in such a way that the scaffold and hydrogel-encapsulating cells are mutually interspersed as described herein is further advantageous since there is no need for the cells to migrate within the scaffold. This can be compared to some conventionally engineered tissues in which cells are applied to a previously-formed scaffold and in which the cells do not migrate to interior portions of the scaffold, or tend to migrate poorly to interior portions of the scaffold, for example due to small pore sizes of the scaffold or other reasons.

[0013] Furthermore, the gel component of the construct allows for loading proteins or drugs or other biological materials that will not be readily washed out of the construct. Conventionally, proteins to be added to a nano- or micro-fiber scaffold have been chemically linked to the polymer before electrospinning or suspended in the solution to be electrospun into nanofibers, both of which can compromise the ability for the solution to be electrospun or form with controllable material properties. In this new system, proteins can be added in both the conventional way and also by adding them to the hydrogel.

[0014] The collector is formed on the interior of a closed-walled structure arranged to surround the nozzle(s) and rotate relative thereto, whereby the substances discharged from the nozzle(s) are applied to an interior surface of the closed-walled structure. For example, the collector may be the interior wall of a cylindrical structure that is placed surrounding the nozzle, and the nozzle is in the center of the cylinder and horizontally equidistant (though not necessarily equidistant) from any point on the interior of the cylindrical structure so that discharge from the nozzle lands on the interior of the cylinder. By providing a device in which the discharged substance is directed to an inward facing surface (e.g. the interior of the cylinder), the shape of the surface in combination with the motion of the surface tends to retain the discharged fluid on the surface. This is in contrast to some conventional electrospinning devices in which a nozzle is placed outside the circumference of the rotating collector, thereby discharging substances onto or toward an outer surface of a rotating drum or conveyor belt, and the inertia of discharged fluid tends to be force the discharged fluid off the outer surface due to the rotation of the surface.

[0015] Among other advantages, the device and method disclosed herein provide the following

- They permit the successful combination of one or more nano- or micro-fiber structural components, hydrogel components, and any desired added chemical or biological components, including live cells. This has immense promise in drug delivery, tissue engineering (for replacement purposes or for ex-vivo tissue/disease modeling purposes), tissue regeneration, cell delivery therapies, wound dressing/sealants/healing, and guides for nerve regeneration.
- The construction of the fiber-reinforced hydrogel composite includes concurrent formation of the fiber scaffold with the hydrogel, which allows for a uniform distribution of components throughout the composite structure, including cells and/or drugs. In some embodiments, addition of the components can also be modulated, for patterning purposes.
- The fibers in this fiber-gel construct are generally aligned in parallel, yielding predictable and anisotropic strength properties.
- The fibers in this fiber-gel composite may also be randomly oriented, or partially aligned in parallel.
- The solutions being used are inherently confined to controllable areas: nano- or micro-fibers are confined by selective PDMS insulation and solutions are confined by the use of centrifugal force and the morphology of the scaffold lay-down area.

[0016] Modes for carrying out the present invention are explained below by reference to an embodiment of the present invention shown in the attached drawings. The above-mentioned object, other objects, characteristics and advantages of the present invention will become apparent from the detailed description of the embodiment of the invention presented below in conjunction with the attached drawings.

BRIEF DESCRIPTION OF THE FIGURES

[0017] Fig. 1 is a perspective view of a portion of an apparatus for forming an electrospun nano- or micro-fiber reinforced hydrogel construct.

[0018] Fig. 2 is a schematic side view of the apparatus for forming an electrospun nano- or micro-fiber reinforced hydrogel construct.

[0019] Fig. 3 is a top view of the portion of the apparatus of Fig. 1.

[0020] Fig. 4 is a schematic side view of an alternative embodiment apparatus for forming an electrospun nano- or micro-fiber reinforced construct.

[0021] Fig. 5 is a schematic side view of another alternative embodiment apparatus for forming an electrospun nano- or micro-fiber reinforced construct.

[0022] Fig. 6 is a side sectional view of a portion of the housing of the apparatus of Fig. 1.

[0023] Fig. 7 is a schematic diagram illustrating the feed assembly for the apparatus of Fig. 1.

[0024] Fig. 8A is an image of an aligned arrangement of electrospun fibers.

[0025] Fig. 8B is an image of a random arrangement of electrospun fibers.

[0026] Fig. 9A is a sectional view of an integrated composite construction of aligned nanofibers and the hydrogel.

[0027] Fig. 9B is a sectional view of a conventional laminar composite construction of nanofibers and the hydrogel.

[0028] Fig. 10 is a side sectional view of a portion of an alternative housing for the apparatus of Fig. 1.

[0029] Fig. 11 is a side sectional view of a portion of another alternative housing for the apparatus of Fig. 1.

[0030] Fig. 12 is a flow diagram illustrating a method for forming a nano-fiber hydrogel composite.

DETAILED DESCRIPTION

[0031] Referring to Figs. 1 and 2, an apparatus 10 for forming an electrospun nano- or micro-fiber hydrogel composite includes a platform 12 suspended within a housing 70. The platform 12 supports nozzles 20, 22, 24 that are directed toward an inner surface 78 of the housing 70. The housing 70 rotates about the platform 12 while substances are discharged from one or more of the nozzles 20, 22, 24 and deposited on a collector 108 provided on the housing inner surface 78, as discussed further below. The apparatus 10 may also include a chamber 1 that encloses the platform 12 and the housing 70 to permit control of the environment in which the composite is formed. For example, the chamber 1 may also enclose a heater 2, a ultra-violet light 3, humidifier 4, etc. as well as corresponding sensors and/or controllers (not shown) to monitor and control the environment within the chamber 1.

[0032] Each nozzle 20, 22, 24 discharges a unique constituent of an engineered tissue or construct, such as a nano- or micro-fiber, hydrogel, cross-linking solution, growth medium and/or other constituent substances. At least one of the nozzles (e.g., 24) is provided at a different electrical potential than the collector 108, so that the substance (e.g., the fiber) discharged from the nozzle is electrospun. Due to the relative movement of the housing 70 with respect to the nozzles 20, 22, 24, the electrospun fiber is deposited on the collector 108 so as to be generally aligned with the circumferential direction of the housing. The hydrogel is discharged from another nozzle (e.g, 22) and is deposited on the collector 108 including the fiber. Another constituent substance such as a bioactive fluid may be discharged from still another nozzle (e.g., 20) and deposited on the fiber and hydrogel. After multiple rotations of the housing 70, a composite is formed on the collector 108 in which the fiber, hydrogel and other constituent substances are each interspersed throughout the thickness of the construct, as discussed further below. The apparatus 10 provides a composite construct in which a hydrogel is reinforced with nano- or micro-fibers or a structural polymer. The resulting construct has sufficient structural integrity to generally maintain its shape and withstand forces after implantation, and the mechanical properties of the composite can be tailored according to its intended use.

[0033] As used herein, the term hydrogel refers to a gellable solution that includes biological cells, biological molecules, proteins, other substances such as polysaccharides (e.g., alginate), synthetic polymers (e.g., polyethylene glycol (PEG)), or natural polymers (e.g., silk), and/or chemical compounds including drugs. Hydrogels are a convenient means for loading protein, chemicals and cells into a semi-solid shape for implantation. However, a gelled hydrogel by itself is generally insufficient for implantation since it often is easily deformed and lacks sufficient strength and rigidity to permit retention in a desired location and/or to withstand forces after implantation. In some embodiments, the hydrogel includes synthetic polymers such as PEG. In other embodiments, the hydrogel includes natural polymers such as silk, collagen, hyaluronic acid, fibrin, or others. In some embodiments, the hydrogel includes polysaccharides such as alginate or others. In some embodiments, the hydrogel includes a blend of any of the above components.

[0034] The housing 70 is a hollow cylinder having a sidewall 72 that includes first edge 74, and a second edge 76 that is opposed to the first edge 74. The housing 70 is open at the first edge 74 and includes a base 82 that closes the housing 70 at its second edge 76.

[0035] The apparatus 10 also includes a motor 120 used to rotate the housing 70 relative to the platform 12. The motor 120 is disposed outside the housing 70, and an output shaft 122 of the motor 120 is fixed to an outer surface 80 of the housing at the center of the housing base 82, so that the rotational axis of the housing 70 is defined by a longitudinal axis 124 of the output shaft 122. In the illustrated embodiment, the motor 120 is a variable speed motor.

[0036] The platform 12 is suspended within the housing 70, for example via a rod 60 having a rod longitudinal axis 62. The platform 12 is rectangular, and therefore includes a first end surface 26, a second end surface 28 opposed to the first end surface 26, and four side surfaces 30, 32, 34, 36 that extend between the first end surface 26 and the second end surface 28. The platform includes the first nozzle 20 that protrudes outward from the first side surface 30, the second nozzle 22 that protrudes outward from the second side surface 32, and the third nozzle 24 that protrudes outward from the third side surface 34. Although the illustrated embodiment includes three nozzles 20, 22, 24, it is understood that a fewer or greater number of nozzles can be supported by the platform 12. The platform 12 also includes passageways 14, 16, 18 that open at the platform first end surface 26 and communicate with a corresponding one of the respective nozzles 20, 22, 24. In the illustrated embodiment, tubes 40, 42, 44 from a feed system (described below) extend through the respective passageway 14, 16, 18 to provide constituent substances to the nozzles. In other embodiments, the tubes 40, 42, 44 may be connected to the corresponding passageway using a luer type connector (not shown).

[0037] The nozzles 20, 22, 24 are provided as separate structures that are independent of the platform and connectable to the platform, permitting individual nozzle selection and customization depending on the substances to be discharged and the desired properties of the discharged substance. In this case, each nozzle 20, 22, 24 is connected to the corresponding passageway 14, 16, 18 in the platform 12, for example via a press fit or a luer lock-type connection. In the illustrated embodiment, the nozzles 20, 22, 24 include a single lumen, and have a lumen diameter selected depending on the substance to be discharged therefrom.

[0038] Referring to Fig. 3, the nozzles are supported within the platform 12 so as to extend in a direction transverse to the rod axis 62. More specifically, the nozzles 20, 22, 24 lie generally within a single plane P (shown in Fig. 6), extend radially outward relative to the rod axis 62, and are angled relative to each other about the rod axis 62. In the illustrated embodiment, the second nozzle 22 is oriented at a first angle θ1 of 90 degrees relative to the first nozzle 20, and the third nozzle 24 is angled at a second angle θ2 of 180 degrees relative to the first nozzle 20. Each nozzle includes an input end 46 configured to be connected to the platform 12 and/or the corresponding feed tube 40, 42, 44, and a discharge end 48 that faces an interior surface 78 of the housing 70.

[0039] Referring to again to Fig. 2, in the illustrated embodiment the apparatus 10 is arranged so that the longitudinal axis 62 of the rod 60 that supports the platform 12 is coaxial with the rotational axis 124 of the housing. In this configuration, it is possible (but not required) to have an arrangement of nozzles 20, 22, 24 in which the discharge end of each nozzle is equidistant from the interior surface 78 of the housing 70. In addition, the rod 60 is fixed in space, and the housing 70 is driven by the motor 120 to rotate about the platform 12. Since the platform 12 is stationary, this arrangement advantageously simplifies connection to a feed system 140 (shown in Fig. 7) that provides constituent substances to the nozzles 20, 22, 24 via the tubes 40, 42, 44.

[0040] Referring to Fig. 4, it is understood that the apparatus 10 could have an alternative arrangement in which the housing 70 is fixed in space, and platform 12 is connected to the output shaft 122 of the motor 120, and driven by the motor 120 to rotate within the housing 70. In this case, the feed system 140 could also be rotated along with the platform 12, or could be connected thereto using rotary fluid couplings.

[0041] Referring to Fig. 5, it is also understood that there is no requirement that the apparatus 10 be arranged so that the longitudinal axis 62 of the rod 60 that supports the platform 12 is coaxial with the rotational axis 124 of the housing. For example, in some embodiments, the longitudinal axis 62 of the rod 60 is parallel and offset relative to the rotational axis 124 of the housing 70 (Fig. 5). Since some properties of an electrospun fiber, for example fiber diameter, depend in part upon the distance between the nozzle discharge end 48 and collector 108, this arrangement permits the relative offset between the rod axis 62 and the rotational axis 124 to be optimized for a particular application.

[0042] Referring to Fig. 6, the housing 70, including the sidewall 72 and base 82, is formed of metal, for example stainless steel. The interior surface 78 of the sidewall 72 and base 82 are coated with a layer 102 of an electrically insulating material, for example polydimethylsiloxane (PDMS), except within a circumferentially extending region 110 that is located between the sidewall first edge 74 and the sidewall second edge 76. Within the region 110, the metal substrate is exposed, forming a collector 108 to receive electrospun substances and other materials discharged from the nozzles 20, 22, 24. The housing 70 is electrically grounded to promote collection of electrospun materials on the collector 108, as discussed further below.

[0043] Because the region 110 results from a discontinuity in the PDMS coating, the collector 108 is recessed relative to an inner surface of the PDMS coating, and extends continuously about the circumference of the sidewall 72 to form a ring thereon. Providing a collector 108 that is recessed is advantageous, since the recess-facing edges 102a of the PDMS serve to retain the discharged substances in the vicinity of the collector 108 despite the rotation of the housing 70.

[0044] The platform 12 is suspended within the housing 70 so that the nozzles 20, 22, 24 are oriented so as to direct a discharged substance toward the recessed region 110, and particularly toward the collector 108. In the illustrated embodiment, the platform 12 is located relative to the housing sidewall 72 so that a line defined by the intersection of the housing sidewall 72 with the plane P that includes the nozzles 20, 22, 24 resides within the region 110.

[0045] As discussed above, at least one nozzle is provided at different electrical potential than the collector 108, and this nozzle is used to provide an electrospun substance. In the illustrated embodiment, the third nozzle 24 is electrically connected to a high voltage, direct current power supply 160a, and the collector 108 is electrically connected to ground. For example, in some embodiments, the power supply 160a provides a relative charge of up to± 60 kV to the third nozzle 24. In operation, the third nozzle 24 is fed with a fiber-forming material via tube 44, whereby a very fine (e.g. micro scale or nano scale) fiber is drawn from the nozzle 24 and collected on the collector 108. Forming fine fibers by electrospinning is advantageous not just because of the scale of the fiber diameters, unattainable by conventional methods, but also since the process does not require coagulation chemistry or high temperatures to produce solid threads from solution, although it is possible to spin a fiber from a melted substance to avoid use of solvents.

[0046] The fiber-forming material that is used may include one or more natural polymers, synthetic polymers, polysaccharides, or a blend of any of the above. Exemplary natural polymers include silk fibroin, collagen, elastin, or other similar natural polymers. Exemplary synthetic polymers include polyethylene oxide, polycaprolactone, polylactic acid, polyglycolic acid, poly-lactic-glycolic acid, polyethylene glycol, or other similar synthetic polymers. In addition, exemplary polysaccharides include alginate and chitosan. In the illustrated embodiment, the fiber-forming material is provided in solution. In some cases, the solution is formed by melting the fiber-forming material, and in other cases the fiber-forming material is dissolved in a solvent to prepare for electrospinning.

[0047] Material is electrospun from the third nozzle 24 in a radially outward direction. It is directed toward the housing interior surface 78, and, due to the positive charge induced by the third nozzle 24, is physically attracted to the relatively-negatively charged collector 108 that extends circumferentially about the housing interior surface 78. During travel of the fiber between the nozzle 24 and the collector 108, the material solvents are evaporated away and the material deposits on the collector 108 in the form of solid polymer fibers. Since the housing sidewall 72 is curved and moving relative to the spun substance (e.g. the nano- or micro-fiber), the spun substance is collected and maintained against the housing interior surface 78.

[0048] Referring to Fig. 7, the apparatus 10 also includes a feed device 140 configured to feed a constituent substance to each respective nozzle 20, 22, 24. In the illustrated embodiment, each nozzle 20, 22, 24 receives a unique constituent substance, although one of skill in the art understands that multiple nozzles can receive the same constituent substance. For each nozzle 20, 22, 24, the feed device 140 includes a fluid reservoir 152 that includes a constituent substance, a supply line 142a, 142b, 142c that includes a fluid pump 144 and corresponding valve(s) 146a, 146b, 146c and delivers the constituent substance to the nozzle via the corresponding tube 40, 42, 44. A power supply 160 is provided for one or more nozzles. In the illustrated embodiment, a power supply 160a is provided for the third nozzle 24, and is electrically connected to the corresponding nozzle, for examplevia a wire 162, so that if desired, the nozzle can be provided at a potential difference relative to the collector 108 of the housing 70. A controller 148a, 148b, 148c is provided for each nozzle 20, 22, 24. The controller 148a, 148b, 148c controls the fluid pump 144a and valve(s) 146a, 146b, 146c to supply the constituent substance at the desired discharge volume and rate. The controller 148a, 148b, 148c also controls the power supply 160a. Thus, in this configuration, the discharge of each nozzle 20, 22, 24 is individually controlled.

[0049] The pump 144a that is used within the supply line 142a depends on the constituent substance flowing within the supply line 142. For example, the pump 144a may be a rotary pump or a syringe pump. In some embodiments, the pump 144a within one of the supply lines may be replaced with a compressor, permitting the constituent substance to be discharged using air pressure. As shown in the illustrated embodiment, use of air or gas pressure to discharge substances can also be achieved by omitting the pump 144a, and storing the constituent substance in a pressurized storage tank (e.g. tank 152b, 152c). The pressure within the storage tank 152b, 152c is maintained by a pressure source 151b, 151c and is controlled by the corresponding valve 150b, 150c via its respective controller 148b, 148c. Flow to the nozzle can be further modulated via the controller 148b, 148c through the valve 146b, 146c.

[0050] During operation, the feed device 140 is controlled so that constituent substances are fed to the nozzles 20, 22, 24 and discharged toward the collector 108. In the illustrated embodiment, the third nozzle 24 is configured to discharge an electrospun nano-fiber, the second nozzle 22 is configured to discharge the hydrogel, including a gellable solution as well as cells, proteins and other substances, and the first nozzle 20 is configured to discharge a growth medium, cross-linker or other solution.

[0051] Referring to Figs. 8A and 8B, during each revolution of the housing 70, the electrospun nano-fiber is collected on the collector along with the hydrogel and other discharged substances. Due to the rotation of the housing, the electrospun nanofiber(s) is generally aligned with the circumferential direction of the housing 70. The accumulated fibers are generally aligned in parallel (Fig. 8A), yielding predictable and anisotropic strength properties. This can be compared to an electrospun construct in which there is no relative motion between the nozzle 24 and the collector 108, yielding a random fiber arrangement (Fig. 8B) that may have unpredictable and isotropic strength properties.

[0052] Referring to Figs. 9A and 9B, after multiple revolutions of the housing 70, the electrospun nano-fiber forms a scaffold that is interspersed with hydrogel and the other discharged substances (Fig. 9A). Thus, the resulting construct will be formed from the bottom up and include an integrated composite construction of aligned nanofibers and the hydrogel. Rather than forming discrete, and perhaps alternating, layers of fiber and hydrogel as found in some conventional fiber-reinforced hydrogel constructs (Fig. 9B), the composite formed in the apparatus 10 has no defined layers, and the hydrogel, being co-formed with the fibers, is well interspersed within, and around, the fibers.

[0053] The gellable solution included in the hydrogel can be gelled incrementally during composite construction, for example by using a gelling agent. As used herein, the term gelling agent refers to chemical agents such as cross-linking chemicals such as calcium carbonate, calcium, chloride, calcium sulfate, glutaraldehyde, acyl azides, glycidyl ethers, diisocyantes, polyethylene glycol diglycidyl ether, pH-altering chemicals and others. Gelling agent can also refer to biological molecules, cells, or enzymes. Gelling agent can also refer to external processes such as application of electromagnetic radiation, heat, electrical current, physical entanglement or physical agitation. Alternatively, the gellable solution can be gelled as a whole after the constituent substances are assembled into the composite. Alternatively, the gellable solution can be pretreated with a gelling agent or gellation process such that gellation occurs as a function of time and is independent of the composite forming steps such that gellation occurs during or after the composite forming steps without the application of a gelling agent as part of the composite formation process.

[0054] By discharging the constituent solutions on the inside of the rotating, closed-sidewall housing 70 and specifically toward the recessed surface 108, centrifugal force will keep the discharged solutions against the surface 108, which is particularly advantageous for non-electrically charged constituent substances that will not be attracted to the collector 108. Moreover, since the surface 108 is recessed, liquid or gellable substances are prevented from creeping away from the target surface 108 during rotation. In some embodiments, partial or complete gelation of the gel solution will be done during composite construction, that is, while the housing 70 is rotating.

[0055] Referring to Fig. 10, an alternative embodiment housing 170 can be used to replace the above described housing 70. Like the above described housing 70, the housing 170 is a hollow cylinder having a sidewall 172 that includes first edge 174, and a second edge 176 that is opposed to the first edge 174. The housing 170 is open at the first edge 174 and includes a base 182 that closes the housing 170 at its second edge 176. However, the sidewall 172 and base 182 of the housing 170 are formed of a non-conductive material, for example plastic. A metal strip 184 is provided on the interior surface 178 of the sidewall 172 at a location midway between the sidewall first edge 174 and the sidewall second edge 176. The metal strip 184 forms a ring about the interior surface 178, and serves as a collector that receives electrospun substances and other materials discharged from the nozzles 20, 22, 24. The metal strip 184 is electrically grounded to promote collection of electrospun materials, as discussed above.

[0056] Referring to Fig. 11, another alternative embodiment housing 270 can be used to replace the above described housing 70. Like the above described housing 70, the housing 270 is a hollow cylinder having a sidewall 272 that includes first edge 274, and a second edge 276 that is opposed to the first edge 274. The housing 270 is open at the first edge 274 and includes a base 282 that closes the housing 270 at its second edge 276. Like the previous embodiment housing 170, the sidewall 272 and base 282 of the housing 270 are formed of a non-conductive material, for example plastic. In this case, the metal strip 284 is provided within a recess 206 formed on the interior surface 278 of the sidewall 272. The recess 206 is located between the sidewall first edge 274 and the sidewall second edge 276. The metal strip 284 serves as a collector that receives electrospun substances and other materials discharged from the nozzles 20, 22, 24. Providing a metal strip (e.g., collector) 284 that is recessed is advantageous, since the recess-facing edges 272a of the sidewall 272 serve to retain the discharged substances in the vicinity of the metal strip 284 despite the rotation of the housing 70.

[0057] The metal strip 284 is electrically grounded to promote collection of electrospun materials, as discussed above. In the illustrated embodiment, this is accomplished by providing a circumferentially-extending recess 208 in the outward facing surface 280 of the sidewall 270, and disposing a second metal strip 286 in the recess. The outward facing surface 288 of the second metal strip 286 resides flush with or protrudes from the outward facing sidewall surface 280. One or more conductors 290 extend through the thickness of the sidewall 272 at spaced locations about the sidewall circumference so as to electrically connect the second metal strip 286 to the metal strip 284 provided on the interior surface 278. The second metal strip 286 is an annular member that extends along the entire circumference of the outward facing surface 280, and is used to simplify control of the electric potential of the interior metal strip (collector) 284. For example, the interior metal strip (collector) 284 can be grounded during rotation of the housing 270 by touching a wire-brush type connector 292 of a grounded wire 294 to the second metal strip 286.

[0058] Referring to Fig. 10, a method of using the apparatus 10 to form a micro- or nano-fiber hydrogel composite that includes a micro- or nano-fiber scaffold that supports a hydrogel will now be described. The method includes the following steps:

[0059] Provide a strand of micro- or nano-fiber by electrospinning a fiber material from the third nozzle 24 (300). During formation of the composite, the fiber material is continuously discharged from the third nozzle 24.

[0060] Deposit a first portion of the strand on the collector 108 (step 301) by providing the collector 108 at a different electric potential than the third nozzle 24, and directing the first nozzle toward the collector 108. The electrically charged electrospun fiber material discharged from the third nozzle 24 is attracted to the collector 108. In the apparatus 10, the housing 70 is rotating relative to the platform 12 that supports the nozzles 20, 22, 24. Due to the motion of the collector 108 relative to the third nozzle 24, the deposited fiber is generally aligned on the collector in the direction of movement.

[0061] As the housing 70 rotates, the first portion of the strand deposited on the collector 108 moves to a location corresponding to the discharge end of the second nozzle 22.

[0062] Apply the hydrogel to the first portion of the strand (step 302) by discharging the hydrogel from the second nozzle 22 toward the collector 108. The hydrogel is thereby applied to the first portion of the strand. During formation of the composite, the hydrogel is continuously discharged from the second nozzle 22.

[0063] As the housing 70 rotates, the first portion of the strand deposited on the collector 108, as well as the hyrogel applied to the first portion, move to a location corresponding to the discharge end of the first nozzle 20.

[0064] Apply another constituent substance of the composite to the first portion of the strand (step 303) by discharging the constituent substance from the first nozzle 20 toward the collector 108. The constituent substance discharged from the first nozzle 20 is thereby applied to the strand and the hydrogel. In some embodiments, the constituent substance discharged from the first nozzle 20 is a bioactive fluid configured to change cellular behavior. In other embodiments, the constituent substance discharged from the first nozzle 20 may include one or more of a growth medium, a crosslinker or other solution beneficial for formation of the composite. During formation of the composite, the constituent substance is continuously discharged from the first nozzle 20.

[0065] As the housing 70 continues to rotate, the first portion of the strand deposited on the collector 108 and including the hydrogel and additional constituent substance moves to a location corresponding to the discharge end of the third nozzle 24, completing one full revolution of the housing 70.

[0066] Deposit another portion of the strand on the collector 108 (step 304). Since the fiber material is continuously discharged from the third nozzle 24, this portion of the strand is deposited on the collector 108 in the vicinity of the first portion. Due to the rotation of the housing 70, this portion of the strand is deposited on the collector 108 in an orientation that is generally aligned with the first portion.

[0067] As the housing 70 rotates, the two deposited portions of the strand, as well as the previously applied hydrogel and other constituent substance, move to a location corresponding to the discharge end of the second nozzle 22.

[0068] Apply hydrogel to the deposited portions of the strand (step 305) by discharging the hydrogel from the second nozzle 22 toward the collector 108. The hydrogel is thereby applied to the deposited portions of the strand.

[0069] As the housing 70 rotates, the two deposited portions of the strand, the two applied portions of hydrogel, and the applied constituent substance move to a location corresponding to the discharge end of the first nozzle 20.

[0070] Apply additional constituent substance to the deposited portions of the strand (step 306) by discharging the constituent substance from the first nozzle 20 toward the collector 108.

[0071] As the housing 70 continues to rotate, the two deposited portions of the strand as well as the hydrogel and additional constituent substance moves to a location corresponding to the discharge end of the third nozzle 24, completing two full revolutions of the housing 70.

[0072] Repeat the steps of depositing another portion of the strand on the collector, applying hydrogel to the deposited portions of the strand, and applying additional constituent substance to the deposited portions of the strand (step 307), forming a three-dimensional micro- or nano-fiber hydrogel composite that includes a micro- or nano-fiber scaffold that supports a hydrogel.

[0073] Although the step of applying the other constituent substance occurs following the application of the hydrogel, the method is not limited to this ordering of steps. For example, in some embodiments, the step of applying the other constituent substance occurs before the application of the hydrogel.

[0074] In addition, although the current method includes continuous deposit of the fiber and application of hydrogel and other constituent substance to the collector 108, the method is not limited to this. For example, the fiber can be deposited continuously, while one or both of the hydrogel and other constituent substance may be applied intermittently.

[0075] Gelate the three-dimensional micro- or nano-fiber hydrogel composite (step 308).

[0076] As discussed above, in some embodiments, the gellable solution included in the hydrogel is partially or completely gelled subsequent to formation of the composite. This can be achieved by exposing the composite as a whole to a gelling agent.

[0077] Alternatively, in some embodiments, the gellable solution included in the hydrogel is partially or completely gelled incrementally during composite construction, for example by using a gelling agent. In these embodiments, the method further includes a step of exposing the deposited strands and applied hydrogel and other constituent substance to a gelling agent. For example, in some embodiments, the other constituent substance applied in steps 4 and 7 is the gelling agent (e.g, a crosslinking solution). In other embodiments, the gelling agent is applied as an additional substance (e.g, via a fourth nozzle). In still other embodiments, a electromagnetic radiation is applied to the construct intermittently during its formation, for example following steps 3 and 6 or is applied continuously during all steps. In still other embodiments, heat is applied to the construct intermittently or continuously during its formation, or after its formation. In still other embodiments, electrical current is applied to the construct after its formation. The manner of crosslinking of the hydrogel varies according to what is appropriate for the constituents of the hydrogel and includes but is not limited to the addition of chemical compounds, biological molecules, cells or enzymes, electromagnetic radiation, heat, changes in pH, and sonication.

[0078] Upon completion of formation of the composite material, the rotation of the housing 70 is stopped and the composite material is removed from the collector 108 (step 309), for example by peeling it off the collector 108. In some cases, completion of hydrogel gelation, and/or cell culture may be performed in a separate system.

[0079] Selected illustrative embodiments of the apparatus and method are described above in some detail. While this working example of the present invention has been described above, the present invention is not limited to the working example described above, but various design alterations may be carried out without departing from the present invention as set forth in the claims.

[0080] Although the method described here employs only a single electrospinning nozzle, the method is not limited to this configuration. For example, more than one nozzle can be configured to discharge an electrospun fiber. In some embodiments, both elecrospun fibers may be formed of the same material. In other embodiments, the electrospun fibers may be formed of different materials. In addition, in some embodiments, a single electrospinning nozzle can be operated intermittently to provide multiple fibers within the same construct.

[0081] Although the platform 12 is disclosed as being rectangular, the platform 12 is not limited to this configuration. For example, the platform may be a short cylinder, a sphere, an ellipsoid or other convenient shape for supporting the nozzles, passageways and connectors for connecting to feed tubing that are required for discharging the constituents of the composite.

[0082] In the illustrated embodiment, the nozzles 20, 22, 24 include a single lumen. However, it is understood that one or more of the nozzles 20, 22, 24 can be provided with a dual, coaxially arranged lumens, for example to provide an electrospun fiber having a core material surrounded by a sheath material.

[0083] In the illustrated embodiment, the nozzles 20, 22, 24 are formed as separate structures from the platform 12. However, in other embodiments, the nozzles 20, 22, 24 may be formed integrally with the platform 12.

[0084] Although the nozzles 20, 22, 24 are disclosed as being arranged such the second nozzle 22 is oriented at a first angle θ1 of 90 degrees relative to the first nozzle 20, and the third nozzle 24 is angled at a second angle θ2 of 180 degrees relative to the first nozzle 20, the nozzles 20, 22, 24 are not limited to this configuration. For example, in some embodiments the nozzles may be arranged so that the angles θ1 and θ2 may be greater or smaller, and in other embodiments the nozzles may be arranges so that the nozzles are mutually parallel and discharge in the same direction (e.g., angles θ1=θ2=0).

[0085] Although the nozzles 20, 22, 24 are disclosed as being generally coplanar, the nozzles 20, 22, 24 are not limited to being arranged to lie in a single plane P, and may instead be arranged to lie in parallel planes, or non-parallel planes. Moreover, the nozzles 20, 22, 24 are not limited to being oriented transverse to the rod axis 62, and instead may be angled relative to this transverse direction, so long as the nozzles are arrange to discharge in the direction of the collector 108.

[0086] In the illustrated embodiment, each nozzle 20, 22, 24 discharges a unique constituent of the engineered construct. However, the apparatus 10 is not limited to this configuration, and in some embodiments, multiple nozzles can provide the same constituent. For example, multiple nozzles can provide the electrospun fiber and be arranged in an alternating manner with other nozzles that provide hydrogel and/or other construct constituents.

[0087] In addition, it should be understood that only structures considered necessary for clarifying the present invention have been described herein. Other conventional structures, and those of ancillary and auxiliary components of the system, are assumed to be known and understood by those skilled in the art.

What is claimed is,

1. An apparatus for forming an electrospun nano- or micro-fiber hydrogel composite, the apparatus comprising

a platform,

a housing including a sidewall that surrounds a periphery of the platform;

a motor connected to one of the housing and the platform so as to rotate the one of the housing and the platform relative to the other of the housing and the platform about a rotational axis that extends parallel to the sidewall of the housing,

a nozzle configured to discharge an electrospun fiber, the nozzle supported by the platform and including

a discharge end that faces an interior surface of the housing, and

an input end configured to receive a dischargeable substance.

2. The apparatus of claim 1, wherein the platform is stationary, and the housing rotates about the platform.

3. The apparatus of claim 1, wherein the housing is stationary, and the platform rotates within the housing.

4. The apparatus of claim 1, wherein the platform is suspended within the housing.

5. The apparatus of claim 1, wherein the apparatus further includes a power supply connected to the nozzle, the power supply configured to provide the nozzle with an electrical charge.

6. The apparatus of claim 1, wherein the nozzle and at least a portion of the interior surface of the housing are at different electrical potentials.

7. The apparatus of claim 1, wherein the nozzle is at a higher electrical potential than the portion of the interior surface of the housing.

8. The apparatus of claim 1, wherein the nozzle is a single-lumen nozzle.

9. The apparatus of claim 1, wherein the nozzle is double-lumen nozzle in which the lumens are arranged coaxially.

10. The apparatus of claim 1, wherein the nozzle is triple (or greater)-lumen nozzle in which the lumens are arranged triaxially (or greater).

11. The apparatus of claim 1, wherein the nozzle includes a longitudinal axis that extends in a direction transverse to the rotational axis.

12. The apparatus of claim 1, wherein the nozzle comprises a first nozzle longitudinal axis, and the apparatus further includes a second nozzle supported by the platform, the second nozzle including a second nozzle longitudinal axis that lies in the same plane as the first nozzle longitudinal axis or in a plane that is parallel to a plane defined by the first nozzle longitudinal axis, and is non-parallel relative to the first nozzle longitudinal axis.

13. The apparatus of claim 12, wherein the apparatus further comprises a third nozzle supported by the platform, the third nozzle including a third nozzle longitudinal axis that lies in the same plane as the first nozzle longitudinal axis or in a plane that is parallel to a plane defined by the first nozzle longitudinal axis, and is non-parallel relative to one of the first nozzle longitudinal axis and the second nozzle longitudinal axis.

14. The apparatus of claim 13, wherein the apparatus further comprises additional nozzles supported by the platform each with a nozzle longitudinal axis that lies in the same plane as another nozzle longitudinal axis or in a plane that is parallel to a plane defined by another nozzle longitudinal axis, and may be parallel or not parallel relative to the longitudinal axis of another nozzle.

15. The apparatus of claim 1, wherein the apparatus further comprises a feed device

connected to the nozzle using tubing, the feed device configured to feed a solution to the nozzle.

16. The apparatus of claim 15, wherein the feed device includes a syringe pump.

17. The apparatus of claim 15, wherein the feed device includes a rotary pump.

18. The apparatus of claim 15, wherein the feed device includes a compressor that is configured to apply air pressure within the tubing.

19. The apparatus of claim 15 wherein the feed device includes a pressurized fluid storage tank that is configured to apply fluid pressure within the tubing.

20. The apparatus of claim 1, wherein the housing sidewall comprises an inner surface arranged to face the platform, the housing sidewall bounded by a first end and a second end that is spaced apart from the first end, the inner surface comprising a metal substrate coated with an insulation layer, wherein the insulation layer is discontinuous such that a region of the metal substrate is exposed, the region extending continuously about a circumference of the housing sidewall at a location spaced apart from each of the first end and the second end.

21. The apparatus of claim 20, wherein the region is recessed.

22. The apparatus of claim 20, wherein the nozzle is oriented relative to the region so as to direct a discharged substance toward the region.

23. The apparatus of claim 20, wherein the region is located relative to the housing sidewall so as to include a line defined by the intersection of the housing sidewall with a plane that includes the nozzle.

24. The apparatus of claim 1, wherein the housing sidewall comprises an inner surface

arranged to face the platform, the housing sidewall bounded by a first end and a second end that is spaced apart from the first end, the inner surface comprising a non-electrically conducting substrate and an electrically-conductive band disposed on the substrate, the band extending continuously about a circumference of the sidewall at a location spaced apart from each of the first end and the second end, and the band is at a different electric potential than the nozzle.

25. The apparatus of claim 24, wherein the band is recessed relative to an interior-facing surface of the substrate.

26. The apparatus of claim 24, wherein the nozzle is oriented relative to the region so as to direct a discharged substance toward the region.

27. A method of forming a micro- or nano-fiber hydrogel composite that comprises a micro- or nano-fiber scaffold that supports a hydrogel, the method comprising providing a strand of micro- or nano-fiber; depositing a first portion of the strand on a receiving surface; applying the hydrogel to the first portion of the strand, depositing another portion of the strand on the receiving surface in an orientation that is generally aligned with the first portion; applying the hydrogel to the another portion of the strand; and repeating the depositing another portion step and the applying hydrogel to the another portion step until a three dimensional composite is achieved.

28. The method of claim 27 wherein the composite consists of more than one type of fiber being deposited and/or more than one type of hydrogel being deposited.

29. The method of claim 27 wherein there are more than one fibers being deposited that may or may not be composed of the same materials.

30. The method of claim 27 wherein there are more than one hydrogels being deposited that may or may not be composed of the same materials.

31. The method of claim 27 further comprising a step of applying second substance to the corresponding portion of the strand, the second substance comprising at least one of a bioactive fluid or chemoactive fluid.

32. The method of claim 31 wherein the step of applying second substance occurs after each application of the hydrogel.

33. The method of claim 31 wherein the step of applying second substance occurs before each application of the hydrogel.

34. The method of claim 27 wherein the fibers are formed of a natural polymer.

35. The method of claim 27 wherein the fibers are formed of a synthetic polymer.

36. The method of claim 27 wherein the fibers are formed of a blend of natural polymers and synthetic polymers.

37. The method of claim 27 wherein one or more of the fiber-forming materials contains cells or other bioactive materials.

38. The method of claim 27 wherein one or more of the fiber-forming materials contains chemoactive materials

39. The method of claim 27 wherein the hydrogel includes one or more of a gellable solution, biological cells, biological molecules, proteins, and chemical molecules.

40. The method of claim 27 wherein one or more of the hydrogels has either the same composition as other hydrogels or differs in composition than other hydrogels by any of the gellable solution, biological cells, biological molecules, proteins, chemical molecules or other constituents.

41. The method of claim 27 wherein one or more of the nanofibers has either the same composition as other nanofibers or differs in composition than other nanofibers by any of the polymer solution, biological cells, biological molecules, proteins, chemical molecules or other constituents.

42. The method of claim 27 wherein the second substance includes a bioactive fluid.

43. The method of claim 27 further comprising a step of exposing the scaffold to a chemoactive or gelling agent.

44. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent occurs concurrently with the depositing and applying steps.

45. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent occurs after completion of all the depositing and applying steps, whereby the scaffold as a whole is exposed to the gelling agent.

46. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent occurs before the depositing and applying steps in a manner such that gelation is a timed process that occurs at some time after the application of the gelling agent.

47. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent comprises exposure to ultra violet light.

48. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent comprises application of crosslinking chemicals to the scaffold.

49. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent comprises changing temperature, humidity or other environmental conditions.

50. The method of claim 43 wherein the step of exposing the scaffold to a gelling agent

comprises changing the pH within or surrounding the scaffold.

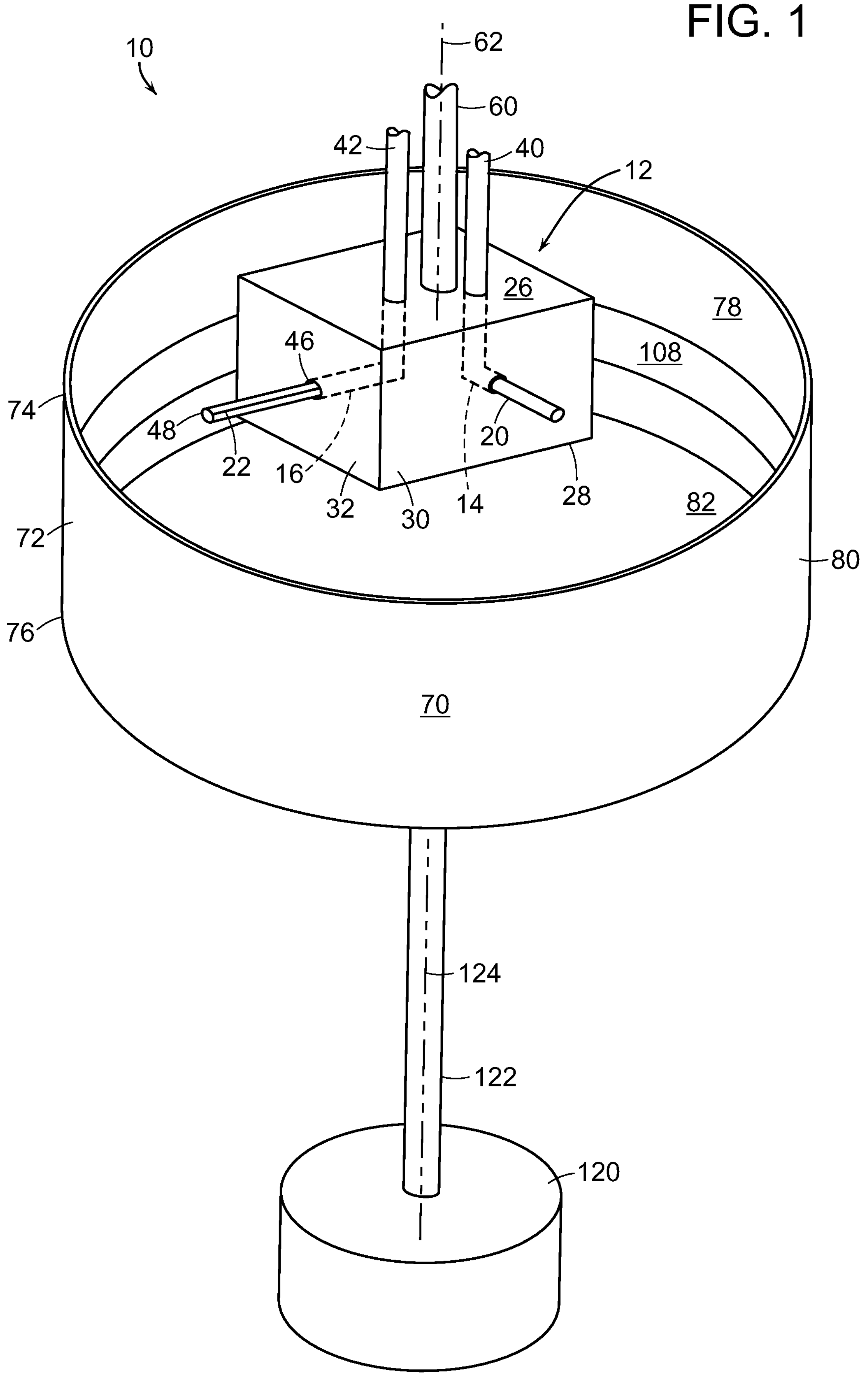
51. The method of claim 43 further comprising providing a second strand of micro- or nano-fiber; depositing a first portion of the second strand on the receiving surface; applying the hydrogel to the first portion of the second strand, depositing another portion of the second strand on the receiving surface in an orientation that is generally aligned with the first portion of the second strand; applying the hydrogel to the another portion of the second strand; repeating the depositing another portion step and the applying hydrogel to the another portion step.

52. The method of claim 51 wherein the step of providing a second strand occurs subsequent to the step of providing the first strand.

53. The method of claim 51 wherein the step of applying the hydrogel occurs continuously.

54. The method of claim 46 wherein the step of applying the hydrogel occurs intermittently or for a specific period of time.

FIG. 1



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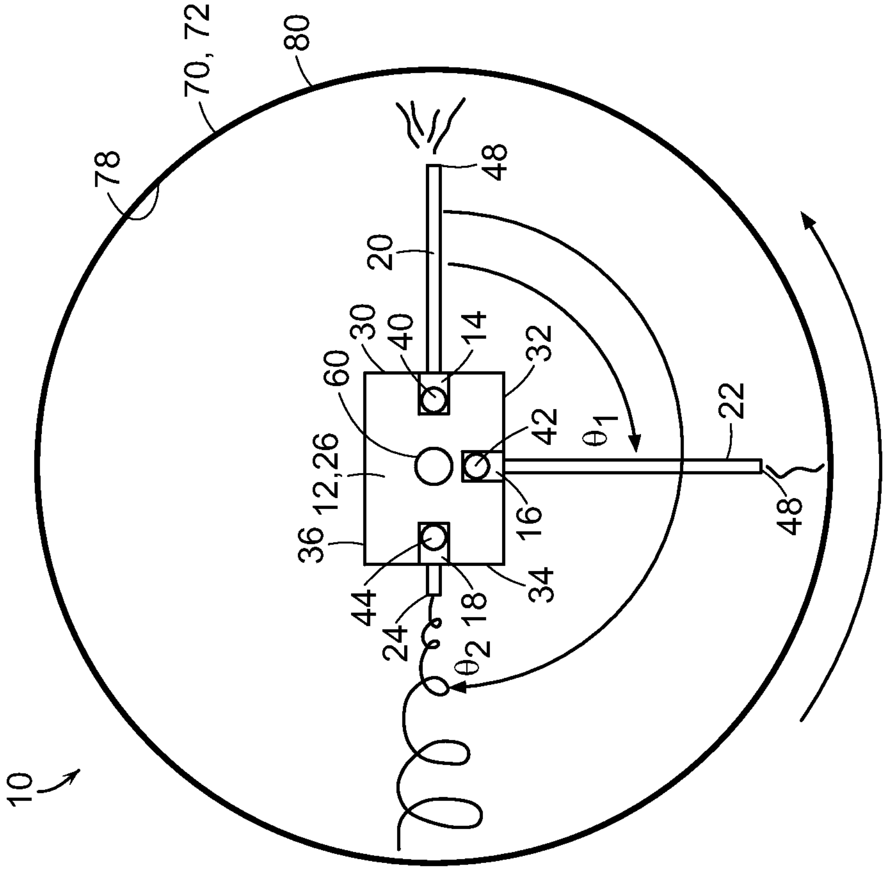

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30
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θ1
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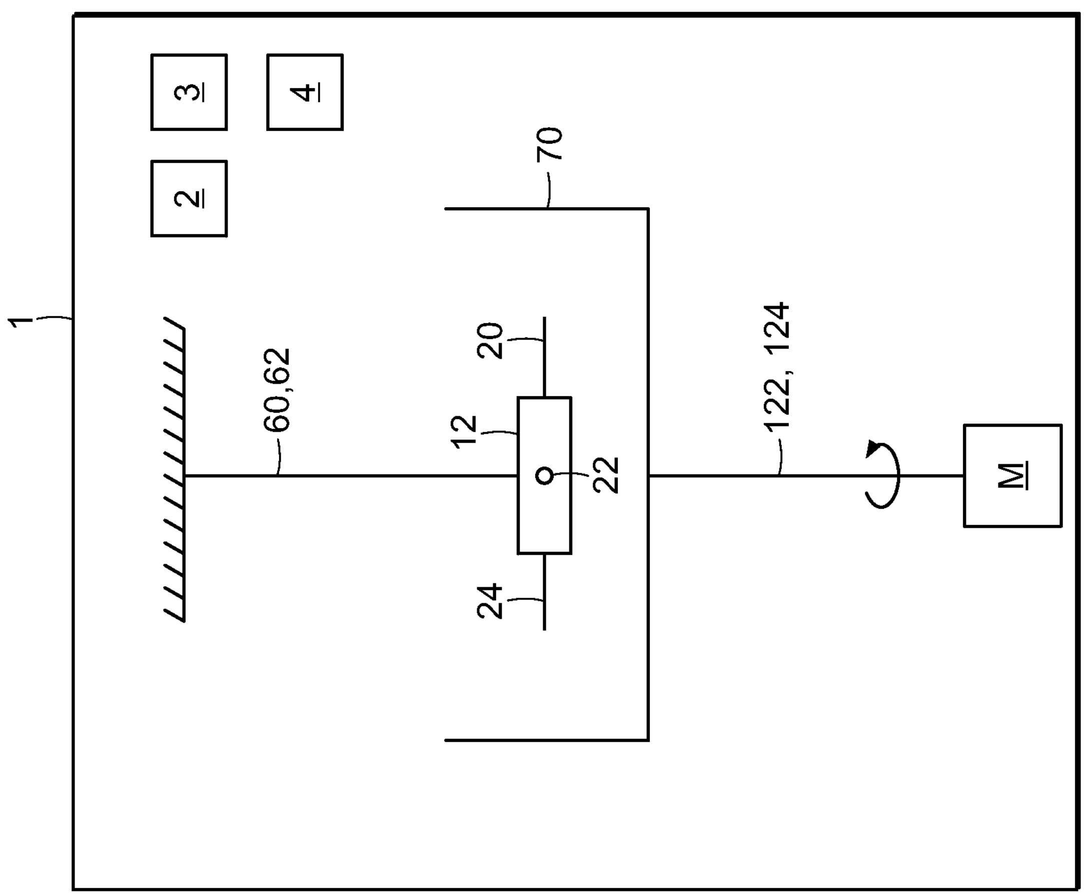
24
θ2
10

FIG. 3



1
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4
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60,62
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12
22
24
122, 124
M

FIG. 2

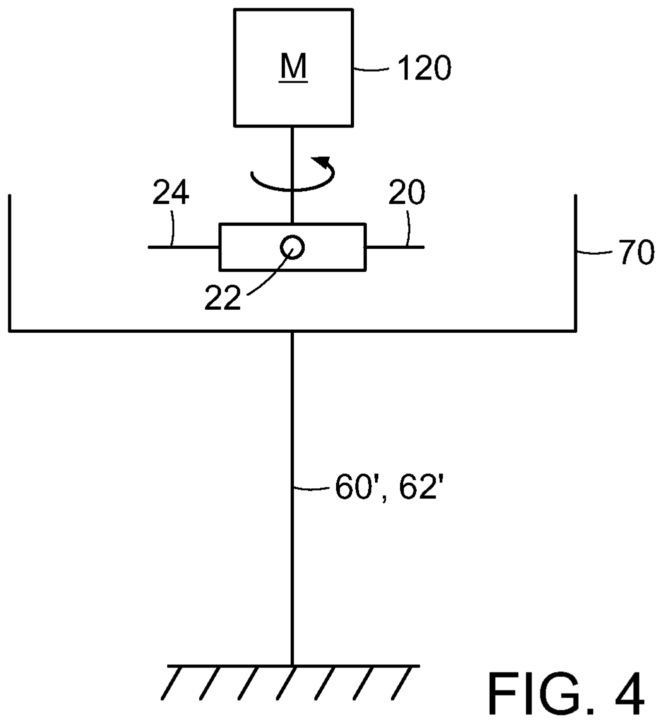


FIG. 4

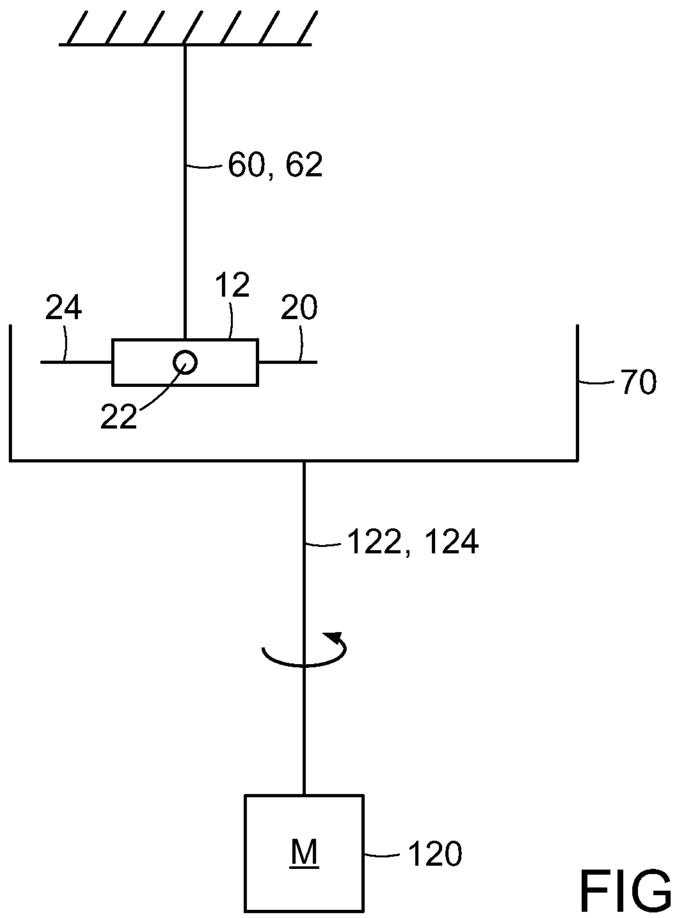
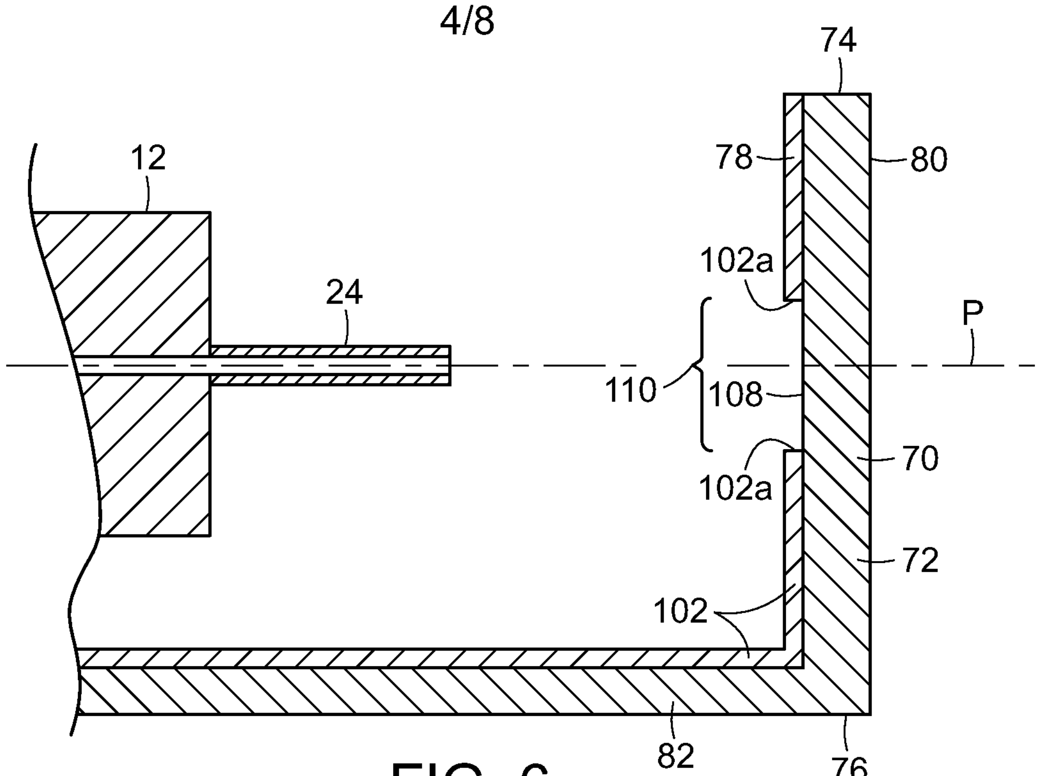


FIG. 5



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24
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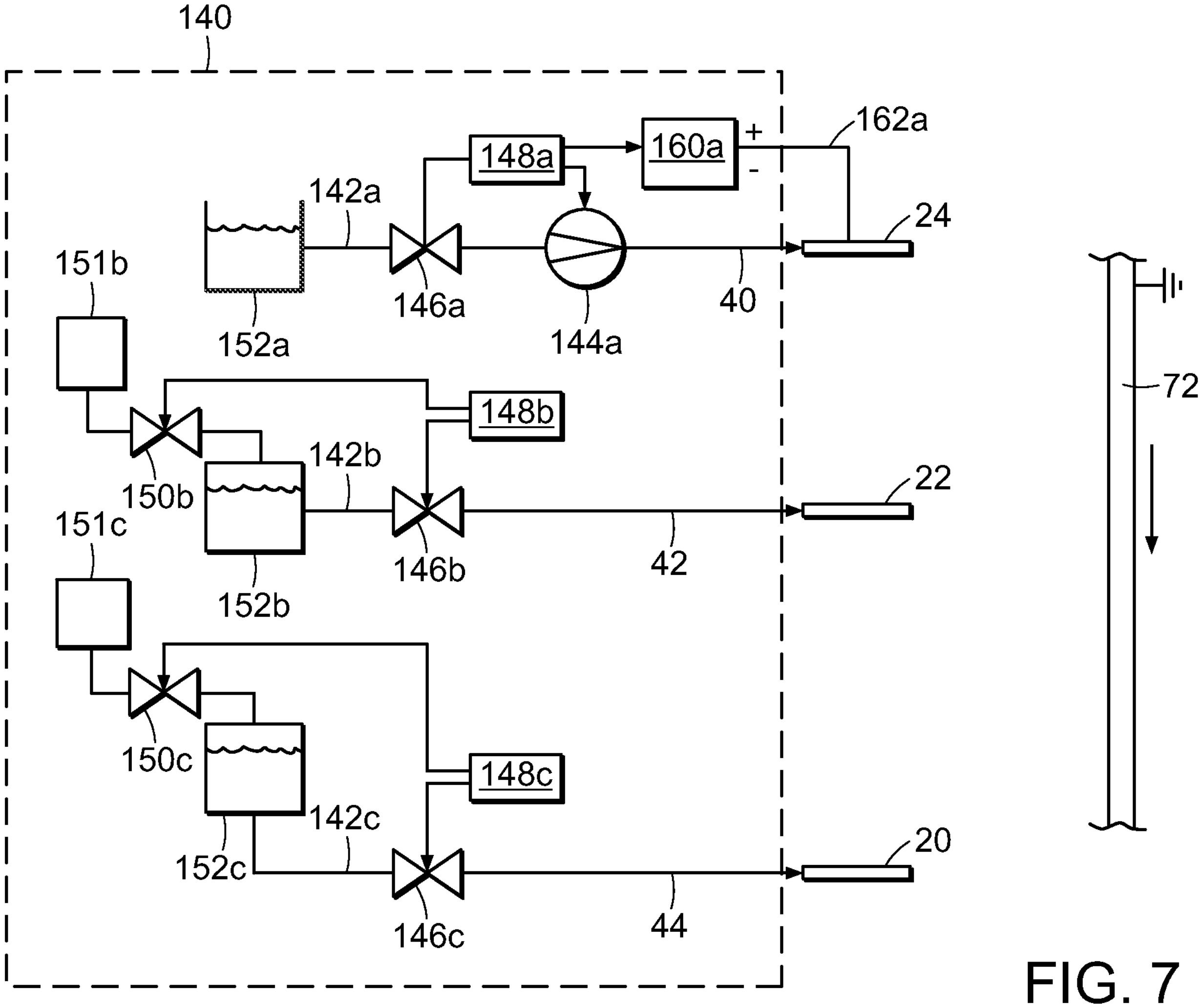
102
82
76

FIG. 6



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+
-
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151b
146a
144a
40
152a
148b
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146b
42
151c
152b
150c
148c
142c
20
152c
146c
44

FIG. 7

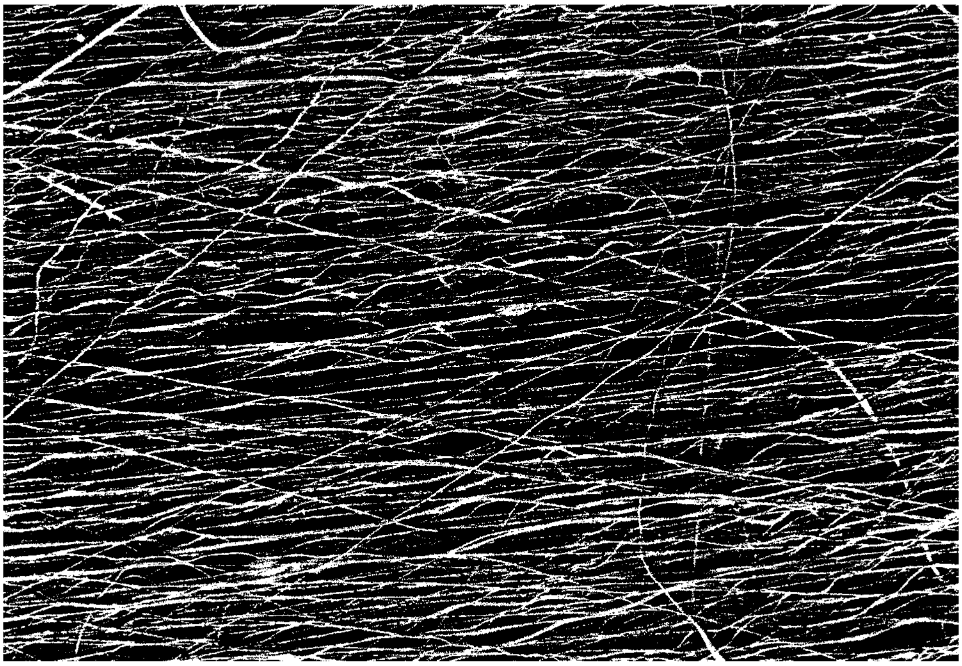


FIG. 8A

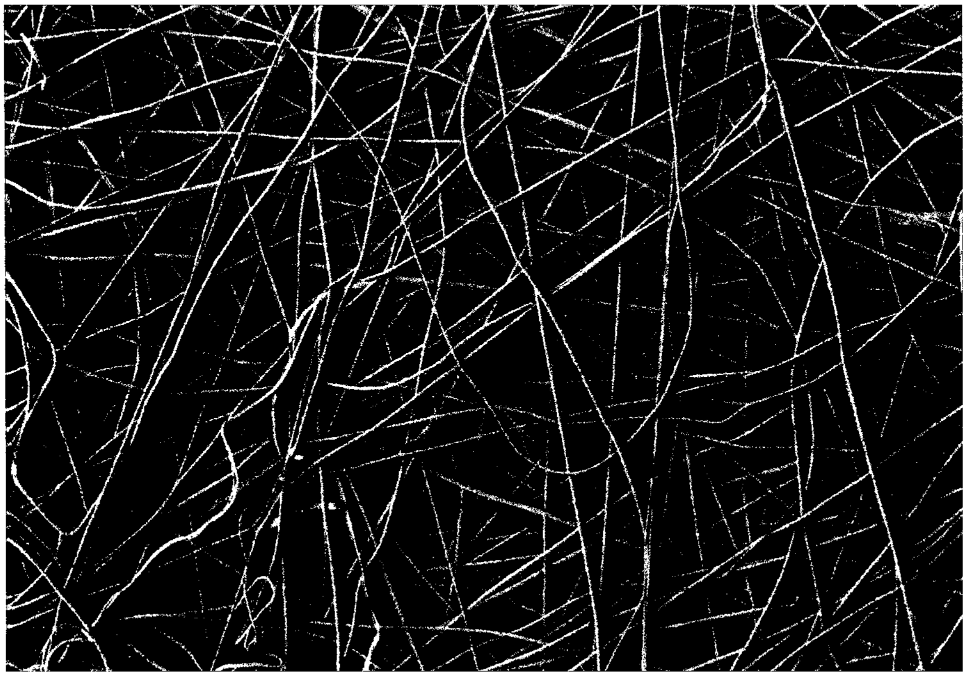


FIG. 8B

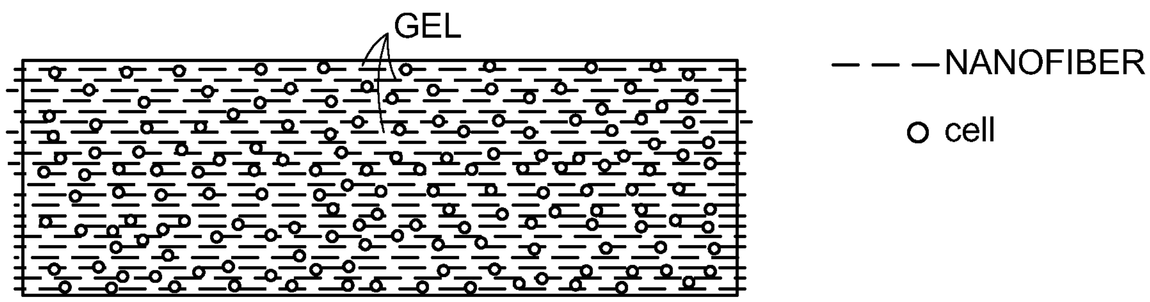
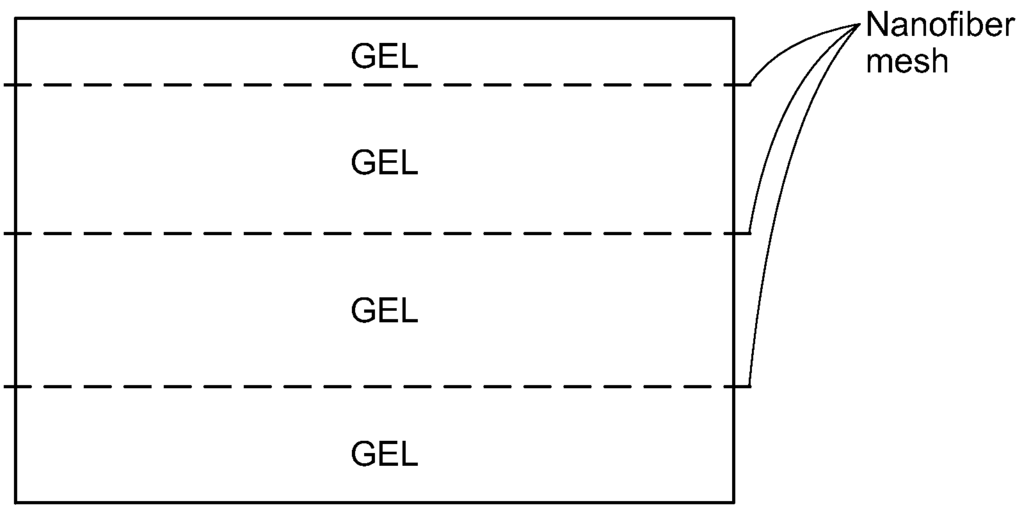


FIG. 9A



PRIOR ART

FIG. 9B

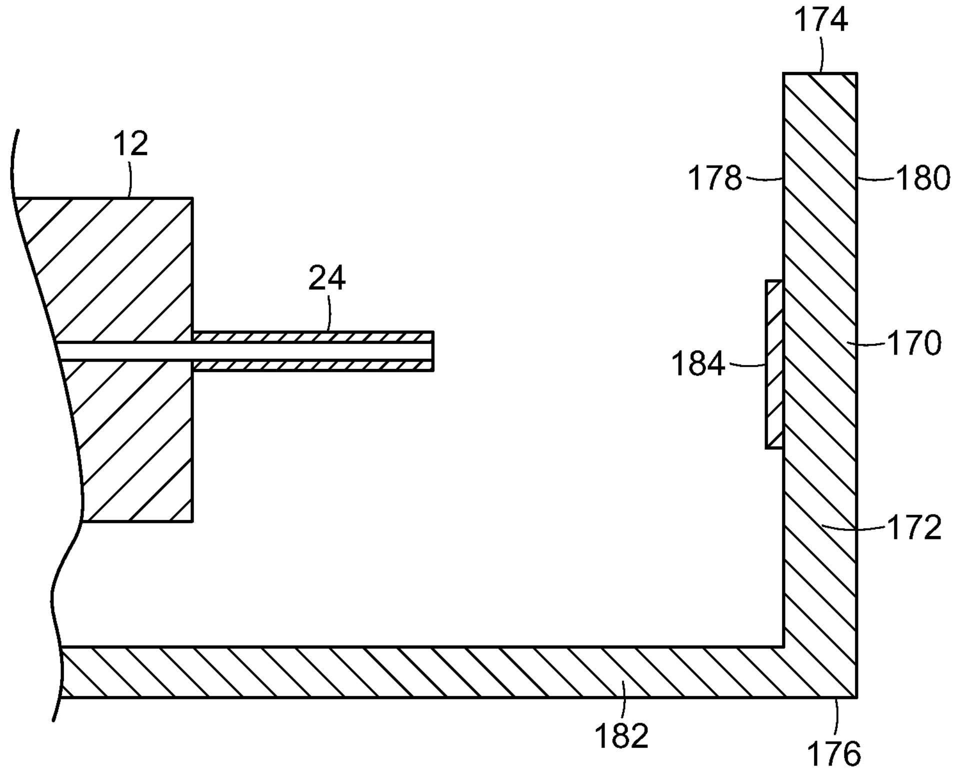
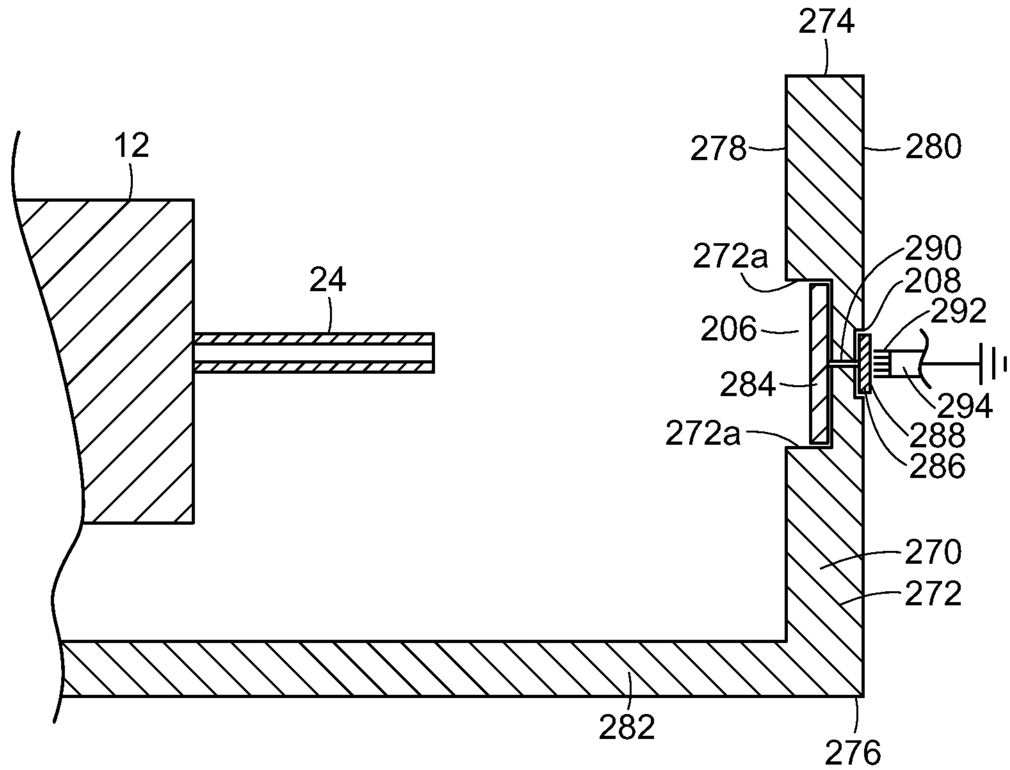

174
12
178
180
24
184
170
172
182
176

FIG. 10


274
278
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272a
208
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206
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294
272a
288
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272
282
276

FIG. 11

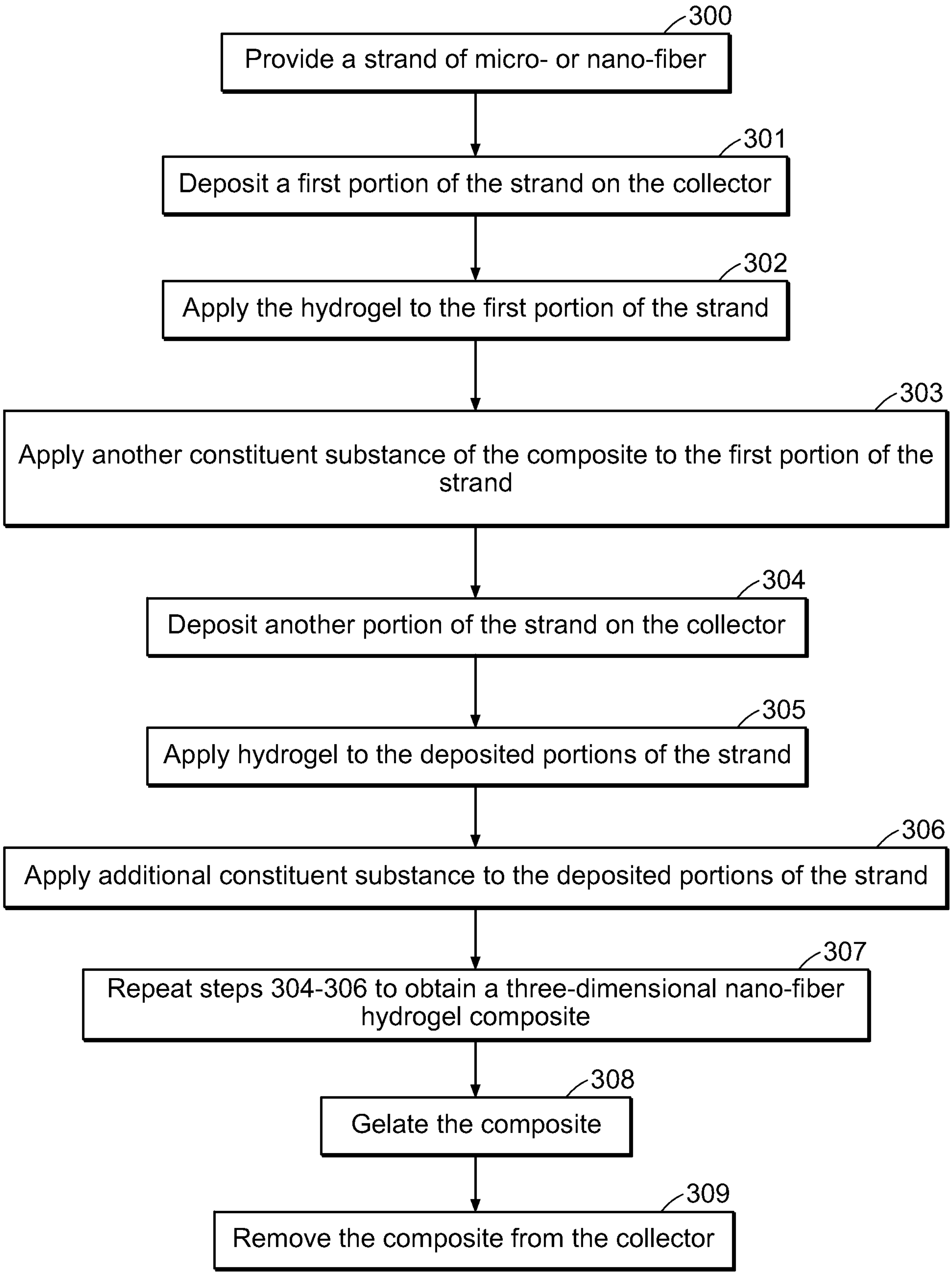
300
Provide a strand of micro- or nano-fiber
301
Deposit a first portion of the strand on the collector
302
Apply the hydrogel to the first portion of the strand
303
Apply another constituent substance of the composite to the first portion of the strand
304
Deposit another portion of the strand on the collector
305
Apply hydrogel to the deposited portions of the strand
306
Apply additional constituent substance to the deposited portions of the strand
307
Repeat steps 304-306 to obtain a three-dimensional nano-fiber hydrogel composite
308
Gelate the composite
309
Remove the composite from the collector

FIG.12