



- (51) International Patent Classification:
B29C 39/02 (2006.01) *C08L 89/06* (2006.01)
- (21) International Application Number:
PCT/US2018/056642
- (22) International Filing Date:
19 October 2018 (19.10.2018)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
62/574,524 19 October 2017 (19.10.2017) US
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- (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,
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,

(54) Title: METHOD OF MAKING, MANUFACTURING OR PRODUCING ORTHOPEDIC SOFT TISSUE

Figure 1

Figure 1A

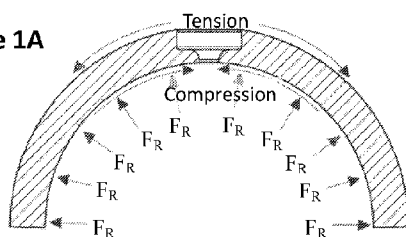


Figure 1B

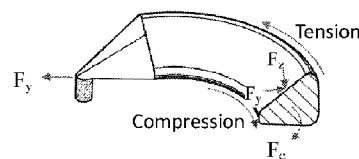


Figure 1C

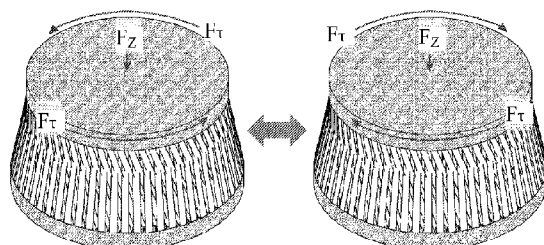
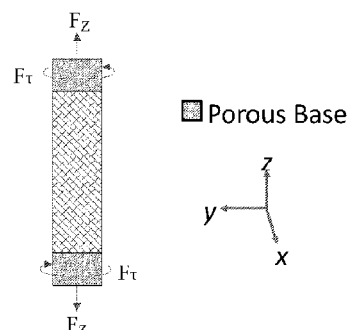


Figure 1D



(57) Abstract: An improved method of making, manufacturing and/or producing engineered orthopedic soft tissue including cartilage, meniscus, annulus fibrosus, and tendon/ligaments which results in engineered soft tissue in which the fibers are aligned the same or nearly the same as naturally occurring tissue. The present invention also includes molds and other apparatus for carrying out the methods of the invention and kits.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*

METHOD OF MAKING, MANUFACTURING OR PRODUCING ORTHOPEDIC SOFT TISSUE

RELATED APPLICATION

5 The present application claims priority to U.S. Patent Application Serial No. 62/574,524, filed October 19, 2017, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

10 The present invention relates to an improved method of making, manufacturing and/or producing engineered orthopedic soft tissue including cartilage, meniscus, annulus fibrosus, and tendon/ligaments using synthetic and natural polymers. The method results in engineered soft tissue in which the fibers are aligned the same or nearly the same as naturally occurring tissue.

15 The present invention also includes molds and other apparatus for carrying out the methods of the invention.

BACKGROUND OF THE INVENTION

20 Joint loading plays an important role in the development and properties of tissue in the body. One place where this is most evident is the extracellular matrix of cartilage where there are distinct zones of collagen fiber alignment through the depth of the tissue. Cartilage comprises three zones: a superficial tangential zone furthest from the bone which is about 10-20% of the tissue; a middle zone which is about 40-60% of the tissue; and a deep zone which is about 30% of the tissues. While physiological forces have been applied to try to recreate this alignment, to date, there has been no success in fully recapitulating this structure. Observations
25 of cartilage postnatally have shown the development of this defined architecture *in situ* before the application of loading, opening the possibility that other constraints are driving the architecture. One such factor is the rapid cellular growth and maturation postnatally.

30 The current invention uses these observations to create an engineered cartilage and other orthopedic soft tissue that mimics native tissue.

SUMMARY OF THE INVENTION

The present invention overcomes the problems in the art by providing a novel method for making, manufacturing and/or producing engineered orthopedic soft tissue comprising applying force(s) to a partially crosslinked polymer and applying additional crosslinking to the polymer while the force(s) is applied, under conditions and for a time to allow the fiber orientation of the engineered orthopedic soft tissue to mimic or recapitulate the fiber orientation of naturally occurring orthopedic soft tissue.

In some embodiments, the orthopedic soft tissue is cartilage and the cartilage is articular.

In further embodiments, the orthopedic soft tissue is meniscus, annulus fibrosus, or tendon/ligament.

In some embodiments, the orthopedic soft tissue is independent. In some embodiments, the orthopedic soft tissue is attached to another cryogel, polymer, or hydrogel, to at least one rigid porous base made of materials such as porous PEEK or porous titanium, and/or to native tissue.

In some embodiments, the invention provides for a method to make, manufacture and/or produce engineered cartilage, comprising the steps of:

- a. placing a uniform layer of a polymer on the surface of a mold, wherein the mold is shaped as a hemisphere;
- b. partially crosslinking the polymer;
- c. placing the partially crosslinked polymer and the mold onto an expander, wherein the expander is shaped as a hemisphere and is capable of radially expanding the polymer on the mold;
- d. crosslinking the polymer while the expander is radially expanding the polymer; and
- e. removing the polymer from the mold.

In some embodiments, the invention provides for a method to make, manufacture and/or produce engineered meniscus, comprising the steps of:

- a. casting a polymer in a negative mold, wherein the mold contains an inlet port in which to inject the polymer, an area to contain the polymer, and an area for at least one rigid porous base;
- b. partially crosslinking the polymer;
- c. removing the polymer and the at least one rigid porous base from the mold;
- d. placing the partially crosslinked polymer and the at least one rigid porous base onto a loading frame, wherein the loading frame contains a post and at least one screw for

keeping the polymer and rigid porous base in place, and a crank for applying force to the polymer;

e. crosslinking the polymer; and

f. removing the polymer and the rigid porous base from the loading frame.

5 In some embodiments, the polymer is crosslinkable and includes but is not limited to poly(vinyl alcohol), polyurethane, polycarbonate urethane, ultrahigh molecular weight polyethylene, polyacrylic acid, collagen, chitosan, hyaluronic acid or any other synthetic or natural polymer.

10 In some embodiments, the crosslinking is done by one or more freeze/thaw cycles, the application of a chemical crosslinking agent, and/or UV light exposure.

The present invention also provides for the engineered orthopedic soft tissue made, manufactured and/or produced by the methods described herein.

The present invention also provides for molds and expanders and other apparatus for carrying out the methods.

15 The present invention also provides for kits.

BRIEF DESCRIPTION OF THE DRAWINGS

20 For the purpose of illustrating the invention, there are depicted in drawings certain embodiments of the invention. However, the invention is not limited to the precise arrangements and instrumentalities of the embodiments depicted in the drawings.

Figure 1 shows schematics of applying forces to polymers when making, manufacturing or producing engineered articular orthopedic soft tissue. Figure 1A shows the forces being applied to recreate cartilage. Figure 1B shows the forces being applied to recreate meniscus. Figure 1C shows the forces applied to recreate the annulus fibrosus. Figure 1D shows the forces
25 required to recreate tendon and ligament.

Figure 2 illustrates an exemplary mold for use in making, manufacturing or producing engineered cartilage. Figure 2A is a cross-sectional side view of the mold. Figure 2B illustrates an exemplary container with the exemplary mold inside.

30 Figure 3 illustrates an exemplary expander for use in making, manufacturing or producing engineered cartilage.

Figure 4 illustrates an exemplary mold for use in making, manufacturing or producing engineered cartilage using radial forces in the four units that make up the mold. Figure 4A is an exploded view of the mold components. Figure 4B is a cross-sectional view of the mold assembled.

Figure 5 shows that the engineered cartilage of the invention is similar in fiber alignment to naturally occurring cartilage. Figure 5A is an 8 mm biopsy punch of the polymer after expansion showing three distinct layers. Figure 5B is a cross-section of expanded polymer with red box denoting the location imaged under polarized light. Figure 5C is a polarized light image of the expanded polymer showing high birefringence on the top and bottom layers. Figure 5D is a representative image of the birefringence pattern of cartilage from newborn porcine articular cartilage.

Figure 6 illustrates an exemplary mold for use in making, manufacturing or producing engineered meniscus. Figure 6A is a top view of the mold. Figure 6B is a side cross-sectional view of the mold. Figure 6C is the polymer and rigid porous base after removal from the mold. Figure 6D is a cross-section of the polymer after removal from the mold.

Figure 7 illustrates an exemplary loading frame for use in making, manufacturing or producing engineered meniscus. Figure 7A is a top view of the loading frame. Figure 7B is a side view of the loading frame. Figure 7C is a top view of the loading frame showing the turning of the crank. Figure 7D shows the forces applied to the polymer when the loading frame is used.

Figure 8 is a polarized image of a 100 μm thick slice of an engineered meniscus.

DETAILED DESCRIPTION OF INVENTION

Definitions

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the methods of the invention and how to use them. Moreover, it will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of the other synonyms. The use of examples anywhere in the specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the invention or any exemplified term. Likewise, the invention is not limited to its preferred embodiments.

The terms “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system, *i.e.*, the degree of precision required for a particular purpose, such as a pharmaceutical formulation.

5 For example, “about” can mean within 1 or more than 1 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where
10 particular values are described in the application and claims, unless otherwise stated, the term “about” meaning within an acceptable error range for the particular value should be assumed.

The term “polymer” means a large molecule composed of repeating structural units often connected by covalent chemical bonds. Polymers can be natural or synthetic.

The term “cryogel” means a polymer material that is crosslinked for example by
15 repeated freeze/thaw cycles ranging from 1 to 10 cycles.

As discussed above, there has been no success to date in engineering cartilage and other orthopedic soft tissue that recapitulates or mimics the fiber alignment of naturally occurring tissue. It was hypothesized that the rapid growth and maturation of the cells in the cartilage is
20 a factor in the development of cartilage architecture and that as cells divide and mature the forces on the cartilage will dictate the growth and alignment of the collagen, *i.e.*, fibers, in cartilage.

Using this concept, methods and systems were created which apply force, *e.g.*, compression, tension, etc., to polymers during which the polymers are being crosslinked.
25 These methods and systems result in engineered cartilage and other orthopedic soft tissue which recapitulates or mimics naturally occurring orthopedic soft tissues including cartilage (see Figure 5) and engineered meniscus that recapitulates or mimics naturally occurring meniscus (see Figure 8).

30 Methods for Making, Manufacturing and/or Producing Engineered Soft Orthopedic Tissue

The current method of the invention of applying forces in multiple directions on partial crosslinked cryogels, chemically crosslinked polymers, and UV crosslinked polymers can be used to mimic the fiber alignment of various orthopedic soft tissues and can be used to make, manufacture and/or produce various engineered orthopedic soft tissues including but not

limited to including cartilage (Figure 1A), meniscus (Figure 1B), annulus fibrosus (Figure 1C), and tendon/ligament (Figure 1D). The methods of the invention can be performed to cryogels and crosslinkable polymers independently as well as those attached to another cryogel/polymer and/or to at least one rigid porous base, such as porous PEEK or porous titanium.

5 As shown in Figure 1, various forces are necessarily applied to the polymer while crosslinking the polymers, either partially or completely, depending upon the type of orthopedic soft tissue being made.

Figure 1A shows the forces that can be applied when crosslinking the polymers and making, manufacturing and/or producing engineered cartilage including articular. These forces
10 include radial force (F_R) applied under the polymer as well as those that create tension on the surface of the polymer and compression at the base of the polymer. The method exemplified in Example 2 use these forces while crosslinking the polymer resulting in an engineered cartilage with fiber alignment similar to native cartilage (Figure 5).

Figure 1B shows the forces that can be applied when crosslinking the polymers and
15 making, manufacturing and/or producing engineered meniscus. These forces include circumferential (F_C), forces in the Y (F_Y) and Z (F_Z) direction, as well as tension and compression. Additionally when making engineered meniscus, the polymer can be attached to at least one rigid porous base. The method exemplified in Example 4 use these forces while crosslinking the polymer resulting in an engineered meniscus with fiber alignment similar to
20 native meniscus (Figure 8).

Figure 1C shows the forces applied when crosslinking the polymers and making, manufacturing and/or producing engineered annulus fibrosus including torsional (F_T) and forces in the Z direction (F_Z).

Figure 1D shows the forces applied when crosslinking the polymers and making,
25 manufacturing and/or producing engineering tendons and ligaments including torsional (F_T) and forces in the Z direction (F_Z). Additionally when making engineered tendons and ligaments, the polymer can be attached to at least one porous base.

In all of the above methods, the polymer used should be crosslinkable and include but is not limited to poly(vinyl alcohol) (PVA), polyurethane, polycarbonate urethane, ultrahigh
30 molecular weight polyethylene, polyacrylic acid, collagen, chitosan, hyaluronic acid or any other synthetic or natural polymer. PVA is a preferred polymer.

In all of these methods, the crosslinking is done by one or more freeze/thaw cycles, chemical crosslinking, and/or UV light exposure.

In all of these methods, the preferred method for freezing/thawing includes ramping the temperature to about -20°C at a rate of about 0.5°C /minute and freezing the polymer at about -20°C for about 4 to about 24 hours, with 20 hours being preferred and then ramping the temperature to about 20°C at a rate of about 0.5°C /minute and thawing the polymer at about 20°C for about 4 to about 12 hours, with 4 hours being preferred. Again this can be varied by a person of skill in the art. Both the number of hours of freezing and/or thawing can be varied as well as the number of cycles and the amount of strain (see Table 1).

In all of these methods, the preferred method for UV crosslinking is to partially crosslink the polymer using a 405 nm laser with liquid polymer at 30°C to crosslink 60% of the reactive groups. This method can be performed using a negative mold or by stereolithography 3D printing. The amount of strain and the number of strain cycles can be varied to achieve the final structure.

In all of these methods, the preferred method for chemical crosslinking is to partially crosslink the polymer with either 10% formaldehyde, paraformaldehyde, or glutaraldehyde for about 0.5 hours to about 2 hours. Again this can be varied by a person of skill in the art. Both the concentration of chemical crosslinking agent and duration of exposure can be varied.

Additionally, the following parameters can also be varied to dictate the amount of polymer fiber alignment and it is within the skill of the art to determine how to vary the parameters to obtain the desired engineered soft orthopedic tissue with the desired fiber alignment.

Table 1- Parameters for Polymer Fiber Alignment in Making Engineered Soft Orthopedic Tissue

Parameter	Minimum	Maximum	Ideal Values for Cartilage	Ideal Values for Meniscus	Effect
Temperature Ramp Rate	0.2°C/min	120°C/min	0.5°C/min	0.5°C/min	Allows for longer polymer relaxation time decreasing the residual strain in the fibers. Longer temperature ramp

					rates decrease the non-uniform freeze and thaw of the polymers
Strain Rate	1%/sec	1%/30 min	1%/sec	5%/sec	Increasing the strain rate increases alignment and residual strains
% Strain	30%	200%	40%	30%	Increasing strain increases fiber alignment
Multiple Strain Cycles (one per crosslinking cycle)	1	5	1	2	Increasing the number of strain cycles increases the amount of aligned polymer
Polymer Concentration	10%	40%	20%	20%	Increasing polymer concentration increases modulus and also number of aligned fibers

Strain Cycle = how many times the polymer is stretched. For example, generally the polymer is stretched only once after the first freeze/thaw cycle but the polymer could be stretched again after the second freeze/thaw cycle to create more aligned fibers.

5 % Strain = the % deformation of the polymer from its original shape

Strain rate = the % strain to apply over time until the desired % strain is reached

Methods and Systems for Making, Manufacturing and/or Producing Engineered Cartilage

Figure 1A shows the forces that can be applied when crosslinking the polymers and
 10 making, manufacturing and/or producing engineered cartilage, including articular.

A method for making, manufacturing or producing cartilage using radial forces as well as generating tensile and compressive forces (see Figure 1A) can comprise the following steps: (reference to Figures 2 and 3):

- a. placing a mold 200 into a hemispherical container 300 wherein the mold 200 is shaped like a hemisphere and comprises at least one leaflet 210 and preferably a plurality such as 4, and a leaflet collar 220 that receives and surrounds a free end of the leaflet;
- b. pouring a liquid polymer into the container 300 wherein the container 300 surrounds the mold 200;
- c. partially crosslinking the polymer to form a polymer structure 100;
- d. removing the mold 200 and polymer 100 from the container 300 and removing the leaflet collar 220;
- e. placing the mold 200 and the polymer 100 onto an expander 400 wherein the expander contains a central threaded channel 410 (defined in a central boss/protrusion of the expander 400) in which the central post 230 of the mold 200 is threaded
- f. advancing the expander 400 towards the mold 200 with the polymer 100 of the mold 200 on the surface by screwing the expander 400 up the central post 230 until the desired expansion is reached (radial expansion of the mold is possible due to the construction of the mold);
- g. crosslinking the polymer 100 after the expander 400 has radially expanded the mold 200 and polymer 100; and
- h. removing the polymer from the mold.

In some embodiments, the partial crosslinking in step b. is done by freeze/thaw cycle, about 1 to about 3 cycles, preferably 1 cycle, or by UV light exposure for about 10 seconds to about 30 seconds or by exposure to a chemical crosslinking agent.

In some embodiments, the crosslinking in step d. is done by freeze/thaw cycles, about 3 to about 5 cycles, preferably 5 cycles or by UV light exposure preferably about 15 minutes to about 60 minutes or by exposure to a chemical crosslinking agent.

In some embodiments, the leaflet expander is advanced up the central post preferably about 1 mm to about 5 mm. The radius of the expander can be varied to change the strain rate and final percent strain, with a preferable radius of the expander being about 25 mm to about 100 mm.

A system for making, manufacturing or producing cartilage can comprise a mold 200, a container 300, an expander 400, and a polymer 100. Additionally, the system can comprise

subsystems crosslinking the polymer either by freeze/thaw or UV light or chemical crosslinking agent.

In all of the above methods and systems, the polymer used should be crosslinkable and include but is not limited to poly(vinyl alcohol) (PVA), polyurethane, polycarbonate urethane, ultrahigh molecular weight polyethylene, polyacrylic acid, collagen, chitosan, hyaluronic acid or any other synthetic or natural polymer. PVA is a preferred polymer. The concentration of the polymer is ranges from about 10% to about 40%, with 20% being ideal. The polymer is preferably in a liquid state.

In all of these methods, the preferred method for freezing/thawing includes ramping the temperature to about -20°C at a rate of about 0.5°C /minute and freezing the polymer at about -20°C for about 4 to about 24 hours, with 20 hours being preferred and then ramping the temperature to about 20°C at a rate of about 0.5°C /minute and thawing the polymer at about 20°C for about 4 to about 12 hours, with 4 hours being preferred. Again this can be varied by a person of skill in the art. Both the number of hours of freezing and/or thawing can be varied as well as the number of cycles (see Table 1).

The ideal parameters for this method are found in Table 1 in the column for cartilage.

It will be appreciated that other types of devices other than the expander 400 can be used to apply the targeted forces to the polymer in the manner described herein.

Mold and Expander for a Method for Making, Manufacturing and/or Producing Engineered Cartilage

A further aspect of the current invention is the unique mold and expander used in the method of making, manufacturing and/or producing engineered articular cartilage using radial forces.

In one embodiment shown in Figure 2A, the mold is composed of: at least one leaflet 210 containing a leaflet compression spring rod 240 and a tension spring 260; a leaflet collar 220, a central post 230 containing at least one compression spring tube 250 (which has a number of outwardly extending arms about which spring rods 240 are disposed).

In one embodiment shown in Figure 4, the illustrated mold 200 is composed of four leaflet units, each comprising a leaflet 210, a leaflet compression spring rod 240, and a tension spring 260 (the spring tube 250 is also configured to attach to one end of the spring 260, while the other end of spring 260 is coupled to the leaflet 210 so as to apply a biasing force to the leaflet) that are assembled radially and held together by a leaflet collar 220 to form a mold that

is a hemisphere. The collar 220 has an annular shaped groove which receives bottom portions of the leaflets. See also Figure 2B.

The mold is used in conjunction with a hemispherical leaflet expander 400.

5 In one embodiment of the expander shown in Figure 3, the expander 400 contains a threaded channel 410 in which the central post 230 of the mold is threadingly received for coupling the mold 200 to the expander 400.

The mold 200 and expander 400 can be made using traditional manufacturing from Teflon, PEEK, PEKK, polycarbonate or ULTEM with polycarbonate being the preferred material. The mold and expander can also be manufactured using newer technologies such as
10 3D printing.

Additionally, the mold 200 and expander 400 can be made of material able to withstand freezing temperatures, change in temperatures, chemical crosslinking agents, and exposure to UV light.

Each leaflet 210 is made with a preferable radius of about 10 mm to about 50 mm. The
15 expander 400 is made with a preferable radius of about 25 mm to about 100 mm. The inner radius of the hemispherical container can be varied to create different thicknesses of polymer on the surface of the mold, preferably about 11 mm to about 60 mm.

Methods and Systems for Making, Manufacturing and/or Producing Engineered Meniscus

20 Figure 1B shows the forces that can be applied when crosslinking the polymers and making, manufacturing and/or producing engineered meniscus.

A method for making, manufacturing or producing meniscus using forces in the X, Y, and Z direction as well as tensile and compressive (see Figure 1B) can comprise the following steps: (reference to Figures 6 and 7):

- 25 a. injecting a liquid polymer into an inlet port 510 of a mold 500 until the liquid polymer starts to flow from the outlet port 520 of the mold 500, the mold 500 being in the desired shape of the polymer and having the inlet port 510, the outlet port 520, an area (cavity) 530 for the polymer 100 and an area (cavity) 540 for rigid porous bases 600;
- 30 b. partially crosslinking the polymer 100;
- c. removing the partially crosslinked polymer 100 attached to the rigid porous bases 600 on each end (see Figure 6C) from the mold 500;
- d. placing the partially crosslinked polymer 100 and the rigid porous bases 600 into a loading frame 700;

- e. fixing the rigid porous bases 600 to lead screws 710 on either side of the loading frame 700 using set screws 720 (*e.g.*, Figure 7D shows one base 600 including a downwardly extending protrusion (pin) that can be received in a corresponding hole);
- 5 f. turning the crank 730 of the loading frame 700 clockwise (a first direction) to cause the rigid porous bases 600 to move toward the crank 730 (as indicated by the arrows in Figure 7C) which in turn causes a tensile force at the ends of the partially crosslinked polymer 100 and forces the polymer 100 against the post 740 that
- 10 (see Figures 7C and 7D) (as shown, the pulling of the polymer into contact with post 740 results in the polymer wrapping around one end of the post 740 and extending along the sides thereof);
- g. crosslinking the polymer 100; and
- h. removing the polymer 100 from the loading frame 700.

15 In some embodiments, the partial crosslinking in step b. is done by freeze/thaw cycle, about 1 to about 3 cycles, preferably 1 or by UV light exposure 10-30 seconds or by exposure to a chemical crosslinking agent.

In some embodiments, the crosslinking in step d. is done by freeze/thaw cycles, about 3 to about 5 cycles, preferably 5 or by UV light exposure preferably about 15 minutes to about 20 60 minutes or by exposure to a chemical crosslinking agent.

The crosslinking step g. is performed after the polymer has been stretched to about 20% to about 50% strain, preferably 30%.

25 A system for making, manufacturing or producing meniscus can comprise a mold 500, a loading frame 700, at least one rigid porous base 600, and a polymer 100. Additionally, the system can comprise subsystems for injecting the polymer, and crosslinking the polymer either by freeze/thaw or UV light or by exposure to a chemical crosslinking agent.

In all of the above methods and systems, the polymer used should be crosslinkable and include but is not limited to poly(vinyl alcohol) (PVA), polyurethane, polycarbonate urethane, ultrahigh molecular weight polyethylene, polyacrylic acid, collagen, chitosan, hyaluronic acid 30 or any other synthetic or natural polymer. PVA is a preferred polymer. The concentration of the polymer is ranges from about 10% to about 40% with 20% being ideal. Ideally the polymer should be in liquid form.

The polymer can be injected into the inlet port 510 of the mold 500 with a syringe.

In all of these methods, the preferred method for freezing/thawing includes ramping the temperature to about -20°C at a rate of about 0.5°C /minute and freezing the polymer at about -20°C for about 4 to about 24 hours, with 20 hours being preferred and then ramping the temperature to about 20°C at a rate of about 0.5°C /minute and thawing the polymer at about 20°C for about 4 to about 12 hours, with 4 hours being preferred. Again this can be varied by a person of skill in the art. Both the number of hours of freezing and/or thawing can be varied as well as the number of cycles (see Table 1).

The ideal parameters for this method are found in Table 1 in the column for meniscus.

It will be appreciated that other types of devices other than loading frame 700 can be used to apply the targeted forces to the polymer in the manner described herein.

Mold and Loading Frame for a Method for Making, Manufacturing and/or Producing Engineered Meniscus

A further aspect of the current invention is the unique mold and loading frame used in the method of making, manufacturing and/or producing engineered meniscus.

In one embodiment shown in Figure 6, the mold 500 is composed of an inlet port 510, an outlet port 520, a well 530 for holding the polymer 100, and an area 540 for a rigid porous base 600.

After the polymer is removed from the mold 500, the polymer 100 and rigid porous base 600 are loaded on a loading frame 700. In one embodiment shown in Figure 7, the loading frame 700 is composed of a base 760, the post 740, the crank 730, at least one worm 750, at least one worm gear 770, at least one set screw 720 and at least one lead screw 710. The worm 750 and worm gear 760 are mechanical means for causing the controlled movement of the screws 720 based on rotation of the crank 730 so as to impart motion to the rigid porous bases 600 since the bases 600 are fixed (anchored) to the set screws 720 (which in turn are coupled to the set screws 710).

The mold can be made using traditional manufacturing from Teflon, PEEK, PEKK, polycarbonate or ULTEM with polycarbonate being the preferred material. The mold can also be manufactured using newer technologies such as 3D printing.

Additionally, the mold and loading frame can be made of material able to withstand freezing temperatures, change in temperatures, chemical crosslinking agents, and exposure to UV light.

The mold can be created to house a triangular cavity with width of about 5 mm to about 20 mm, height of about 3 to about 8 mm, and length of about 70 mm to about 100 mm. The

loading frame can include a post with a radius of about 5 mm to about 15 mm and the ability to apply from about 10% to about 50% strain to the polymer.

Engineered Orthopedic Soft Tissue

5 The current invention also provides for the engineered orthopedic soft tissue which is made, manufactured and/or produced by the methods and systems disclosed herein.

In one embodiment, the engineered orthopedic soft tissue is cartilage and has the fiber alignment of naturally occurring cartilage including a superficial tangential zone, a middle zone, and a deep zone. See Figure 5.

10 In a further embodiment, the engineered orthopedic soft tissue is meniscus and has the fiber alignment of naturally occurring cartilage. See Figure 8. In some embodiments, the engineered meniscus is attached to at least one rigid porous base.

In a further embodiment, the engineered orthopedic soft tissue is annulus fibrosus and had the fiber alignment of naturally occurring cartilage. In some embodiment, the engineered annulus fibrosus is attached to a hydrogel which mimics or is a replacement for nucleus pulposus.

In yet a further embodiment, the engineered orthopedic soft tissue is tendon and ligament and has the fiber alignment of naturally occurring tendon and ligament.

20 In some embodiments, the engineered orthopedic soft tissue is used alone to treat, repair and/or replace defects and/or injuries to biological tissue, more specifically to musculoskeletal tissue, more specifically orthopedic soft tissue.

In some embodiments, the engineered orthopedic soft tissue is used in combination with or attached to at least one rigid porous base to treat, repair and/or replace defects and/or injuries to biological tissue, more specifically to musculoskeletal tissue including both orthopedic soft tissue and rigid material such as bone.

In some embodiments, the engineered orthopedic soft tissue is used in combination with or attached to another polymer or native tissue by mechanical attachment (*i.e.* sutures, screws, or a rigid material), chemical attachment (*i.e.* formaldehyde, glutaraldehyde), or UV attachment (*i.e.* methacrylation of the polymer and redox reagents).

30 In further embodiments, the engineered orthopedic soft tissue is used in combination with an additional polymer, hydrogel or cryogel to treat, repair and/or replace defects and/or injuries to biological tissue, more specifically to musculoskeletal tissue.

Kits

The current invention also provides for kits which includes the materials needed to practice the novel method of making, manufacturing and/or producing engineered cartilage.

In one embodiment, the kit comprises a mold 200 and the leaflet expander 400 of the invention for making, manufacturing and/or producing engineered cartilage.

5 In another embodiment, the kit comprises a mold 500 and loading frame 700 of the invention for making, manufacturing and/or producing engineered meniscus.

In further embodiments, the kit provides starting materials for the methods including polymers and when necessary rigid porous bases.

10 In some embodiments, instructions are included in the kit. Such instruction can include information regarding the assembly of the mold, and expander or loading frame, with the various components, and parameters for crosslinking the polymer either by freezing and thawing including time, temperature and number of cycles, chemical concentration and exposure time, or exposure to UV light.

15 **Examples**

The present invention may be better understood by reference to the following non-limiting examples, which are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed to limit the broad scope of the invention.

20

Example 1- Assembly of Mold for Making Engineered Cartilage using Radial Forces

The mold used in this method comprises four leaflets. The first step of the method is assembling the four leaflets radially into one hemispherical shaped mold.

25 As shown in Figures 2 and 4, the four leaflet units contain a leaflet 210, a leaflet compression spring rod 240, and a tension spring 260 that are assembled radially and held together by a leaflet collar 220 to form a mold that is a hemisphere (Figure 2). To assemble the mold, each leaflet 210 was attached to the central post 230 through a rod on the leaflet 240 that attached to a tube 250 in the central post 230 (Figure 5B). The leaflets were then held together with a leaflet collar 220 (Figures 2A and 4B).

30

Example 2- Making Engineered Cartilage using Radial Forces

Once the mold was assembled, it was placed into a hemispherical container 300 (Figure 2B). Five (5) ml of 10% liquid poly(vinyl alcohol) was poured into the container 300 such that it surrounded the mold and formed a layer of uniform thickness on the surface of the mold 200.

The liquid polymer then underwent one freeze/thaw cycles which entailed the following steps: 1) ramping down to -20°C at a rate of 0.5°C/min; 2) when a temperature of -20°C was reached, the chamber was held at -20°C for 20 hours after which; 3) the temperature was ramped to 20°C at a rate of 0.5°C/min and held for 4 hours. This creates a partially crosslinked polymer network with randomly aligned polymer chains.

The partially crosslinked polymer 100 and mold 200 were removed from the container 300 and the leaflet collar 220 removed. The central post 230 of the mold 200 was then screwed onto the hemispherical leaflet expander 400. The leaflet expander contained a central threaded component 410 that acted to advance the hemisphere up the central post (Figure 3) and radially expanded the polymer 100 cast on the mold 200. The expansion rate was controlled to create a strain rate of 1%/sec until a strain of 40% was reached. The expanded mold then underwent five remaining freeze/thaw cycles as described.

Example 3- Removal of PVA from the Mold and Testing of Fiber Alignment

After expanding the PVA, the randomly aligned polymer chains crosslinked in the partially crosslinked PVA aligned with directions of strain and were fixed in their strained positions by further crosslinking which created new randomly crosslinked chains.

After completion of the remaining crosslinking processes, the PVA was removed from the mold and kept hydrated in water. After expansion, the engineered cartilage shows three distinct layers similar to naturally occurring cartilage (Figure 5A).

Cross-sections of the PVA (Figure 5B) were imaged under polarized light. 100 µm sections of the PVA were taken by first cutting the expanded and crosslinked PVA into eighths along its radius and then freezing one of the eight pieces containing the cut edges on a freezing stage attached to a sledge microtome. After the PVA slice was completely frozen, a 100 µm section was taken and placed on a microscope slide. The slide was then imaged under circular polarized light to visualize the alignment of the polymer chains.

As shown in Figure 5, the engineered cartilage made in Example 2 (Figure 5C) has distinct regions under polarized light which resembled the alignment of naturally occurring articular cartilage (Figure 5D).

Example 4- Making Engineered Meniscus

A negative mold 500 shown in Figures 6A and 6B was used to cast the polymer into the desired shape. The mold consists of an inlet port 510, outlet port 520, and areas to place rigid porous bases for integration with the polymer 540 and the polymer 530.

Liquid PVA (20%) was injected with a syringe into the inlet port 510 until liquid polymer began to flow from the outlet port 520. The mold with liquid polymer was then placed into an environmental chamber and underwent one freeze/thaw cycles which entailed the following steps: 1) ramping down to -20°C at a rate of $0.5^{\circ}\text{C}/\text{min}$; 2) when a temperature of -20°C was reached, the chamber was held at -20°C for 20 hours after which; 3) the temperature was ramped to 20°C at a rate of $0.5^{\circ}\text{C}/\text{min}$ and held for 4 hours. After 1 freeze/thaw cycle, the partially crosslinked polymer was removed from the mold. Figures 6C and 6D show the shape of the polymer and its cross section once removed from the mold.

The partially crosslinked PVA polymer was then placed into the loading frame 700 and the rigid porous bases were fixed to the lead screws 710 on either side of the loading frame by set screws 720 (Figure 7A). The crank was turned clockwise to cause the rigid porous bases 600 to move toward the crank (Figure 7C). This caused a tensile force at the ends of the partially crosslinked polymer 100 and forced the polymer 100 against the post 740 that creates radial (F_y), axial (F_z), circumferential (F_c), compressive and tensile forces (Figure 7D) that acts on the polymer. After stretching to 30% strain the PVA on the loading frame was placed by in the environmental chamber and underwent 5 more freeze/thaw cycles as described above.

Once the freeze/thaw cycles were complete, the fully crosslinked PVA cryogel was removed from the loading frame.

Polarized images of a 100 μm thick slice of the cryogel shows circumferential alignment of the PVA cryogel (Figure 8).

CLAIMS:

1. A method of making, manufacturing and/or producing engineered orthopedic soft tissue comprising applying force(s) to a polymer and crosslinking the polymer while the force(s) is applied under conditions and for a time to allow the fiber orientation of the engineered orthopedic soft tissue to mimic or recapitulate the fiber orientation of naturally occurring orthopedic soft tissue.
2. The method of claim 1, wherein the engineered orthopedic soft tissue is cartilage and the forces to be applied are radial force (F_R), compressive force, tensile force, and combinations thereof.
3. The method of claim 1, wherein the engineered orthopedic soft tissue is meniscus and the forces to be applied are circumferential (F_C), forces in the Y (F_Y) and Z (F_Z) direction compressive force, tensile force, and combinations thereof.
4. The method of claim 1, wherein the engineered orthopedic soft tissue is annulus fibrosus and the forces are torsional (F_τ), forces in the Z direction (F_Z), and combinations thereof.
5. The method of claim 1, wherein the engineered orthopedic soft tissue is tendons and ligaments and the forces are torsional (F_τ), forces in the Z direction (F_Z), and combinations thereof.
6. The method of claim 1, wherein the polymer is crosslinkable.
7. The method of claim 1, wherein the polymer is chosen from the group consisting of poly(vinyl alcohol) (PVA), polyurethane, polycarbonate urethane, ultrahigh molecular weight polyethylene, polyacrylic acid, collagen, chitosan, hyaluronic acid and any other synthetic or natural polymer.
8. The method of claim 1, wherein the polymer concentration ranges from about 10% to about 40%.
9. The method of claim 1, wherein the crosslinking is done by one or more freeze/thaw cycles, exposure to a chemical agent, or exposure to UV light.

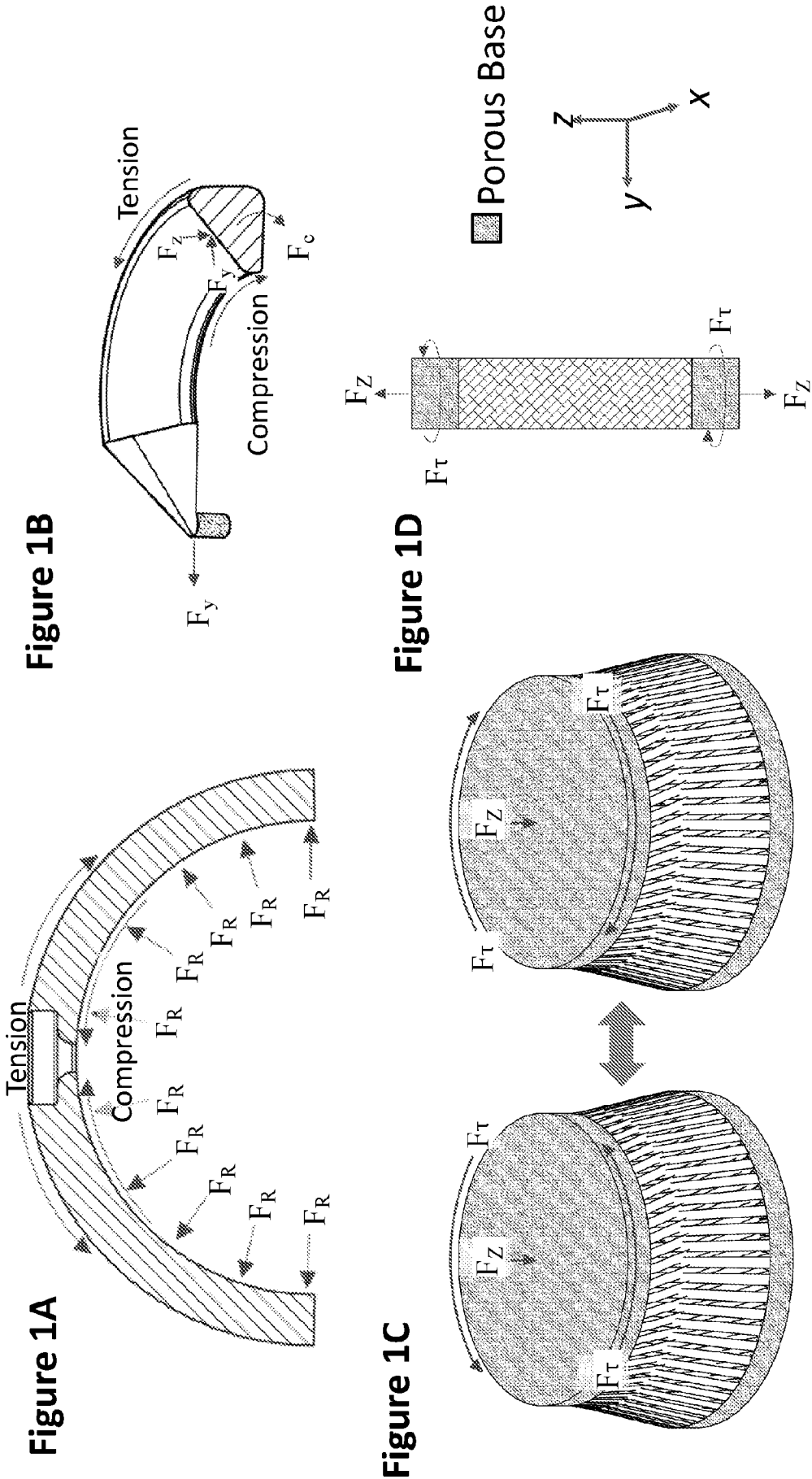
10. The method of claim 9, wherein the freeze / thaw cycle comprises the steps:
- a. ramping the temperature to about -20°C at a rate of about 0.5°C /minute;
 - b. freezing the polymer at about -20°C for about 4 to about 24 hours;
 - c. ramping the temperature to about 20°C at a rate of about 0.5°C /minute; and
 - 5 d. thawing the polymer at about 20°C for about 4 to about 12 hours.
11. The method of claim 1, wherein the polymer is attached or connected to an additional polymer, cryogel, or hydrogel, a rigid porous base, native tissue, or combinations thereof.
- 10
12. An engineered orthopedic soft tissue manufactured by the method of claim 1.
13. A method for making, manufacturing and/or producing cartilage, comprising the steps of:
- 15 a. placing a mold into a hemispherical container, wherein the mold comprises at least four leaflets which when held together by a leaflet collar around a central post, comprise a hemisphere;
 - b. pouring a liquid polymer into the container, wherein the container surrounds the mold;
 - 20 c. partially crosslinking the polymer;
 - d. removing the mold and polymer from the container;
 - e. placing the mold and the polymer onto an expander, wherein the expander contains a central threaded channel in which the central post of the mold is threaded;
 - 25 f. advancing the mold with the polymer on the expander;
 - g. crosslinking the polymer while the expander is radically expanding the mold and polymer; and
 - h. removing the polymer from the mold.
- 30
14. The method of claim 13, wherein the polymer is poly(vinyl alcohol) (PVA).
15. The method of claim 14, wherein the polymer concentration is about 20%.

16. The method of claim 13, wherein the crosslinking is done by one or more freeze/thaw cycles, exposure to a chemical agent, or exposure to UV light.
17. The method of claim 16, wherein the freeze / thaw cycle comprises the steps:
- 5 a. ramping the temperature to about -20°C at a rate of about 0.5°C /minute
 - b. freezing the polymer at about -20°C for about 4 to about 24 hours;
 - c. ramping the temperature to about 20°C at a rate of about 0.5°C /minute; and
 - d. thawing the polymer at about 20°C for about 4 to about 12 hours.
18. The method of claim 13, wherein the partial crosslinking of step c. is done by one freeze/thaw cycle or exposure to UV light for about 10 to about 30 seconds.
19. The method of claim 13, wherein the crosslinking of step g. is done by five freeze/thaw cycles or exposure to UV light for about 60 minutes.
20. An engineered cartilage manufactured by the method of claim 13.
21. A method for making, manufacturing and/or producing meniscus, comprising the steps of: can comprise the following steps:
- 20 a. injecting a liquid polymer into an inlet port of a mold until the liquid polymer starts to flow from an outlet port, the mold being in the desired shape of the polymer and having an inlet port, an outlet port, an area for the polymer and an area for at least one rigid porous base and containing at least one rigid porous base;
 - b. partially crosslinking the polymer;
 - 25 c. removing the polymer attached to the at least one rigid porous base from the mold;
 - d. placing the partially crosslinked polymer and the at least one rigid porous base into a loading frame comprising at least one screw, a crank, and a post;
 - e. fixing the rigid porous base to the screw on the loading frame;
 - f. turning the crank of the loading frame clockwise to cause the rigid porous base to
 - 30 move toward the crank which in turn forces the polymer against the post;
 - g. crosslinking the polymer; and
 - h. removing the polymer from the loading frame.
22. The method of claim 21, wherein the polymer is poly(vinyl alcohol) (PVA).

23. The method of claim 22, wherein the polymer concentration is about 20%.
24. The method of claim 21, wherein the crosslinking is done by one or more freeze/thaw cycles, exposure to a chemical agent, or exposure to UV light.
- 5
25. The method of claim 24, wherein the freeze / thaw cycle comprises the steps:
- a. ramping the temperature to about -20°C at a rate of about 0.5°C /minute
 - b. freezing the polymer at about -20°C for about 4 to about 24 hours;
 - c. ramping the temperature to about 20°C at a rate of about 0.5°C /minute; and
 - 10 d. thawing the polymer at about 20°C for about 4 to about 12 hours.
26. The method of claim 21, wherein the partial crosslinking of step b. is done by one freeze/thaw cycle or exposure to UV light for about 10 to about 30 seconds.
- 15
27. The method of claim 21, wherein the crosslinking of step g. is done by five freeze/thaw cycles or exposure to UV light for about 60 minutes.
29. An engineered meniscus manufactured by the method of claim 21.
- 20
30. A mold for performing the method of claim 13, comprising:
- a. at least one leaflet, wherein the leaflet comprises a leaflet compression spring rod and a tension spring;
 - b. a leaflet collar; and
 - c. a central post with at least one compression spring tube, wherein the number of
 - 25 compression spring tubes corresponds to the number of leaflets.
31. The mold of claim 30, wherein the mold comprises four leaflets and the leaflets are assembled radially from the central post and held together by the leaflet collar.
- 30
32. An expander for performing the method of claim 13, comprising a threaded channel in which a central post of a mold is threaded.

33. A kit for making, manufacturing and/or producing engineered cartilage, comprising the mold of claim 31, the leaflet expander of claim 32, and a hemispherical container to hold the assembled mold.
- 5 34. The kit of claim 33, further comprising a crosslinkable polymer and instructions.
35. A mold for performing the method of claim 21, comprising an inlet port, an outlet port, an area for holding a polymer, and an area for holding at least one rigid porous base, wherein the mold is able to withstand freezing temperature, change in temperatures,
10 chemical crosslinking agents, and exposure to UV light.
36. A loading frame for performing the method of claim 21, comprising a base, a post, a crank, at least one worm, at least one worm gear, and at least one screw.
- 15 37. A kit for making, manufacturing and/or producing engineered meniscus, comprising the mold of claim 35 and the loading frame of claim 36.
38. The kit of claim 37, further comprising a crosslinkable polymer, at least one rigid
20 porous base, and instructions.

Figure 1



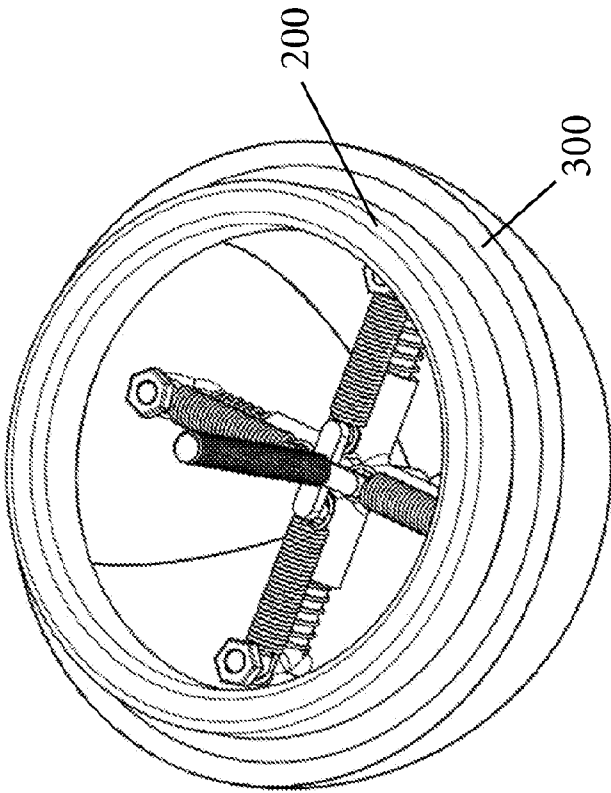


Figure 2B

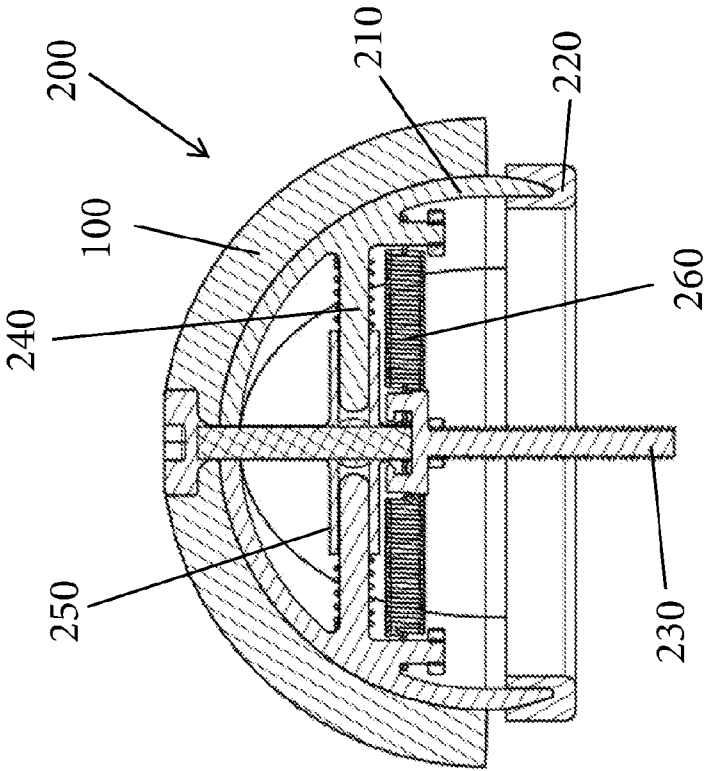


Figure 2A

Figure 2

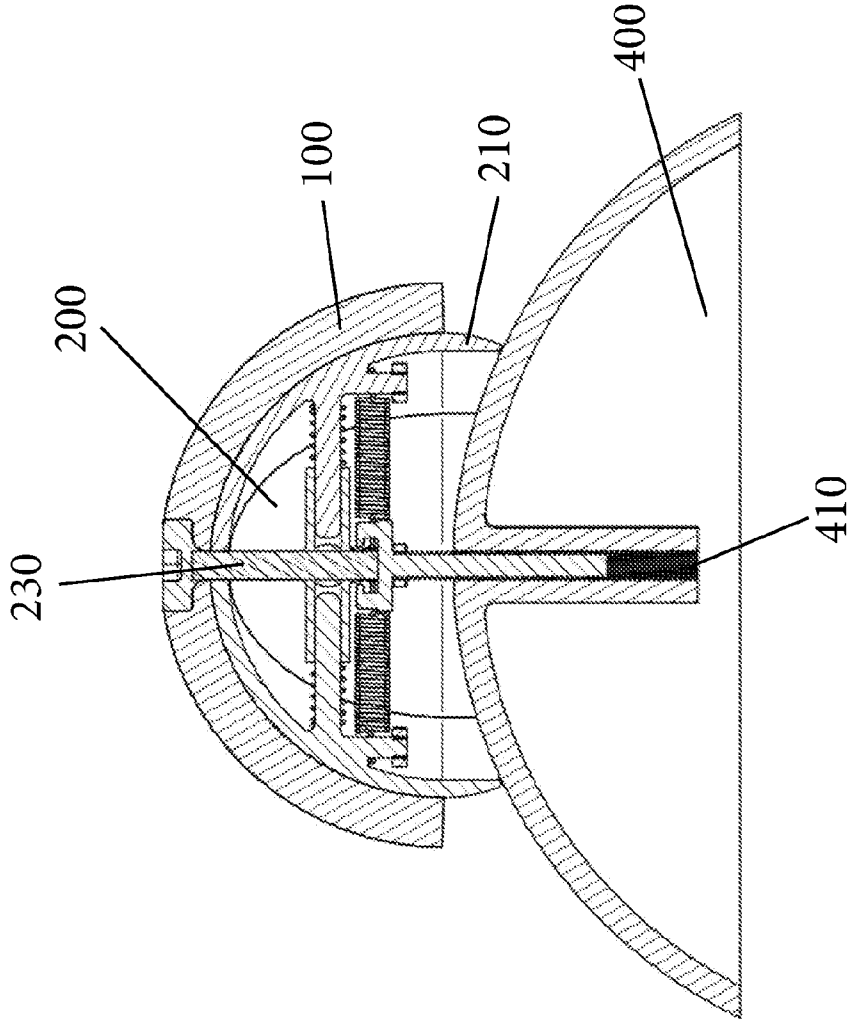


Figure 3

Figure 4

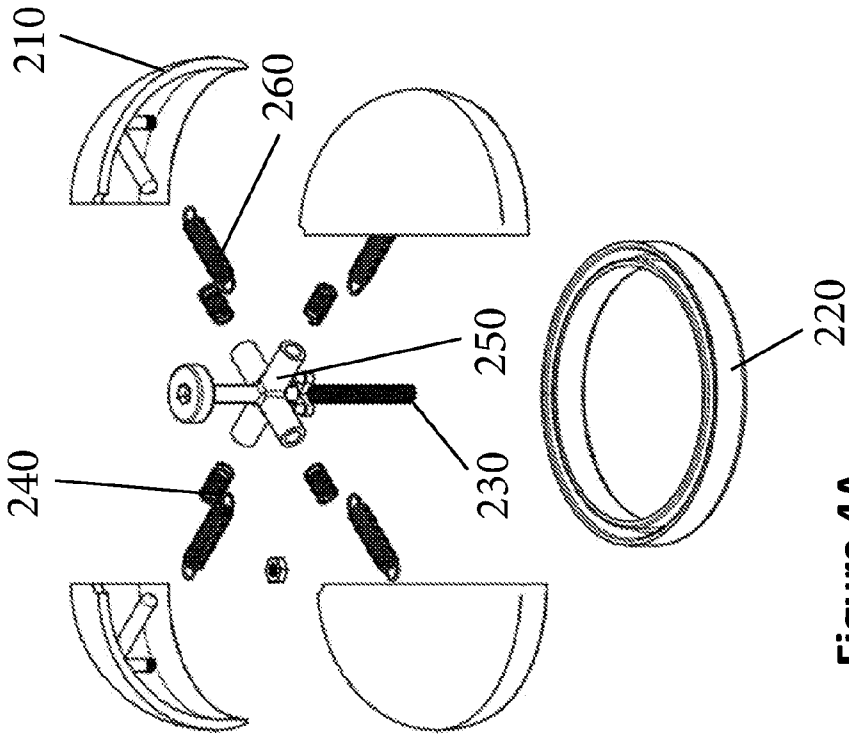


Figure 4A

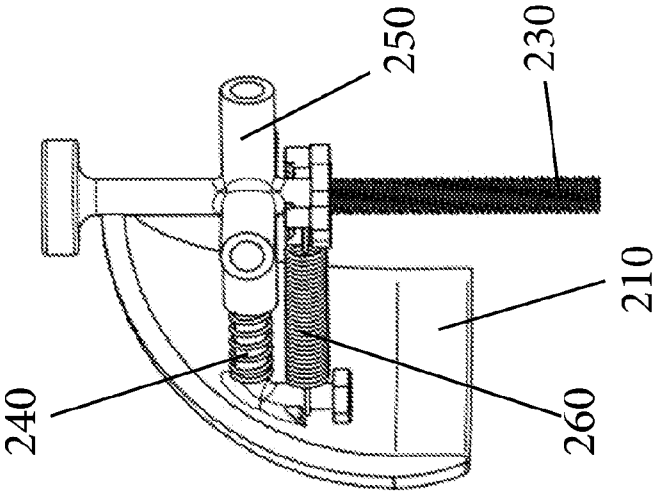


Figure 4B

Figure 5

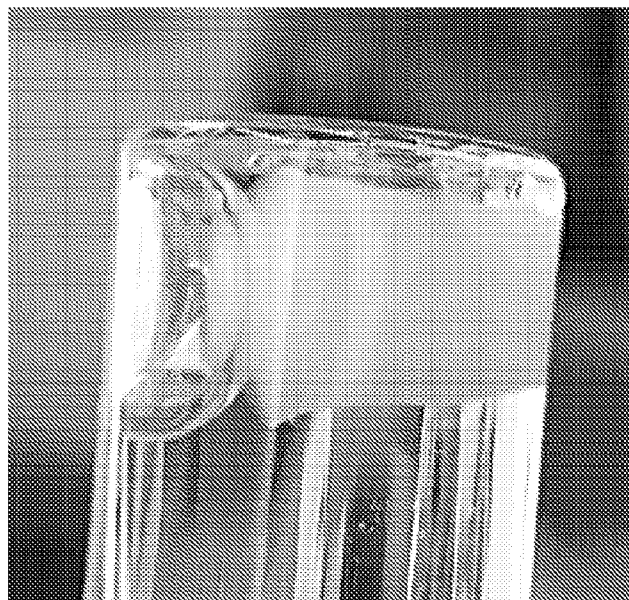


Figure 5A

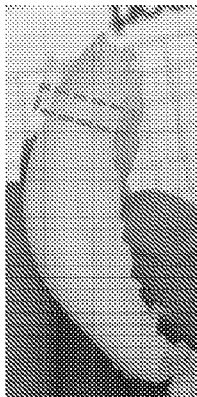


Figure 5B

Dilated PVA

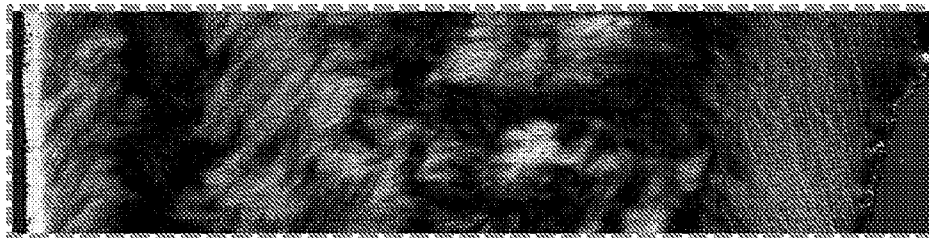


Figure 5C

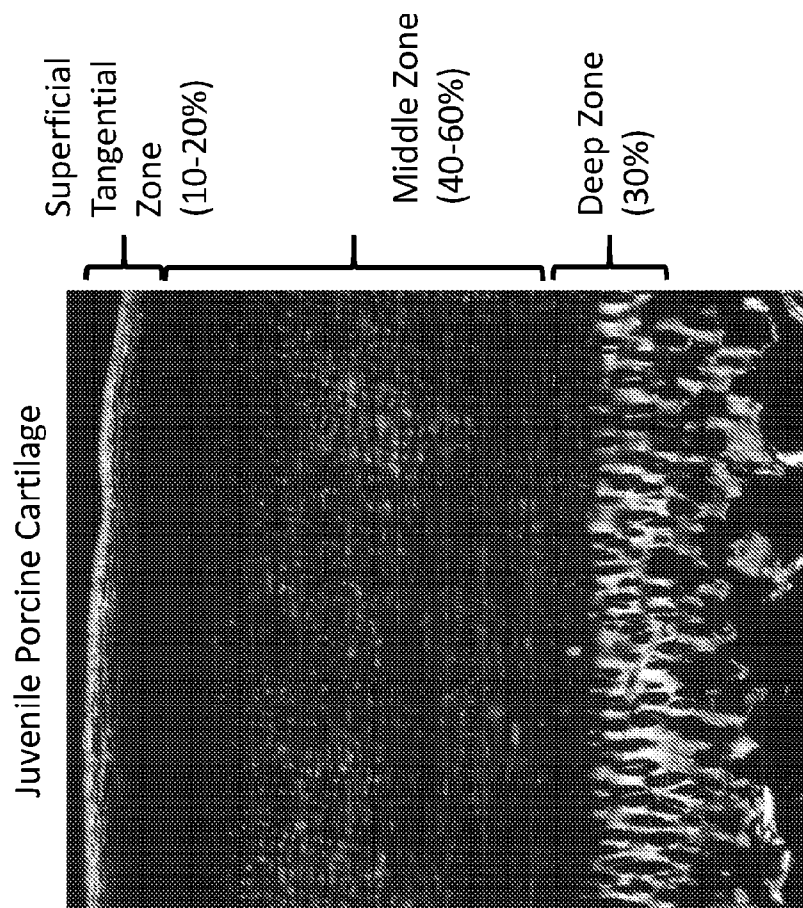


Figure 5D

Figure 6

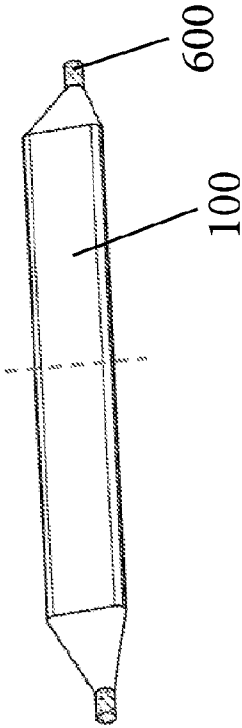
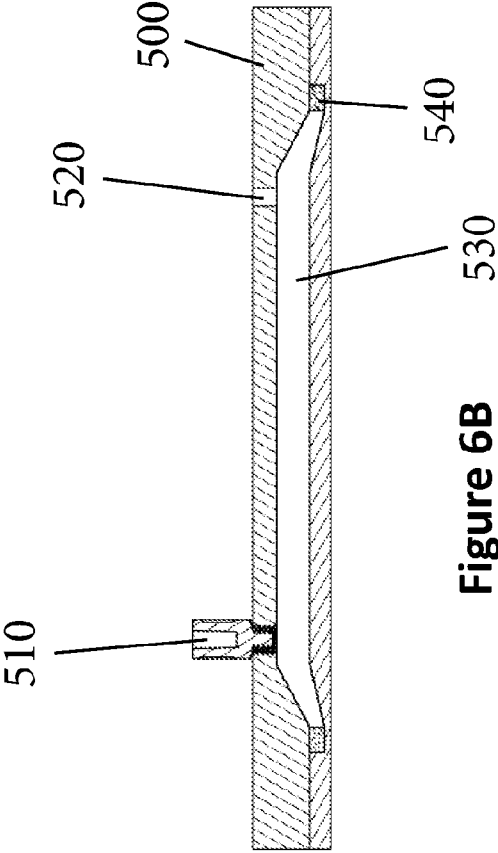
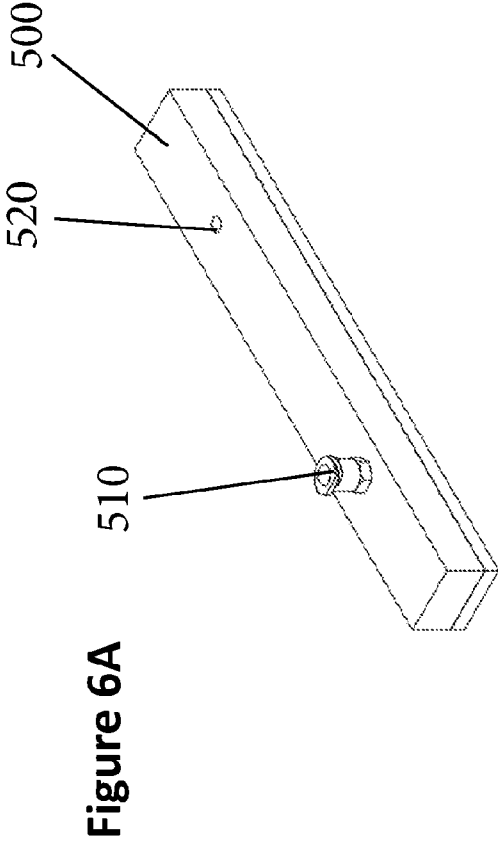


Figure 7

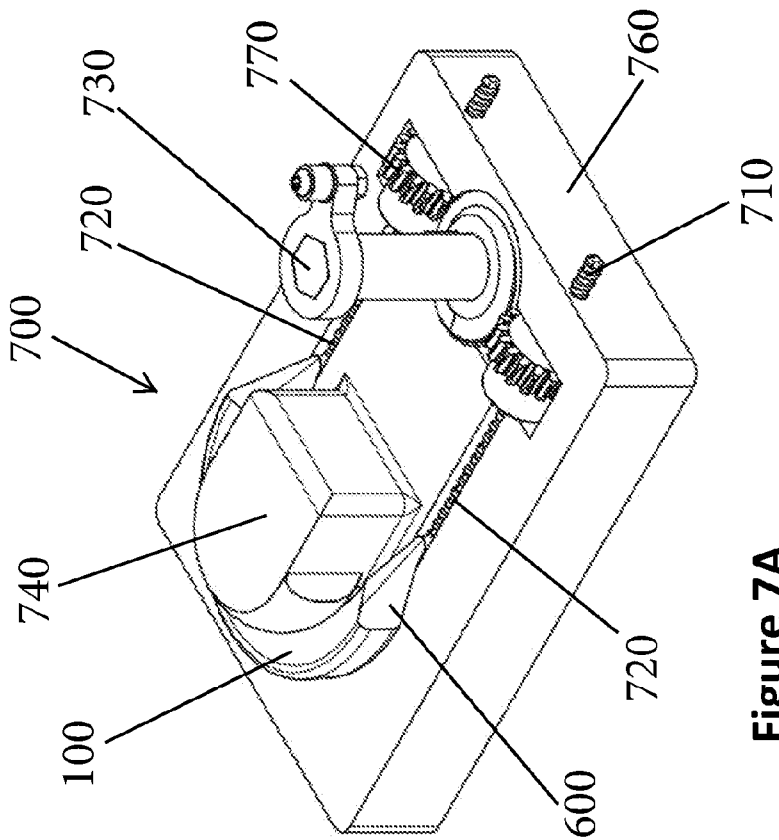


Figure 7A

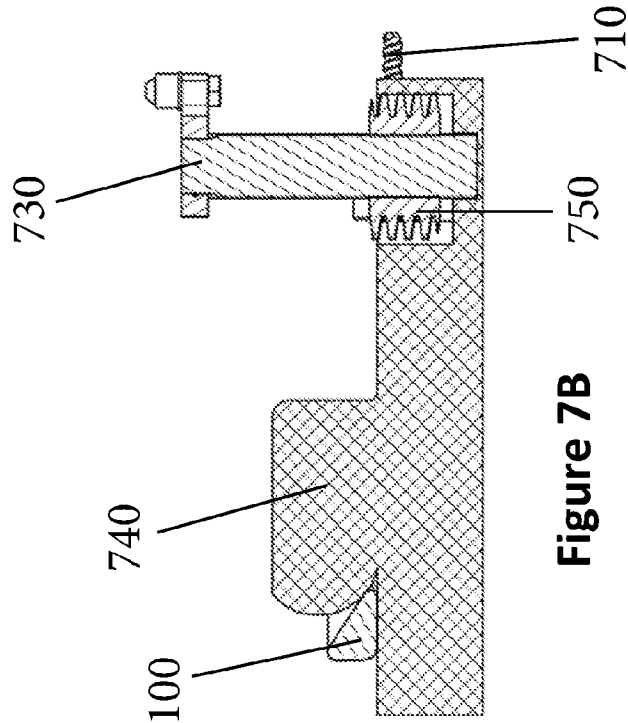


Figure 7B

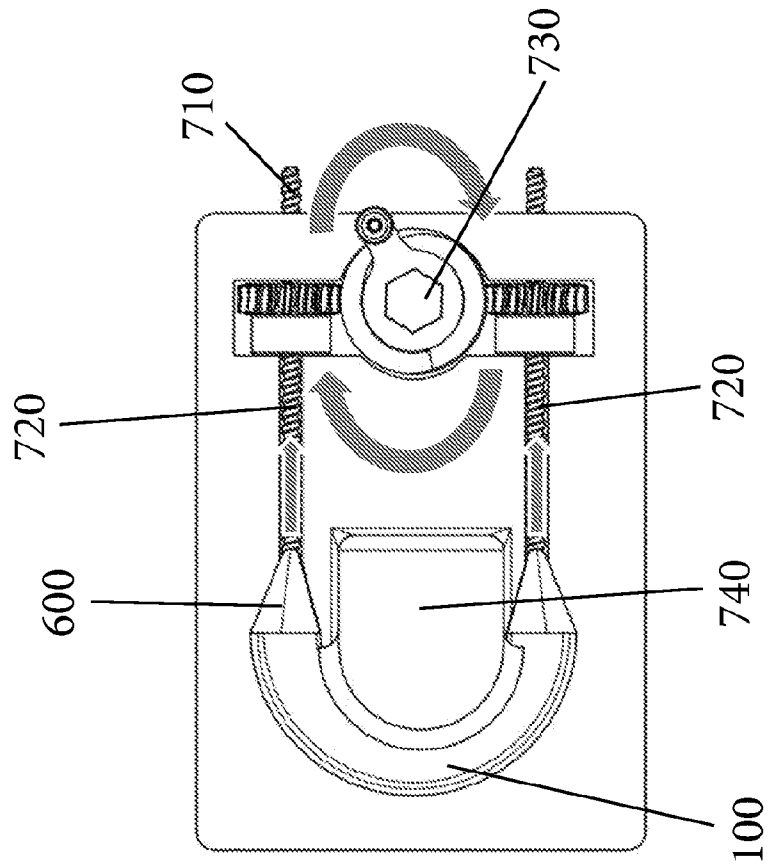


Figure 7C

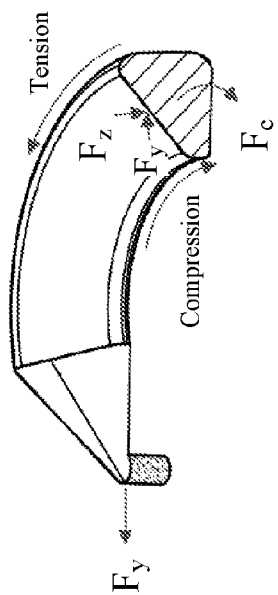


Figure 7D

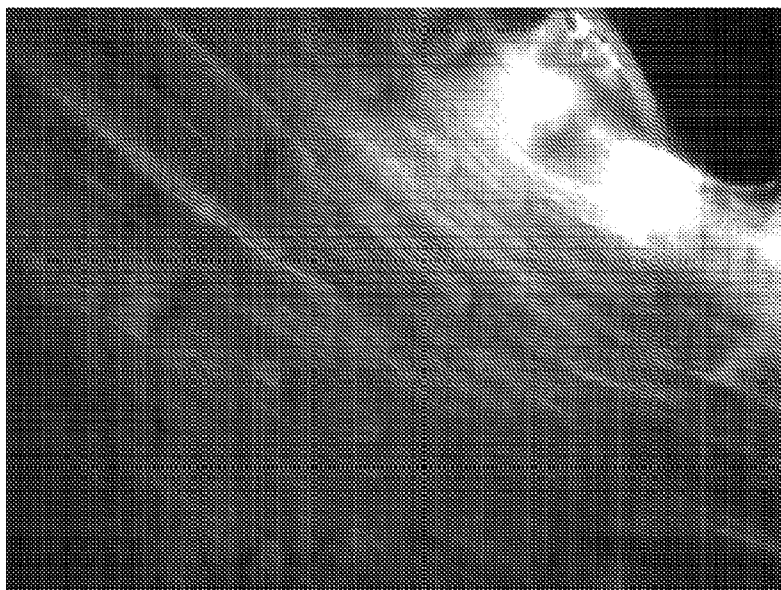


Figure 8