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(54) **APPARATUSES AND METHODS FOR TREATING BIOLOGICAL TISSUE TO MITIGATE CALCIFICATION**

(75) Inventors: **Sophie Carpentier**, Paris (FR); **Alain F. Carpentier**, Paris (FR); **Lillian J. Quintero**, Chester, NY (US); **Victor S. Packham**, Costa Mesa, CA (US); **Jong H. Wang**, Rancho Palos Verdes, CA (US); **Stefan G. Schreck**, Vista, CA (US)

(73) Assignee: **Edwards Lifesciences Corporation**, Irvine, CA (US)

(*) Notice: This patent is subject to a terminal disclaimer.

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(22) Filed: **Apr. 3, 2003**

Related U.S. Patent Documents

Reissue of:

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Issued: **Apr. 3, 2001**
Appl. No.: **09/283,596**
Filed: **Apr. 10, 1999**

U.S. Applications:

(62) Division of application No. 08/874,180, filed on Jun. 13, 1997, now Pat. No. 5,931,969, which is a continuation-in-part of application No. 08/812,506, filed on Mar. 7, 1997, now abandoned, which is a continuation of application No. 08/282,358, filed on Jul. 29, 1994, now abandoned.

(51) **Int. Cl.**
A61F 2/04 (2006.01)
A01N 1/00 (2006.01)

(52) **U.S. Cl.** ..... **435/284.1**; 8/94.11; 8/94.1 R; 600/36; 623/2.13; 623/23.72; 623/915; 435/289.1; 435/297.1; 435/297.2; 435/297.5; 435/303.1; 435/307.1

(58) **Field of Classification Search** ..... 8/94.1 R, 8/94.11; 600/36; 623/2.13, 23.72, 915; 435/284.1, 435/289.1, 297.1, 297.2, 297.5, 303.1, 307.1
See application file for complete search history.

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Primary Examiner—Brian P Mruk
(74) *Attorney, Agent, or Firm*—Rajiv Yadav; Guy Cumberbatch

(57) **ABSTRACT**

An apparatus for treating fixed biological tissue to inhibit calcification of the biological tissue following implantation thereof in a mammalian body. The apparatus includes a container for placing the biological tissue in contact with a treatment solution, structure to induce relative tissue/solution movement, and structure to heat the solution. The relative movement may be induced by shaking a container in which the tissue is immersed in the treatment solution, or by stirring the solution within the container. The movement may also be induced by flowing a treatment solution past the tissue to be treated. The tissue may be free to move in the treatment container, or may be restrained from gross movements. The flow may be part of a circulation system having a reservoir, with a heater being provided to heat the treatment solution in the reservoir. Alternatively, a treatment apparatus, including a fluid circulation system if desired, may be enclosed in an incubator. The tissue may be mounted in a planar configuration generally parallel to the direction of fluid flow. A flow column having a plurality of sections divided by perforated baffles may be used to treat multiple tissues at once.

72 Claims, 9 Drawing Sheets

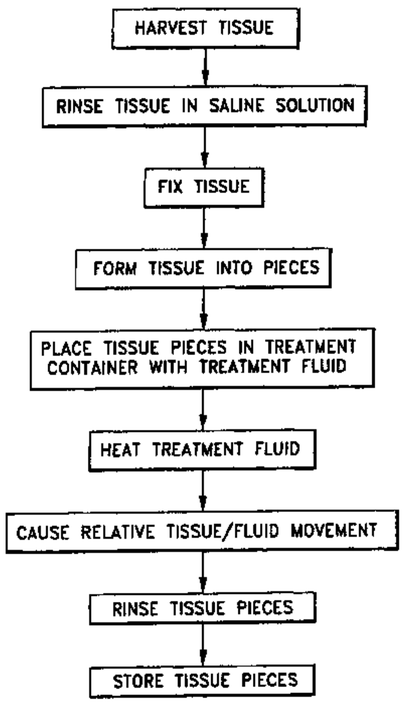

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FIG. 1

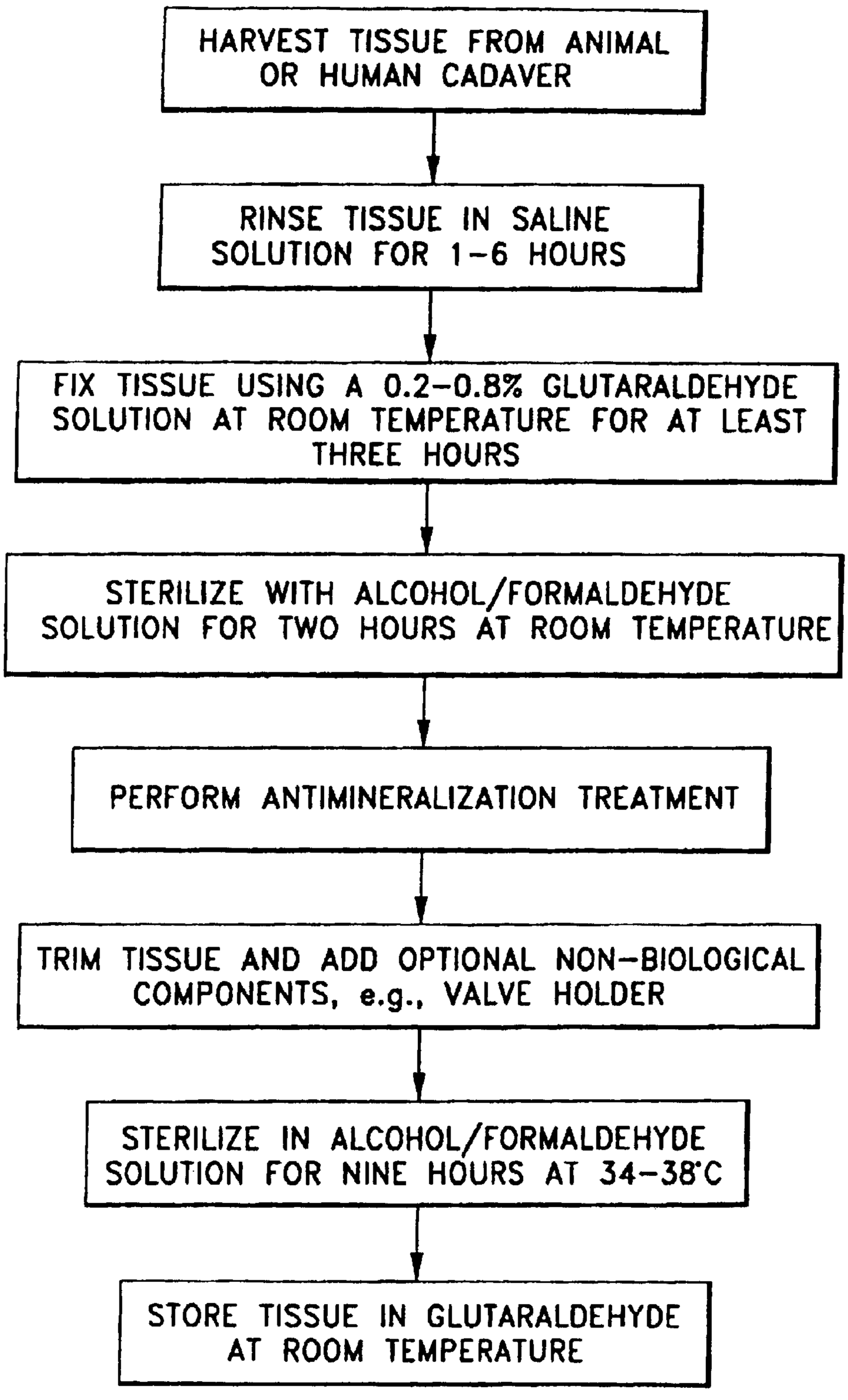

HARVEST TISSUE FROM ANIMAL OR HUMAN CADAVER
RINSE TISSUE IN SALINE SOLUTION FOR 1-6 HOURS
FIX TISSUE USING A 0.2-0.8% GLUTARALDEHYDE SOLUTION AT ROOM TEMPERATURE FOR AT LEAST THREE HOURS
STERILIZE WITH ALCOHOL/FORMALDEHYDE SOLUTION FOR TWO HOURS AT ROOM TEMPERATURE
PERFORM ANTIMINERALIZATION TREATMENT
TRIM TISSUE AND ADD OPTIONAL NON-BIOLOGICAL COMPONENTS, e.g., VALVE HOLDER
STERILIZE IN ALCOHOL/FORMALDEHYDE SOLUTION FOR NINE HOURS AT 34-38°C
STORE TISSUE IN GLUTARALDEHYDE AT ROOM TEMPERATURE

FIG. 2

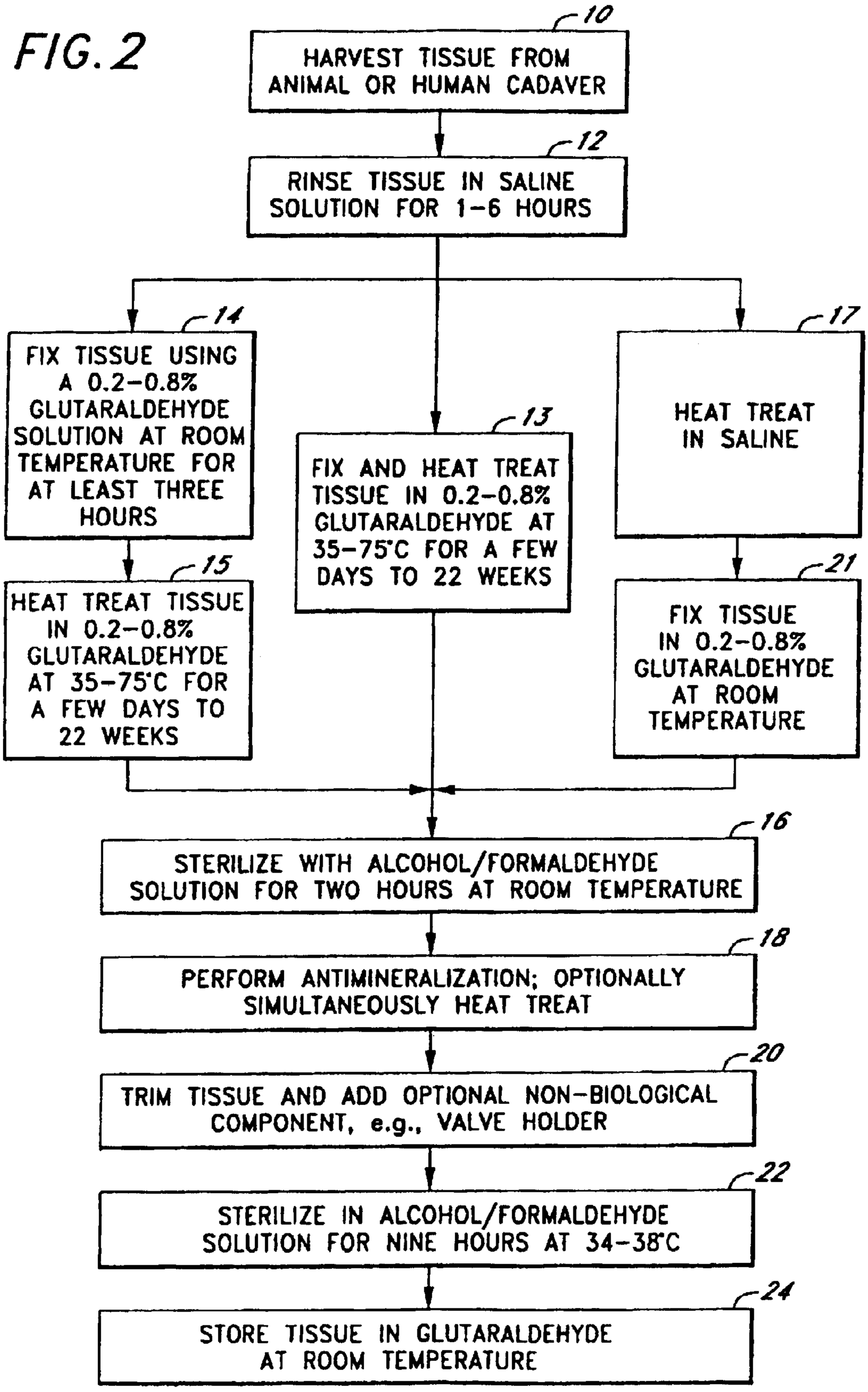

10
HARVEST TISSUE FROM ANIMAL OR HUMAN CADAVER
12
RINSE TISSUE IN SALINE SOLUTION FOR 1–6 HOURS
14
FIX TISSUE USING A 0.2–0.8% GLUTARALDEHYDE SOLUTION AT ROOM TEMPERATURE FOR AT LEAST THREE HOURS
15
HEAT TREAT TISSUE IN 0.2–0.8% GLUTARALDEHYDE AT 35–75°C FOR A FEW DAYS TO 22 WEEKS
13
FIX AND HEAT TREAT TISSUE IN 0.2–0.8% GLUTARALDEHYDE AT 35–75°C FOR A FEW DAYS TO 22 WEEKS
17
HEAT TREAT IN SALINE
21
FIX TISSUE IN 0.2–0.8% GLUTARALDEHYDE AT ROOM TEMPERATURE
16
STERILIZE WITH ALCOHOL/FORMALDEHYDE SOLUTION FOR TWO HOURS AT ROOM TEMPERATURE
18
PERFORM ANTIMINERALIZATION; OPTIONALLY SIMULTANEOUSLY HEAT TREAT
20
TRIM TISSUE AND ADD OPTIONAL NON-BIOLOGICAL COMPONENT, e.g., VALVE HOLDER
22
STERILIZE IN ALCOHOL/FORMALDEHYDE SOLUTION FOR NINE HOURS AT 34–38°C
24
STORE TISSUE IN GLUTARALDEHYDE AT ROOM TEMPERATURE

FIG. 3

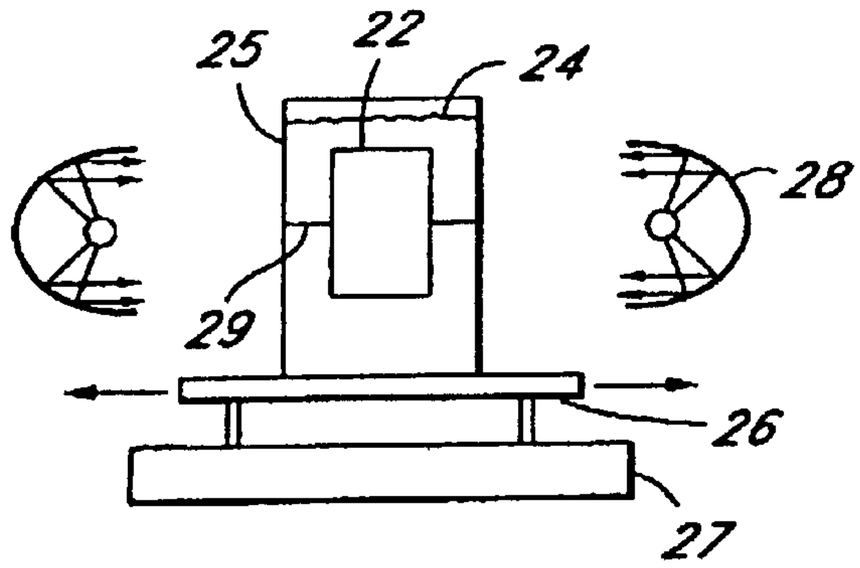


FIG. 4

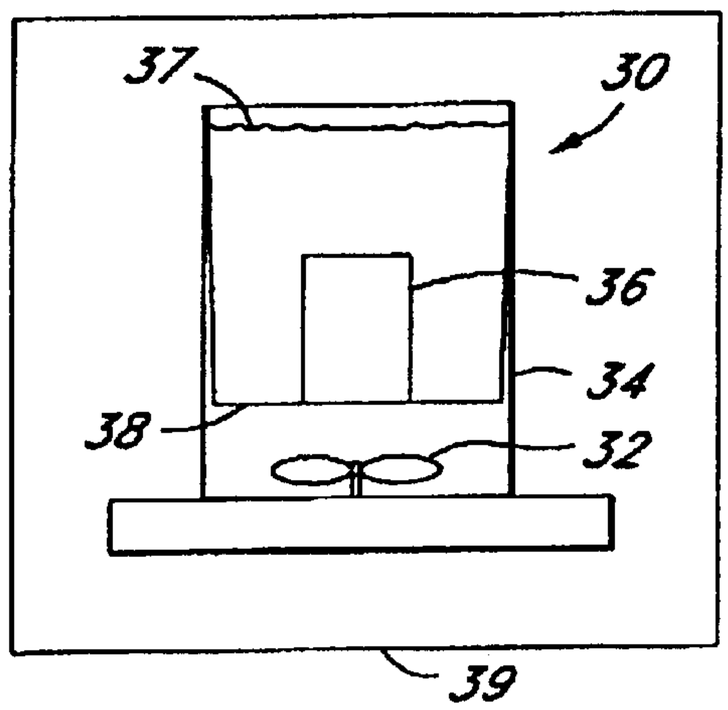


FIG. 6

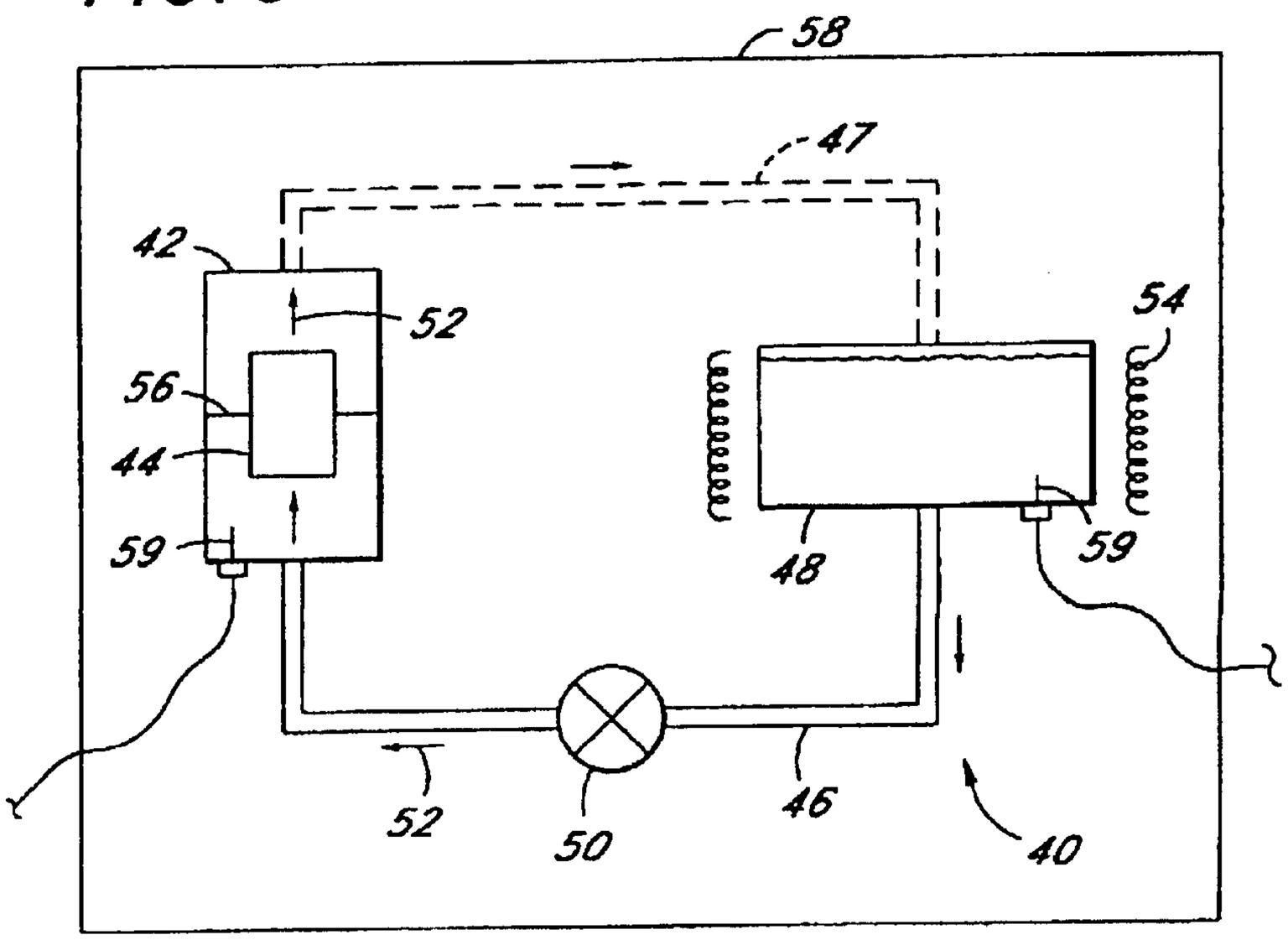


FIG. 5

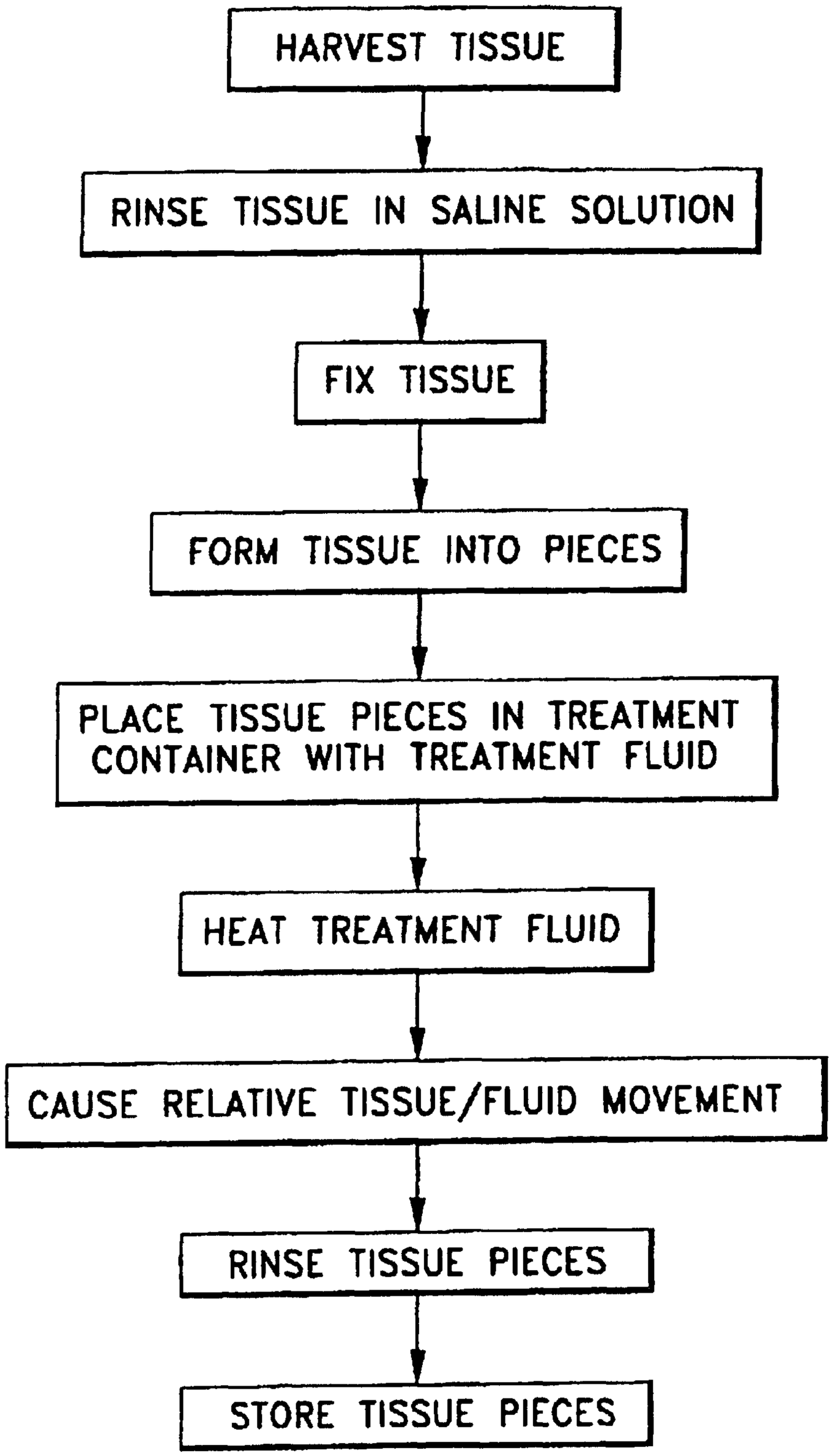

HARVEST TISSUE
RINSE TISSUE IN SALINE SOLUTION
FIX TISSUE
FORM TISSUE INTO PIECES
PLACE TISSUE PIECES IN TREATMENT CONTAINER WITH TREATMENT FLUID
HEAT TREATMENT FLUID
CAUSE RELATIVE TISSUE/FLUID MOVEMENT
RINSE TISSUE PIECES
STORE TISSUE PIECES

FIG. 7

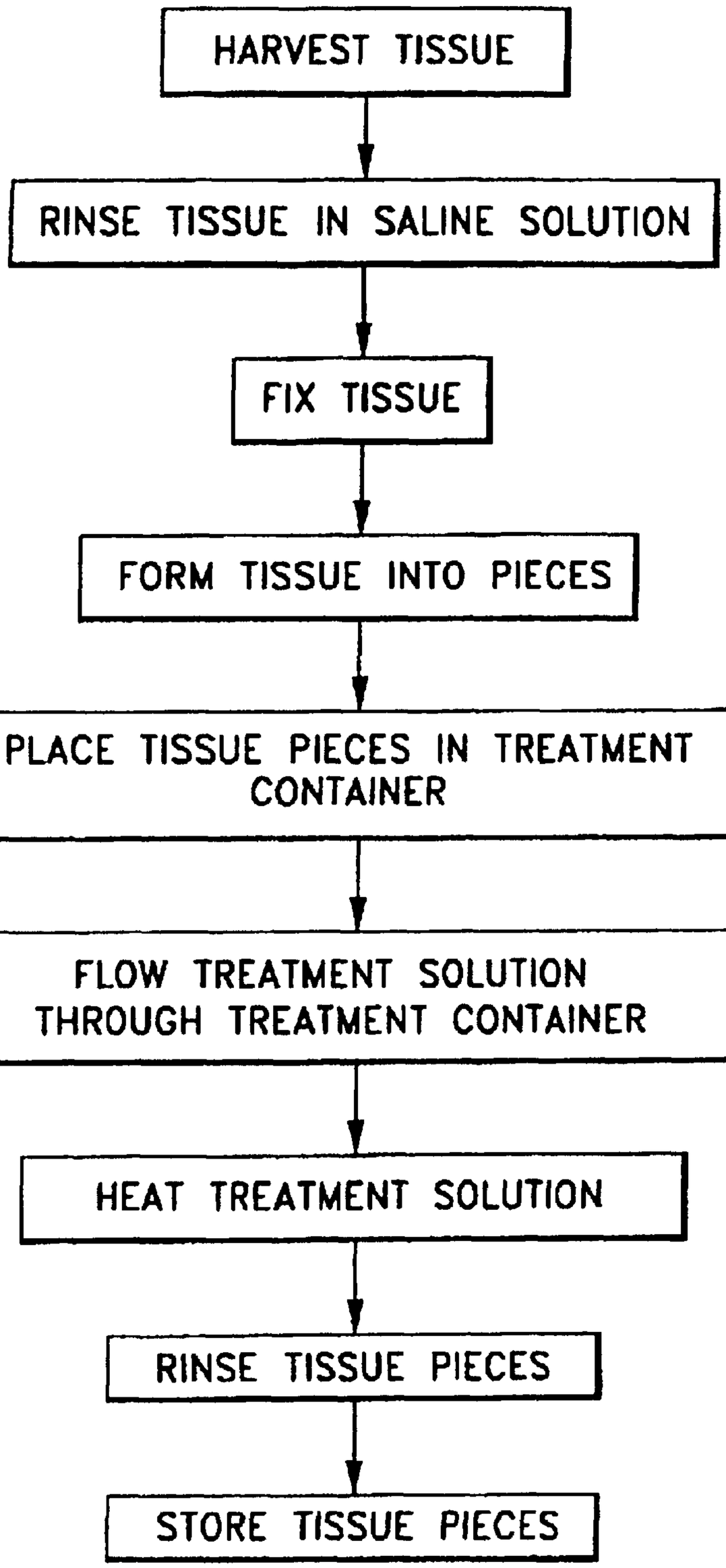

HARVEST TISSUE
RINSE TISSUE IN SALINE SOLUTION
FIX TISSUE
FORM TISSUE INTO PIECES
PLACE TISSUE PIECES IN TREATMENT CONTAINER
FLOW TREATMENT SOLUTION THROUGH TREATMENT CONTAINER
HEAT TREATMENT SOLUTION
RINSE TISSUE PIECES
STORE TISSUE PIECES

FIG. 8

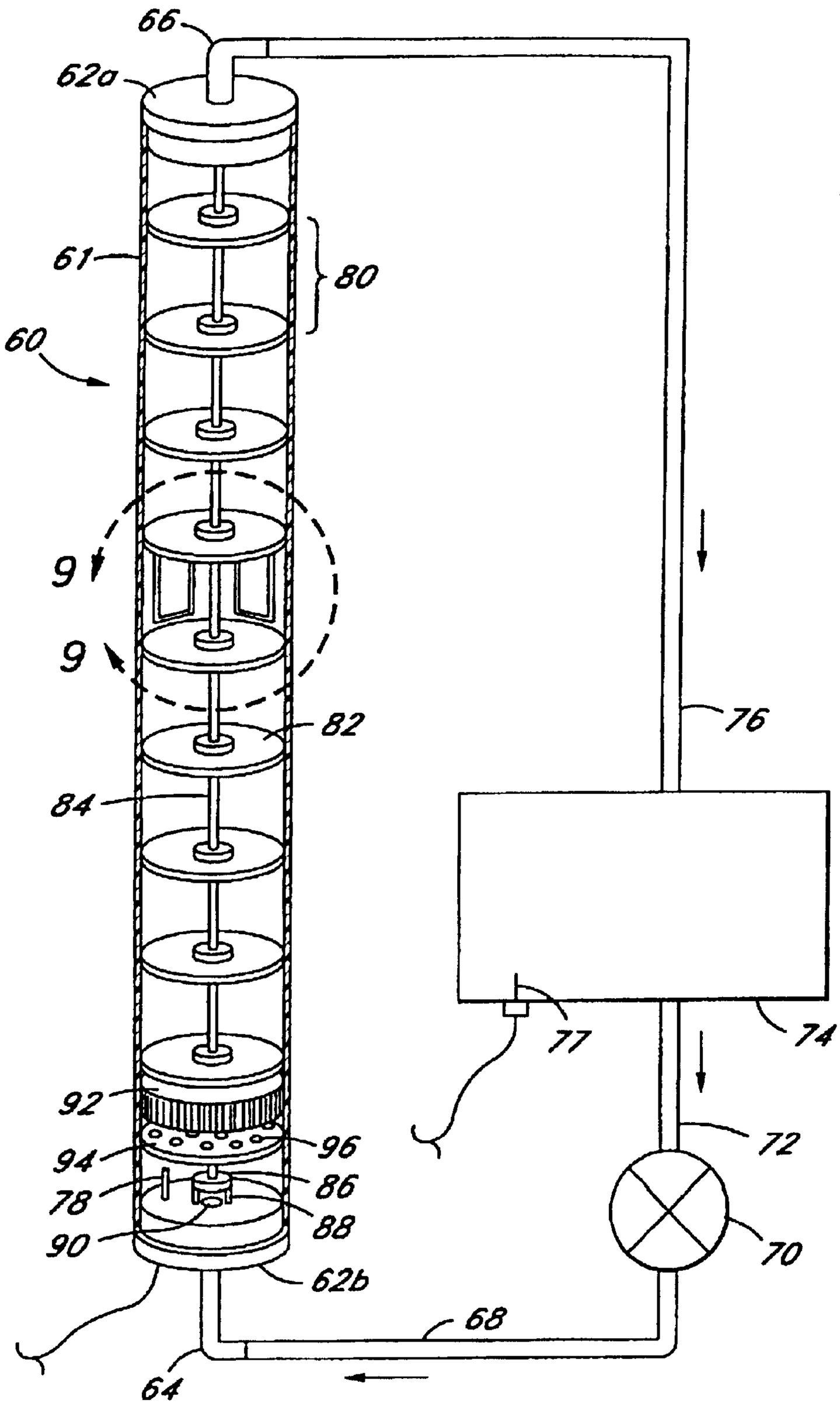


FIG. 9

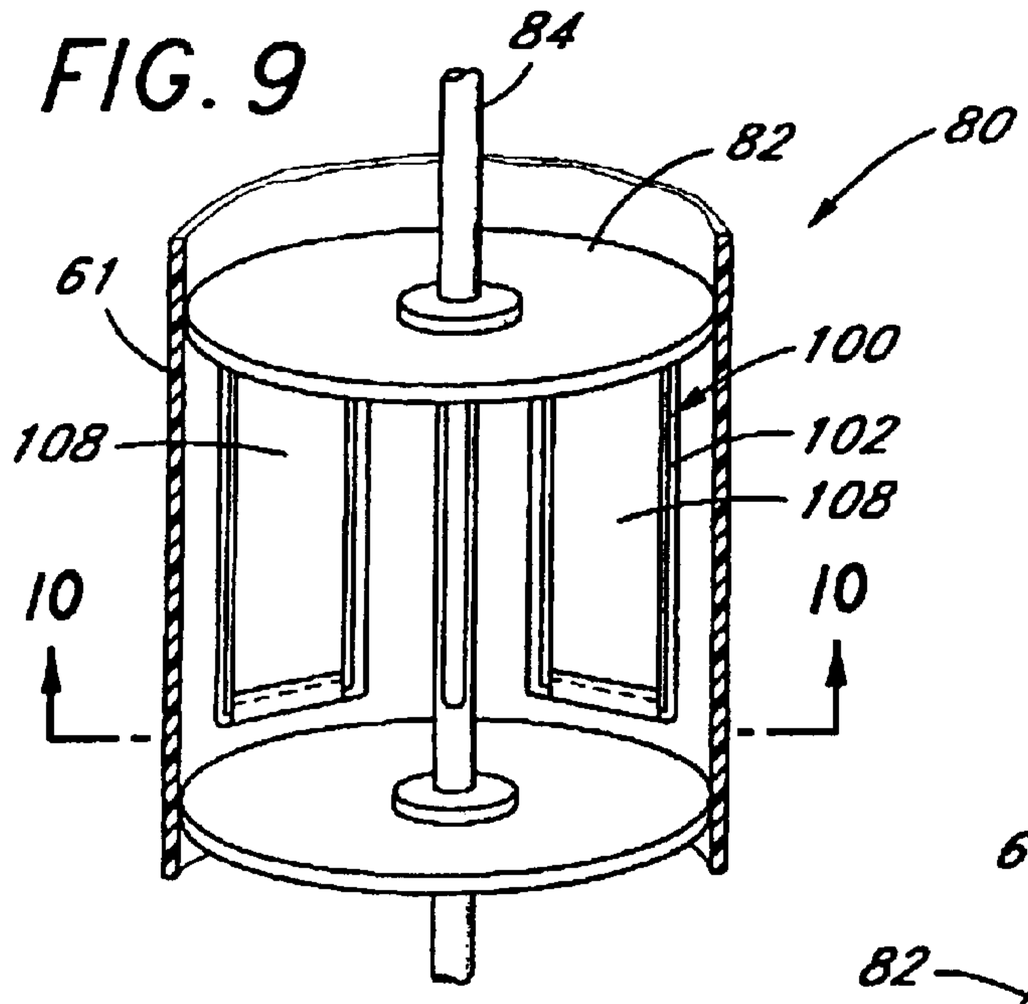


FIG. 10

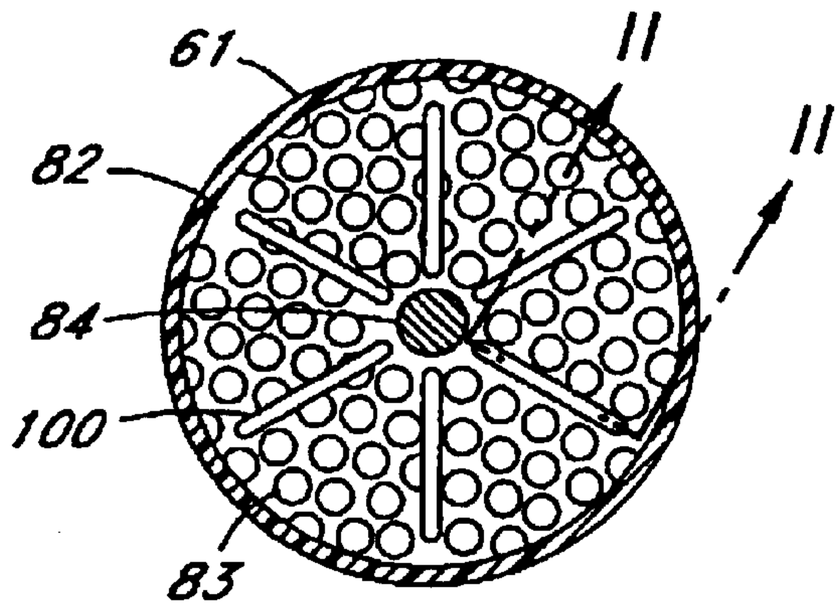


FIG. 11

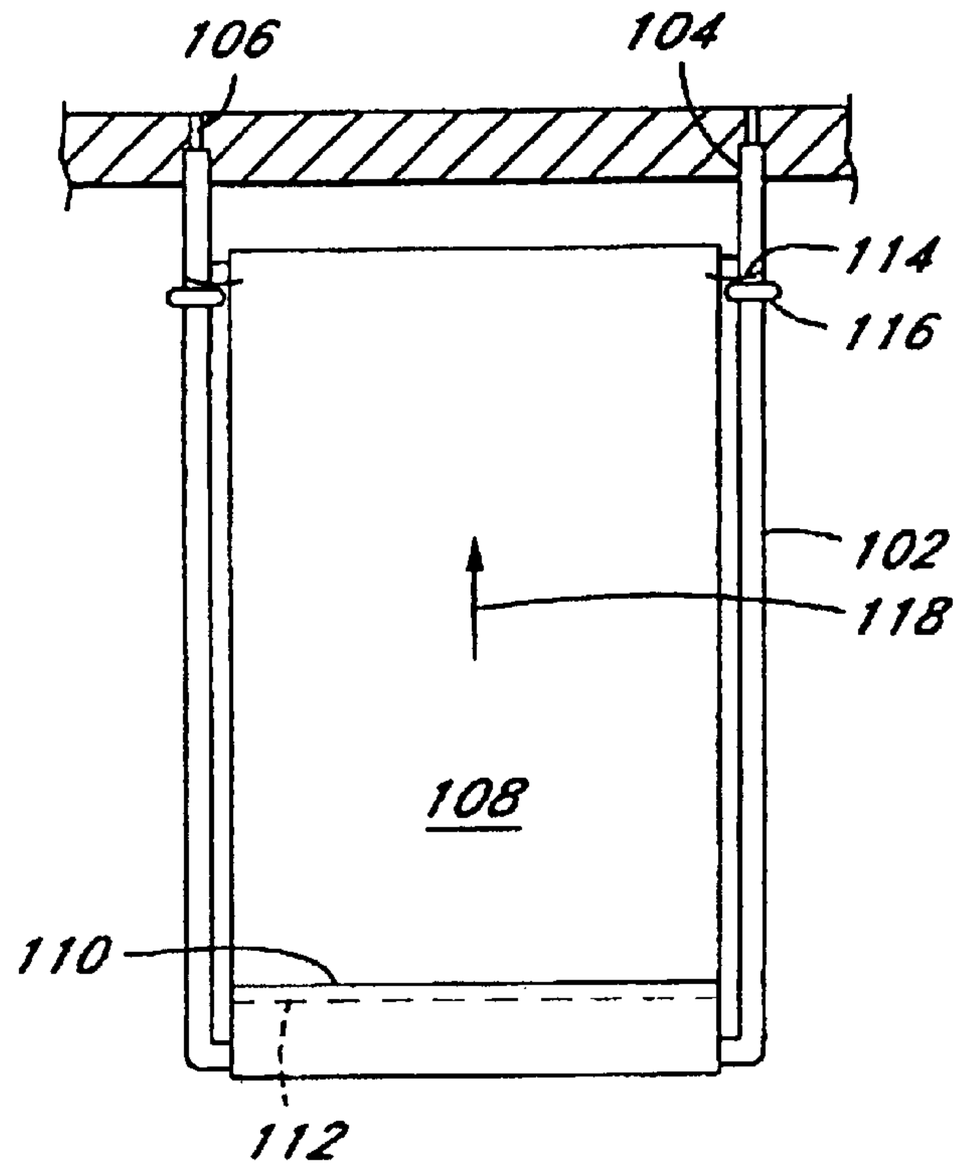


FIG. 12

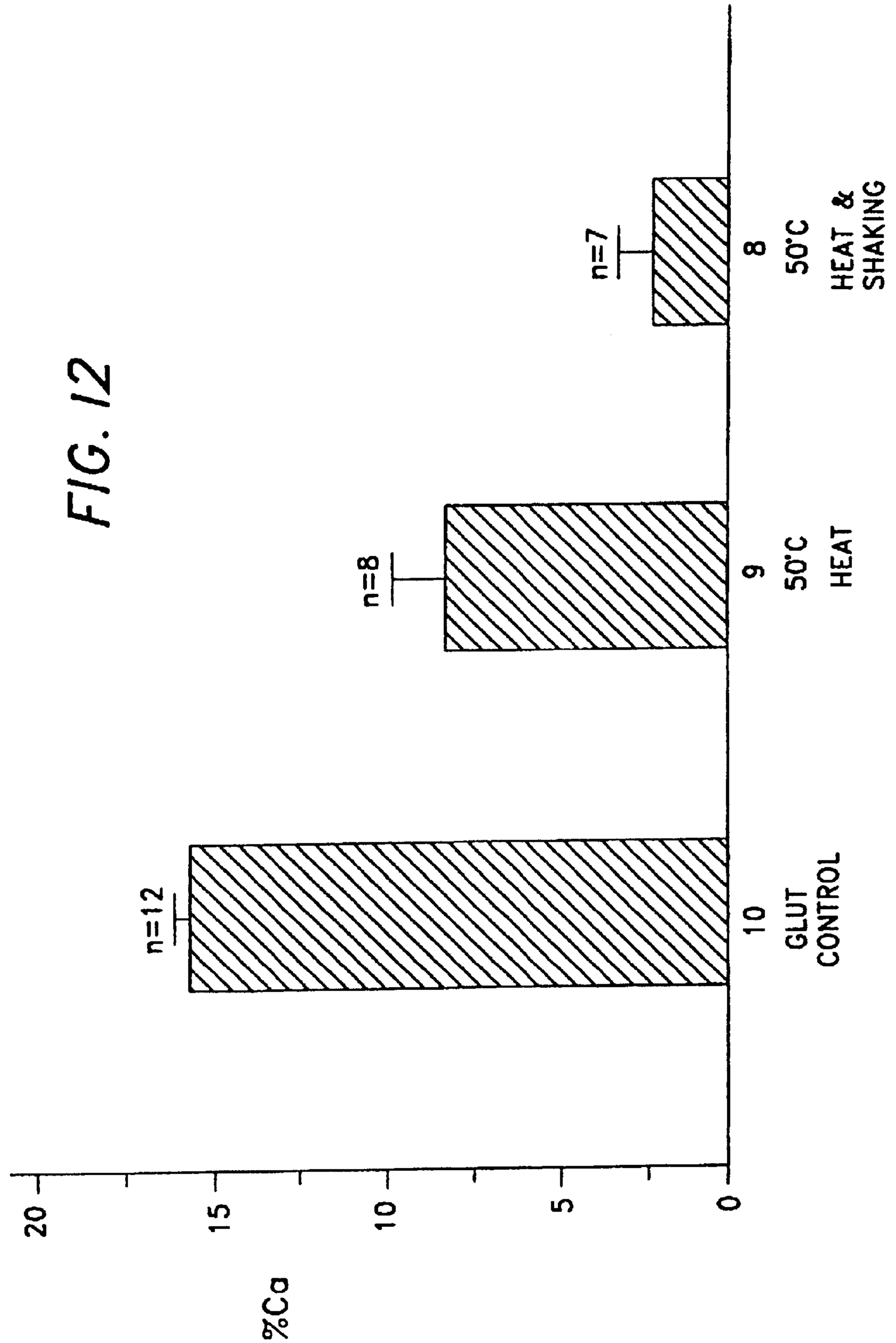
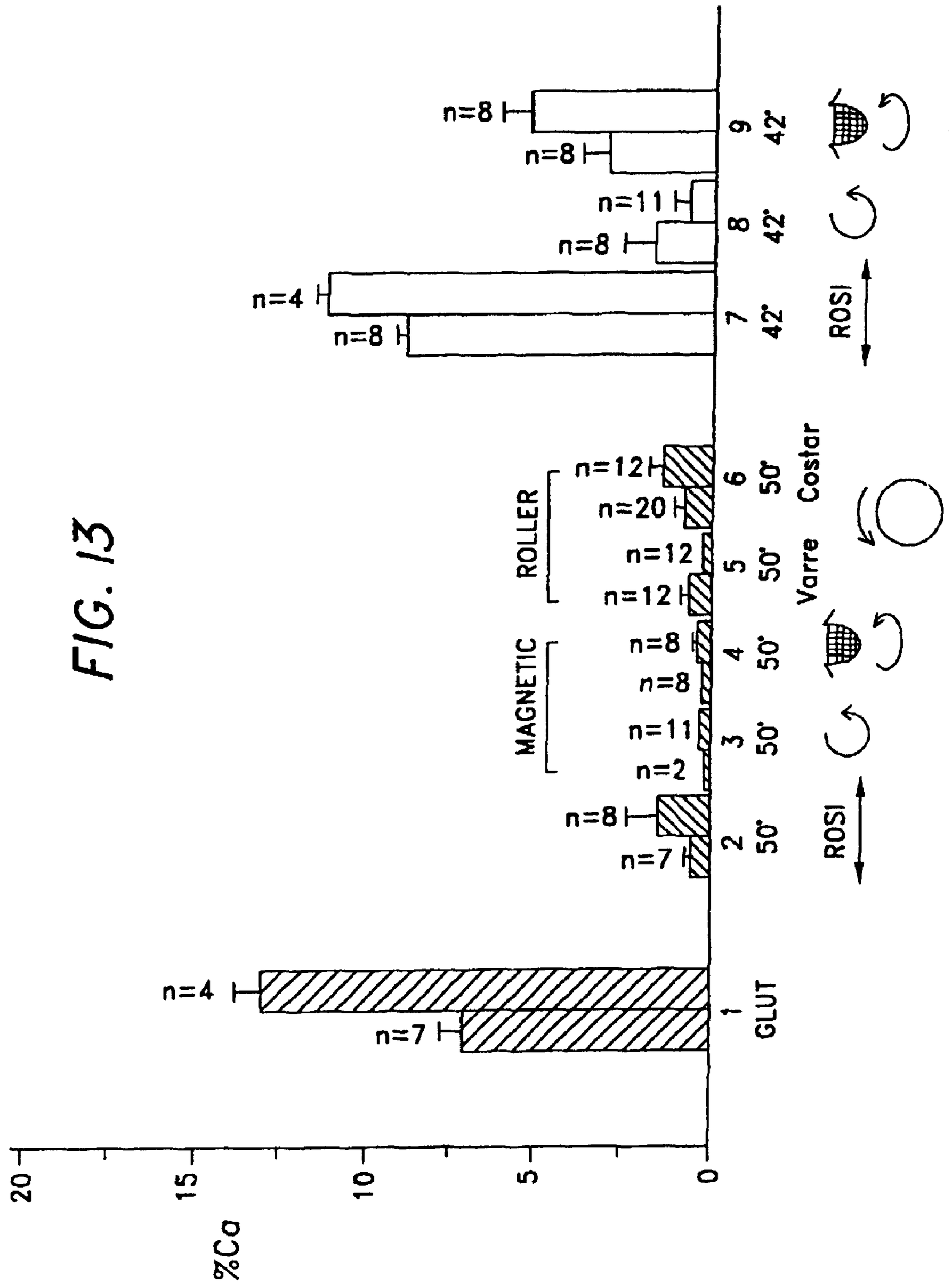

%Ca
20
15
10
5
0
n=12
10
GLUT
CONTROL
n=8
9
50°C
HEAT
n=7
8
50°C
HEAT &
SHAKING

FIG. 13


n=8
n=8
9
42°
n=11
n=8
8
42°
n=4
n=8
7
42°
ROSI
n=12
n=20
6
50°
Costar
ROLLER
n=12
n=12
5
50°
Varre
n=8
n=8
4
50°
MAGNETIC
n=11
n=2
3
50°
n=8
n=7
2
50°
ROSI
n=4
n=7
1
GLUT
20
15
10
5
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%Co

APPARATUSES AND METHODS FOR TREATING BIOLOGICAL TISSUE TO MITIGATE CALCIFICATION

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

RELATED APPLICATIONS

The present application is a divisional of U.S. application Ser. No. 08/874,180, filed Jun. 13, 1997, now U.S. Pat. No. 5,931,969, entitled "Methods and Apparatuses for Treating Biological Tissue to Mitigate Calcification," which is a *continuation-in-part of U.S. application Ser. No. 08/812,506, filed Mar. 7, 1997, now abandoned, which is a* continuation of U.S. application Ser. No. 08/282,358, filed Jul. 29, 1994, now abandoned.

Notice: More than one reissue application has been filed for the reissue of U.S. Pat. No. 6,210,957. The reissue applications are application Ser. Nos. 10/406,354 (the present application), and 10/834,399, all of which are divisional reissues of U.S. Pat. No. 6,210,957.

FIELD OF THE INVENTION

The present invention pertains generally to apparatuses for preparing biomedical materials, and more particularly to an apparatus for preparing preserved biological tissue, such as bovine pericardium, for implantation in a mammalian body that induces relative treatment fluid/tissue motion.

BACKGROUND OF THE INVENTION

The prior art has included numerous methods for preserving or fixing biological tissues, to enable such tissues to be subsequently implanted into mammalian bodies. Examples of the types of biological tissues that have heretofore been utilized for surgical implantation include cardiac valves, vascular tissue, skin, dura mater, pericardium, ligaments and tendons.

The term "grafting" as used herein is defined as the implanting or transplanting of any living tissue or organ (See Dorlands Illustrated Medical Dictionary, 27th Edition, W.B. Saunders Co. 1988). Biological tissues which are grafted into the body of a mammal may be xenogeneic (i.e., a xenograft) or allogeneic (i.e., an allograft). The term "bioprosthesis" defines many types of biological tissues chemically pretreated before implantation (Carpentier—See Ionescu (editor), Biological Tissue in Heart Valve Replacement, Butterworths, 1972). As opposed to a graft, the face of a bioprosthesis is based upon the stability of the chemically treated biological material and not upon cell viability or host cell ingrowth. Chemical pretreatment includes the "fixing" or tamning of the biological tissue. Such fixing or tanning of the tissue is accomplished by exposing the tissue to one or more chemical compounds capable of cross-linking collagen molecules within the tissue.

Various chemical compounds have been utilized to fix or cross-link biological tissues including formaldehyde, glutaraldehyde, dialdehyde starch, hexamethylene diisocyanate and certain polyepoxy compounds.

In particular, glutaraldehyde has proven to be relatively physiologically inert and suitable for fixing various biological tissues for subsequent surgical implantation (Carpentier, A., J. Thorac. Cardiovasc. Surg. 58:467–68 (1969)). In particular, examples of the types of biological tissues which have heretofore been subjected to glutaraldehyde fixation include porcine bioprosthetic heart valves and bovine pericardial tissues.

Clinical experience has revealed that glutaraldehyde-fixed bioprosthetic tissues may tend to become calcified. Such calcification of glutaraldehyde-fixed bioprosthetic tissues has been reported to occur most predominantly in pediatric patients see, Carpentier et al. and "Continuing Improvements in Valvular Bioprostheses, J. Thorac Cardiovasc. Surg. 83:27–42, 1982. Such calcification is undesirable in that it may result in deterioration of the mechanical properties of the tissue and/or tissue failure. In view of this, surgeons have opted to implant mechanical cardiovascular valves into pediatric patients, rather than to utilize glutaraldehyde-preserved porcine valves. However, pediatric patients who receive mechanical valve implants require long term treatment with anticoagulant medications and such anticoagulation is associated with increased risk of hemorrhage.

The mechanism by which calcification occurs in glutaraldehyde-fixed bioprosthetic tissue has not been fully elucidated. However, factors which have been thought to influence the rate of calcification include:

a) patient's age

b) existing metabolic disorders (i.e., hypercalcemia, diabetes, kidney failure . . .)

c) dietary factors

d) race

e) infection

f) parenteral calcium administration

g) dehydration

h) distortion/mechanical factors

i) inadequate coagulation therapy during initial period following surgical implantation; and

j) host tissue chemistry

Methods for treating fixed biological tissue so as to inhibit calcification thereof following implantation in a mammalian body tend to substantially increase the usable life of such tissue subsequent to implantation in a mammalian body, thereby mitigating the requirements for subsequent tissue replacement. As those skilled in the art will appreciate, such tissue replacement frequently causes substantial trauma to the patient, occasionally resulting in the patient's death. As such, it greatly beneficial to be able to either avoid or postpone the need for the replacement of implanted biological tissue.

Various efforts have been undertaken to find ways to mitigating calcification of glutaraldehyde fixed bioprosthetic tissue. Included among these calcification mitigation techniques are the methods described in U.S. Pat. No. 4,885,005 (Nashef et al.) SURFACTANT TREATMENT OF IMPLANTABLE BIOLOGICAL TISSUE TO INHIBIT CALCIFICATION; U.S. Pat. No. 4,648,881 (Carpentier et al.) IMPLANTABLE BIOLOGICAL TISSUE AND PROCESS FOR PREPARATION THEREOF; U.S. Pat. No. 4,976,733 (Girardot) PREVENTION OF PROSTHESIS CALCIFICATION; U.S. Pat. No. 4,120,649 (Schechter) TRANSPLANTS; U.S. Pat. No. 5,002,2566 (Carpentier) CALCIFICATION MITIGATION OF BIOPROSTHETIC IMPLANTS; EP 103947A2 (Pollock et al.) METHOD FOR INHIBITING MINERALIZATION OF NATURAL TISSUE DURING IMPLANTATION; WO84/01879 (Nashef et al.) SURFACTANT TREATMENT OF IMPLANTABLE BIOLOGICAL TISSUE TO INHIBIT CALCIFICATION;

U.S. Pat. No. 5,595,571 (Jaffe) BIOLOGICAL MATERIAL PRE-FIXATION TREATMENT; and WO 95/11047 (Levy et. al.) METHOD OF MAKING CALCIFICATION-RESISTANT BIOPROSTHETIC TISSUE.

Although some researchers believe that glutaraldehyde actually increases the risk of calcification, it is still the most accepted fixation solution. For example, the Levy patent application noted above utilizes an alcohol treatment for mitigating calcification, in addition to a glutaraldehyde fixation

There is significant research occurring into the extent the mechanisms mentioned above cause calcification. Many processes are believed to mitigate calcification, without their proponents knowing exactly why. Indeed, the Levy patent does not offer a mechanism why alcohol is effective in calcification mitigation, other than it is preferred over aldehydes.

A number of tests are conventionally used to gauge the efficiacy of various calcification mitigation treatments. The most reliable test is actual implantation into a living organisms, preferably a human. Of course, such host studies are by their nature long-term and the results somewhat skewed by the variations present in each individual host. Researchers are therefore constrained to predict the ultimate calcification mitigation benefits of a particular treatment by using laboratory tests on treated tissue, such as calcium uptake studies. Ultimately, there is a substantial amount of extrapolation from the empirical data of such laboratory tests, and to date there is no one predominant mechanism recognized for multigrating calcification.

There remains a need for the development of new methods for inhibiting or mitigrating calcification of chemically fixed biological tissue.

SUMMARY OF THE INVENTION

These, as well as other advantages of the present invention will be more apparent from the following description and drawings. It is understood that changes in the specific structure shown and the described may be made within the scope of the claims without departing from the spirit of the invention.

The present invention provides a method for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in mammalian body, comprising immersing the tissue in a treatment solution, inducing relative and repeated tissue/solution movement, and beating the solution during the step of inducing. The step of inducing may comprise flowing treatment fluid across the tissue and restraining the immersed tissue from gross movement, or enclosing the treatment solution in a container and either shaking the container or stirring the solution within the container, with the immersed tissue floating free or being restrained from gross movement within the container. The step of heating may be applying beat to the outside of the container to indirectly heat the solution therein, or placing the treatment container in an enclosure and heating the enclosure. Alternatively, the step of heating may comprise applying heat directly to the treatment solution.

The present invention also includes a method for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising positioning the tissue in a flow container; restraining the tissue from gross movement within the container, flowing treatment solution through the flow container into contact with the tissue, and heating the solution during the step of flowing. The step of restraining may comprise mounting the tissue in a planar configuration substantially parallel to the direction of flow of the flowing solution. The tissue may be positioned within a flow container having a cross-section oriented substantially normal to the direction of flow of the flowing solution, the tissue being positioned downstream of a baffle to create a substantially uniform downstream flow profile over the cross-section. In one embodiment, treatment solution is supplied to an inlet of the flow container from a reservoir, and fluid is expelled from an outlet of the flow container to the reservoir. The treatment solution may be heated in the reservoir. Preferably, the treatment fluid flows upward through the flow container from the inlet to the outlet and into contact with the tissue.

In accordance with the invention, an apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body is provided. The apparatus comprises a flow container, a supply of treatment fluid, a fluid input to the container, a fluid output from the container, a tissue mount for positioning the at least partially fixed biological tissue within the container between the input and output and restrain its gross movement therein, and means for heating the fluid. The flow container is preferably divided into at least two sections in series separated by perforated baffles, with at least one tissue mount in each section. The flow container may be an elongated tube and the baffles circular. The tissue mount may be configured to mount the tissue in a planar configuration substantially parallel to the direction of flow of the solution flowing through the container. The apparatus may additionally include at least one baffle positioned in the flow container and upstream of the tissue mount, the baffle being configured to create a substantially uniform downstream flow profile over a cross-section of the flow container.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a flow diagram illustrating the prior art process for preparing biological tissue for implantation within a mammalian body comprising fixing of the biological tissue with a glutaraldehyde solution;

FIG. **2** is a flow chart of the preparation of biological tissue for implantation in a mammalian body comprising a method for inhibiting calcification of the biological tissue according to the present invention;

FIG. **3** is a schematic view of an exemplary tissue treatment apparatus including a closed treatment container and container movement device;

FIG. **4** is a schematic view of another exemplary tissue treatment apparatus including an open treatment container and fluid stirring rod;

FIG. **5** is a flow chart of the preparation of biological tissue using the system of FIG. **3** or **4** including the application of heat and motion to a treatment solution;

FIG. **6** is a schematic view of an exemplary tissue treatment apparatus including a treatment container positioned in a flow stream;

FIG. **7** is a flowchart of the preparation of biological tissue using the system of FIG. **5** including the application of heat and flow of treatment solution past the tissue;

FIG. **8** is a perspective view of another preferred tissue treatment apparatus including an upstanding flow column and a plurality of vertical sections within which tissues to be treated are mounted;

FIG. **9** is an enlarged perspective view of one vertical segment of the flow column of FIG. **8** illustrating a piece of tissue suspended from a baffle in a flow stream;

FIG. **10** is a horizontal cross section taken along line **10**—**10** of FIG. **9** through one vertical section of the flow column;

FIG. **11** is a vertical cross section taken along line **11**—**11** of FIG. **10** and through a baffle and tissue suspension mount;

FIG. **12** is a bar graph comparing the measured calcium uptake in bovine pericardium tissues treated in a conventional manner, solely with heat, and with heat and motion; and

FIG. **13** is a bar graph comparing the measured calcium uptake in bovine pericardium tissues treated in a conventional manner and with heat and motion from various sources.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The detailed description set forth below in connection with the appended drawings is intended as a description of the presently preferred embodiment of the invention, and is not intended to represent the only form in which the present invention may be constructed or utilized. The description sets forth the functions and sequence of steps for constructing and operating the invention in connection with the illustrated embodiment. It is to be understood, however, that the same or equivalent functions and sequences may be accomplished by different embodiments that are also intended to be encompassed within the spirit and scope of the invention.

One method for treating glutaraldehyde fixed biological tissue to inhibit calcification thereof following implantation in a mammalian body is illustrated in FIG. **2** which depicts a flow chart of the presently preferred embodiment of the invention. FIG. **1** depicts a flow chart of the prior art method for preparing biological tissue for implantation within a mammalian body.

Referring now to FIG. **1**, the prior art process for preparing biological tissue for implantation within a mammalian body comprises first harvesting the tissue from an animal or human cadaver **10**. As those skilled in the art will recognize, various different types of tissue are routinely harvested from different animals and/or human cadavers. For example, heart valves are routinely harvested from pigs, pericardium is routinely harvested from cows or pigs, and skin is routinely harvested from human cadavers. Those skilled in the art will further recognize the new tissues are, from time to time, being found to be implantable within a mammalian body.

After harvesting, the biological tissue is carried in saline solution, typically for a period of 1–6 hours **12**.

The tissue is next fixed using a buffered glutaraldehyde solution of adequate concentration, for example between 0.2% and 0.8%, at room temperature for at least 3 hours **14**. As is well known, glutaraldehyde effects cross-linking of the proteins, e.g., collagen, within the tissue. Such cross-linking tends to make the tissue more durable and effects preservation thereof. It is known that cross-linked protein exhibits increased resistance to proteolytic cleavage and further that one of the major processes by which circulating blood may destroy tissue is via enzymatic activity which involves unfolding of the protein substrate in order to facilitate enzymatic hydrolysis. Cross-linking of the protein of a tissue makes the tissue resistant to such unfolding, and consequently tends to prevent deterioration thereof due to the enzymatic activity of blood.

The tissue is next sterilized, preferably with an alcohol/formaldehyde solution for 2 hours at room temperature **16**. The preferred solution for effecting sterilization of the tissue comprises approximately 12 ml/l of Tween 80; approximately 2.65 gms/l of MgCl2.H2O; approximately 108 ml/l of formaldehyde (37%); approximately 220 ml/l of ethyl alcohol (100%) and approximately 4.863 gms/l of HEPES buffer. The balance of the solution comprises double filtered H2O. The pH of the solution is typically adjusted to 7.4 via the addition of NaOH. Those skilled in the art will recognize various other sterilization solutions are likewise suitable.

Antimineralization treatment **18** is optionally performed so as to inhibit the accumulation of mineral deposits upon the biological tissue after implantation of a mammalian body. As those skilled in the art will recognize, various different antimineralization treatments are utilized so as to prevent the deposition of various different minerals upon the biological tissue.

The tissue is trimmed and any non-biological components are then added thereto **20**. For example, it is common to sew a heart valve to a valve holder which aids in the handling thereof and which may additionally function as a mount for the valve when implanted into a mammalian body.

Next, the biological tissue is once again sterilized **22**, preferably in an alcohol/formaldehyde solution as discussed above. Since preparation of the biological tissue is substantially complete and the biological tissue will next likely be stored for an extended period of time, a more rigorous sterilization procedure from that previously utilized is typically employed. At this stage, the biological tissue is typically sterilized for approximately 9 hours at 34–38° C.

After sterilization, the biological tissue is stored in glutaraldehyde at room temperature **24**.

Tissue Treatment Using Heat

Referring now to FIG. **2**, a method for treating glutaraldehyde fixed biological tissue to inhibit calcification thereof following implantation in a mammalian body comprises the additional step of heating preferably when the glutaraldehyde is in contact with the biological tissue, to approximately 35–75° C. for approximately 4–22 weeks, and more preferably for a period of a few days to 22 weeks.

The treatment fluid should be heated to a temperature greater than body temperature (37° C.) but not high enough to damage either the tissue or the treatment fluid. Thus, the preferred heat range is between 35–75° C. However, the temperature affects the amount of calcification mitigation, and the process time, and is preferably between 45° C. and 55° c., and more preferably between 50° C.±1° C.

Heating of the biological tissue may be performed at any time after harvesting the tissue from the animal or human cadaver and prior to implanting the tissue within a mammalian body. However, heating of the biological tissue is preferably performed at a point in the process for preparing the biological tissue when the biological tissue is already disposed within a bath of glutaraldehyde solution, as occurs at various stages of the process according to the prior art. Thus, the method for treating glutaraldehyde fixed biological tissues according to the present invention is preferably performed either during fixing thereof with a glutaraldehyde solution, immediately after fixing thereof with the glutaraldehyde solution, or alternatively just prior to or after being stored in a glutaraldehyde solution.

As a further alternative, a method for treating glutaraldehyde fixed biological tissues may be performed during antimineralization treatment by adding glutaraldehyde to the antimineralization solution and heating the solution, preferably to approximately 35–75° C. for approximately 4–22 weeks. More preferably, the tissue is heat treated at 50° C.±1° C. for a period of a few days to 22 weeks.

For example, after fixing tissue using a buffered glutaraldehyde solution of adequate concentration, for example between 0.2% and 0.8%, at room temperature for at least 3 hours **14**, the biological tissue may be heat treated in either the same or different glutaraldehyde solution, preferably at approximately 35–75° C. for a few days to 22 weeks **15**.

As one of the alternative discussed above, the biological tissue is fixed and heat-treated simultaneously **13** in the 0.2–0.8% glutaraldehyde solution, again preferably at approximately 35–75° C. for approximately a few days to 22 weeks. Another alternative is to heat the tissue in saline **17** prior to fixation **21**.

As the other alternative discussed above, the biological tissue may simultaneously undergo antimineralization treatment and heat treatment **19**. Glutaraldehyde is added to the antimineralization solution so as to effect the inhibition of calcification of the tissue following implantation in a mammalian body.

Tissue Treatment Using Relative Tissue/Fluid Movement

FIG. **3** illustrates one preferred embodiment of a tissue treatment system **20** of the present invention. One or more pieces of tissue **22** of leaflets are immersed in a treatment solution **24** within a closed container **25**. The container **25** rests on a shaker table **26** which reciprocates relative to a base **27** in one or more directions. One particularly preferred type of shaking device is an orbital shaker. In one exemplary embodiment, the orbital shaker **26** is actuated at a rotational speed of approximately 55 RPM. The container **25** and contents therein may be subjected to heating, such as with radiant heaters **28** as illustrated. Of course, any number of means for heating the container **25** are known, such as resistance heaters, convective flow, and the like.

The solution **24** is preferably a buffered glutaraldehyde, but may be any chemical solution, such as Denacol® or others, which performs substantially the same in this context. The shaking and/or heat may be applied during fixation or after. The tissue is preferably at least partially fixed prior to being subjected to the calcification mitigation treatment described herein, and more preferably the tissue is fully fixed prior to the treatment. The treatment thus can be designed to complete the fixation process. In a preferred embodiment, tissue that has been fixed for a period of between thirty minutes to fourteen days is placed in the container **25** with a buffered clutaraldehyde solution of adequate concentration, for example between 0.2% and 0.8%. The solution is then shaken for thirty minutes after which the container **25** remains static for fourteen days.

The tissue **22** may be sheets of bovine pericardium tissue, precut leaflets, or fully formed porcine heart valves. One potential disadvantage of using precut leaflets or porcine heart valves is the tissue's nonuniform capacity for shrinkage during calcification mitigation treatment. It can be difficult, though not impossible, to consistently and accurately compensate for this phenomenon. A detailed map of the fiber orientation, thickness and other properties of each individual leaflet may be required to predict the final form of the leaflet after treatment. Therefore, the preferred procedure is to place sheets or pieces of tissue in the container and subject it to the shaking and/or heat. Afterwards, the leaflets are cut from the treated tissue.

It will be noted that the tissue **22** within the solution **24** may be allowed to move about freely. In another embodiment, and as will be described below with respect to the embodiment of FIG. **6**, the tissue may be restrained from gross movement but allowed to freely shrink, such as with a device schematically shown at **29**.

In another variation on the shaking, a treatment system **30** is shown in FIG. **4** wherein a stirring rod **32** is positioned in a container **34** to replace the shaking table **28**. The stirring rod is preferably actuated magnetically through the container, but may also comprise a shaft driven apparatus. The stirring rod **32** is preferably designed so as not to batter the tissue **36** but instead just to cause gentle movement of the fluid **37** relative to tissue. Therefore, in the illustrated embodiment, a piece of filter paper **38**, or other such similar porous substrate or mesh, is draped over the top rim of the container and the tissue pieces **36** placed therein. In this way, the stirring rod **32** imparts rotational or other momentum to the fluid **37** in the container **34**, but the tissue **36** remains above the damaging action of the rotating rod. Also shown in FIG. **4** is a heated enclosure or incubator **39** within which is placed the entire apparatus **30**.

In another version of shaking, multiple flasks or containers holding the treatment fluid and tissue samples are clamped to a rotating ferris-wheel apparatus. The apparatus includes a wheel rotating about a tilted axis so that the flasks follow a tilted circular trajectory. In this manner, the fluid within the flasks gently washes over the tissue pieces as the wheel rotates.

The containers **25** and **34** in FIGS. **3** and **4** may be open or closed, primarily depending on the nature of the treatment fluid. Glutaraldehyde is a toxic substance which evaporates to create a dangerous gas. Thus, treatment with glutaraldehyde is preferably done in a closed container. On the other hand, some substances like Denacol® may be less hazardous and the container may be left open under a hood, for example.

Relative movement between the tissue and the treatment fluid is believed to enhance calcification mitigation. A mechanism for this result has not been fully formulated, although mass transport of the fluid surrounding the tissue may be relevant. Indeed, one theory is that certain cell material, for example, proteins, is extracted or removed from the tissue by the treatment fluid, which removal is enhanced relative to static treatment methods by the movement of the fluid. In other words, the relative movement of the tissue within the fluid repeatedly replenishes the fluid surrounding any one portion of tissue. Test results shown in FIGS. **12** and **13** for samples of tissue treated in a variety of ways in accordance with the present invention indicate that the combination of heat and relative tissue/fluid movement decreases the amount of calcium uptake after implantation in rats, suggesting that such treatment will mitigate calcification in long or short term implantation in humans.

FIG. **5** is a flowchart showing a preferred method for treating tissue using the system shown in FIGS. **3** or **4**. Many of the specific pre- and post-treatment steps described with respect to FIGS. **1** and **2** have been left out for clarity, but remain applicable. Initially, the tissue is harvested, rinsed, fixed and cut into pieces, preferably squares or rectangles, from which leaflets may be formed. The pieces of tissue are then immersed in the treatment fluid within the container, and the fluid heated to a predetermined temperature. Relative movement between the tissue pieces and surrounding treatment medium is induced and continued for a predetermined time. Inducing relative tissue/fluid movement may be accomplished by any of the configurations shown herein, such as shaking or vibrating a container for the tissue and fluid, or by flowing treatment fluid onto the tissue. Finally, the tissue pieces are removed from the container, rinsed and stored for later use. Of course, rather than storing the tissue, it may be formed directly into leaflets and assembled into a heart valve directly after the treatment process.

The solution is heated indirectly through the surrounding air, such as with the radiant heaters **28** shown in FIG. **3**, to a temperature of about 50° C. plus or minus 1° C. The container is shaken or the fluid is stirred to cause relative tissue/fluid movement. The treatment time ranges between fourteen days to two months, but is preferably closer to two months. The container **25** is preferably a glass tissue culture

flask having a volume of approximately 250 ml., and the solution is a buffered glutaraldehyde solution of adequate concentration, for example between 0.2% and 0.8%. As mentioned above, a number of pieces of tissue **22** may be treated at a single time within the container **25**. One proposed ratio of tissue to solution is approximately 12 leaflets or leaflet-sized pieces of tissue per every 150 ml of solution.

Tissue Treatment Using Relative Tissue/Fluid Flow

FIG. **6** illustrates schematically another variation on a treatment system **40** which utilizes flow past the tissue as opposed to shaking a container or stirring the fluid in which the tissue is placed. A flow creates the relative motion between the treatment solution and the tissue which is believed to result in the beneficial calcification mitigation effects.

The system **40** comprises a flow container **42** within which tissue **44** is placed. A number of conduits **46** connect one end of the flow container **42** to a pump **50** and then to a solution reservoir **48**. Conduit **47**, shown in dashed line, may be coimected between the other end of the flow container **42** and the reservoir **48** to complete a closed circulation loop. The pump propels treatment solution through the system **40** in the direction shown by the arrows **52**. The tissue **44** is preferably restrained within the flow container **42** using means schematically illustrated at **56**. Resistance heaters **54** are illustrated surrounding the reservoir **48**. If immersion heaters are used, they must be able to withstand the extended exposure to sometimes caustic treatment fluid. Of course, one or both of the resistance heating elements **54** may be removed from around the reservoir, or alternative heating devices may be used. For example, treatment system **40**, and the system **20** or **30** shown in FIGS. **3** and **4**, for that matter, may be enclosed in a larger enclosure or room **58** which is heated to the preferred temperature by internal or external heaters. In the illustrated embodiment, thermocouples **59** are provided to sense the temperature within both the flow container **42** and the reservoir **48**. The thermocouple **59** in the reservoir is preferably connected to feedback electronics for controlling the heaters **56** based on the temperature of the fluid in the reservoir. This is so that the temperature does not rise too high to a level which might be detrimental to the tissue. The temperature within the flow container is monitored using a thermocouple both as a safety, and to record the precise temperature profile of the treatment fluid.

The basic elements of a method for treating tissue using the system **40** are illustrated in FIG. **7**. Initially, the tissue is harvested, rinsed, fixed and cut into pieces, preferably squares or rectangles, from which leaflets may be formed. The tissue (or leaflets in some instances) may be placed within the flow container **42** and subjected to flow during or after fixation. In a preferred embodiment, the tissue **44** is at least partially fixed before being subjected to the flow within the system **40**, and more preferably the tissue is fully fixed prior to the treatment. The pieces of tissue are then placed in the treatment container, and the solution caused to flow therethrough, initiating relative movement between the tissue pieces and surrounding treatment medium which is continued for a predetermined time. The solution is heated directly outside of the container, or indirectly by heating the container. Finally, the tissue pieces are removed from the container, rinsed and stored for later use. Of course, rather than storing the tissue, it may be formed directly into leaflets and assembled into a heart valve directly after the treatment process.

With reference to FIG. **6**, the tissue is first fixed for a period of between thirty minutes to fourteen days and placed in the flow container **42**. In an alternative, the tissue may be first placed within the container **25** shown in FIG. **3** and shaken for a period of thirty minutes. After the fixation (or after the shaking, if desired), the tissue is placed in the flow container **42** and subjected to solution flow of between ten and fifteen gallons per minute (38–57 lpm) for a period of between fifteen to sixty days. The solution is preferably heated directly within the reservoir **48** to a temperature of about 50° C. (122° F.). The solution is preferably a 0.2–0.8% buffered glutaraldehyde, and the tissue **44** is restrained from movement but allowed to shrink.

In an alternative method of treating tissue in the system **40**, the treatment time is between thirty and sixty days. The flow rate is approximately 7.4 gallons per minute (28 lpm) on average, and is uniform throughout a cross section normal to the flow within the flow container **42**. The tissue **44** is preferably a rectangle of bovine pericardium of about 2 inches by 4 inches in dimension. This size of tissue sample may be used to form one or two leaflets after treatment.

Those with skill in the art will recognize that variations to the above mentioned systems and processes for moving the fluid and/or heating the tissue are available. For example, the flow of solution past the tissue may be combined with a vibrational or shaking motion of the flow container **42** to enhance any calcification mitigation benefits derived from either method. Additionally, though the system **40** is shown as a closed circulation device, fresh solution may be pumped to the flow container **42** and discharged after passing through the container (thus the conduit **47** is shown as optional). Of course, this will require a significant amount of treatment solution which may be prohibitively expensive. Nevertheless, one of the theoretical mechanisms for the beneficial aspects of the present treatment method including flow is that the solution is constantly replenished in the region surrounding the tissue so that a maximum mass transport of chemicals and/or biological material such as protein is realized from the tissue to the solution. Thus, a system which inputs fresh treatment solution, rather than recycling it through a reservoir, would theoretically be more effective in this regard.

Flow Column Apparatus

FIG. **8** illustrates a perspective view of a flow column **60** which may represent the flow container **42** illustrated schematically in FIG. **6**. The column **60** is preferably a clear acrylic tube **61** having an inner diameter of approximately six inches (15.2 cm), a height of about six feet (1.8 m), and a capacity of about ten gallons (38 l). The top and bottom ends of the cylinder **60** are closed by caps **62**a and **62**b, respectively, which are sealed against the inner surface of the cylinder **60** with O-rings (not shown). A lower inlet fitting **64** centered in the cap **62**b provides a conduit for introducing treatment fluid to the lower end of the cylinder **60**. Likewise, an upper fitting **66** connected to the cap **62**a provides an outlet for the treatment fluid. A length of hose **68** connects the lower fitting **64** to a fluid pump **70**, which is in turn connected by a hose **72** to a fluid reservoir **74**. A length of hose **76** connecting the upper fitting **66** to the reservoir **74** completes the circulatory treatment system. Those with skill in the art will understand the fluid connections and requirements, which will not be described further herein.

As mentioned above, the solution within the reservoir **74** is preferably directly heated to the desired treatment temperature. Although not illustrated, the reservoir is desirably provided with one or more immersion resistance heaters. A thermocouple **77** senses the temperature of the reservoir and is preferably connected to feedback electronics for controlling the immersion heater so that the solution temperature does not rise too high to a level which might be detrimental

to the tissue. The temperature within the flow container is monitored using a thermocouple **78** both as a safety, and to record the precise temperature profile of the treatment fluid. Excessive temperatures can detrimentally affect the treatment solution itself, and thus the heating must be done gradually and with a heater having good temperature control.

The vertical flow column or cylinder **60** is segmented into a plurality of vertical sections **80** (seen enlarged in FIG. **9**) by a number of regularly spaced baffles **82** having perforations **83**. The baffles are substantially circular perforated disks positioned horizontally within the vertical cylinder **60**, normal to the fluid flow. The outer diameter of each baffle **82** contacts, or comes into close proximity with, the inner surface of the tube **61**. Although the flow column **60** is illustrated vertically, other arrangements will work. However, the vertical flow orientation is preferred to help purge bubbles from the flow column at start up. In other words, the bubbles naturally migrate out of the flow column in a very short time, as opposed to a horizontal flow path, for example. It should be also noted that the perforations are not shown in FIGS. **8** and **9** for clarity, but are shown in FIG. **10**.

The baffles **82** are commonly mounted on a vertical support rod **84** extending along the axis of the cylinder **60**. The support rod **84** contacts the lower ceiling cap **62**b and extends upward into close proximity to the upper cap **62**a. As seen at the lower end of FIG. **8**, the support rod **84** preferably terminates in a stand member **86** having a pair of bifurcated legs **88** which contact the top surface of lower cap **62**b on either side of an inlet aperture **90**. In this manner, the support rod **84** can be positioned along the axis of the cylinder **60** while not occluding inlet flow from the pump **70**.

As mentioned above, the baffles **82** divide the cylinder **60** into a plurality of vertical sections **80**. In this respect, the vertical sections **80** include the region between two baffles **82**. In the illustrated embodiment, there are eight such vertical sections **80** having a height of between seven and eight inches (17.8–20.3 cm). The entire height of the column **60** is approximately 6 feet (1.8 m), and thus there is some space left above the top baffle and below the bottom baffle. The baffles **82** are slidably mounted on the support rod **84** to enable adjustment of the spacing therebetween, if desired. Furthermore, the tissue pieces **82** can be easily mounted when the baffles **82** are removed from the system, whereupon the baffles are slid over the support rod which is then positioned within the tube **61**. The tissue pieces to be treated are mounted in a particular manner in a circumferential array about the support rod **84**, as will be apparent from the description of FIGS. **9**–**11**.

At the top of the cylinder **60** a vertical space is created between the upper baffle and the upper cap **62**a, in which the central support rod **84** terminates. The space is needed to insure that the flow passing through upper baffle **82** is not unduly disturbed so that the flow within the upper vertical section **80** remains uniform in a horizontal cross section. Indeed, the uniformity of flow across any horizontal cross section between the baffles is important in the present configuration to insure that the flow past any one piece of tissue is equal to the flow past other tissues. The primary mechanism for insuring such uniform flow is the baffles **82** themselves. Preferably, the perforations **83** are sufficiently numerous and have a sufficient diameter so that the cross-sectional area of the baffles **82** has less structural material than open flow channels. The baffles **82** are thus designed to maintain a uniform, non-laminar upward flow stream through each flow section **80**.

At the lower end of the cylinder **60**, below the lowest baffle **82**, a flow straightener **92** is positioned just above a velocity reducer plate **94**. Inlet flow through the aperture **90** thus passes upward through the velocity reducer plate **94** and flow straightener **92** to impinge on the lowest baffle **82**. The velocity reducer plate **94** is a disc like plate having a plurality of apertures **96** formed therein. The apertures are relatively widely spaced in the plate **94** to create a drag on the flow and slow its velocity. The flow straightener **92** resembles a honeycomb structure with a relatively densely spaced number of individual flow channels, and has a vertical dimension greater than the velocity reducer plate **94** or baffles **82**. Flow enters the column **60** through the aperture **90** and continues upward through the velocity reducer plate **94** and straightener **92**. After flow passes through the straightener **92**, it impinges on the lowest baffle **82**. The treatment solution flows upward through each baffle **82** into each successive section **80** and out the top of the column **60**. The column **60** is initially filled with air which is forced out as the surface of the upwardly advancing treatment solution flow passes upward through the column.

Now with reference to FIG. **9**, a vertical section **80** is enlarged illustrating a plurality of tissue mounts **100** depending from the upper baffle **82**. The tissue mounts **100** comprise U-shaped members **102**, more clearly shown in FIG. **11**. FIG. **10** shows the circumferential array of mounts **100** surrounding the central support rod **84**. Each mount **100** has a generally rectangular configuration and is oriented radially in the baffle **82**. That is, free ends of the U-shaped members **102** insert within similarly sized downwardly opening apertures **104** in the baffle **82**. One of the apertures **104** for each mount **100** is positioned close to the support rod **84**, while the other is positioned close to the tube **61**. The apertures **104** extend approximately halfway through the thickness of the baffle **82** and a smaller diameter through hole **106** continuous upward to the top surface of the baffle. This hole **106** is needed to push the mounts **100** from the apertures **104** when treatment is finished. Preferably, the legs of the U-shaped members **102** are spread outward a slight amount so that they have to be squeezed together to fit into the two apertures **104**. This ensures a tight fit so the mounts **100** will not fall out of the apertures **104**.

Rectangular tissue pieces **108** are attached to the mounts with sutures or other similar expedient. In the illustrated embodiment, a lower edge **110** of each tissue piece **108** loops around the bridge portion of the U-shaped member **102** and is sewn to the main body of the tissue piece along line **112**. In this way, the leading edge of the tissue piece **108** in the upward flow stream is rounded, and thus protected from friction induced tearing or wear. One or more sutures **114** connect the upper corners of the tissue piece **108** to the upper ends of the legs of the U-shaped members **112**. Preferably the tissue piece **108** is only connected at one or two locations along its vertical length to prevent gross movement or flapping of the tissue, while allowing the maximum freedom for the tissue to shrink. An O-ring **116** or other such device placed on each leg of the member **112** prevents the sutures **114** from sliding down the leg. The upward flow **118** of treatment solution also assists in maintaining the generally planar configuration of each tissue piece **108**.

Mounting the tissue pieces **108** in a planar configuration substantially parallel to the direction of flow of the solution ensures that an even amount of solution contacts both sides of the tissue. That is, is the tissue pieces were canted with respect to the flow, the backsides would be exposed to less direct flow, and eddy currents and the like might be set up, further making the fluid exposure nonuniform. In addition, the preferred parallel orientation minimizes any stretching of the tissue during the extensive treatment period, such as might occur if the fluid was directed to one face of the tissue or the other.

The radial orientation of the plane of each tissue piece **108** desirably ensures uniform contact with treatment solution during flow through the column **60**. Ideally, the baffles **82** include perforations **120**, seen in FIG. **10**, which create the uniform, nonlinear flow. The same velocity of solution is produced at any radial point from the support rod **84** outward. Of course, different piece of tissue **108** have been shown to possess widely different properties, even from the same pericardial sac. Nevertheless, the present treatment configuration is designed to maximize the uniformity of conditions seen by each piece of tissue **108**. There may be some variation in treatment conditions between the top and bottom reaches of the container due to fluid head differences, but applicants believe that such variations are minimal for the six foot tall column **60** described herein.

There are preferably eight vertical sections **80** in which six tissue pieces **108** are mounted for a total of forty-eight tissue pieces being treated at once. Of course, other numbers of sections and tissue pieces per section are possible. The present flow column is extremely well-suited for consistently manufacturing high quality treated bioprosthetic tissue. The segmented flow column with uniform flow, and vertical orientation of each tissue piece **108** provides high uniformity of treatment. The modular nature of the column with the entire support rod **84** having all of the baffles **82** attached thereto is a significant advantage in manufacturing. One batch of tissues may be treated, and then removed so that after flushing the system a new batch can be ready for installation and treatment. Furthermore, the flow column lends itself to a high degree of control over the system parameters such as the relative tissue/fluid velocity and the temperature. Significantly, there are no large stagnant zones of flow within the column, and especially not within each vertical segment **80**.

Rat Subcutaneous Studies

FIGS. **12** and **13** are results of calcium uptake measurements from tissue treated in a variety of ways, implanted subcutaneously in rats for several months, and then removed. These graphs indicate that heat alone reduces calcium uptake in comparison with a control, and that heat and motion reduces the calcium update even further. A number of shaking, stirring or movement apparatuses were used at two different temperatures, with the same general results.

FIG. **12** shows the results from three groups of samples of untreated and treated bovine pericardium tissue. The first group (GLUT CONTROL) exhibited an average measurement of about 16% calcium from 12 tissue samples which were subjected to a post-fixation treatment of unheated and static glutaraldehyde. The second group (HEAT) exhibited an average measurement of about 7% calcium from 8 tissue samples which were subjected to a post-fixation treatment of static glutaraldehyde heated to a temperature of 50° C. Finally, the third group (HEAT AND SHAKING) exhibited an average measurement of about 4% calcium from 7 tissue samples which were subjected to a post-fixation treatment of static glutaraldehyde heated to a temperature of 50° C. The treatment solution for all three groups was identical—0.6% HEPES-glutaraldehyde at a pH of 7.4—and the treatment period was equal—2 months. The third group was shaken in a bottle or container using a reciprocal orbital shaker actuated at 80 RPM. The rats were all approximately 12 days old, and the tissue samples were left implanted for eight weeks.

FIG. **13** shows the results from a number of groups of samples of untreated and treated bovine pericardium tissue. The calcium uptake results for the groups are indicated by bars with different shading depending on the overall treatment regimen. Thus, the black bars for group **1** are the control (no heat or shaking), the middle shaded bars are for samples subjected to shaking and heat treated to 50° C., and the right-hand white bars are for samples subjected to shaking and heat treated to 42° C.

Group **1** on the left is a control and shows results for two subgroups of 7 and 4 samples each. The control samples were treated for 2 months in 0.6% HEPES-glutaraldehyde at a pH of 7.4 with no heat or movement. Each sample was implanted in 16 day old rats, and left implanted for a period of between 3 and 4 months before being removed to test for calcium.

Groups **4**–**6** in the middle were all heat treated at 50° C. in the same treatment solution as group **1** for the same period. The differences between the treatment regimen for groups **2**–**6** are the methods used to induce relative tissue/fluid movement. The methods are shown graphically below each group. Group **2**–**7** includes two subgroups of 7 and 8 samples each subjected to reciprocal orbital shaking. Group **3**–**7** includes two subgroups of 2 and 11 samples each placed in a flask with a magnetic stirring bar in the bottom. Group **4** is the same method as group **3** but with two subgroups of 8 samples each placed on a filter instead of being allowed to float around the flask. Group **5** included two subgroups of 12 samples each placed in a first container and subjected to a rolling motion, using a tilted ferris wheel arrangement. Group **6** included two subgroups of 20 and 12 samples each placed in a second container and also subjected to a rolling motion.

Groups **7**–**9** on the right were all heat treated at 42° C. in the same treatment solution as groups **1**–**8** and for the same period. Again, the differences between the treatment regimen for groups **7**–**9** are the methods used to induce relative tissue/fluid movement, shown graphically below each group. Group **7** includes two subgroups of 8 and 4 samples each subjected to reciprocal orbital shaking. Group **8** includes two subgroups of 8 and 11 samples each placed in a flask with a magnetic stirring bar in the bottom. Group **9** is the same method as group **8** but with two subgroups of 8 samples each placed on a filter instead of being allowed to float around the flask.

It is apparent from these tests that the shaking and heat treatment reduced calcium intake over the control group, as well as over the heat treatment alone. Also, treatment at 50° C. was substantially more effective than treatment at 42° C. Comparisons of the different shaking/stirring methods indicates that stirring with a magnetic rod within the flask produced the least amount of calcium uptake, regardless of temperature, although perhaps not by a significant margin at 50° C.

It is understood that the exemplary methods and apparatuses for treating glutaraldehyde fixed biological tissue described herein and shown in the drawings represent only presently preferred embodiments of the present invention. Indeed, various modifications and additions may be made to such embodiments without departing from the spirit and scope of the invention. For example, various fixing agents, such as Denacol® or aldehydes other than glutaraldehyde, may exhibit properties similar to those glutaraldehyde so as to make them suitable for use in the present invention and, thus, may likewise be utilized. Accordingly, these and other modifications and additions may be obvious to those skilled in the art and may be implemented to adapt the present invention for use in a variety of different applications. Furthermore, the scope of the invention should be determined with reference to the appended claims.

What is claimed is:

1. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a flow container;

a reservoir containing treatment fluid suitable for fixing the tissue;

a fluid input to the container;

a fluid output from the container;

a tissue mount for positioning the at least partially fixed biological tissue within the container between the input and **[**ouput**]** *output* and restrain its gross movement therein; and

means for heating the fluid to a temperature greater than body temperature (>37° C.) comprising a heater for heating fluid in the reservoir.

2. The apparatus of claim **1**, wherein the flow container is divided into at least two sections in series separated by perforated baffles, with at least one tissue mount in each section.

3. The apparatus of claim **2**, wherein the flow container is an elongated tube and the baffles are circular.

4. The apparatus of claim **1**, wherein the tissue mount is configured to mount the tissue in a planar configuration substantially parallel to the direction of flow of the solution flowing through the container.

5. The apparatus of claim **1**, further including at least one baffle positioned in the flow container and upstream of the tissue mount, the baffle being configured to create a substantially uniform downstream flow profile over a cross-section of the flow container.

6. The apparatus of claim **5**, wherein the baffles are perforated plates oriented substantially normal to the direction of flow of the solution flowing through the container, and the flow container is divided into at least two sections in series, each two adjacent sections being separated by a baffle, with at least one tissue mount in each section removably secured to one of the baffles.

7. The apparatus of claim **1**, further including a sensor for monitoring the fluid temperature in the reservoir, and a feedback control loop responsive to the sensed temperature for adjusting the heater temperature.

8. The apparatus of claim **1**, wherein the flow container comprises an upstanding tube, the fluid input being located at the lower end of the tube and the fluid output being located at the upper end of the tube, the apparatus further comprising a velocity reducer above the fluid input, and a flow straightener above the velocity reducer and below the first tissue mount.

9. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a piece of at least partially fixed biological tissue;

a container suitable for containing tissue treatment fluid and immersing the piece of at least partially fixed biological tissue in the fluid;

a shaker for causing treatment fluid movement within the container;

means for heating the treatment fluid and tissue within the container; and

means for restraining the immersed tissue from gross movement within the container.

10. The apparatus of claim **9**, wherein the piece of at least partially fixed biological tissue is a bioprosthetic heart valve leaflet.

11. The apparatus of claim **9**, wherein the shaker is an orbital shaker.

12. An apparatus for treating at least partially **[**faced**]** *fixed* biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a piece of at least partially fixed biological tissue,

a container suitable for containing tissue treatment fluid and immersing the piece of at least partially fixed biological tissue in the fluid,

means for causing treatment fluid movement within the container comprising a stirrer immersed in the treatment fluid,

means for heating the treatment fluid; and

means for restraining the immersed tissue from gross movement within the container.

13. The apparatus of claim **12**, wherein the stirrer is a stirring rod.

14. The apparatus of claim **13**, further including a shaft drive for the stirring rod.

15. The apparatus of claim **13**, further including a magnetic drive for the stirring rod.

16. The apparatus of claim **12**, wherein the means for restraining tissue from gross movement within the container comprises a porous substrate separating the tissue from the stirrer.

17. The apparatus of claim **16**, wherein the container has an open mouth and the porous substrate is draped over the open mouth and separates the container into an upper portion for receiving the tissue and a lower portion for receiving the stirrer.

18. The apparatus of claim **12**, wherein the means for heating comprises a heater adjacent to the container that applies heat to the outside of the container and indirectly heats the treatment fluid therein.

19. The apparatus of claim **18**, wherein the heater comprises a resistive heater.

20. The apparatus of claim **12**, wherein the means for heating comprises a heater that applies heat directly to the treatment fluid.

21. The apparatus of claim **12**, wherein the heater is external to the container.

22. The apparatus of claim **12**, wherein the heater comprises a convective flow heater.

23. An apparatus for treating an at least partially fixed sheet of biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

treatment fluid suitable for fixing the tissue;

a container suitable for containing the tissue treatment fluid having a fluid input and a fluid output;

a system for continuously flowing the treatment fluid through the flow container between the input and output;

a tissue mount for positioning the at least partially fixed sheet of biological tissue within the container between the input and output and restrain its gross movement therein, the tissue mount being adapted to mount the tissue sheet in a planar configuration substantially parallel to the direction of flow of the treatment fluid, the sheet of biological tissue being immersed in the continuous flow of treatment fluid; and

means for heating the treatment fluid.

24. The apparatus of claim **23**, wherein the flow container has a cross-section oriented substantially normal to the direction of flow of the treatment fluid, the apparatus further including a baffle positioned upstream of the mount which

creates a substantially uniform downstream flow profile over the container cross-section in the region of the mount.

25. The apparatus of claim **24**, further comprising a plurality of perforated baffles dividing the flow container into a series of sections, and a plurality of said mounts in each section for mounting multiple tissue pieces within each section.

26. The apparatus of claim **23**, further comprising a reservoir external to the container, the flow container having an inlet and an outlet, and a pump for circulating treatment fluid from the reservoir to the flow container and expelling fluid from the flow container outlet back to the reservoir.

27. The apparatus of claim **26**, wherein the means for heating heats the treatment solution in the reservoir.

28. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a flow container divided into at least two sections in series separated by perforated baffles;

a supply of treatment fluid;

a fluid input to the container;

a fluid output from the container;

at least one tissue mount in each section for positioning the at least partially fixed biological tissue within the container between the input and output and restrain its gross movement therein; and

means for heating the fluid.

29. The apparatus of claim **28**, wherein the flow container is an elongated tube and the baffles are circular.

30. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a flow container;

a supply of treatment fluid;

a fluid input to the container;

a fluid output from the container;

a tissue mount for positioning the at least partially fixed biological tissue within the container between the input and output and restrain its gross movement therein;

means for heating the fluid; and

at least one baffle positioned in the flow container and upstream of the tissue mount, the baffle being configured to create a substantially uniform downstream flow profile over a cross-section of the flow container.

31. The apparatus of claim **30**, wherein the baffle is a perforated plate oriented substantially normal to the direction of flow of the solution flowing through the container, and the flow container is divided into at least two sections in series, each two adjacent sections being separated by a baffle, with at least one tissue mount in each section removably secured to one of the baffles.

32. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a flow container comprising an upstanding tube;

a supply of treatment fluid;

a fluid input to the container at a lower end of the tube;

a fluid output from the container at an upper end of the tube;

a tissue mount for positioning the at least partially fixed biological tissue within the container between the input and output and restrain its gross movement therein;

means for heating the fluid; and

a velocity reducer above the inlet aperture, and a flow straightener above the velocity reducer and below the first tissue mount.

33. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a container suitable for containing tissue treatment fluid;

means for causing treatment fluid movement within the container including a stirrer immersed in the treatment fluid;

means for heating the treatment fluid; and

means for restraining the immersed tissue from gross movement within the container.

34. The apparatus of claim **33**, wherein the means for restraining tissue from gross movement within the container comprises a porous substrate separating the tissue from the stirrer.

35. The apparatus of claim **34**, wherein the container has an open mouth and the porous substrate is draped over the open mouth and separates the container into an upper portion for receiving the tissue and a lower portion for receiving the stirrer.

36. An apparatus for treating at least partially fixed biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, comprising:

a flow container suitable for containing tissue treatment fluid;

a system for flowing treatment fluid through the flow container, wherein the flow container has a cross-section oriented substantially normal to the direction of flow of the treatment fluid;

means for restraining the tissue from gross movement within the flow container comprising a mount for mounting the tissue in a planar configuration substantially parallel to the direction of flow of the treatment fluid;

a baffle positioned upstream of the mount which creates a substantially uniform downstream flow profile over the container cross-section in the region of the mount; and

means for heating fluid.

37. The apparatus of claim **36**, further comprising a plurality of perforated baffles dividing the flow container into a series of sections, and a plurality of said mounts in each section for mounting multiple tissue pieces within each section.

38. A method for treating biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, the method comprising:

fixing the tissue in a fixative solution; and

prior to implantation, heat treating the fixed tissue in a glutaraldehyde solution at a temperature in the range of 45–55° C. for a period of time.

39. The method of claim 38, wherein heat treating is done at a temperature in the range of 50° C.±1° C.

40. The method of claim 38, wherein the period of time is in the range of about a few days to 22 weeks.

41. The method of claim 38, wherein the period of time is in the range of about 4 to 22 weeks.

42. The method of claim 38, wherein the period of time is in the range of about 14 days to 2 months.

43. The method of claim 38, wherein the period of time is in the range of about 30 days to 60 days.

44. The method of claim 38, further including immersing the tissue in a solution comprising an alcohol, formaldehyde and Tween 80.

45. The method of claim 44, including immersing the tissue in the solution comprising an alcohol, formaldehyde and Tween 80 for about 2 hours at room temperature so as to sterilize the tissue.

46. The method of claim 38, further including inducing relative and repeated tissue/solution movement during the step of heat treating.

47. The method of claim 46, further including enclosing the treatment solution in a container.

48. The method of claim 46, wherein the step of inducing comprises shaking the container.

49. The method of claim 46, wherein the step of inducing comprises rolling the container.

50. A method for treating biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, the method comprising:

fixing the tissue in a glutaraldehyde solution while heating the glutaraldehyde solution to a temperature in the range of about 50° C.±1° C. for a period of time.

51. The method of claim 50, wherein the tissue is heat treated in a glutaraldehyde solution.

52. The method of claim 50, wherein the period of time is in the range of about a few days to 22 weeks.

53. The method of claim 50, wherein the period of time is in the range of about 4 to 22 weeks.

54. The method of claim 50, wherein the period of time is in the range of about 14 days to 22 months.

55. The method of claim 50, wherein the period of time is in the range of about 30 days to 60 days.

56. The method of claim 50, further including immersing the tissue in a solution comprising an alcohol, formaldehyde and Tween 80.

57. The method of claim 56, including immersing the tissue in the solution comprising an alcohol, formaldehyde and Tween 80 for about 2 hours at room temperature so as to sterilize the tissue.

58. The method of claim 50, further including inducing relative and repeated tissue/solution movement during the step of heat treating.

59. The method of claim 58, further including enclosing the treatment solution in a container.

60. The method of claim 58, wherein the step of inducing comprises shaking the container.

61. The method of claim 58, wherein the step of inducing comprises rolling the container.

62. A method for treating biological tissue to inhibit calcification of the tissue following implantation in a mammalian body, the method comprising:

prior to fixing the tissue, heat treating the tissue in a saline solution only to a temperature of about 50° C.±1° C. for a period of time.

63. The method of claim 62, wherein the period of time is in the range of about a few days to 22 weeks.

64. The method of claim 62, wherein the period of time is in the range of about 4 to 22 weeks.

65. The method of claim 62, wherein the period of time is in the range of about 14 days to 2 months.

66. The method of claim 62, wherein the period of time is in the range of about 30 days to 60 days.

67. The method of claim 62, further including immersing the tissue in a solution comprising an alcohol, formaldehyde and Tween 80.

68. The method of claim 67, including immersing the tissue in the solution comprising an alcohol, formaldehyde and Tween 80 for about 2 hours at room temperature so as to sterilize the tissue.

69. The method of claim 62, further including inducing relative and repeated tissue/solution movement during the step of heat treating.

70. The method of claim 69, further including enclosing the treatment shown in a container.

71. The method of claim 69, wherein the step of inducing comprises shaking the container.

72. The method of claim 69, wherein the step of inducing comprises rolling the container.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : RE 40,570 E
APPLICATION NO. : 10/406354
DATED : November 11, 2008
INVENTOR(S) : Sophie Carpentier et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 16, line 43 of the Patent, change "12" to --21--.

Column 19, line 23 of the Patent, change "22" to --2--.

Signed and Sealed this

Fourth Day of May, 2010

David J. Kappos
Director of the United States Patent and Trademark Office