Title: HIGH STRENGTH, HIGH STIFFNESS, RECONSTITUTED COLLAGEN MEMBRANES FOR BIOMEDICAL IMPLANTATION

Abstract: A method of preparing a bioimplantable collagen structure having a controlled porosity and a high strength and tensile modulus by casting a solution of reconstituted collagen against a membrane, lyophilizing the membrane to foam, applying an alcohol/water solution, and air drying, and bioimplantable collagen structures produced thereby having a high strength and stiffness.
High Strength, High Stiffness, Reconstituted Collagen Membranes for Biomedical Implantation

BACKGROUND OF THE INVENTION

Collagen molecules are present in virtually all animals. Collagen for medical implants is most often derived as a natural protein from animal sources, typically cow, pigs, horses or even cadaveric human tissue. Collagen composes approximately 35% of vertebrates by weight. Therefore, it is an abundantly available for use as allografts or xenografts in medical devices.

Although the largest portion of the basic collagen molecule is not immunogenic, the ends of the collagen molecules contain a telopeptide which is immunogenic and can initiate an inflammatory response. In addition, other components (e.g., structural proteins and cells) within the transplant tissue can initiate significant inflammatory responses, causing rejection and the failure to repair and regenerate.

Collagen membranes are conventionally manufactured by one of two processes: transplanting of connective tissue or reconstituted, purified collagen. Tissue directly obtained or transplanted from the donor maintains the complex composite nature of the connective tissue. Although these membranes principally contain collagen, they have other components, such as elastin which provides some of the strength, toughness and stiffness. They may also contain remnant cells and other immunogenic molecules. Theses biomaterials be extensively processed and cross-linked to further increase their strength and stability. These biomaterials are not highly purified. Consequently, they may contain immunogenic components that cause a tissue reaction in patients.

Sheets or membranes containing high concentrations of collagen are currently used by reconstructive surgeons as implantable medical devices. They serve as either barriers or trellis for a multitude of indications, including
tissue repair and tissue regeneration. For many of these indications, collagen membranes must have significant biomechanical strength and stiffness.

Collagen membranes can be manufactured by a variety of methods. Transplanted connective tissues serve as membranes with high strength and high stiffness, but these membranes can initiate an inflammatory reaction because they are immunogenic. In addition, they are expensive and difficult to manufacture into precise shapes and dimensions. To decrease the immunogenicity and to increase the manufacturing efficiency, collagen can be reconstituted to its primary molecular form. When the collagen is purified by conventional, contemporary methods, the resulting membranes lack strength and stiffness.

Collagen implants typically are made of highly porous, reconstituted bovine (i.e., cow) collagen. These collagen implants are commercially sold to surgeons as rectilinear sheets with uniform thicknesses and porosity. Their low density, high porosity makes these collagen membranes supple and conformable. However, for the collagen membrane to function effectively as a containment device, it is a requirement that the membrane exhibit an adequate degree of stiffness. Unfortunately conventional collagen membranes have inadequate tensile strength and stiffness for many surgical applications, particularly after wetting with saline or blood.

SUMMARY OF THE INVENTION

The present invention relates to a process for producing collagen membranes for biomedical implantation which is capable of producing implantable collagen membranes with high strength and high stiffness in a variety of shapes and sizes by casting a solution of reconstituted collagen against a membrane, lyophilizing the membrane to foam, applying an alcohol/water solution, and air drying. Appropriate adjustments in the composition of the formulation of alcohol/water solution, the method for applying the solution, the air drying time and temperature allow the operator to control the mechanical properties and porosity of resulting collagen membranes to adapt them for specific applications.
The collagen membranes produced by the method of the invention may be used by reconstructive surgeons to replace or help repair torn ligament, tendons or fascia. In addition, the collagen membranes produced in accordance with the present invention may be used to constrain structures or materials during the healing phase. The collagen membranes of the invention also may be used to contain implanted bone grafting materials at a desired position during the healing phase. High strength and stiff collagen provides structure for containing or retaining cells, growth factors or particulate matrices.

Collagen is a helical trimer, composed of monomers each containing approximately 1000 amino acids. The triple helix is bundled into fibrils which are bundled into fibers. This complex provides strength and stiffness to tissues. It also serves as a matrix or substructure for tissue formation. Thus collagen is inherently an excellent biomaterial for use by reconstructive surgeons to regenerate tissues.

Reconstituted, purified collagen is produced by a process involving the isolation and purification of the collagen in a suspension. To purify the collagen, the most immunogenic portions are cleaved. These immunogenic portions of the tissue are removed and disposed. The collagen is then precipitated to form a membrane by casting it against a mold. The material derived from this manufacturing process is referred to as reconstituted collagen. It is called reticulated collagen if the structure has high porosity or reticulation. Unfortunately, porosity decreases the strength, toughness and stiffness of the membrane. Thus, these types of reconstituted, reticulated collagen membranes have limited utility for many of the most desirable applications by surgeons.

For many bone reconstructive indications, a biocompatible and resorbable membrane is desirable. These membranes or sheets serve four possible functions. First, they act as a trellis for tissue regeneration. Second, the function as a barrier for separating tissues. Third, they serve as a structural biomaterial for containing biomaterials. Fourth, collagen membranes can also be used for load bearing, typically in tension.

Trellises of porous biomaterials (i.e., matrices) serve as a framework on which and through which tissue grow. Most tissues proliferate only by
attaching to a structure or matrix. Cells then multiply and expand on pre-existing cells, extra-cellular matrix or biomaterials. Therefore, these matrices must have porosity. However, porosity generally decreases strength, typically non-linearly such that a small amount of porosity disproportionately decreases mechanical properties. The optimal porosity has been characterized in the musculoskeletal field, for various principal regenerative tissues. For neovascular tissue (i.e., new blood vessels), pore diameters must be larger than 20 micrometers. For osteoid (non-mineralized bone), pore diameters must be larger than 50 micrometers. For bone formation, pore diameters must be larger than 100 micrometers.

Tissue regeneration is a race between competing tissues. Whichever tissue fills the space first, will dominate. Fibrovascular tissues proliferate faster than bone tissue. Consequently, fibrovascular tissue can preferentially fill in a defect where bone is desired, resulting in scar tissue. A membrane of collagen can block fibrovascular tissue, giving more time for bone formation to occur. Therefore, bone surgeons can exclude fibrovascular (i.e., scar tissue) from bone defects using biocompatible, resorbable membranes as barriers to tissue regeneration. However, these membranes must be strategically shaped and implanted.

Collagen has been used as an implantable biomaterial for more than 50 years. The collagen used for biomedical implants is either derived from animals (e.g., cows, pigs, horses) and humans, or it is manufactured in vitro using recombinant engineering. Collagen is thus well-recognized as a biocompatible material.

Depending on the extent of cross linking, collagen biomaterials can be manufactured to resorb over a prescribed range of time, typically from 6 weeks to one year. Collagen implants are resorbed and remodeled like natural tissues, via cellular and enzymatic processes. Consequently, collagen is an ideal biomaterial for implantable medical devices to aid in tissue regeneration and repair.

Collagen membranes according to the invention with three dimensional shapes can be produced to facilitate tissue regeneration, particularly bone. These three dimensional shapes are manufactured by casting collagen in male
and female molds and lyophilizing, to form a highly porous structure. The collagen membranes are then collapsed and cross linked to provide high strength, stiff membranes. Collagen membranes can be formed into a variety of three dimensional shapes, such as capsules, wedges or balloons. For examples, capsules have been formed to contain and retain bone grafting materials to their desired location. These capsules can aid in the reconstruction of the buccal plate after tooth extraction.

The high strength collagen membranes of the invention are initially produced by casting a suspension of purified collagen. The collagen desirably may have a native fibrous structure and an average fiber length of from about 0.2 to about 3 mm.

Collagen suitable for use in the membranes of the invention may be obtained by known techniques, for example, from bovine tendons. The collagen may be suitably purified for use by the process described in Nimni et al., US patent no. 5,374,539, the entire disclosure of which is hereby incorporated herein by reference. The collagen fibers may also be treated for implantation by the process of Cheung, US patent no.7,008,763, the entire disclosure of which is likewise incorporated herein by reference.

A typical collagen suspension will contain from 10 to 60 mg/ml of purified collagen in a mixture of 5 to 25% alcohol in water. Three-dimensional shapes, reticulated and reconstituted collagen membranes can be manufactured using male/female molds by conventional casting methods. The space between the molds is filled with a collagen suspension. After casting, the collagen membrane or sponge is then lyophilized or freeze dried to produce a reticulated foam.

The next step in the process of producing high strength and stiff membranes is to prepare an appropriate alcohol/water treatment solution for subsequent rehydration/drying treatment of the membrane to densify the membrane. The alcohol concentration in the solution may range from 40 to 90%, preferably from 50 to 80 or 85%. It has been found that to produce a useful, high stiffness high strength membrane for most applications, an optimal alcohol/water concentration is about 70%.
If desired, the reticulated foam membrane may optionally be removed from mold template prior to rehydration/drying by alcohol/water treatment. The membrane is then subject to rehydration treatment by applying the alcohol/water solution to the surface of the cast, reticulated membrane by spaying the solution onto the surface of the collagen membrane. The ultimate strength and stiffness of the membrane will vary as a function of the alcohol concentration.

The treated (i.e., rehydrated) membrane is then dried. Drying may advantageously be effected by air drying at a temperature of 25°C to 37°C. Air drying at ambient temperature is ordinarily sufficient. If desired, the membrane may be subject to vacuum drying in addition to, or in lieu of, the air drying step.

The air dried membrane is then subjected to heat treatment. Heating is carried out at a temperature in the range from 100°C to 160°C, preferably from 120°C to 140°C, particularly preferably at about 130°C. Heating time may range from about 15 minutes to 24 hours or more, preferably 15 minutes to 2 hours. For most applications, a heating time of about 30 minutes is sufficient.

The alcohol/water content is a controlling variable for strength/stiffness. Decreasing the alcohol/water ratio increases the strength/stiffness. Generally, the greater the proportion of water, the greater the density and the higher the strength and stiffness will be. However, too much water can cause the collagen to degrade. Generally, a water content of 40-50% is optimal for super high strength/stiff membranes.

If desired, membrane having varying properties such as porosity and/or tensile strength in different portions of the membrane (multiphase porosity) may be produced by selectively rehydrating different portions of the membrane with alcohol/water solutions containing different amounts of alcohol or with different amount of alcohol/water solution.

Excessively high alcohol/water ratios can cause the membrane to dry more rapidly, with the result that the foam does not collapse as much. This makes the membrane more reticulated and more porous, but less strong and stiff.

As a rule, increasing the drying temperature and/or time increases the strength/stiffness of the resulting collagen membrane. However, excessive
heat/time also may degrade the collagen. The appropriate drying
temperature/time profile for a given application can be selected by a person
skilled in the art by routine experimentation.

The collagen membrane of the invention produced by the process of the
invention has a number of important advantages over conventional collagen
membranes.

A first advantage is high strength. The membrane of the invention
assures that the optimal mechanical properties are provided in collagen
membranes so that they will contain bone graft materials at the optimal
location.

Another advantage is high toughness. The membrane of the invention is
non-fragile and exhibits sufficient sturdiness to enable it to be readily handled
during implantation surgery without danger of damage of disintegration.

A further advantage is high stiffness. The membrane of the invention
exhibits sufficient rigidity that it can be formed by folding or bending to a
desired configuration to fit a surgical site and will retain that configuration.

Yet another advantage of the membrane of the invention is convenience
for the surgeon because it can be formed with a desired uniform or non-uniform
degree of porosity. Although it is possible for a surgeon to make holes in
collagen membranes to achieve a desired degree or distribution of porosity, the
precision and continuity of the holes would be difficult for the surgeon to make
with typical surgical tools. Plus, attempts to perforate the membranes manually
may result in the membranes being excessively cut or penetrated so that they
no longer retain the desired mechanical attributes for use in graft containment
and/or tissue separation.

The membrane of the invention also is biocompatible so that it may
safely be used in implantation surgery. Thus the membrane may be safely
incorporated into a patient's body with tissue regeneration materials to contain
the tissue regeneration materials at a desired implantation site and/or
segregate the tissue implantation materials from surrounding tissues. No
surgery for subsequent removal is necessary because the collagen membranes
are naturally resorbed by the body. Moreover, by appropriately adjusting the
degree of cross-linking and the thickness of the membrane the effective lifetime
of the membrane may be varied from a few weeks to up to a year. Typical resorption times for most surgical applications will vary from four weeks to six months, advantageously from six weeks to four months.

EXAMPLE

A major problem incurred by dentists, particularly oral surgeons and periodontists, is restoration of the jaw bone that has undergone resorption due to the loss of the teeth. If a natural tooth is lost either due to must be extraction because of a functional or cosmetic defiance, it may be replaced with a dental implant. Dental implants require bone to biologically anchor the metal surface into the mandible or maxilla. This biological process is called osteointegration. After tooth extraction, the surgeon must regenerate the maximum amount of bone to provide adequate osteointegration of the dental implant. Resorption occurs because the bone is not stressed normally, according to Wolff's law. Before dental implants can be implanted, the alveolar ridge must be regenerated with new bone. To accomplish this, bone graft must be placed on the ridge and retained during the healing process. The bone graft can be stabilized with a high stiffness reconstituted collagen membrane. The membrane can be fixed to the existing bone with bone tacks. The stiff membrane retains the form and function of the bone graft until bone and connective tissue regenerates. After several months of healing, the collagen membrane resorbs. Even if some membrane is still present, surgeon can then easily drill through the membrane to place the dental implants.

A capsule of relatively stiff, high density, low porosity collagen is ideal for containing bone grafting material and placing into the socket. The capsule restrains the bone grafting material to exactly the correct location for maximal bone formation.

Step 1: Casting

A suspension of 15 mg/ml purified collagen in a mixture of 10% ethanol in water was formed. The fibers had a native fibrous structure with an average fiber length of about 1.5 mm. After removal of air bubbles from collagen suspension, a fixed amount of the suspension was poured into a mold.
comprised of mating male and female mold members defining a mold chamber between them. The main frame of the mold was tightly attached to an elastic bottom plate. The filled mold was then placed in a freezer at -70°C, and the suspension was frozen. After the collagen suspension had solidified, one of the two vertical mold plates holding the frozen collagen was removed. The other vertical mold plate with the frozen collagen attached was also removed and subjected to freeze-drying in a freeze-dryer. After freeze drying was complete, the resulting collagen membrane was removed from the freeze dryer for subsequent densification.

Step 2: Densification
The freeze-dried (lyophilized) collagen membrane was then treated by spraying with an alcohol/water solution composed of 70% ethanol in water. Then the treated membrane was air dried at ambient temperature of approximately 25°C. The air dried material was then heated at a temperature of approximately 130°C for 30 minutes. The heat treated material was allowed to cool, and then trimmed to desired size.

The resulting collagen web had tensile strength of approximately 3600 g/mm² (35 MPa), a tensile modulus of 95,000 g/mm² (932 MPa), pore diameters of less than 50 microns and porosity of less than 20%.

EXAMPLE 2
Following an analogous procedure, but varying the alcohol concentration of the rehydration solution and the time of the heat treatment, a collagen membrane was obtained having a a tensile strength of about 35 g/mm², a tensile modulus of about 560 g/mm², a porosity of more than 50%, and pore diameters of greater than 50 microns.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting. Since modifications of the described embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations within the scope of the appended claims and equivalents thereof.
CLAIMS:

1. A method of producing a collagen membrane comprising:
   (a) forming a collagen membrane on a template;
   (b) subjecting the collagen membrane to freeze drying;
   (c) treating the freeze-dried membrane with a water/alcohol mixture.
   (d) drying the treated membrane; and
   (e) heat treating the dried membrane.

2. A method as claimed in claim 1, wherein the collagen membrane is formed by:
   - producing a suspension of purified collagen in an alcohol/water mixture;
   - filing the collagen suspension into a mold; and
   - freezing the suspension to solidify the collagen into a membrane.

3. A method as claimed in claim 1, wherein step (c) is effected by spraying the freeze-dried membrane with a mixture of from 40 to 90% ethanol in water.

4. A method as claimed in claim 3, wherein said mixture comprises from 50 to 70% ethanol in water.

5. A method as claimed in claim 1, wherein step (e) is effected by subjecting the dried membrane to a temperature in the range from 100°C to 160°C for a period of from 15 minutes to 24 hours.

6. A method as claimed in claim 5, wherein the dried membrane is subject to a temperature of from 120°C to 140°C for a period of from 15 minutes to 2 hours.

7. A collagen membrane produced by the process of claim 1.

8. A collagen membrane having a tensile strength of about 3600 g/mm², a tensile modulus of about 95,000 g/mm², pore diameters of less than 50 microns, and porosity of less than 20%.
9. A collagen membrane having a tensile strength of about 35 g/mm², a tensile modulus of about 560 g/mm², a porosity of more than 50%, and pore diameters of greater than 50 microns.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/47772

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61 F 2/06 (2010.01)
USPC - 623/1 .47

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 623/1 .47

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 623/1.1, 1.47, 917; 606/151; 424/443 (keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PUBWEST(PGPB, USPT, EPAB, JPAB): Google
Search Terms Used: high, large, tensile, strength, bone, modulus, collagen, pore, porosity, matrix, collagen, spray$, ethanol, heat$, freeze$, treat$, dry$

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C.

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search
08 October 2010 (08.10.2010)

Date of mailing of the international search report
14 OCT 2010

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)