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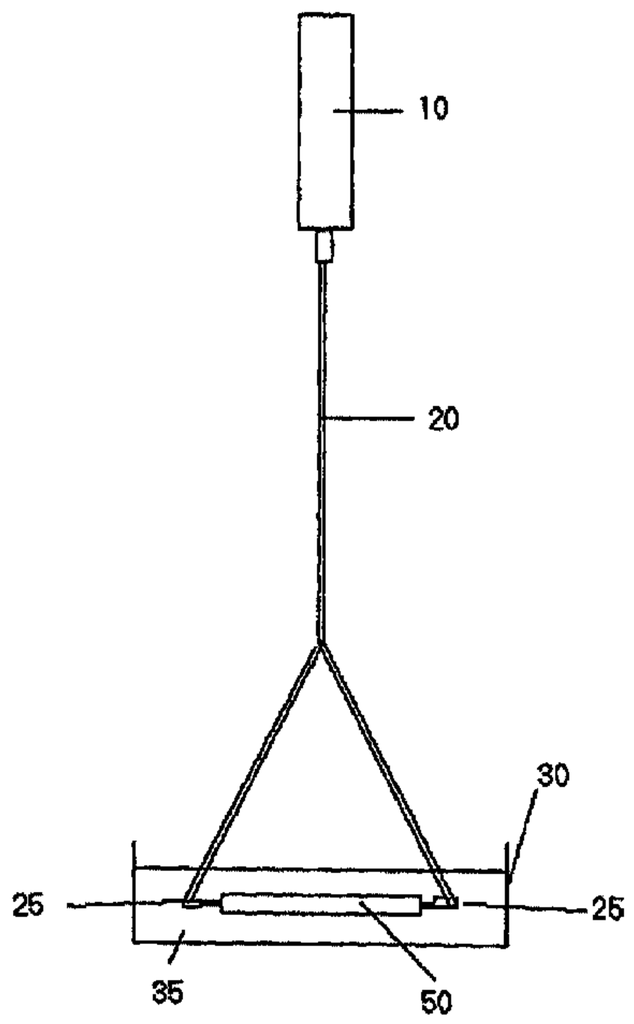
(54) **PROTHESES DE GREFFAGE VASCULAIRE PREPAREES A L'AIDE DE TECHNIQUES BIOLOGIQUES**

(54) **BIOENGINEERED VASCULAR GRAFT PROSTHESES**



(57) La présente invention concerne des prothèses de greffage préparées à partir de matériau tissulaire propre, d'origine animale, à l'aide de techniques biologiques. Les prothèses de greffage de l'invention sont préparées en utilisant des techniques qui, pour la matrice tissulaire traitée, préservent la compatibilité cellulaire, la résistance et la faculté de se régénérer en milieu vivant. Les prothèses de greffage préparées à l'aide de techniques biologiques sont utilisées dans les implantations, les réparations de blessures, ou utilisées chez un mammifère hôte.

(57) The invention is directed to bioengineered graft prostheses prepared from cleaned tissue material derived from animal sources. The bioengineered graft prostheses of the invention are prepared using methods that preserve cell compatibility, strength, and bioremodelability of the processed tissue matrix. The bioengineered graft prostheses are used for implantation, repair, or use in a mammalian host.

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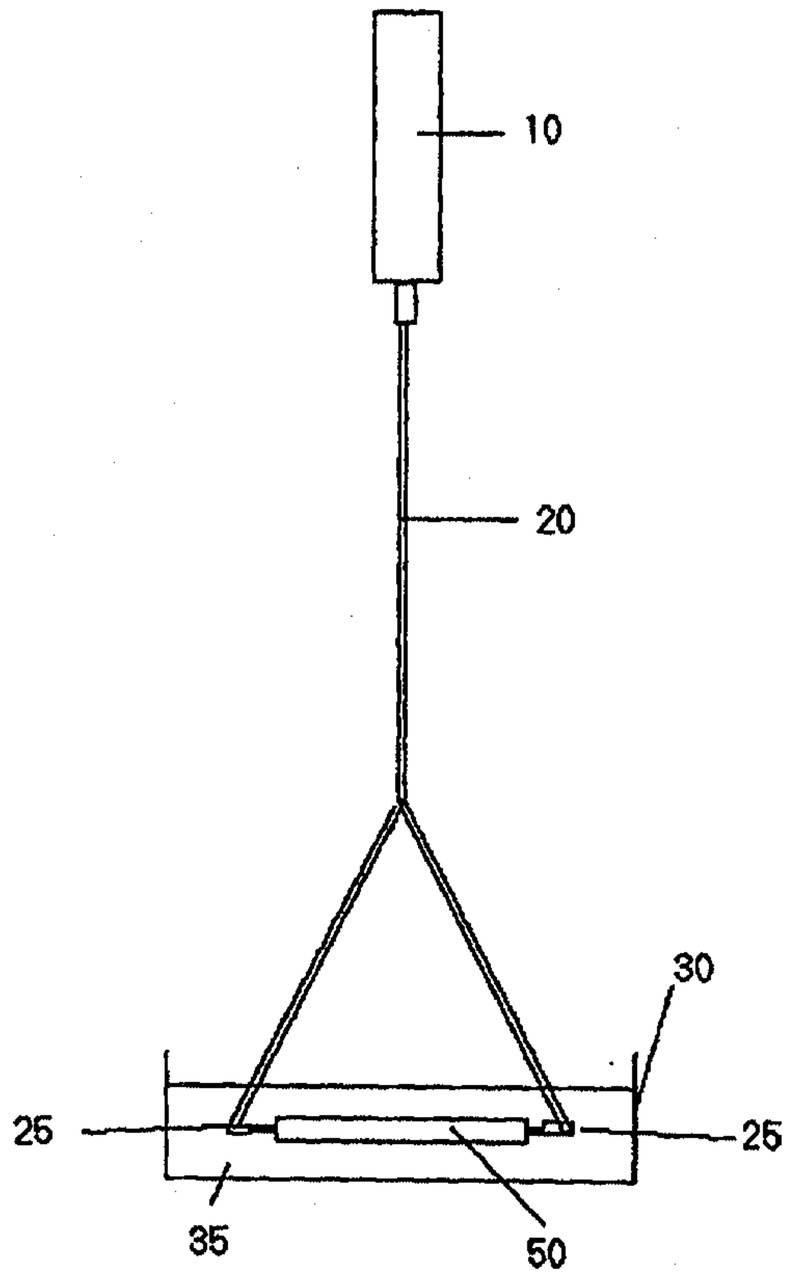
(54) **Title:** BIOENGINEERED VASCULAR GRAFT PROSTHESES

(57) **Abstract**

The invention is directed to bioengineered graft prostheses prepared from cleaned tissue material derived from animal sources. The bioengineered graft prostheses of the invention are prepared using methods that preserve cell compatibility, strength, and bioremodelability of the processed tissue matrix. The bioengineered graft prostheses are used for implantation, repair, or use in a mammalian host.



BIOENGINEERED VASCULAR GRAFT PROSTHESES

1. Field of the Invention:

This invention is in the field of tissue engineering. The invention is directed to bioengineered graft prostheses prepared from cleaned tissue material derived from animal sources. The bioengineered graft prostheses of the invention are prepared using methods that preserve cell compatibility, strength, and bioremodelability of the processed tissue matrix. The bioengineered graft prostheses are used for implantation, repair, or use in a mammalian host.

2. Brief Description of the Background of the Invention:

The field of tissue engineering combines the methods of engineering with the principles of life science to understand the structural and functional relationships in normal and pathological mammalian tissues. The goal of tissue engineering is the development and ultimate application of biological substitutes to restore, maintain, and improve tissue functions.

Collagen is the principal structural protein in the body and constitutes approximately one-third of the total body protein. It comprises most of the organic matter of the skin, tendons, bones, and teeth and occurs as fibrous inclusions in most other body structures. Some of the properties of collagen are its high tensile strength; its ion exchanging ability, its low antigenicity, due in part to masking of potential antigenic determinants by the helical structure; and its low extensibility, semipermeability, and solubility. Furthermore, collagen is a natural substance for cell adhesion. These properties and others make collagen a suitable material for tissue engineering and manufacture of implantable biological substitutes and bioremodelable prostheses.

Methods for obtaining collagenous tissue and tissue structures from explanted mammalian tissues and processes for constructing prosthesis from the tissue, have been widely investigated for surgical repair or for tissue or organ replacement. It is still a continuing goal of researchers to develop prostheses that can successfully be used to replace or repair mammalian tissue.

SUMMARY OF THE INVENTION

Biologically derived collagenous materials such as the intestinal submucosa have been proposed by many of investigators for use in tissue repair or replacement. Methods for mechanical and chemical processing of the proximal porcine jejunum to generate a single, acellular layer of intestinal collagen (ICL) that can be used to form laminates for bioprosthetic

applications are disclosed. The processing removes cells and cellular debris while maintaining the native collagen structure. The resulting sheet of processed tissue matrix is used to manufacture multi-layered laminated constructs with desired specifications. We have investigated the efficacy of laminated patches for soft tissue repair as well as the use of entubated ICL as a vascular graft. This material provides the necessary physical support, while generating minimal adhesions and is able to integrate into the surrounding native tissue and become infiltrated with host cells. In vivo remodeling does not compromise mechanical integrity. Intrinsic and functional properties of the implant, such as the modulus of elasticity, suture retention and UTS are important parameters which can be manipulated for specific requirements by varying the number of ICL layers and the crosslinking conditions.

DESCRIPTION OF THE FIGURES

Figure 1 is a diagram of the apparatus used for the deposition of dense fibrillar collagen to the lumenal surface of the ICL tube.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to a tissue engineered prostheses, which, when implanted into a mammalian host, can serve as a functioning repair, augmentation, or replacement body part or tissue structure, and will undergo controlled biodegradation occurring concomitantly with remodeling by the host's cells. The prosthesis of this invention, when used as a replacement tissue, thus has dual properties: First, it functions as a substitute body part, and second, while still functioning as a substitute body part, it functions as a remodeling template for the ingrowth of host cells. In order to do this, the prosthetic material of this invention is a processed tissue matrix developed from mammalian derived collagenous tissue that is able to be bonded to itself or another processed tissue matrix to form a prosthesis for grafting to a patient.

The invention is directed toward methods for making tissue engineered prostheses from cleaned tissue material where the methods do not require adhesives, sutures, or staples to bond the layers together while maintaining the bioremodelability of the prostheses. The terms, "processed tissue matrix" and "processed tissue material", mean native, normally cellular tissue that has been procured from an animal source, preferably a mammal, and mechanically cleaned of attendant tissues and chemically cleaned of cells, cellular debris, and rendered substantially free of non-collagenous extracellular matrix components. The processed tissue matrix, while substantially free of non-collagenous components, maintains much of its native matrix structure, strength, and shape. Preferred compositions for preparing the bioengineered grafts of the invention are animal tissues comprising collagen, including,

but not limited to: intestine, fascia lata, pericardium, dura mater, and other flat or planar structured tissues that comprise a collagenous tissue matrix. The planar structure of these tissue matrices makes them able to be easily cleaned, manipulated, and assembled in a way to prepare the bioengineered grafts of the invention. Other suitable collagenous tissue sources with the same flat sheet structure and matrix composition may be identified by the skilled artisan in other animal sources.

A more preferred composition for preparing the bioengineered grafts of the invention is an intestinal collagen layer derived from the tunica submucosa of small intestine. Suitable sources for small intestine are mammalian organisms such as human, cow, pig, sheep, dog, goat, or horse while small intestine of pig is the preferred source.

The most preferred composition for preparing the prosthesis of the invention is a processed intestinal collagen layer derived the tunica submucosa of porcine small intestine. To obtain the processed intestinal collagen layer, the small intestine of a pig is harvested and attendant mesenteric tissues are grossly dissected from the intestine. The tunica submucosa is preferably separated, or delaminated, from the other layers of the small intestine by mechanically squeezing the raw intestinal material between opposing rollers to remove the muscular layers (tunica muscularis) and the mucosa (tunica mucosa). The tunica submucosa of the small intestine is harder and stiffer than the surrounding tissue, and the rollers squeeze the softer components from the submucosa. In the examples that follow, the tunica submucosa was mechanically harvested from porcine small intestine using a Bitterling gut cleaning machine and then chemically cleaned to yield a cleaned tissue matrix. This mechanically and chemically cleaned intestinal collagen layer is herein referred to as "ICL".

Importantly, the bioremodelability of the tissue matrix is preserved in part by the cleaning process as it is free of bound detergent residues that would adversely affect the bioremodelability of the collagen. Additionally, the collagen molecules have retained their telopeptide regions as the tissue has not undergone treatment with enzymes during the cleaning process.

The collagen layers of the prosthetic device may be from the same collagen material, such as two or more layers of ICL, or from different collagen materials, such as one or more layers of ICL and one or more layers of fascia lata.

The processed tissue matrices may be treated or modified, either physically or chemically, prior to fabrication of a bioengineered graft prosthesis. Physical modifications such as shaping, conditioning by stretching and relaxing, or perforating the cleaned tissue matrices may be performed as well as chemical modifications such as binding growth factors,

selected extracellular matrix components, genetic material, and other agents that would affect bioremodeling and repair of the body part being treated, repaired, or replaced.

As ICL is the most preferred starting material for the production of the bioengineered graft prostheses of the invention, the methods described below are the preferred methods for producing bioengineered graft prostheses comprising ICL and dense fibrillar collagen.

In the most preferred embodiment, the tunica submucosa of porcine small intestine is used as a starting material for the bioengineered graft prosthesis of the invention. The small intestine of a pig is harvested, its attendant tissues removed and then mechanically cleaned using a gut cleaning machine which forcibly removes the fat, muscle and mucosal layers from the tunica submucosa using a combination of mechanical action and washing using water. The mechanical action can be described as a series of rollers that compress and strip away the successive layers from the tunica submucosa when the intact intestine is run between them. The tunica submucosa of the small intestine is comparatively harder and stiffer than the surrounding tissue, and the rollers squeeze the softer components from the submucosa. The result of the machine cleaning was such that the submucosal layer of the intestine solely remained.

After mechanical cleaning, a chemical cleaning treatment is employed to remove cell and matrix components, preferably performed under aseptic conditions at room temperature. The intestine is then cut lengthwise down the lumen and then cut into approximately 15 cm square sheet sections. Material was weighed and placed into containers at a ratio of about 100:1 v/v of solution to intestinal material. In the most preferred chemical cleaning treatment, such as the method disclosed in International PCT Application WO 98/49969, the disclosure of which is incorporated herein, the collagenous tissue is contacted with a chelating agent, such as ethylenediaminetetraacetic tetrasodium salt (EDTA) under alkaline conditions, preferably by addition of sodium hydroxide (NaOH); followed by contact with an acid where the acid contains a salt, preferably hydrochloric acid (HCl) containing sodium chloride (NaCl); followed by contact with a buffered salt solution such as 1 M sodium chloride (NaCl)/10 mM phosphate buffered saline (PBS); finally followed by a rinse step using water.

Each treatment step is preferably carried out using a rotating or shaking platform. After rinsing, the water is then removed from each container and the ICL may be stored frozen at -80 °C, at 4 °C in sterile phosphate buffer, or dry until use in fabrication of a prosthesis. If stored dry, the ICL sheets are flattened on a surface such as a flat plate, preferably of relatively inert or nonreactive material, such as a polycarbonate, and any lymphatic tags from the abluminal side of the material are removed using a scalpel, and the

ICL sheets are allowed to dry in a laminar flow hood at ambient room temperature and humidity.

The ICL is a planar sheet structure that can be used to fabricate various types of constructs to be used as a prosthesis with the shape of the prosthesis ultimately depending on its intended use. To form prostheses of the invention, the constructs must be fabricated using a method that preserves the bioremodelability of the processed matrix material but also is able to maintain its strength and structural characteristics in its performance as a replacement tissue. The structural layer of the prosthesis is a tubular construct formed from a single, generally rectangular sheet of processed tissue matrix. The tissue matrix sheet is rolled so that one edge meets and overlaps an opposing edge of the sheet. The overlap serves as a bonding region. As used herein, "bonding region" means an area of contact between tow or more layers of the same or difference processed tissue matrix treated in a manner such that the layers are superimposed on each other and are sufficiently held together by self-lamination and chemical linking. The bonding region must be able to withstand suturing and stretching while being handled in the clinic, implantation and during the initial healing phase until the patients cells populate and subsequently bioremodel the prosthesis to form a new tissue. When used as a conduit or a duct, the bonding region must be able to withstand pressures of the matter it contains or is passing, particularly when used as a vascular graft under the systolic and diastolic pressures of systemic blood flow.

In a preferred embodiment, a processed tissue matrix is formed into a tubular prosthesis coated with a layer of fibrillar collagen on either the luminal surface, the abluminal surface, or both.

In a more preferred embodiment, a processed tissue matrix is derived from tunica submucosa of small intestine and is formed into a tube structure and is provided with a coating of dense fibrillar collagen.

In an even more preferred embodiment, a processed tissue matrix comprises processed ICL formed into a tubular structure and is provided with a coating of dense fibrillar collagen on the luminal surface.

In the most preferred embodiment, a processed tissue matrix comprises processed ICL formed into a tubular structure and is provided with a coating of dense fibrillar collagen fibrillar collagen on the luminal surface which is in turn provided with an antithrombogenic agent, such as heparin to form a vascular prosthesis.

The ICL tube may be fabricated in various diameters, lengths, and number of layers and may incorporate other components depending on the indication for its use. The tubular

ICL construct may be used as a vascular graft. For this indication, the graft comprises at least one layer with at least a 5% overlap to act as a bonding region that forms a tight seam and the luminal surface is preferably treated with heparin or an agent that prevents thrombosis. In another vascular indication, the tubular ICL construct may be used as an external stent and cases where vein autografts are transplanted within the body and external support for the transplanted vein is desired. In still another vascular indication, the tubular ICL construct is formed on a metal stent to provide a cover for the stent. When implanted, the ICL benefits the recipient by providing a smooth protective covering for the stent, to prevent additional damage to host tissue during deployment. Tubular ICL prostheses may also be used to repair or replace other normally tubular structures such as gastrointestinal tract sections, urethra, ducts, etc. It may also be used in nervous system repair when fabricated into a nerve growth tube packed with extracellular matrix components, growth factors, or cultured cells.

To form a tubular construct, a mandrel is chosen with a diameter measurement that will determine the diameter of the formed construct. The mandrel is preferably cylindrical or oval in cross section and made of glass, stainless steel or of a nonreactive, medical grade composition. The mandrel may be straight, curved, angled, it may have branches or bifurcations, or a number of these qualities. The number of layers intended for the tubular construct to be formed corresponds with the number of times an ICL is wrapped around a mandrel and over itself. The number of times the ICL can be wrapped depends on the width of the processed ICL sheet. For a two layer tubular construct, the width of the sheet must be sufficient for wrapping the sheet around the mandrel at least twice. It is preferable that the width be sufficient to wrap the sheet around the mandrel the required number of times and an additional percentage more as an overlap to serve as a bonding region. Between about 5% to about 20% of the mandrel circumference is sufficient to serve as a bonding region and to form a tight seam. Similarly, the length of the mandrel will dictate the length of the tube that can be formed on it. For ease in handling the construct on the mandrel, the mandrel should be longer than the length of the construct so the mandrel, and not the construct being formed, is contacted during the procedure for fabricating the construct.

The ICL has a sidedness quality from its native tubular state. The ICL has two opposing surfaces: an inner mucosal surface and outer serosal surface. It has been found that these surfaces have characteristics that can affect post-operative performance of the prosthesis but can be leveraged for enhanced device performance. In the formation of a tubular construct for use in as a vascular graft, it is preferred that the mucosal surface of the material be the luminal surface of the tubular graft when formed. Having the mucosal surface contact

the bloodflow provides an advantage as it has some nonthrombogenic properties that are preferred to prevent occlusion of the graft when it has been implanted in a patient.

It is preferred that the mandrel is provided with a covering of a nonreactive, medical grade quality, elastic, rubber or latex material in the form of a sleeve. While a tubular ICL construct may be formed directly on the mandrel surface, the sleeve facilitates the removal of the formed tube from the mandrel and does not adhere to, react with, or leave residues on the ICL. To remove the formed construct, the sleeve may be pulled from one end off the mandrel to carry the construct from the mandrel with it. Because the processed ICL only lightly adheres to the sleeve and is more adherent to other ICL layers, fabricating ICL tubes is facilitated as the tubulated contract may be removed from the mandrel without stretching or otherwise stressing or risking damage to the construct. In the most preferred embodiment, the sleeve comprises KRATON® (Shell Chemical Company), a thermoplastic rubber composed of styrene-ethylene/butylene-styrene copolymers with a very stable saturated midblock.

For simplicity in illustration, a two-layer tubular construct with a 4 mm diameter and a 10% overlap is formed on a mandrel having about a 4 mm diameter. The mandrel is provided with a KRATON® sleeve approximately as long as the length of the mandrel and longer than the construct to be formed on it. A sheet of ICL is trimmed so that the width dimension is about 28 mm and the length dimension may vary depending on the desired length of the construct. In the sterile field of a laminar flow cabinet, the ICL is then formed into ICL collagen tubes by the following process. The ICL is moistened along one edge and is aligned with the sleeve-covered mandrel and, leveraging the adhesive nature of the ICL, it is "flagged" on the sleeve and dried in position for at least 10 minutes or more. The flagged ICL is then hydrated and wrapped around the mandrel and then over itself one full revolution plus 10% of the circumference to provide a tight seam.

For the formation of single layer tubular construct, the ICL must be able to wrap around the mandrel one full revolution and at least about a 5% of an additional revolution as an overlap to provide a bonding region that is equal to about 5% of the circumference of the construct. For a two-layer construct, the ICL must be able to wrap around the mandrel at least twice and preferably an additional 5% to 20% revolution as an overlap. While the two-layer wrap provides a bonding region of 100% between the ICL surfaces, the additional percentage for overlap ensures a tight, impermeable seam. For a three-layer construct, the ICL must be able to wrap around the mandrel at least three times and preferably an additional 5% to 20% revolution as an overlap. The construct may be prepared with any number of layers depending on the specifications for a graft required by the intended indication. Typically, a

tubular construct will have 10 layers or less, preferably between 2 to 6 layers and more preferably 2 or 3 layers with varying degrees of overlap. After wrapping, and air bubbles, folds, and creases are smoothed out from under the material and between the layers.

ICL may be rolled either manually or with the assistance of an apparatus that allows for even tensioning and smoothing out air or water bubbles or creases that can occur under the mandrel or between the layers of ICL. The apparatus would have a surface that the mandrel can contact along its length as it is turned to wrap the ICL.

The layers of the wrapped ICL are then bonded together by dehydrating them while in wrapped arrangement on the sleeve-covered mandrel. While not wishing to be bound by theory, dehydration brings the extracellular matrix components, such as collagen fibers, in the layers together when water is removed from the spaces between the fibers in the matrix. Dehydration may be performed in air, in a vacuum, or by chemical means such as by acetone or an alcohol such as ethyl alcohol or isopropyl alcohol. Dehydration may be done to room humidity, normally between about 10% Rh to about 20% Rh, or less; or about 10% to 20% moisture by weight. Dehydration may be easily performed by angling the mandrel with the ICL layers up into the oncoming sterile airflow of the laminar flow cabinet for at least about 1 hour up to 24 hours at ambient room temperature, approximately 20 °C, and at room humidity. The ICL tube is then removed from the sleeve by removing the sleeve from the mandrel carrying the ICL tube with it and the sleeve removed from the lumen of the construct by pulling the sleeve from the ends and sliding it off the mandrel. The constructs are then rehydrated in an aqueous rehydration agent, preferably water, by transferring them to a room temperature sterile container containing rehydration agent for at least about 10 to about 15 minutes to rehydrate the layers without separation or delamination.

In another preferred embodiment, a collagen coating may be added to either the luminal surface or abluminal surface, or both the luminal or abluminal surfaces of the formed tube. Collagen may be deposited on surfaces of the ICL as described in Example 5 of U.S. Patent 5,256,418, incorporated herein by reference. This deposited collagen layer is characterized as "dense fibrillar collagen", also termed as "DFC", and is a layer of fibrillar telopeptide collagen formed from collagen solution. The DFC layer serves as a smooth flow surface on the internal surface of the structural ICL layer when the construct is used as a vascular graft. The deposition step may also be accomplished as described in the above-referenced patent application using a hydrostatic pressure head formed using the apparatus in Figure 1. Figure 1 shows a collagen reservoir, 10, a bifurcated tube, 20, luer fittings, 25, a tubular construct of bonded ICL, 50, and container, 30, containing PEG, 35. To create a

hydrostatic pressure head, the collagen reservoir, 10, containing collagen solution is mounted a distance between 3-8 feet above the tubular construct, 50, and is connected to the construct via the bifurcated tubing, 20, attached to the luer fittings, 25, secured at either end of the construct. Briefly, after the ICL is tubulated, the multi-layered fabric is fitted at one end by luer fittings, 25, rehydrated with deionized water and the collagen from the collagen reservoir, 10, fills the tube. The inner layer of collagen can also be deposited by flowing collagen into both ends of the tube simultaneously. A preferred collagen composition is solubilized acid extracted collagen concentration between about 1 to about 5 mg/mL in acetic acid. The tube is then placed into a bath of buffered 20% w/v polyethylene glycol 600-650 mmol/Kg Molecular Wt. 8000 (PEG) in isotonic phosphate buffered saline (PBS), neutral pH. The grafts are mounted on a frame during the collagen deposition to provide for a hydrostatic pressure of between about 100 to about 150 mmHg, preferably at about 120 mmHg. The osmotic gradient between the internal collagen solution and outer PEG solution in combination cause a simultaneous concentration and deposition of the collagen along the lumen of the internal structural layer wall. DFC deposition is allowed to occur for a time preferably between about 10 minutes to 6 hours, more preferably between about 10 minutes to about 60 minutes, most preferably about 30 minutes. A 10 minute deposition will yield a DFC coating of about 15 to about 20 μm; a 30 minute deposition, about 40 to about 50μm. A longer deposition time will yield a thicker DFC coating. The tube is then removed from the PEG bath, flushed with PBS and the lumen of the construct is maintained while the prosthesis is allowed to dry. One means for keeping the lumen is to insert a second mandrel with a diameter smaller than the first into the lumen. A preferred means for maintaining a lumen is to force air into the construct. The prosthesis is then allowed to dry. The tube is then rehydrated in isotonic phosphate buffered saline (PBS). This process allows the deposited collagen layer to fill slight irregularities in the intestinal structural layer, thus resulting in a layer having a smooth surface and approximate uniform thickness. The procedure also facilitates the bonding of the collagen gel to the intestinal collagen layer. A collagenous layer of varying thickness and density can be produced by simply changing the deposition conditions such as: the concentration or pH of the collagen solution, the concentration or pH of the PEG solution, time, and temperature. The parameter changes can be easily determined and by the skilled routineer in the collagen field. The same procedures can be used to apply the collagen to the outer surface of the ICL to create a three-layer prosthesis.

To bond the ICL and DFC layers together, the prosthesis is hydrated and dehydrated for at least two cycles of hydration and dehydration. Hydration is preferably in a hydration

agent such as either water or an isotonic phosphate buffered saline (PBS) with a neutral pH. Dehydration is performed by exposing the prosthesis to air, preferably flowing air under aseptic conditions such as in the oncoming airflow of a laminar flow cabinet and allowed to dry for about 18 ± 2 hours in the cabinet at room temperature, approximately 20 °C to room humidity or less. Dehydration can also be accomplished by means of a solvent, such as ethanol, acetone or isopropanol.

The prosthesis is chemically crosslinked using a chemical crosslinking agent. A preferred crosslinking agent is EDC in water, at a concentration between about 0.1mM –to about 100mM, more preferably at a concentration between about 1.0mM –to about 10 mM, most preferably at a concentration between about 0.1mM –to about 1 mM. The most preferred crosslinking agent is 1 mM EDC in water. EDC is 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Besides EDC, other carbodiimides and their adjuncts such as fibrin-based glues or medical grade adhesives such as cyanoacrylates, polyurethane, vinyl acetate or polyepoxy may be used as a crosslinker. Water, phosphate buffered saline, or (2-[N-morpholino]ethanesulfonic acid) (MES) buffer may be used to dissolve the crosslinker. The hydrated ICL tubes are then transferred to a container such as a test-tube or shallow pan and the crosslinking agent is gently decanted in to the pan while ensuring that the layers were both covered and free-floating and that no air bubbles were present inside the tubes. The pan was covered and allowed to sit for about 18 ± 2 hours in a fume hood after which time the crosslinking solution is decanted and disposed. After crosslinking, the tubes are rinsed. ICL tubes were rinsed three times with sterile water in a test-tube or pan for about five minutes for each rinse.

The prostheses are then chemically sterilized using a chemical sterilant in liquid or vapor phase. Constructs are sterilized in dilute peracetic acid solution as described in U.S. Patent No. 5,460,962, incorporated herein by reference. Other sterilization systems for use with collagen are known in the art and can be used. In the preferred method, and in the examples that follow, the ICL was disinfected with 0.1% peracetic acid solution and stored until use at 4°C. If the sterilant residues remain after contact with the sterilant, the constructs are rinsed. ICL tubes are rinsed in a test-tube or pan three times with sterile water for about 5 to about 15 minutes for each rinse.

The vessel may be rendered non-thrombogenic by applying heparin to the lumen of the formed tube. Heparin can be applied to the prosthesis, by a variety of well-known techniques. For illustration, heparin can be applied to the prosthesis in the following three ways. First, benzalkonium heparin (BA-Hep) isopropyl alcohol solution is applied to the

prosthesis by vertically filling the lumen or dipping the prosthesis in the solution and then air-drying it. This procedure treats the collagen with an ionically bound BA-Hep complex. Second, EDC can be used to activate the heparin, then to covalently bond the heparin to the collagen fiber. Third, EDC can be used to activate the collagen, then covalently bond protamine to the collagen and then ionically bond heparin to the protamine. Many other coating, bonding, and attachment procedures are well known in the art that could also be used. When fabrication of the constructs is complete, they are placed in sterile containers until use.

Constructs are then terminally sterilized using means known in the art of medical device sterilization. A preferred method for sterilization is by contacting the constructs with sterile 0.1% peracetic acid (PA) treatment neutralized with a sufficient amount of 10 N sodium hydroxide (NaOH), according to US Patent No. 5,460,962, the disclosure of which is incorporated herein. Decontamination is performed in a container on a shaker platform, such as 1 L Nalge containers, for about 18 ± 2 hours. Constructs are then rinsed by contacting them with three volumes of sterile water for 10 minutes each rinse.

Tubular prostheses may be used, for example, to replace cross sections of tubular organs such as vasculature, esophagus, trachea, intestine, and fallopian tubes. These organs have a basic tubular shape with an outer surface and an inner luminal surface. Flat sheets may also be used for organ support, for example, to support prolapsed or hypermobile organs by using the sheet as a sling for the organs, such as bladder or uterus. In addition, flat sheets and tubular structures can be formed together to form a complex structure to replace or augment cardiac or venous valves.

The bioengineered graft prostheses of the invention may be used to repair or replace body structures that have been damaged or diseased in host tissue. While functioning as a substitute body part or support, the prosthesis also functions as a bioremodelable matrix scaffold for the ingrowth of host cells. "Bioremodeling" is used herein to mean the production of structural collagen, vascularization, and cell repopulation by the ingrowth of host cells at a functional rate about equal to the rate of biodegradation, reforming and replacement of the matrix components of the implanted prosthesis by host cells and enzymes. The graft prosthesis retains its structural characteristics while it is remodeled by the host into all, or substantially all, host tissue, and as such, is functional as an analog of the tissue it repairs or replaces.

The shrink temperature (°C) of the tissue matrix prosthesis is an indicator of the extent of matrix crosslinking. The higher the shrink temperature, the more crosslinked the material. Non-crosslinked ICL has a shrink temperature of about 68 ± 0.3 °C. In the preferred

embodiment, EDC crosslinked prostheses should have a shrink temperature between about 68 ± 1 °C to about 75 ± 1 °C.

The mechanical properties include mechanical integrity such that the prosthesis resists creep during bioremodeling, and additionally is pliable and suturable. The term "pliable" means good handling properties for ease in use in the clinic.

The term "suturable" means that the mechanical properties of the layer include suture retention which permits needles and suture materials to pass through the prosthesis material at the time of suturing of the prosthesis to sections of native tissue, a process known as anastomosis. During suturing, such prostheses must not tear as a result of the tensile forces applied to them by the suture, nor should they tear when the suture is knotted. Suturability of prostheses, i.e., the ability of prostheses to resist tearing while being sutured, is related to the intrinsic mechanical strength of the prosthesis material, the thickness of the graft, the tension applied to the suture, and the rate at which the knot is pulled closed. Suture retention for a 2 layer tubular prosthesis crosslinked in 1 mM EDC in water is about 3.9 N ± 0.8 N. The preferred lower suture retention strength is about 2 N for a crosslinked flat 2 layer prosthesis; a surgeon's pull strength when suturing is about 1.8 N.

As used herein, the term "non-creeping" means that the biomechanical properties of the prosthesis impart durability so that the prosthesis is not stretched, distended, or expanded beyond normal limits after implantation. As is described below, total stretch of the implanted prosthesis of this invention is within acceptable limits. The prosthesis of this invention acquires a resistance to stretching as a function of post-implantation cellular bioremodeling by replacement of structural collagen by host cells at a faster rate than the loss of mechanical strength of the implanted materials due from biodegradation and remodeling.

The processed tissue material of the present invention is "semi-permeable," even though it has been layered and bonded. Semi-permeability permits the ingrowth of host cells for remodeling or for deposition of agents and components that would affect bioremodelability, cell ingrowth, adhesion prevention or promotion, or blood flow. The "non-porous" quality of the prosthesis prevents the passage of fluids intended to be retained by the implantation of the prosthesis. Conversely, pores may be formed in the prosthesis if a porous or perforated quality is required for an application of the prosthesis.

In some embodiments, after ICL is reformed into a construct for tissue repair or replacement and has the dense fibrillar collagen layer deposited on it, it may be populated with cells to form a cellular tissue construct comprising bonded layers of ICL and cultured

cells. Cellular tissue constructs can be formed to mimic the organs they are to repair or replace.

Cell cultures are established from mammalian tissue sources by dissociating the tissue or by explant method. Primary cultures are established and cryopreserved in master cell banks from which portions of the bank are thawed, seeded, and subcultured to expand cell numbers. To populate an acellular ICL construct with cells, the construct is placed in a culture dish or flask and contacted by immersion in media containing suspended cells. Because collagen is a natural substance for cell adhesion, cells bind to the ICL construct and proliferate on and into the collagenous matrix of the construct.

Preferred cell types for use in this invention are derived from mesenchyme. More preferred cell types are fibroblasts, stromal cells, and other supporting connective tissue cells, or human dermal fibroblasts. Human fibroblast cell strains can be derived from a number of sources, including, but not limited to neonate male foreskin, dermis, tendon, lung, umbilical cords, cartilage, urethra, corneal stroma, oral mucosa, and intestine. The human cells may include but need not be limited to: fibroblasts, smooth muscle cells, chondrocytes and other connective tissue cells of mesenchymal origin. It is preferred, but not required, that the origin of the matrix-producing cell used in the production of a tissue construct be derived from a tissue type that it is to resemble or mimic after employing the culturing methods of the invention. For instance, a multilayer sheet construct is cultured with fibroblasts to form a living connective tissue construct; or myoblasts, for a skeletal muscle construct. More than one cell type can be used to populate an ICL construct, for example, a tubular ICL construct can be first cultured with smooth muscle cells and then the lumen of the construct populated with the first cell type is cultured with vascular endothelial cells as a second cell type to form a cellular vascular replacement device. Similarly, a urinary bladder wall patch prosthesis is similarly prepared on multilayer ICL sheet constructs using smooth muscle cells as a first cell type and then urinary endothelial cells as a second cell type. Cell donors may vary in development and age. Cells may be derived from donor tissues of embryos, neonates, or older individuals including adults. Embryonic progenitor cells such as mesenchymal stem cells may be used in the invention and induced to differentiate to develop into the desired tissue.

Although human cells are preferred for use in the invention, the cells to be used in the method of the are not limited to cells from human sources. Cells from other mammalian species including, but not limited to, equine, canine, porcine, bovine, ovine, and murine sources may be used. In addition, genetically engineered cells that are spontaneously,

chemically or virally transfected may also be used in this invention. For those embodiments that incorporate more than one cell type, mixtures of normal and genetically modified or transfected cells may be used and mixtures of cells of two or more species or tissue sources may be used, or both.

Recombinant or genetically-engineered cells may be used in the production of the cell-matrix construct to create a tissue construct that acts as a drug delivery graft for a patient needing increased levels of natural cell products or treatment with a therapeutic. The cells may produce and deliver to the patient via the graft recombinant cell products, growth factors, hormones, peptides or proteins for a continuous amount of time or as needed when biologically, chemically, or thermally signaled due to the conditions present in the patient. Cells may also be genetically engineered to express proteins or different types of extracellular matrix components which are either 'normal' but expressed at high levels or modified in some way to make a graft device comprising extracellular matrix and living cells that is therapeutically advantageous for improved wound healing, facilitated or directed neovascularization. These procedures are generally known in the art, and are described in Sambrook et al, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), incorporated herein by reference. All of the above-mentioned types of cells may be used in this invention for the production of a cellular tissue construct formed from an acellular construct formed from bonded ICL layers.

The following examples are provided to better explain the practice of the present invention and should not be interpreted in any way to limit the scope of the present invention. It will be appreciated that the device design in its composition, shape, and thickness is to be selected depending on the ultimate indication for the construct. Those skilled in the art will recognize that various modifications can be made to the methods described herein while not departing from the spirit and scope of the present invention.

EXAMPLES

Example 1: Chemical Cleaning of Mechanically Cleaned Porcine Small Intestine

The small intestine of a pig was harvested and mechanically stripped, using a Bitterling gut cleaning machine (Nottingham, UK) which forcibly removes the fat, muscle and mucosal layers from the tunica submucosa using a combination of mechanical action and washing using water. The mechanical action can be described as a series of rollers that compress and strip away the successive layers from the tunica submucosa when the intact intestine is run between them. The tunica submucosa of the small intestine is comparatively

harder and stiffer than the surrounding tissue, and the rollers squeeze the softer components from the submucosa. The result of the machine cleaning was such that the submucosal layer of the intestine solely remained. The remainder of the procedure was performed under aseptic conditions and at room temperature. The chemical solutions were all used at room temperature. The intestine was then cut lengthwise down the lumen and then cut into 15 cm sections. Material was weighed and placed into containers at a ratio of about 100:1 v/v of solution to intestinal material.

A. To each container containing intestine was added approximately 1 L solution of 0.22 µm (micron) filter sterilized 100 mM ethylenediaminetetraacetic tetrasodium salt (EDTA)/10 mM sodium hydroxide (NaOH) solution. Containers were then placed on a shaker table for about 18 hours at about 200 rpm. After shaking, the EDTA/NaOH solution was removed from each bottle.

B. To each container was then added approximately 1 L solution of 0.22 µm filter sterilized 1 M hydrochloric acid (HCl)/1 M sodium chloride (NaCl) solution. Containers were then placed on a shaker table for between about 6 to 8 hours at about 200 rpm. After shaking, the HCl/NaCl solution was removed from each container.

C. To each container was then added approximately 1 L solution of 0.22 µm filter sterilized 1 M sodium chloride (NaCl)/10 mM phosphate buffered saline (PBS). Containers were then placed on a shaker table for approximately 18 hours at 200 rpm. After shaking, the NaCl/PBS solution was removed from each container.

D. To each container was then added approximately 1 L solution of 0.22 µm filter sterilized 10 mM PBS. Containers were then placed on a shaker table for about two hours at 200 rpm. After shaking, the phosphate buffered saline was then removed from each container.

E. Finally, to each container was then added approximately 1 L of 0.22 µm filter sterilized water. Containers were then placed on a shaker table for about one hour at 200 rpm. After shaking, the water was then removed from each container.

Treated samples were cut and fixed for histological analyses. Hemotoxylin and eosin (H&E) and Masson's trichrome staining was performed on both cross-section and long-section samples of both control and treated tissues. Treated tissue samples appeared free of cells and cellular debris while untreated control samples appeared normally and expectedly very cellular.

Example 2: Comparative Study of Other Cleaning Treatments for Collagenous Tissue

Other methods for disinfecting and sterilizing collagenous tissues described in US Patent No. 5,460,962 to Kemp were compared to similar methods described by Cook, et al. in International PCT application WO 98/22158. Examples 1, 2, and 3, from Kemp, in addition to a non-buffered peracetic acid method were done.

Small intestines were harvested from 4 large pigs. Intestines were procured, the outer mesenteric layer was stripped, and the intestines were flushed with water.

The study included seven conditions: Condition A was carried out according to the disclosure of Example 1 in Cook, et al. in International PCT Application WO 98/22158. Condition B was a variation of A in that the intestinal material was mechanically cleaned before employing the disclosed chemical treatment. Conditions C, D, and E were carried out according to the methods of Examples 1, 2, and 3 in U.S. Patent No. 5,460,962 to Kemp. In all conditions, a ten-to-one ratio of solution to material is used, that is, 100 g of tissue material is treated with 1 L of solution.

A. Material from each of the 4 intestines were placed into separate bottles containing a one liter solution of 0.2% peracetic acid in 5% ethanol (pH 2.56) and agitated on a shaker platform. After two hours of agitation, condition A was mechanically cleaned on the Bitterling gut cleaning machine.

For the other six conditions, B through G, intestine was mechanically cleaned using the Bitterling gut cleaning machine prior to chemical treatment. After mechanical cleaning, representative pieces from the 4 intestines were placed into bottles containing solution for chemical treatment. Bottles were shaken 18 ± 2 hours on a platform. The remaining six conditions, B through G, were as follows:

B. A one liter solution of 0.2% peracetic acid in 5% ethanol (pH 2.56).

C. A one liter solution of 0.1% peracetic acid in phosphate buffered saline (pH 7.2).

D. A one liter solution of 0.1% peracetic acid and 1M sodium chloride (NaCl) (pH 7.2).

E. A one liter solution of 0.1% peracetic acid and 1M NaCl (pH 2.9).

F. One liter solution of "chemical cleaning" solutions as mentioned above in Example 1.

G. A one liter solution of 0.1% peracetic acid in deionized water, buffered to pH 7.0.

After chemical and mechanical treatments, all conditions were rinsed for a total of 4 times with filtered sterile purified water. The mechanically and chemically treated material

was grossly stained to examine cellular debris with Mayer's hematoxylin. Morphological assessment included Hematoxylin & Eosin, Masson's Trichrome, and Alizarin Red staining techniques. Histological results from the various treatments show that the method of condition A yielded a material where it was difficult to remove mucosal layers on Bitterling after chemical treatment. The material had to be run through Bitterling about an extra 10-12 times. The material was very swollen at first and had a significantly large amount of cellular debris on surface and in the vasculature of the material. The method of condition B was also very swollen and also demonstrated a significantly large amount of cellular debris on surface and in the vasculature of the material. The methods of conditions C and D yielded a non-swollen material having minimal cellular debris in vasculature. Condition E yielded a material that was slightly swollen and contained minimal cellular debris in the vasculature.

A DNA/RNA isolation kit (Amersham Life Sciences) was used to quantify the residual DNA/RNA contained in the cleaned tissues. The results are summarized in Table 1.

Table 1: DNA/RNA Isolation kit Results (µg DNA/mg tissue)

Condition	A	B	C	D	E	F	G
Average± Std. Dev.	2.16±0.32 (n=5)	2.1±0.48 (n=5)	0.32±0.11 (n=3)	1.92±0.28 (n=3)	0.32±0.23 (n=3)	0±0 (n=4)	1.42±0.03 (n=2)

Morphological analysis correlates with the DNA/RNA quantification to show that the cleaning regimens of conditions A and B result in a collagenous tissue matrix that remains highly cellular and contain residual DNA as a result. The cleaning methods of Kemp are much more effective for the removal of cells and cellular debris from collagenous tissue matrices. Finally, the chemical cleaning method of Condition F, described in International PCT Application No. WO 98/49969 to Abraham, et al. and outlined in Example 1, above, removes all cells and cellular debris and their DNA/RNA to a level undetectable by these methods.

Example 3: Method for Making Tubular ICL/DFC graft

In the sterile field of a laminar flow cabinet, the ICL was formed into ICL collagen tubes by the following process. Lymphatic tags were trimmed from the serosal surface of the ICL. The ICL was blotted with sterile absorbent towelettes to absorb excess water from the material and then spread on a porous polycarbonate sheet and allowed to dry in the oncoming airflow of the laminar flow cabinet. Once dry, ICL was cut into 28.5 mm x 10 cm pieces for a

2 layer graft with approximately a 10% overlap. To support the ICL in the formation of the tubes, a cylindrical stainless steel mandrel with a diameter of about 4 mm was covered with KRATON®, an elastic sleeve material that facilitates the removal of the formed collagen tube from the mandrel and does not adhere or react with the ICL. The long edge of the ICL was then moistened with sterile water and adhered to the mandrel and allowed to dry for about 15 minutes to form a "flag". Once adhered, the ICL was rolled around the mandrel and over itself one complete revolution. After rolling was complete, air bubbles, folds, and creases were smoothed out from under the material and between the layers. The rolled constructs (on mandrels) were allowed to dry in the airflow of the laminar flow cabinet for about an hour in the cabinet at room temperature, approximately 20 °C. The formed ICL tube and the Kraton were removed together from the mandrel and the ICL tube was removed from the Kraton.

To deposit the dense fibrillar collagen (DFC) on the luminal surface of the tube, luer barbs were attached to both ends of the tube for attaching tubing to facilitate delivery of collagen and deposition agent to the interior of the tube. Acid extracted collagen solution in acetic acid at approximately 2.5 mg/ml was added through a luer barb until the lumen of the tube was full of collagen solution under static internal pressure of 120 mmHg or under forced flowing air for a time 10, 30, or 60 minutes, depending on desired thickness for final coating of DFC. When the air was displaced from the interior of the tube by the collagen solution, the second end was connected to a branch in the tube supplying collagen to the first end. The tube was submerged in a bath of circulating polyethylene glycol (PEG) N600, MW 8000 of approximately 500 mL. Through the luer barbs the tubes were then flushed with phosphate buffered saline to neutralize the collagen volumes approximately 60 cc with hydrostatic pressure, so as to not damage the newly deposited luminal covering of DFC. Then the luer barb was attached to a dry sterile filtered air source and the tube was dried with the flowing air under static internal pressure of 120 mmHg for desired time about 2-3 hours, or until reaches approximately room relative humidity. The tube was rehydrated in PBS at least one hour and dried again for at least 2 hours to overnight (18± 2 hours), using the same air drying method. The tubes were each then chemically crosslinked in a about 50 mL crosslinking agent of 1mM EDC in sterile water for about 18 ± 2 hours. The tubes were then rinsed with three equal volumes of sterile water to remove residual crosslinking agent and reaction products. The tubes were then terminally sterilized in a solution of 0.1% peracetic acid in deionized water, neutralized according to Kemp, to a pH of 6.9 to 7.1. The tubes were then rinsed in two volumes of sterile water to remove residual sterilant. Finally tubes were hung

vertically and the lumen was filled with BA-HEP for at least one minute, drained and allowed to dry for at least one hour. This procedure is repeated twice for a total of three treatments of benzalkonium heparin. Tube constructs were then packaged in sterile polycarbonate test tubes until use.

Example 4: Mechanical testing of ICL Tube Prostheses

Various mechanical properties of a 2 layer ICL tubular construct formed from a single sheet of ICL wrapped around a mandrel with 20% overlap, crosslinked at 1mM EDC in water was measured. Suture retention, burst, porosity, and compliance testing were done in accordance with the "Guidance for the Preparation of Research and Marketing Applications for Vascular Graft Prostheses", FDA Draft Document, August 1993. Suture retention, burst and compliance analyses were performed using a servohydraullic MTS testing system with TestStar-SX software.

Briefly, the suture retention test consisted of a suture being pulled 2.0 mm from the edge of a graft at a constant rate. The peak force when the suture ripped through the graft was measured. The average measurement obtained was above required limits indicating that the construct can withstand the physical pressures of suturing in the clinic.

In the burst test, pressure was applied to the graft in 2.0 psi increments for one minute intervals until the graft burst. For reference, systolic pressure is approximately 120mmHg (16.0 kPa) in a normotensive person. The burst strength obtained by the testing demonstrated that the construct could maintain pressures about 7.75 times systolic pressure thus indicating that the construct can be grafted for vascular indications and withstand the rigors blood circulation.

For compliance testing, the graft was brought to 80 and 120 mmHg in succession. The diameter of the graft was then measured at each pressure using image analysis software and the compliance calculated as $(D_{120}-D_{80})/(D_{80} \times 40mmHg) \times 100\%$. Compliance of a rabbit carotid artery is approximately 0.07%/mmHg, human artery is about 0.06%/mmHg and human vein is about 0.02%/mmHg, indicating that the construct exhibits the requisite compliance to serve as a vascular graft.

To measure porosity, PBS under hydrostatic pressure of 120 mmHg is applied to the graft. The volume of PBS that permeated through the graft over a 72 hour period was normalized to the time and surface area of the graft to calculate the porosity.

The shrink temperature is used to monitor the extent of crosslinking in a collagenous material. The more crosslinked a graft, the more energy is required for denaturation, thus a

higher shrink temperature. A differential scanning calorimeter was used to measure the heat flow to and from a sample under thermally controlled conditions. The shrink temperature was defined as the onset temperature of the denaturation peak in the temperature-energy plot.

The suture retention was well above the 2N suggested for suturing a prosthesis in place. The measured burst strength was over seven times systolic pressure. The compliance was in the range of human arteries and veins. The porosity of the ICL tube was low compared to a woven graft and does not require pre-clotting. The shrink temperature was close to that of non cross-linked ICL indicating a low amount of cross-linking.

Table 1: Summary of Mechanical Properties

Mechanical Test	Result
Suture Retention Test	3.7 ± 0.5 N
Burst Test	18.0 ± 5.4 psi (124 ± 37 kPa)
Porosity	4.26 x 10^{-3} ml/cm^2/min
Shrink Temperature	68.4 ± 0.4 °C
Compliance (between 80 and 120 mmHg)	0.05 %/mmHg

Example 5: Animal Study Using the ICL/DFC Prosthesis

Two-layer wrapped ICL constructs bonded with 1 mM EDC in deionized water and having the mucosal side of the ICL as the luminal surface coated with DFC were prepared. Two DFC layer thicknesses were tested: a 10 minute deposition having a DFC layer of approximately 15 to 20μm and a 30 minute deposition having a DFC layer of approximately 40 to 50μm thick. The grafts were used as a carotid replacement graft in a rabbit model.

New Zealand White rabbits weighed between about 2.0 kg to about 2.5 kg just prior to surgery and anesthesia was induced using ketamine hydrochloride (30-60 mg/kg) and Xylazine (3-6 mg/kg) injected subcutaneously. Anesthesia was maintained with Ketamine at 50% of induction dose. The surgical area was clipped, prepared and draped to maintain field sterility. Baytril (10 mg/kg) was administered intramuscularly preoperatively and the animals were kept warm during surgery with a heating pad. Through a right cervical oblique incision, the jugular vein was mobilized, ligated and divided to expose the carotid artery. Following complete mobilization of the right carotid artery and prior to arterial clamping, heparin (200 IU/kg) was given intravenously. Atraumatic arterial clamps were then placed proximal and distal to the arteriotomy sites to occlude flow and the proximal arteriotomy was made. An end-to side proximal anastomosis of artery to graft was performed using 10-0 Ethilon sutures. After a quick

flush of the graft, the distal anastomosis was done in a similar manner. The segment of carotid artery between the anastomoses was excised leaving an interposition graft 3-4 cm long. Arterial flow was reestablished and closure was done in layers using 3-0 Vicryl for the fascia and 4-0 Ethilon for the skin. Immediately postoperatively, the animals were housed individually and observed every 15 minutes until sternal recumbence was maintained. All surgical wounds were checked at least once a day for any signs of complications. Any necessary corrective actions were taken and noted in the record.

Prior to sacrifice, a general physical examination to evaluate body weight and general condition of the animals was performed. The graft pulse was assessed by palpation and peripheral circulation estimated. Anesthesia was induced as described for implantation. The previous incision was reopened. After the graft was mobilized, heparin was administered as described above. The proximal carotid was cannulated and the animal was euthanized with Pentobarbital (100 mg/kg) administered intravenously. Immediately after euthanasia, the graft was flushed with Hanks' balanced salt solution at physiologic pressure (80 mm Hg) in situ. Graft segments to be analyzed histologically were fixed with McDowell-Trump solution at the same pressure for 30-60 minutes. Analysis of the mechanical properties and physiologic responsiveness was carried out using explants that have not been fixed. The grafts were excised with minimal handling to avoid periadventitial tissue removal. A necropsy examination was conducted and any abnormal lesions or tissues were placed in neutral formalin for analysis.

All vascular graft prostheses with a DFC layer of approximately 40 to 50μm thick were patent (n=20). Prostheses having a DFC layer of approximately 10 to 20 μm thick were 50% patent (n=4). Histology by hematoxylin and eosin staining and by immunohistochemistry showed smooth muscle ingrowth and vascular endothelial cell migration in and along the patent explants. These results demonstrate that the vascular constructs were able to be bioremodeled by host cells while serving a vascular prosthesis.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious to one of skill in the art that certain changes and modifications may be practiced within the scope of the appended claims.

WE CLAIM:

1. A bioremodelable tubular prosthesis comprising a first layer of processed tissue matrix formed into a tube having a luminal surface and abluminal surface and a second layer of dense fibrillar collagen on the luminal surface of the first layer.

2. A bioremodelable tubular prosthesis comprising a first layer of processed tissue matrix formed into a tube having a luminal surface and abluminal surface wherein opposing edges of the layer overlap to form a bonding region between about 5% and 20% of the circumference of the tube and a second layer of dense fibrillar collagen on the luminal surface of the first layer.

3. The bioremodelable tubular prosthesis of claims 1 and 2 wherein the processed tissue matrix is derived from the tunica submucosa of small intestine.

4. The bioremodelable tubular prosthesis of claims 1 and 2 wherein the prosthesis is crosslinked with EDC.

5. A method for producing a bioremodelable prosthetic tube construct comprising at least two layers of processed tissue matrix wherein the method comprises:

(a) flagging the processed tissue matrix around a sleeve-covered mandrel by hydrating one edge of the processed tissue matrix and contacting the sleeve-covered mandrel to the hydrated edge of the processed tissue matrix;

(b) drying the processed tissue matrix to the sleeve-covered mandrel;

(c) rehydrating the processed tissue matrix with a hydrating agent to form hydrated processed tissue matrix;

(d) rotating the mandrel twice to wrap the hydrated processed tissue matrix to form two wrapped hydrated layers of processed tissue matrix;

(e) dehydrating the layers of the processed tissue matrix;

(f) removing the processed tissue matrix from the mandrel;

(g) contacting the processed tissue matrix with a crosslinking agent to crosslink the collagen.

(h) depositing a dense fibrillar collagen layer on the luminal surface of the tube.

6. A method for replacing a damaged or diseased portion of vasculature with a bioremodelable tubular prosthesis comprising a two layers of processed tissue matrix and a layer of dense fibrillar collagen on the luminal surface of the tubular prosthesis wherein the method comprises:

(a) anastomosizing a segment of vasculature;

(b) removing the segment of vasculature;

(c) replacing the segment of removed vasculature with a bioremodelable tubular prosthesis;

(d) suturing the bioremodelable tubular prosthesis; and,

(e) removing the anastomoses to resume blood flow.

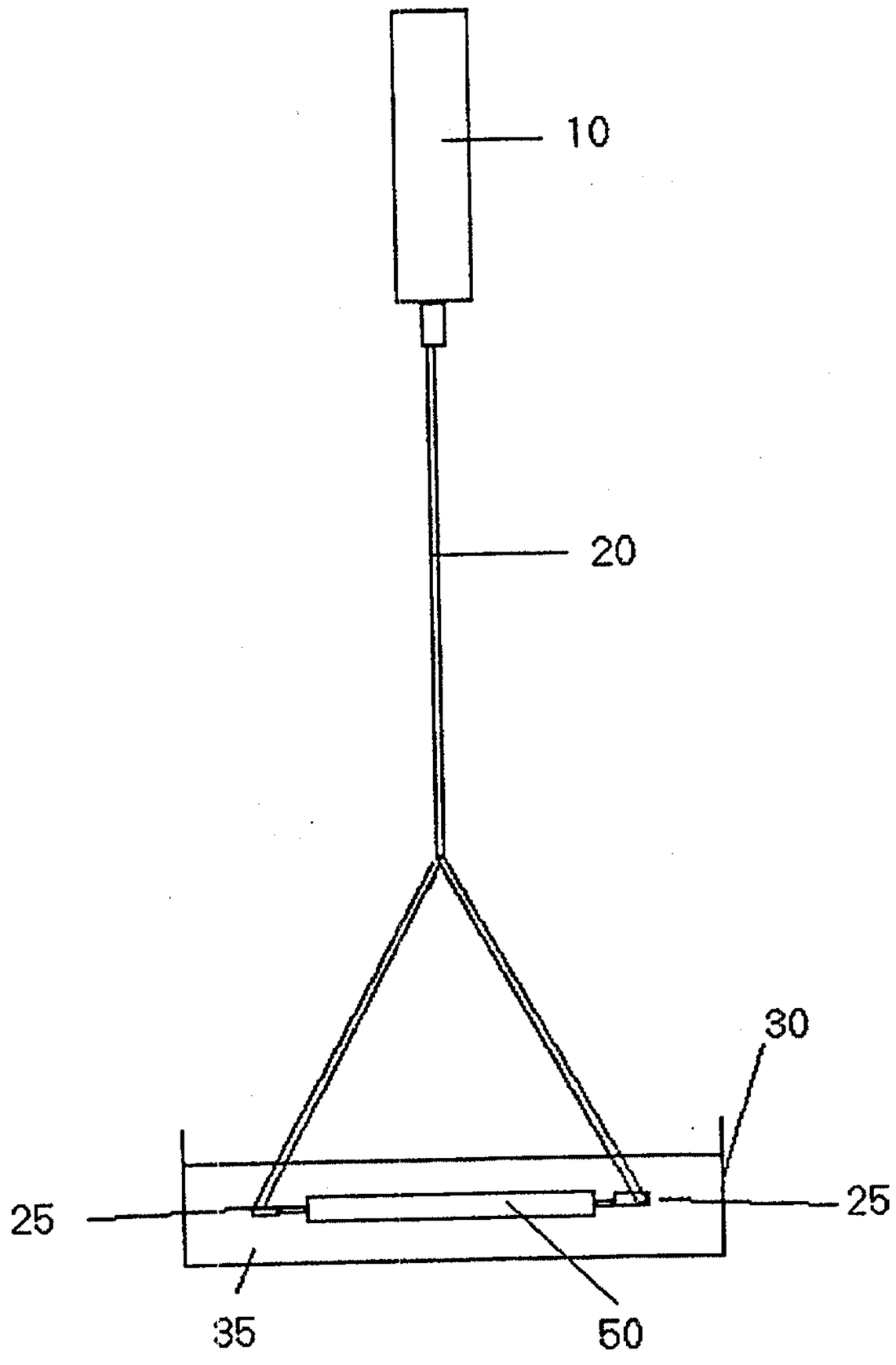
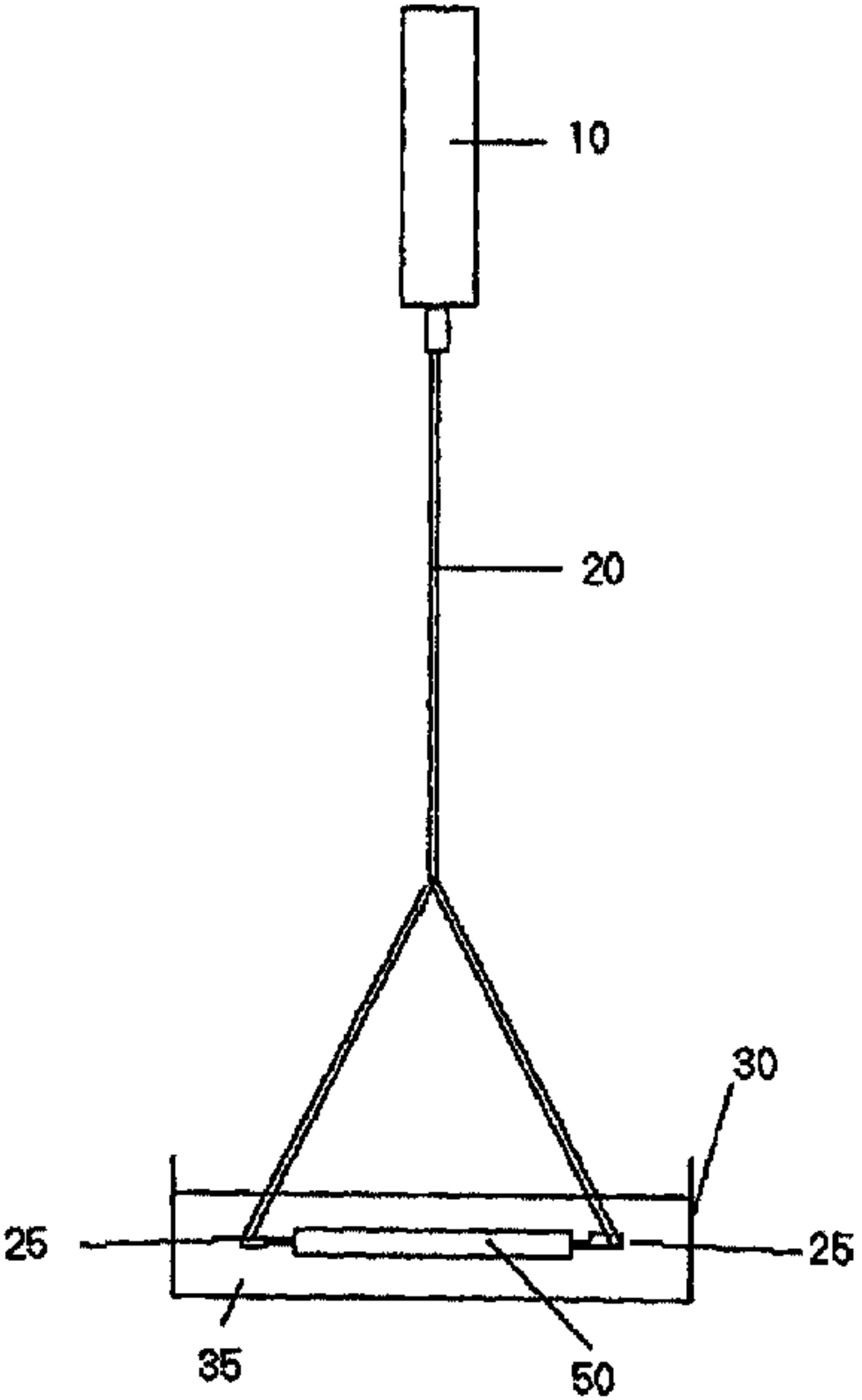


FIG. 1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