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(54) Titre : PROCEDE DE PRODUCTION D'ELASTINE, DE MATERIAUX A BASE D'ELASTINE ET DE MATERIAUX A  
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(54) Title: METHOD OF PRODUCING ELASTIN, ELASTIN-BASED BIOMATERIALS AND TROPOELASTIN MATERIALS

(57) **Abrégé/Abstract:**

A method is provided for producing elastin or elastin-based materials or tropoelastin materials which have a substantially reduced level of calcification. These materials are typically capable of being formed into a fused layer. The subject method comprises providing unreacted elastin or elastin-based biomaterials or tropoelastin materials, and pretreating the unreacted elastin or elastin-based biomaterials or tropoelastin materials with an aliphatic alcohol prior to reaction thereof. In this way, when the pretreated unreacted elastin or elastin-based biomaterials or tropoelastin materials is reacted subsequently, elastin or elastin-based materials or tropoelastin materials are produced which have a substantially reduced level of calcification as compared with elastin or elastin-based materials or tropoelastin materials produced from non-pretreated counterpart unreacted elastin or elastin-based biomaterials or tropoelastin materials.

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<b>(21) International Application Number:</b> PCT/US98/27790 <b>(22) International Filing Date:</b> 29 December 1998 (29.12.98) <b>(30) Priority Data:</b> 09/000,604                      30 December 1997 (30.12.97)      US <b>(71) Applicant (for all designated States except US):</b> SISTERS OF PROVIDENCE IN OREGON [US/US]; 520 Pike Street, Seattle, WA 98111-9038 (US). <b>(71)(72) Applicant and Inventor:</b> GREGORY, Kenton, W. [US/US]; 3737 S.W. Council Crest Drive, Portland, OR 97201 (US). <b>(74) Agents:</b> MARGER, Jerome, S. et al.; Marger Johnson & McCollom, P.C., 1030 S.W. Morrison Street, Portland, OR 97205 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHOD OF PRODUCING ELASTIN, ELASTIN-BASED BIOMATERIALS AND TROPOELASTIN MATERIALS <b>(57) Abstract</b> <p>A method is provided for producing elastin or elastin-based materials or tropoelastin materials which have a substantially reduced level of calcification. These materials are typically capable of being formed into a fused layer. The subject method comprises providing unreacted elastin or elastin-based biomaterials or tropoelastin materials, and pretreating the unreacted elastin or elastin-based biomaterials or tropoelastin materials with an aliphatic alcohol prior to reaction thereof. In this way, when the pretreated unreacted elastin or elastin-based biomaterials or tropoelastin materials is reacted subsequently, elastin or elastin-based materials or tropoelastin materials are produced which have a substantially reduced level of calcification as compared with elastin or elastin-based materials or tropoelastin materials produced from non-pretreated counterpart unreacted elastin or elastin-based biomaterials or tropoelastin materials.</p>		



## **METHOD OF PRODUCING ELASTIN, ELASTIN-BASED BIOMATERIALS AND TROPOELASTIN MATERIALS**

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### **TECHNICAL FIELD**

The present invention relates to elastin and elastin-based biomaterials, to tropoelastin materials, particularly to methods of producing such materials, and more particularly to methods of using same in tissue repair and replacement.

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### **BACKGROUND OF THE INVENTION**

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Elastic fibers are responsible for the elastic properties of several tissues such as skin and lung, as well as arteries, and are composed of two morphologically distinct components, elastin and microfibrils. Microfibrils make up the quantitatively smaller component of the fibers and play an important role in elastic fiber structure and assembly.

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The most abundant component of elastic fibers is elastin. The entropy of relaxation of elastin is responsible for the rubber-like elasticity of elastic fibers. Elastin is an extracellular matrix protein that is ubiquitous in mammals. Elastin is found, for example, in skin, blood vessels, and tissues of the lung where it imparts strength, elasticity and flexibility. In addition, elastin, which is prevalent in the internal elastic lamina (IEL) and external elastic lamina (EEL) of the normal artery, may inhibit the migration of smooth muscle cells into the intima. Elastin in the form of solubilized peptides has been shown to inhibit the migration of smooth muscle cells in response to platelet-derived factors (Ooyama et al, Arteriosclerosis 7:593 (1987). Elastin repeat hexapeptides attract bovine aortic endothelial cells (Long et al, J. Cell. Physiol. 140:512 (1989) and elastin nonapeptides have been shown to attract fibroblasts (USP 4,976,734). The present invention takes advantage of these physical and biochemical properties of elastin.

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Thirty to forty percent of atherosclerotic stenoses are opened with balloon angioplasty restenose as a result of ingrowth of medial cells. Smooth muscle

ingrowth into the intima appears to be more prevalent in sections of the artery where the IEL of the artery is ripped, torn, or missing, as in severe dilatation injury from balloon angioplasty, vessel anastomoses, or other vessel trauma that results in tearing or removal of the elastic lamina. While repair of the arterial wall occurs following injury, the elastin structures IEL and EEL do not reorganize. Since these components play major structural and regulatory roles, their destruction is accompanied by muscle cell migration. There are also diseases that are associated with weakness in the vessel wall that result in aneurysms that can ultimately rupture, as well as other events that are, at least in part, related to abnormalities of elastin.

In vertebrates elastin is formed through the secretion and crosslinking of tropoelastin, the 72-kDa biosynthetic precursor to elastin. This is discussed, for example, in an article entitled "Oxidation, Cross-linking, and Insolubilization of Recombinant Crosslinked Tropoelastin by Purified Lysyl Oxidase" by Bedell-Hogan, et al in the Journal of Biological Chemistry, Vol. 268, No. 14, on pages 10345-10350 (1993).

In vascular replacement and repair, the best current option is to implant autologous veins and arteries where the obvious limit is the supply of vessels which can be sacrificed from the tissues they were intended to service.

Autologous vein replacements for damaged arteries also tend to be only a temporary measure since they can deteriorate in a few years in high pressure arterial circulation.

When autologous graft material is not available, the surgeon must choose between sacrificing the vessel, and potentially the tissue it sub-served, or replacing the vessel with synthetic materials such as Dacron\* or Gore-tex\*. Intravascular compatibility indicate that several "biocompatible polymers", including Dacron\*, invoke hyperplastic response, with inflammation particularly at the interface between native tissue and the synthetic implant. Incomplete healing

\* Trade-marks



is also due, in part, to a compliance mismatch between currently used synthetic biomaterials and native tissues.

As described in US patent 5,990,379 issued November 23, 1999; US patent 5,989,244 issued November 23, 1999; US patent 6,087,552 issued July 11, 2000 and  
5 US patent 6,110,212 issued August 29, 2000, elastin and elastin-based biomaterials, or tropoelastin materials, can be used in a number of medical applications. For example, these materials can be employed to provide a method of effecting repair or replacement or supporting a section of a body tissue, as a stent, such as a vascular stent, or as conduit replacement, or as an artery, vein or a ureter replacement, or as a  
10 stent or conduit covering or coating or lining. It can also provide a graft suitable for use in repairing a lumen wall, or in tissue replacement or repair in, for example, interior bladder replacement or repair, intestine, tube replacement or repair such as fallopian tubes, oesophagus such as for oesophageal varicies, ureter, artery such as for aneurysm, vein, stomach, lung, heart such as congenital cardiac repair, or colon repair  
15 or replacement, or skin repair or replacement, or as a cosmetic implantation or breast implant.

### **SUMMARY OF THE INVENTION**

A problem with using elastin or elastin-based materials or tropoelastin  
20 materials or tropoelastin materials in the aforementioned applications is that when are implanted in vivo, calcification thereof will occur. The effect of calcification of the implanted materials is that it prevents the effective and efficient operation of these material for the intended in vivo use. This can happen, for example, when one of the first and second outer surfaces of the elastin or elastin-based materials or tropoelastin  
25 material, and a tissue substrate, are fused together. The present invention also relates to a method of pretreating elastin and elastin-based

materials or tropoelastin materials which can be employed in the repairing, replacing or supporting a section of a body tissue.

5 A method is therefore provided for producing elastin or elastin-based materials or tropoelastin materials which, on implantation, has a substantially reduced level of in vivo calcification as compared with elastin or elastin-based materials or tropoelastin materials produced from non-pretreated counterpart unreacted elastin or elastin-based biomaterials or tropoelastin materials. These materials are typically capable of being formed into a fused layer.

10 The subject method comprises providing unreacted elastin or elastin-based biomaterials or tropoelastin materials, and pretreating the unreacted materials with an aliphatic alcohol prior to reaction thereof. When the pretreated unreacted elastin or elastin-based biomaterials or tropoelastin materials are reacted subsequently, elastin or elastin-based materials or tropoelastin materials are implanted, they exhibit a substantially reduced level of in vivo calcification as compared to the level of vivo calcification for elastin or elastin-based materials or tropoelastin materials produced from non-pretreated counterpart unreacted elastin or elastin-based biomaterials or tropoelastin materials.

15 The aliphatic alcohol employed in present invention is typically a lower aliphatic alcohol, preferably a lower aliphatic alcohol comprises from one to eight carbon atoms, more preferably a lower aliphatic alcohol comprises from two to four carbon atoms, the most preferred lower aliphatic alcohol being ethanol.

20 The method of this invention can comprise positioning elastin and elastin-based materials or tropoelastin materials at the site of the section and bonding the biomaterial to the site or to the tissue surrounding the site. The bonding is effected by contacting the elastin material and the site, or tissue surrounding the site, at the point at which the bonding is to be effected, with an energy absorbing agent. The agent is then exposed to an amount of energy

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absorbable by the agent sufficient to bond the elastin material to the site or to the tissue surrounding the site.

5 The absorbing material can comprises a bio-compatible, more preferably an energy absorbing dye. In one form of the present invention, the energy absorbing material is substantially dissipated when the elastin or elastin-based materials or tropoelastin material and the tissue substrate are fused together. In another form of this invention, the energy absorbing material comprises a material for staining the first or second surface of the elastin or elastin-based materials or tropoelastin material. The energy absorbing material can also be applied to one of the outer  
10 surfaces of the biomaterial by doping a separate elastin layer with an energy absorbing material and then fusing the doped separate elastin layer to the elastin and elastin-based materials or tropoelastin materials. In any case, the energy absorbing layer is preferably substantially uniformly applied to at least one of the outer surfaces, typically in a manner wherein the energy absorbing material  
15 substantially covers the entire outer surface of the elastin or elastin-based materials or tropoelastin material.

Some of the key properties which effect the method of the present invention regarding fusing the elastin or elastin-based materials or tropoelastin material and tissue substrate include the magnitude of the wavelength, energy  
20 level, absorption, and light intensity during irradiation with light energy of the energy absorbing material, and the concentration of the energy absorbing material. These properties are arranged so that the temperature during irradiation with light energy for period of time which will cause fusing together of one of the first and second outer surfaces of the elastin or elastin-based materials or tropoelastin  
25 material and the tissue substrate is from about 40 to 140 degrees C., and more preferably from about 50 to 100 degrees C., but if well localized to the biomaterial tissue interface can be as high as 600 degrees C. Furthermore, the average

thickness of the energy absorbing material in the preferred method of this invention is from about 0.5 to 300 microns.

According to one aspect of the invention, there is provided a method for  
5 producing elastin or elastin-based materials or tropoelastin materials which having a substantially reduced level of calcification, and which are capable of being formed into a fused layer, comprising:

providing unreacted elastin or elastin-based biomaterials or tropoelastin  
materials; and

10 pretreating the unreacted elastin or elastin-based biomaterials or tropoelastin materials with an aliphatic alcohol prior to reaction thereof; and

reacting the pretreated unreacted elastin or elastin-based biomaterials or  
tropoelastin materials to produce the elastin or elastin-based materials or tropoelastin  
materials which, on implantation, has a substantially reduced level of in vivo  
15 calcification as compared with elastin or elastin-based materials or tropoelastin  
materials produced from non-pretreated counterpart unreacted elastin or elastin-based  
biomaterials or tropoelastin materials.

According to another aspect of the invention, there is provided a method for  
producing elastin or elastin-based materials or tropoelastin materials having a  
20 substantially reduced level of calcification, comprising:

providing unreacted elastin or elastin-based biomaterials or tropoelastin  
materials; and

pretreating the unreacted elastin or elastin-based biomaterials or tropoelastin  
materials with an aliphatic alcohol prior to reaction thereof; and

25 reacting the pretreated unreacted elastin or elastin-based biomaterials or  
tropoelastin materials to produce the elastin or elastin-based materials or tropoelastin  
materials which, on implantation, has a substantially reduced level of in vivo  
calcification as compared with elastin or elastin-based materials or tropoelastin  
materials produced from non-pretreated counterpart unreacted elastin or elastin-based  
30 biomaterials or tropoelastin materials.

According to a further aspect of the invention, there is provided a method for  
producing elastin or elastin-based materials or tropoelastin materials which having a  
substantially reduced level of calcification, and which are capable of being formed



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into a fused layer, comprising:

providing unreacted elastin or elastin-based biomaterials or tropoelastin materials;

5       pretreating the unreacted elastin or elastin-based biomaterials or tropoelastin materials with an aliphatic alcohol prior to reaction thereof;

reacting the pretreated unreacted elastin or elastin-based biomaterials or tropoelastin materials to produce the elastin or elastin-based materials or tropoelastin materials which, on implantation, has a substantially reduced level of in vivo  
10       calcification as compared with elastin or elastin-based materials or tropoelastin materials produced from non-pretreated counterpart unreacted elastin or elastin-based biomaterials or tropoelastin materials;

forming a layer of the elastin or elastin-based materials or tropoelastin materials; and

15       fusing the layer of the elastin or elastin-based materials or tropoelastin materials.

According to another aspect of the invention, there is provided a method for producing elastin or elastin-based materials or tropoelastin materials, which, on implantation, has a substantially reduced level of in vivo calcification, and which can  
20       be fused onto a tissue substrate, comprising:

providing a sheet of elastin or elastin-based materials or tropoelastin materials having a first and second outer major surface and a tissue substrate having a first and second outer major surface which has been pretreated with an aliphatic alcohol; and

25       applying an energy absorbing material, which is energy absorptive within a predetermined range of light wavelengths, to a selected one of the first and second outer surfaces of the pretreated sheet of the pretreated elastin or elastin-based materials or tropoelastin materials in an amount which will cause fusing together of one of the first and second outer surfaces of the pretreated elastin or elastin-based materials or tropoelastin material and an outer surface of

30       the tissue substrate, the energy absorbing material penetrating into the interstices of the pretreated elastin or elastin-based materials or tropoelastin materials,

irradiating the energy absorbing material with light energy in the predetermined wavelength range with an intensity sufficient to fuse together one of

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the first and second outer surfaces of the pretreated elastin or elastin-based materials or tropoelastin materials and the tissue substrate; and

5 fusing together one of the first and second outer surfaces of the preformed sheet of the pretreated elastin or elastin-based materials or tropoelastin materials and the tissue substrate.

In accordance with an aspect of the invention, a method for producing elastin or tropoelastin materials which have a substantially reduced level of calcification, comprises:

10 providing biomaterials consisting essentially of elastin or tropoelastin; pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohol, prior to reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation, having a substantially reduced level of in vivo calcification as compared with elastin or tropoelastin biomaterials produced from non-pretreated counterpart

15 elastin or tropoelastin biomaterials;

producing a layer of said elastin or tropoelastin biomaterials having a substantially reduced level of calcification.

In accordance with another aspect of the invention, a method for producing elastin or tropoelastin biomaterials which have a substantially reduced level of

20 calcification, comprises:

providing biomaterials consisting essentially of elastin or tropoelastin; pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohols, prior to reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation, having a substantially reduced level of in vivo calcification as compared with elastin

25 or tropoelastin biomaterials produced from non-pretreated counterpart elastin or tropoelastin biomaterials; and

producing a layer of said elastin or tropoelastin biomaterials having a substantially reduced level of calcification having a thickness of approximately 200 microns to 5 mm.

30 In accordance with a further aspect of the invention, a method for producing a fused layer of elastin or tropoelastin biomaterials which have a substantially reduced level of calcification, comprises:



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providing biomaterials consisting essentially of elastin or tropoelastin;  
pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohol, prior to  
reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation,  
5 having a substantially reduced level of in vivo calcification as compared with elastin  
or tropoelastin biomaterials produced from non-pretreated counterpart elastin or  
tropoelastin biomaterials;

forming a layer of said elastin or tropoelastin biomaterials having a  
substantially reduced level of calcification; and

10 fusing said layer of said elastin or tropoelastin biomaterials.

In accordance with another aspect of the invention, a method for producing  
elastin or tropoelastin biomaterials, which, on implantation, has a substantially  
reduced level of in vivo calcification, and which can be fused onto a tissue substrate,  
comprises:

15 providing a sheet of elastin or tropoelastin biomaterials having a first and  
second outer major surface and a tissue substrate having a first and second outer  
major surface which has been pretreated with an aliphatic alcohol, wherein the  
pretreated sheet of elastin or tropoelastin biomaterials comprises a thickness in a  
range between approximately 200 microns to 5 mm;

20 applying an energy absorbing material, which is energy absorptive within a  
predetermined range of light wavelengths, to a selected one of said first and second  
outer surfaces of the pretreated sheet of the pretreated elastin or tropoelastin  
biomaterials in an amount which will cause fusing together of one of said first and  
second outer surfaces of the pretreated elastin or tropoelastin biomaterials and an  
25 outer surface of said tissue substrate, said energy absorbing material penetrating into  
the interstices of said pretreated elastin or tropoelastin biomaterials,

irradiating the energy absorbing material with light energy in the  
predetermined wavelength range with an intensity sufficient to fuse together one of  
the first and second outer surfaces of the pretreated elastin or tropoelastin biomaterials  
30 and the tissue substrate; and

fusing together one of the first and second outer surfaces of the preformed  
sheet of the pretreated elastin or tropoelastin biomaterials and the tissue substrate.

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According to an aspect of the present invention, there is provided a method for producing one of elastin, elastin-based materials, and tropoelastin materials, which, on implantation, has a substantially reduced level of in vivo calcification, and which are fuseable onto a tissue substrate, comprising:

5        providing a sheet of the one of the elastin, elastin-based materials, and tropoelastin materials having a first and second outer major surface and a tissue substrate having a first and second outer major surface which has been pretreated with an aliphatic alcohol; and

10        applying an energy absorbing material, which is energy absorptive within a predetermined range of light wavelengths, to at least one of the first and second outer surfaces of the pretreated sheet of the one of the pretreated elastin, elastin-based materials, and tropoelastin materials, in an amount which will cause fusing together of one of the first and second outer surfaces of the one of the pretreated elastin, elastin-based materials, and tropoelastin materials and an outer surface of the tissue substrate  
15        when the energy absorbing material is irradiated with light energy in the predetermined range of light wavelengths, the energy absorbing material penetrating into interstices of the one of the pretreated elastin, elastin-based materials, and tropoelastin materials.

20        Further objects of aspects and advantages of the invention will be clear from the description that follows.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1. Application of laser energy to biomaterial and exposed native tissue.

FIGURE 2. Placement of elastin biomaterial into artery.

25        FIGURE 3. Use of biomaterial as intestinal patch.

FIGURE 4. Scanning electron micrograph of elastin-based biomaterial (prepared using elastin, fibrinogen and thrombin) fused to porcine aorta using continuous wave diode laser.

30        FIGURE 5. Light microscopic picture of elastin-based biomaterial fused to porcine aorta using a pulsed diode laser. E = elastin biomaterial; A = aorta.

FIGURE 6. Light microscopic photomicrograph of elastin-based biomaterial derived from arterial digest welded to porcine carotid artery. E = elastin biomaterial;



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A = aorta.

FIGURE 7. Histological examination of elastin heterograph, without pretreatment, after 30 days of subcutaneous implantation in rats.

5 FIGURE 8. Histological examination of elastin heterograph, with aliphatic alcohol pretreatment, after 30 days of subcutaneous implantation in rats.

FIGURE 9. Light microscopy of implanted elastin tissue, without pretreatment, after six months of subderthal implantation in mice.

FIGURE 10. Light microscopy of implanted elastin tissue, with aliphatic alcohol pretreatment, after six months of subdermal implantation in mice.

**DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT**

The present invention relates to elastin or elastin-based materials or tropoelastin materials which having a substantially reduced level of calcification. Elastin-based biomaterials suitable for use in the present invention can be prepared, for example, from elastin (e.g. from bovine nuchal ligament), fibrinogen and thrombin as described by Rabaud et al (USP 5,223,420). (See also Aprahamian et al, J. Biomed. Mat. Res. 21:965 (1987); Rabaud et al, Thromb. Res. 43:205 (1986); Martin, Biomaterials 9:519 (1988).) Such biomaterials can have associated thrombogenic property that can be advantageous in certain types of tissue repair. Elastin-based biomaterials suitable for use in the invention can also be prepared from elastin and type III collagen, also as described by Rabaud and co-workers (Lefebvre et al, Biomaterials 13(1):28-33 (1992). Such preparations are not thrombogenic and thus can be used for vascular stents, etc. A further type of elastin-based biomaterial suitable for use in the present invention is prepared as described by Urry et al (see, for example, USP 4,132,746 and 4,500,700) (See also USP's 4,187,852, 4,589,882, 4,693,718, 4,783,523, 4,870,055, 5,064,430, 5,336,256). Elastin matrixes resulting from digestion of elastin-containing tissues (eg arteries) can also be used. Digestion results in the removal of cells, proteins and fats but maintenance of the intact elastin matrix. The biomaterial used will depend on the particular application.

Elastin-based biomaterial of the invention prepared from soluble elastin (see Rabaud et al above) can be molded so as to render it a suitable size and shape for any specific purpose. Molded biomaterial can be prepared as follows. Elastin (eg soluble elastin (MW 12-32,000 daltons) is washed and swollen in buffer. Fibrinogen or cryoglobulins (prepared, for example, according to Pool et al, New Engl. J. Med. 273 (1965 are added to the swollen elastin, followed by thiourea, with or without a protease inhibitor (such as aprotinin), and collagen. Thrombin is added with stirring and the resulting mixture is immediately poured into an



appropriate mold. The mold is then incubated (for example, at 37°C) while polymerization of the fibrin/elastin material is allowed to proceed, advantageously, for from between 15 minutes to 1 hour, 30 minutes being preferred. The reaction can be carried out at temperatures less than 37°C., but the reaction proceeds more rapidly at 77°C. Heating the reaction to over 40°C, however, can result in denaturation of the thrombin. Cooling of the mixture while stirring allows more time for mixing to occur. For polymerization to occur, it is important to have calcium and magnesium in the buffer and to use undenatured thrombin.

Following polymerization in the mold, the resulting biomaterial can be further cross-linked using gamma radiation or an agent such as glutaraldehyde (a solution of glutaraldehyde, formic acid and picric acid being preferred). When radiation is used, the samples are, advantageously, subjected to gamma-irradiation from a Cobalt-60 source. The amount of irradiation can range, for example, from 10 to 100MRAD, with 25MRAD being preferred. It has been shown that the amount of gamma-irradiation can affect the strength of the material (Aprahamian, J.Biomed. Mat. Res. 21:965(1987).

Sheets of biomaterial can be prepared that are of a controlled thicknesses by using appropriate molds. Sheets of the biomaterial can be made in thicknesses ranging, for example, from 200 microns to 5 mm. Sheets are generally made as thin as possible to allow for penetration of laser energy while maintaining sufficient strength. By way of example, a sheet suitable for use as an intestinal patch can range in thickness from 200 microns to 5 mm, with about 2 mm being preferred. A patch requiring greater strength, such a patch for use in the bladder, is typically thicker. Arterial stents or patches can be thinner (eg 100  $\mu\text{m}$  - 1000  $\mu\text{m}$ ).

Biomaterial prepared from soluble elastic or insoluble elastin fragments can also be molded into tubular segments for example, by injecting the material into

tubular molds. Crosslinkage of the elastin solution present between the inner and outer tubes can be effected prior to withdrawal of biomaterial from the mold or after the tubes are removed. Tubular segments of different inner and outer diameters, as well as of different lengths, can be prepared using this approach by varying the diameters of the inner and outer tubes. A mold of this type can be made in virtually any size with the inner and outer tubes varying in diameter. A small tube can be used for a coronary arterial stent. A large tube of 1-5 inches in diameter can be made and used as an angularly welded patch for anastomosis of the small intestine or colon. Various molding techniques and molding materials can be used; the foregoing is merely an example.

As indicated above, biomaterial suitable for use in the present invention can be prepared from digests of tissue containing an elastin matrix. Tissues suitable for use as a starting material include arteries (e.g. coronary or femoral arteries, for example, from swine), umbilical cords, intestines, ureters, etc. Preferably, the matrix material is (derived from the species of animal in which the implantation is being performed so that bio-compatibility is increased. Any method of removing (digesting away) cellular material, proteins and fats from the native matrix while leaving the extracellular elastin matrix intact can be used. These methods can involve a combination of acidic, basic, detergent, enzymatic, thermal or erosive means, as well as the use of organic solvents. This may include-incubation in solutions of sodium hydroxide, formic -acid, trypsin, guanidine, ethanol, diethylether, -acetone, t-butanol, and sonication. Typically, the digestion proceeds more quickly at higher temperatures. The optimal temperature and time (of incubation depend on the starting material and digestive agent used and can be readily determined.

One skilled in the art will appreciate that while tubular segments result from digestion of tubular starting materials, those segment can be opened and shaped to yield sheets suitable for use as tissue grafts. Alternatively, such



segments can be opened and then reconstructed as tubular segments having a diameter different than the starting tissue. Preferably, however, when tubular products are sought, the starting material is selected so as to yield a tubular segment after digestion having the appropriate diameter so that subsequent manipulations (other than adjustment of length) can be avoided.

The biomaterial of the invention, whether prepared from elastin powder or from tissues digests, is normally secured to existing tissue. Various techniques for effecting that attachment can be used, including art-recognized techniques.

However, it is preferred that the biomaterial be secured using a tissue welding energy source and an agent that absorbs energy emitted by that source.

Advantageously, the energy source is an electromagnetic energy source, such as a laser, and the absorbing agent is a dye having an absorption peak at a wavelength corresponding to that of the laser. The elastin biomaterial and the tissue to be welded have much less absorption of light at this wavelength and the effect therefore is confined to a zone around the dye layer. A preferred energy source is a laser diode having a dominant wavelength at about 808 nm and a preferred dye is indocyanine green (ICG), maximum absorbance 795-805 nm (see WO 91/(,4073). Other laser/dye combinations can also be used. It is preferred that the dye be applied to that portion of the biomaterial that is to be contacted with and secured to the existing tissue. The dye can also be applied to the surface of the structure to which the elastin biomaterial is to be welded or secured. The dye can be applied directly to the biomaterial or the surface of the biomaterial can first be treated or coated (eg primed) with a composition that controls absorption of the dye into the biomaterial so that the dye is kept as a discrete layer or coating.

Alternatively, the dye can be bound to the elastin biomaterial so that it is secured to the surface and prevented from leeching into the material. The dye can be applied in the form of a solution or the dye can be dissolved in or suspended in a

medium which then can be applied as a thin sheet or film, preferably, of uniform thickness and dye concentration.

Tissue welding techniques employing a soldering agent can be used. Such techniques are known (WO 91/04073). Any proteinaceous material that thermally denatures upon heating can be used as the soldering agent (for example, any serum protein such as albumin, fibronectin, Von Willebrand factor, vitronectin, or any mixture of proteins or peptides). Solders comprising thrombin polymerized fibrinogen are preferred, except where such materials would cause undesirable thrombosis or coagulation such as within vascular lumens. Solders are selected for their ability to impart greater adhesive strength between the biomaterial and the tissue. The solder should be non-toxic and generally biocompatible.

In accordance with the present invention, the laser energy can be directed to the target site (eg, the dye) directly from the laser by exposure of the tissue (eg, during a surgical procedures). In some cases, i.e. endovascular catheter-based treatments where open surgical exposure does not occur, the laser energy is directed to the bonding site via optical fibers. When ICG is used as the dye, targeting media wavelengths of around 800nm can be used. Such wavelengths are not well absorbed by many tissues, particularly vascular tissues, therefore, there will be a negligible effect on these tissues and thermal effects will be confined to the dye layer. The biomaterial of the invention similarly has little optical absorbance in this waveband, as compared to the energy absorbing dye. Thus, the laser energy can pass through either the elastin/elastin biomaterial or the native tissue and be absorbed by the energy absorbing material, i.e., the dye layer as shown in Figure 1. Once the surgeon has exposed the surface or vessel where the biomaterial reinforcement or replacement is to be effected, the dye-containing surface of the biomaterial is placed in contact with the native tissue at the site and laser energy delivered by directing the laser beam to the desired location. The absorbance of the dye (eg ICG) layer is ideally previously or concurrently



determined so that the optimal amount of light for optimal bonding can be delivered. Pressure can be used to ensure adequate approximation of the tissue and biomaterial. With a diode laser source, the diode laser itself, or a condenser or optical fiber based optical delivery system, can be placed against the material to ensure uniform light delivery.

In cases where a new elastin lining or new-internal elastic lamina is required, for example, following an open surgical endarterectomy, once the artery has been surgically cleared of the atheroma or other lesion, the biomaterial is then put in place, dye side down (see Figure 2). The biomaterial can be deployed as a flat patch or as a tubular segment. A tubular segment can be hollow or filled with a material that supports the lumen during placement and that is melted with low grade heat or dissolved or removed with a variety of means. When necessary, a small number of surgical sutures (eg stay sutures) can be used to appose the edges of the vessel together or to sew the vessel. Once the biomaterial is in place, the laser energy is directed through the vessel wall or through the biomaterial to the absorbing dye, the appropriate laser energy having been previously determined based upon the measured absorbance in the biomaterial. Alternatively, the dye can be applied at the time of the surgery to the biomaterial or the vessel wall or both and then laser energy delivered. In this embodiment, absorbance can be determined at the time of the surgery to the biomaterial or the vessel wall or both and then laser energy delivered or with a feedback device that assesses the adequacy of the bonding or thermal effect. (Figure 4 is a SEM of elastin-based biomaterial fused to porcine aorta.)

In addition to the above, the biomaterial of the invention can be used as a patch material for use in intestinal or colon repairs which frequently do not heal well with current techniques, particularly when the patient has nutritional or other problems or when the patient is in shock, such as in the case of multiple gunshot wounds or other abdominal injuries (see Figure 3). The use of such a patch can,

for example, seal off intestinal contents and thereby reduce the likelihood of peritonitis. In addition, a patch can be used on a solid organ, such as the liver, when lacerations have occurred. Similarly, the biomaterial of the invention can be used to repair or replace portions of the urinary system i.e., from the calyces of the kidney on down to the urethra. The patch can also be used to seal a defect in a cardiac chamber, such as an atrial septal defect, as well as bronchial or rectal fistulas. The biomaterial can also be used as a cerebrovascular patch for an aneurysm. The biomaterial can be sealed in place with targeted laser fusion. For applications where direct exposure is not possible or not desirable, a variety of catheter or endoscopic systems can be employed to direct the laser energy to the target site.

The elastin-based biomaterials to which the invention relates can be used in a variety of other clinical and surgical settings to effect tissue repair graft. For delivery of biomaterial in the form of an intravascular stent, the biomaterial can be pre-mounted upon a deflated balloon catheter. The balloon catheter can be maneuvered into the desired arterial or venous location using standard techniques. The balloon can then be inflated, compressing the stent (biomaterial) against the vessel wall and then laser light delivered through the balloon to seal the stent in place (the dye can be present on the outside of the biomaterial). The balloon can then be deflated and removed leaving the stent in place. A protective sleeve (eg of plastic) can be used to protect the stent during its passage to the vessel and then withdrawn once the stent is in the desired location.

The biomaterial of the invention can also be used as a biocompatible covering for a metal or synthetic scaffold or stent. In such cases, simple mechanical deployment can be used without the necessity for laser bonding. Laser bonding can be employed, however, depending upon specific demands, e.g, where inadequate mechanical bonding occurs, such as in stent deployment for abdominal aortic aneurysms. An alternative catheter-based vascular stent deployment



strategy employs a temporary mechanical stent with or without a balloon delivery device.

A further catheter-based vascular stent deployment strategy employs a heat deformable metal (such as nitinol or other similar type metal) scaffold or stent or coating that is incorporated into the catheter tubing beneath the stent biomaterial. The stent is maneuvered into the desired location whereupon the deformable metal of the stent is activated such that it apposes the stent against the vessel wall. Laser light is then delivered via an optical fiber based system, also incorporated into the catheter assembly.

The elastin-based biomaterial can also be used to replace portions of diseased or damaged vascular or nonvascular tissue such as esophagus, pericardium, lung plura, etc. The biomaterial can also be used as a skin layer replacement, for example, in burn or wound treatments. As such, the biomaterial serves as a permanent dressing that acts as a scaffolding for epithelial cell regrowth. The biomaterial can include antibiotics, coagulants or other (drugs desirable for various treatments that provide high local concentrations with minimal systemic drug levels. A drug can be incorporated into the biomaterial thereby decreasing the need for systemic intravenous or oral medications. The elastin biomaterial can be deployed with a dye on the tissue side and then fused with the appropriate wavelength and laser energy.

Furthermore, a drug can be incorporated into the layer of elastin or elastin biomaterial thereby decreasing the need for systemic intravenous or oral medications. Also, photodynamic therapy drugs ("PDT") which are activated with light can be employed herein.

In addition to repair of tubular body structures, the biomaterial of the present invention can also be used in organ reconstruction. For example, the biomaterial can be molded or otherwise shaped as a pouch suitable for use in bladder reconstruction. The biomaterial of the invention can also be molded or

otherwise shaped so as to be suitable for esophageal replacement. Again, metal or synthetic mesh could also be associated with the implant if extra wall support is needed so as to control passage of food from the pharynx to the stomach. This could be used for stenosis of the esophagus, as a covering for bleeding esophageal varicies to prevent bleeding or to treat bleeding, for repair from acid reflux for erosive esophagitis or, more preferably, for refurbishing damaged esophageal segments during or following surgery or chemotherapy for esophageal carcinoma.

For certain applications, it may be desirable to use the biomaterial of the invention in combination with a supporting material having strong mechanical properties. For those applications, the biomaterial can be coated on the supporting material (see foregoing stent description), for example, using the molding techniques described herein. Suitable supporting materials include polymers, such as woven polyethylene terephthalate (Dacron), teflon, polyolefin copolymer, polyurethane polyvinyl alcohol or other polymer. In addition, a polymer that is a hybrid between a natural polymer, such as fibrin and elastin, and a non-natural polymer such as a polyurethane, polyacrylic acid or polyvinyl alcohol can be used (sets Giusti et al, Trends in Polymer Science 1:261 (1993). Such a hybrid material has the advantageous mechanical properties of the polymer and the desired biocompatibility of the elastin based material. Examples of other prostheses that can be made from synthetics (or metals coated with the elastin biomaterial or from the biomaterial/ synthetic hybrids include cardiac valve rings and esophageal stents.

The elastin-based prostheses of the invention can be prepared so as to include drug; that can be delivered, via the prostheses, to particular body sites. For example, vascular stents can be produced so as to include drugs that prevent coagulation, such as heparin, drugs to prevent smooth muscle ingrowth or drugs to stimulate endothelial regrowth. Vasodilators can also be included. Prostheses formed from the elastin based biomaterial can also be coated with viable cells,



preferable, cells from the recipient of the prosthetic device. Endothelial cells, preferably autologous (eg harvested during liposuction), can be seeded onto the elastin bioprosthesis prior to implantation (eg for vascular stent indications). Alternatively, the elastin biomaterial can be used as a skin replacement or repair media where cultured skin cells can be placed on the biomaterial prior to implantation. Skin cells can thus be used to coat elastin biomaterial.

Elastin structures constituting a framework for a three-dimensional, multi-layer cell culture system will provide intact elastic structures not constructed by stromal cells populating synthetic matrices. In vivo elastin production is thought to only occur during development and ceases during childhood (the only exceptions being hypertension and restenosis). Elastogenesis is a complex process and formation of mature elastic structures not likely to be achieved in relatively simple in vitro cell culture systems. However, it has not been reported that such three dimensional cell culture systems can organize elastin into coherent fibrous matrices analogous to those found in elastic tissues. A method by which to produce a living tissue graft with elastic structure and function most similar to tissue which is high in elastin content is by culturing cells in three dimensional frameworks made of elastin or elastin based biomaterials. This insures the presence of biologically important elastic structures in the living tissue grafts.

A method for both organizing elastin and elastin-based biomaterials fibrils and providing a support for fibroblast growth is by coacervating elastin monomers in solution with fibroblasts. Elastin monomers mixed with stromal cells (fibroblasts) in a physiologic buffer aggregate into fibers (coacervation) upon raising the temperature of the solution. In doing so the fibroblasts become trapped in a loose matrix of elastin fibers. The contraction of the fibroblasts bound to the coacervated elastin monomers could preferentially align the elastin fibrils prior to crosslinking.

Certain aspects of the invention are described in greater detail in the non-limiting Examples that follow.

Example 1 Preparation of Sheets of Elastin-  
5 Based Biomaterial from Soluble Peptides

Materials used for biomaterial production:

Phosphate buffer: The phosphate buffer used contained 1 mM sodium phosphate,  
150 mM sodium chloride, 2 mM calcium chloride, 1 mM magnesium (chloride,  
10 pH 7.4.

Soluble elastin peptides: Bovine ligamentum nuchae elastin powder was  
obtained from Sigma, St. Louis, Missouri. The following procedure was used to  
obtain the soluble elastin peptides: 2.7 g elastin powder was suspended in 35 ml  
of a 1M KOL solution in 80% ethanol. The suspension was stirred at 50°C for 2.5  
15 hr. Next, 10 ml deionized water was added and the solution neutralized with  
concentrated 12M HCl to pH 7.4. The solution was cooled at 4°C for 12 hrs. The  
clear solution was decanted from the salt crystals, and the supernatant centrifuged  
for 15 mins at 2000 rpm. The solution was then dialyzed against three changes of  
tap water at two hour intervals and one 15 hr interval using a 10,000 MW cutoff  
20 dialysis tubing. The dialysis was continued with six changes of deionized water at  
two hour intervals and one for 15 hrs. The resulting dialyZate was lyophilized  
and stored at -20°C. The yield was 40%.

Cryoglobulin preparation: A modification of the method of Pool and  
Shannon was used to Produce the cryoglobulins (New Engl. J. Med. 273 (1965).  
25 Cryoglobulins are principally fibrinogen (40 mg/ml) and fibronectin (10 mg/ml)  
(concentrations of fibrinogen and fibronectin will vary). Briefly, blood was  
collected from swine in a standard 500 ml blood collection bag containing  
adenine, citrate and dextrose anticoagulant. The blood was transferred to twelve



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50 ml plastic centrifuge tubes and centrifuged for 15 mins at 1500 rpm. The plasma was decanted from the erythrocyte layer and frozen at -70°C for 12 hrs. The plasma was then thawed at 4°C. The cryoglobulins were collected by centrifugation of the plasma at 4°C for 15 mins at 1500 rpm. The supernatant was decanted and the cryoglobulins collected by removing the precipitate with a pasteur pipette. Each tube was also rinsed with 3 ml of a sodium citrate solution containing 0.9% NaCl, and 0.66% sodium citrate. The cryoglobulins were pooled, frozen at -70°C, lyophilized and stored at -20°C until use.

Thiourea: Reagent grade thiourea. was obtained from Sigma, St. Louis, Missouri. A 0.5 mg/ml solution was used.

Type I collagen: Acid soluble type I collagen was obtained from Sigma. It was preferred from rat tail tendon by a modification of the method of Bornstein. Two mg of collagen was heated in 0.6 ml phosphate buffer to 60°C for 10 minutes until the collagen dissolved. It was then cooled to 37°C and used.

Thrombin: Thrombin from bovine plasma was obtained from Sigma in lyophilized form. When reconstituted with 1 ml water, the solution contained 106 NIH units per ml.

Aprotinin: Aprotinin from bovin lung was obtained from Sigma. It contained 15-30 trypsin inhibitory units (TIU) per ml.

Preparation:

Six molds were made by gluing a 620  $\mu$ m quartz fiber to one side of a glass plate ~40 mm x 25 mm and attaching a second glass plate to the first using a rubber band. Each mold so constructed held about 0.5 ml.

The biomaterial was prepared by successively adding and mixing the following: 200 mg soluble kappa-elastin or kappa-elastin powder in 2ml phosphate buffer (PB) (1 mM P041 150 mM NaCl, 2 mM Ca21 1 mM Mg21 PH 7.4) at 37°C

160 mg cryoglobulin in 1 ml P:B (37°C)

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2 mg collagen in 0.6 ml PB (60°C 37°C)

200  $\mu$ l thiourea (0.5 mg/ml)

200  $\mu$ l aprotinin (5 Units)

5 A 0.6 ml aliquot of the above solution was loaded into a test tube and 50  $\mu$ l  
thrombin solution was added (~6 units). The resulting solution was immediately  
loaded into the mold. Certain of the resulting sheets were crosslinked with  
glutaraldehyde for 2 mins.

10 Results: The sheets prepared as described above were slightly yellowish  
and opaque. The glut-araldehyde-fixed sheets were less stretchy and tore more  
easily than non-fixed sheets. Glutaraldehyde fixed sheets were subjected to  
electron microscopy. These sheets had a smooth cohesive surface appearance at  
100X and 1000X.

15 Example 2      Tissue Welding of Sheets of  
Elastin-Based Biomaterial

20 Pre-welding procedure: A 1 mg/ml ICG solution was applied to fresh  
swine aorta that had been carefully trimmed of adventitia, washed in a sterile  
0.9% NaCl solution, and cut into 1cm<sup>2</sup> squares. The 1mg/ml ICG solution was  
applied to the luminal side of the aorta for ~3 min and wiped off. (ICG was  
obtained from Sigma and contained 90% dye and 10% sodium iodide. Absorption  
coefficient measured at 780 nm with a  $7.25 \times 10^{-6}$  M solution was found to be  
175,000 M<sup>-1</sup>cm<sup>-1</sup>. The adsorption maximum shifts to 805nm when ICG is bound to  
serum proteins (Landsman et al, J. Appl. Physiol. 40 (1976). A small amount of  
25 cryoglobulins, containing approximately 40mg/ml fibrinogen and 10mg/ml  
fibronectin doped with ICG, was also applied and the biomaterial placed on it.



The two materials were placed between two glass slides. This was submerged in a 0.9% saline solution.

Welding Procedure: Sheets of biomaterial made as described in Example 1 were equilibrated in phosphate buffer, pH 7.4, and welded to ICG stained porcine aorta using an aluminum gallium arsenide diode array laser. The maximum output was at 808 +/- 1.5nm. The laser was coupled to a 1µm quartz fiber with polyethylene cladding material. The laser energy was collimated with a focusing lens and coupled to the quartz fiber. The spot size at the distal end of the fiber could be varied from 1mm to 4mm by adjusting the distance between the focusing lens and the proximal end of the fiber. The laser operated continuously, CW, and the output measured at the distal end of the fiber was 1.5W.

The quartz fiber was positioned directly above the glass slide, biomaterial, aorta. Before welding, the spot size of the laser was measured. Welding appeared to occur under saline at irradiances of 0.85W but not 1.32W. Twenty seconds was sufficient time to weld and 40 seconds caused a brown color change and charring of the biomaterial.

### Example 3      Preparation of Elastin-Based Biomaterial from Artery Digest

Fresh 4 cm lengths of porcine carotid artery were dissected clean and washed in two changes of 0.9% saline overnight. Vessels were then placed in 0.5M NaOH and sonicated for 120 minutes (a modified method of Crissman, R. 1987) (Crissman, Rogert S. "Comparison of Two Digestive Techniques for Preparation of Vascular Elastic Networks for SEM Observation", Journal of Electron Microscopy Techniques 6:335-348 (1987). Digested vessels were then washed in distilled water and autoclaved at 225°F for 30 minutes. Digested vessels appear translucent, pearly white in color and collapsed when removed

from water indicating the absence of collagen and other structurally supportive proteins.

Welding of the artery digests to porcine aorta was accomplished through the following methods. Fresh porcine aorta was coated with 5 mJ/ml ICG for 5 minutes. Excess ICG solution was blotted off. One x one cm sections of NaOH-sonicated digested carotid artery elastin segments were placed upon the freshly stained aortas. An array of pulsed aluminum gallium arsenide diode lasers (Star Medical Technologies) was used to weld the segments. Five millisecond pulses at 790-810 light was emitted at 2 joules and applied to the tissue with a condenser that created a uniform beam 4x4 mm which was placed on the elastin digest covered by a glass coverslip. Good welds were achieved with up to 10 pulses. A light microscopic photograph of the elastin digest welded to the porcine aorta is shown in Figure 6.

#### Example 4      Preparation of Elastin-Based Biomaterial & Fusion to Porcine Aorta

Materials: Bovine nuchal elastin powder (Sigma St. Louis MO) Was sifted with a 40  $\mu$ m screen and swollen with phosphate buffer. Elastin fragments were then reacted with 67 mg of fibrinogen (Sigma):in phosphate buffer, 2m acid soluble Type 1 collagen (Sig\_na), 2.8 mg thiourea, 2 mM  $\text{Ca}^{2+}$ , 1mM  $\text{Mg}^{2+}$  and 75 units of thrombin and injected into molds and heated to 77°C. One mm thick sheets and tubes of this bio-material were removed and stored in 33% ethanol for later use.

Indocyanine green dye was dissolved in de-ionized water to provide a 1% solution and applied to the luminal surface of fresh porcine aorta. The dye was in place for 5 minutes then the residual dye was blotted off. The elastin biomaterial was placed on the ICG stained aorta and covered with a glass coverslip. Laser



energy was applied with a condenser which collected the output of an array of gallium arsenide diode lasers emitting light at 800nm in 5 msec pulses. Six mm<sup>2</sup> Spots were irradiated with 2.89 Joules for 1-10 pulses which provided adequate welds. Samples were then bisected and fixed in formalin for microscopic study. Figure 5 is a light microscopic photograph of such a weld stained with an elastin stain. Excellent welding of the elastin biomaterial to porcine aorta is noted with no detectable thermal or other injury to the biomaterial or aorta.

Example 5     Preparation of Elastin-Based Biomaterial  
                  & Fusion to Porcine Aorta

Materials: Bovine ligamentum nuchae elastin, Fibrinogen from porcine plasma, and acid soluble type I collagen from rat tail tendon were obtained from Sigma Chemical Corp. (St. Louis, MS.). Elastin was solubilized in 1M KOL/80% ethanol at 50°C for 2.5 hrs. (Homebreck). Cryoprecipitates were obtained from porcine plasma. Fresh porcine aorta was obtained from Carlton Packaging Co. (Carlton, Ore) and stored at -20°C until thawed for use.

Elastin-fibrin biomaterials were prepared. Patches made of solubilized elastin and cryoprecipates were prepared by successive addition with thorough mixing of 200mg. soluble elastin dissolved in 2 ml buffer, 160 mg. lyophilized cryoprecipitate dissolved in 1 ml buffer, 2 mg type I collagen dissolved in 0.6 ml buffer, and 0.2 ml thiourea solution (0.5 mg/ml H<sub>2</sub>O). 6 units of thrombin were added to 0.5 ml. aliquots of the mixture, thoroughly mixed in a 1 ml syringe, and injected into 4cm<sup>2</sup> glass molds. The molds were incubated at 37°C for 30 min. and subjected to 25 mrad of g-radiation (cobalt source). The biomaterial was stored at 4°C in 33% etOH. Prior to use the biomaterial was washed several times with saline.

Patches were also made with insoluble elastin and fibrinogen. Lyophilized elastin from Sigma was passed through a U.S. no 4000 mesh sieve (Tyler) prior to use. Only the 40  $\mu$ m or smaller particles were used. 28-0mg of the filtered elastin was swollen and washed overnight in an excess of phosphate buffer. the mixture was centrifuged (1000 rpm, 10 min) and the excess buffer discarded. The swollen elastin was suspended in 2 ml of phosphate buffer. Successively added to this suspension are 67 mg. lyophilized fibrinogen dissolved in 1 ml buffer, 2 mg type I collagen dissolved in 0.6 ml buffer, and 0.2 ml thiourea solution (0.5 mg/ml H<sub>2</sub>O). Finally, 33 units of thrombin were added and the mixture was thoroughly vortexed and quickly poured into 3 cm x 7 cm molds. The molds were incubated at 37°C for 30 min. the biomaterial was stored in 4°C in 33% EtOH. Prior to use the biomaterial was washed several times with saline solution.

The soluble elastin-cryoprecipitated patch was fused to porcine aorta using an Aluminum Gallium Arsenide diode array laser emitting 808 nm continuous wave optical radiation. Fresh porcine aorta was washed in 0.9% NaCl and trimmed into 2 cm<sup>2</sup> portions. Indocyanine green (Sigma) in aqueous concentrated of 1 or 5 mg/ml was applied to aorta via a pasteur pipette, left undisturbed for 5 min. and then blotted away. The tissue was then equilibrated in a 0.9% saline solution for 15 minutes to remove any unbound dye. The biomaterial was then applied to the luminal surface of the aorta. The laser beam was directed at the biomaterial surface via a 1  $\mu$ m fused silica fiber (Polymicro Technologies Phoenix, Az.) through a glass coverslip as shown in Figure 1. The spot size of the laser beam varied between 1-4 mm. The laser output measured from the fiber tip was 1.5 Watts and exposed durations varied from 5 to 4 seconds.

The insoluble elastin-fibrinogen patch was fused to porcine aorta using an Aluminum Gallium Arsenide diode array laser emitting 790-810 nm pulsed optical radiation (Star Medical Technologies). Thawed porcine aorta was



prepared and stained with 5mg/ml aqueous ICG solution as previously described for fresh aorta. After applying the biomaterial to the stained luminal surface of the aorta, laser radiation was directed at the biomaterial via a copper coated condenser placed against a glass coverslip. The laser output was set at 2J and 5 msec pulse durations.

Example 6      Preparation of Elastin-Based Biomaterials  
and Fusing of Same

Bovine ligamentum nuchae elastin, fibrinogen from porcine plasma, and acid soluble type I collagen from rat tale tendon were obtained from Sigma Chemical Corp. (St. Louis, Mo).

1 mg. indo cyanine green is dissolved in 1 ml of 24% human serum albumin. 67 mg of fibrinogen was dissolved in 1 ml phosphate buffer (@37°C). Just prior to mixing 16.6 units of thrombin are added to the indocyanine green solution. The mixtures were cooled to 4°C. The two mixtures are rapidly mixed and injected, or poured, into a 3 X 7 cm mold and incubated for 30 min. at 37°C.

Lyophilized elastin from Sigma was passed through a U.S. No. 400 mesh sieve (Tyler) prior to use. Only the 40 µm or smaller particles were used. 210 mg of the filtered elastin was swollen and washed overnight in an excess of phosphate buffer. The mixture was centrifuged (1000 rpm, 10 min.) and the excess buffer discarded. The swollen elastin was suspended in 1.5 ml of phosphate buffer. Successively added to this suspension were 67 mg lyophilized fibrinogen dissolved in 0.75 ml buffer, 2 mg type I collagen dissolved in 0.45 ml buffer, and 0.15 ml thiourea solution (0.5mg/ml H<sub>2</sub>O). Finally, 26 units of thrombin were added and the mixture was thoroughly vortexed and quickly poured onto the fibrin matrix doped with indocyanine green in the 3 cm x 7 cm molds. The molds were

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again incubated at 37°C for 30 minutes. When removed from the mold, the two layers are inseparable and the preparation yields a single patch.

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Example 7. Welding of Elastin Fibrin Biomaterial to Porcine Intestine.

10 Fresh porcine intestine was obtained from Carlton Packing Co. (Carlton, OR). The intestine was rinsed with tap water and stored at -20°C in Ziploc\* freezer bags. Prior to use the intestine is thawed in ambient air and kept on saline soaked gauze to prevent drying out.

15 The elastin fibrin biomaterial prepared as described in Example 4 was fused to porcine intestine using a Aluminum Gallium Arsenide diode array laser (Star Medical Technologies) as follows: Indocyanine green in aqueous concentrations of 5 mg/ml was applied to the serosa of thawed porcine intestine with a pasteur pipette, left undisturbed for 5 minutes and then blotted away with a Kimwipe\*EXL wipe. Elastin-fibrin biomaterial was cut into 1 X 1 cm patches an excess moisture was blotted away with a Kimwipe\*EXL wipe. The biomaterial was then positioned on top of the ICG stained serosa of the intestine and a glass microscope coverlip is positioned on top of the biomaterial. A scale was placed underneath the intestine. Laser radiation was directed at the biomaterial via a 4 X 4 mm copper coated condenser placed against the glass coverslip. Laser output was set at 1.99-2.19 joules and 5 msec pulses. During laser exposure, manual force was applied to the glass coverslip with the condenser. The amount of pressure applied was monitored on the scale placed underneath the intestine. 5 pulses and 500 to 1600 grams of force resulted in successful adhesion of the elastin-fibrin biomaterial to the intestine. Figure 6 (Figure 14 of army grant proposal) is a light microscope slide of elastin fibrin biomaterial welded to porcine intestine (1.99 joules per pulse, 10 pulses, 500g force).

25  
\* Trade-mark



Example 8: Preparation and Welding of Coronary  
Vessel Digests.

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Fresh left anterior descending, right main, and circumflex coronary arteries were excised from a porcine heart. Excess fat and thrombus were removed from the excised vessels. The vessels were cut in half and the distal halves were washed in saline and sonicated in 0.5M NaOH for 45 min at 65°C. The distal halves were then removed from the alkali, immersed in 500 ml distilled water for 30 min, and finally immersed in boiling distilled water for another 30 min. The NaOH-sonicated vessels are hereafter referred to as heterografts. The proximal half of the vessels were saved and stored on saline soaked gauze until use. Right main coronary heterografts were welded to right main and left anterior descending arteries with an Aluminum Gallium Arsenide pulsed dioded laser emitting 790-810 nm optical radiation (Star Medical Technologies). 5 mg of indocyanine green (ICG) was dissolved in 1 ml of distilled water. This solution was then diluted with 4 ml of 25% human serum albumin (HSA) with careful mixing avoiding the formation of excessive air bubbles. The heterografts were coaxed onto a percutaneous transluminal coronary angioplasty balloon measuring 3.0 mm in diameter when inflated. The heterograft covered balloon was inflated to 4 psi and immersed in the ICG-HSA for 5 minutes to stain the heterograft. After removing the heterograft and balloon from the staining solution, the balloon is deflated and inserted into the untreated proximal half of a right main or LAD coronary artery. Following insertion, the balloon is inflated to 8 psi. The inflated balloon/heterograft is placed on a benchtop and a coverslip is placed over the region to be welded. A 4 X 4 mm copper coated condenser is placed against the coverslip. The laser output was set for 2.3 joules of energy and 5 msec pulse

5 durations. After 5 pulses, the balloon is rotated approximately 30 degrees and another region is illuminated with 5 pulses. This procedure is repeated until the entire circumference of the balloon has been illuminated. The balloon is then deflated, leaving behind the heterograft, now fused to the luminal surface of the artery.

Example 9: Preparation of Elastin-Based Materials  
With and Without Pretreatment

10 The following example describes in vivo experiments which compare the effectiveness of pretreatment of elastin materials with an aliphatic alcohol to inhibit in vivo calcification, as compared to non-pretreated counterpart elastin materials.

15 Carotid arteries from domestic swine were obtained for testing purposes. Specimens were stored on ice during transport for testing. Extraneous adipose from the vessels was carefully removed using Metzenbaum dissecting scissors.

20 Ethanol incubated arteries were immersed for 72 hours at 25 Degrees C. in 500 ml of 80% ethanol/ phosphate buffered saline pH 7.4 (PBS). Following ethanol incubation, the vessels were rinsed twice in 500 ml of 0.9% NaCl and then each vessel was individually immersed in 200 ml of 0.5 M NaOH prewarmed to 65 Degrees C. Vessels not incubated in ethanol were washed overnight in PBS, and then individually immersed in 200 ml of 0.5 M NaOH prewarmed to 65 Degrees C.

25 All vessels were sonicated for 60 minutes in 0.5 M NaOH at 65 Degrees C. Following sonication, the digested vessels (heterografts) were removed from the 0.5 M NaOH and bathed twice for 30 minutes in 1000 ml of deionized water.

A dermal biopsy punch (Miltex Instrument Co., New York) was used to cut out uniform 'disks' from the wall of each heterograft. The diameter of each disk was 2 mm. The disks were immersed in vials containing 15 ml of deionized water



and autoclaved at 225 Degrees F for 30 minutes. A sterile 16-gauge needle was used to make incisions in the right flanks of BALB/c mice.

A sterile cannula, preloaded with a elastin biomaterial disc was inserted in vivo through the incision into the skin of a mouse and positioned subcutaneously.

5 An obturator ejected each elastin biomaterial disk from the needle into the subcutaneous layer. The mice were followed to assess any acute or chronic symptoms of rejection of the implants.

The in vivo implants were harvested at 30 days, 60 days, and at six months.

10 Each in vivo implanted heterograft disk was fixed, sectioned and mounted for histological examination. The classic method of von Kossa was used to demonstrate calcification of the mounted heterograft sections. Calcium deposits were stained black using von Kossa's silver method

15 Elastin heterografts implanted in vivo in the subcutaneous layer of BALB/c mice which are not incubated in ethanol prior to implantation, begin to calcify in vivo in as little as thirty days, and are severely calcified at six months. Elastin heterografts which have been incubated in ethanol, prior to sodium hydroxide digestion, showed no sign of in vivo calcification at 30 days, 60 days, or at six months.

20 In FIGS. 7 and 8, photomicrographs at 125 X magnification depict histological examination of elastin heterograph, without pretreatment, after 30 days of subcutaneous in vivo implantation in rats. In FIGS. 9 and 10, photomicrographs at 125 X magnification depict an elastin heterograph, without pretreatment, after six months of subdermal in vivo implantation in mice.

25 More specifically, calcification was observed in 5 of 6 heterografts implanted in vivo for 30 days (see FIG. 8) and was observed in all heterografts implanted in vivo for 60 days (n=6) and 6 months (n=12) (see FIG. 10). None of the ethanol pretreated heterografts calcified in vivo. This includes ethanol

pretreated heterografts (see FIGS. 7 and 9) implanted for 30 days (n=3), 60 days (n=3), and 6 months (n=2).

Therefore, this example clearly demonstrates that pretreatment of elastin materials with an aliphatic alcohol substantially reduces the level of in vivo  
5 calcification in such elastin materials as compared with elastin materials produced from non-pretreated counterpart elastin materials

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.



What is claimed is:

1. A method for producing elastin or tropoelastin materials which have a substantially reduced level of calcification, comprising:
  - 5 providing biomaterials consisting essentially of elastin or tropoelastin; pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohol, prior to reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation, having a substantially reduced level of in vivo calcification as compared with elastin or tropoelastin biomaterials produced from non-pretreated counterpart elastin or
  - 10 tropoelastin biomaterials; producing a layer of said elastin or tropoelastin biomaterials having a substantially reduced level of calcification.
2. The method of claim 1, wherein the aliphatic alcohol is a lower aliphatic
- 15 alcohol.
3. The method of claim 2, wherein the lower aliphatic alcohol comprises from one to eight carbon atoms.
- 20 4. The method of claim 3, wherein said lower aliphatic alcohol comprises from two to four carbon atoms.
5. The method of claim 1, wherein said aliphatic alcohol comprises ethanol.
- 25 6. A method for producing elastin or tropoelastin biomaterials which have a substantially reduced level of calcification, comprising:
  - providing biomaterials consisting essentially of elastin or tropoelastin; pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohols, prior to reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation,
  - 30 having a substantially reduced level of in vivo calcification as compared with elastin or tropoelastin biomaterials produced from non-pretreated counterpart elastin or tropoelastin biomaterials; and

producing a layer of said elastin or tropoelastin biomaterials having a substantially reduced level of calcification having a thickness of approximately 200 microns to 5 mm.

5     7.     The method of claim 6, further comprising incorporating a drug into the sheet of elastin or elastin-based materials or tropoelastin materials for localized treatment of a medical condition.

8.     The method of claim 7, wherein the drug comprises an antibiotic.

10

9.     The method of claim 7, wherein said drug comprises a coagulant.

10.    The method of claim 7, wherein said drug comprises a photodynamic therapy drug.

15

11.    A method for producing a fused layer of elastin or tropoelastin biomaterials which have a substantially reduced level of calcification, comprising:

providing biomaterials consisting essentially of elastin or tropoelastin;  
pretreating the elastin or tropoelastin biomaterials with an aliphatic alcohol, prior to  
20    reaction thereof, said pretreated elastin or tropoelastin biomaterials, on implantation, having a substantially reduced level of in vivo calcification as compared with elastin or tropoelastin biomaterials produced from non-pretreated counterpart elastin or tropoelastin biomaterials;

forming a layer of said elastin or tropoelastin biomaterials having a

25    substantially reduced level of calcification; and

fusing said layer of said elastin or tropoelastin biomaterials.

12.    The method of claim 11, wherein the aliphatic alcohol is a lower aliphatic alcohol.

30

13.    The method of claim 11, wherein the aliphatic alcohol comprises ethanol.



14. The method of claim 11, further comprising incorporating a drug into the fused layer of elastin or elastin-based materials or tropoelastin materials for localized treatment of a medical condition.

5 15. The method of claim 14, wherein said drug is one selected from a group consisting of: an antibiotic, a coagulant, and a photodynamic therapy drug.

16. A method for producing one of elastin, elastin-based materials, and tropoelastin materials, which, on implantation, has a substantially reduced level of in vivo calcification, and which are fuseable onto a tissue substrate, comprising:  
10 providing a sheet of said one of the elastin, elastin-based materials, and tropoelastin materials having a first and second outer major surface and a tissue substrate having a first and second outer major surface which has been pretreated with an aliphatic alcohol; and  
15 applying an energy absorbing material, which is energy absorptive within a predetermined range of light wavelengths, to at least one of said first and second outer surfaces of the pretreated sheet of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials, in an amount which will cause fusing together of one of said first and second outer surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and an outer surface of said tissue  
20 substrate when the energy absorbing material is irradiated with light energy in the predetermined range of light wavelengths, said energy absorbing material penetrating into interstices of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials.

25

17. The method of claim 16, wherein the aliphatic alcohol is a lower aliphatic alcohol.

18. The method of claim 16, wherein the aliphatic alcohol comprises ethanol.

30

19. The method of claim 16, further comprising incorporating a drug into the sheet of said one of the elastin, elastin-based materials, and tropoelastin materials for localized treatment of a medical condition.

5 20. The method of claim 19, wherein said drug is one selected from a group consisting of: an antibiotic, a coagulant, and a photodynamic therapy drug.

21. The method of claim 16, wherein said energy absorbing material is capable of being indirectly irradiated by directing the light energy first through one of the tissue  
10 substrate, the pretreated elastin, the elastin-based materials, and the tropoelastin materials and then to the energy absorbing material.

22. The method of claim 16, wherein said energy absorbing material comprises a biocompatible chromophore.

15

23. The method of claim 16, wherein said energy absorbing material comprises an energy absorbing dye.

24. The method of claim 16, which further comprises the step of substantially  
20 dissipating said energy absorbing material when said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and said tissue substrate are caused to fuse together.

25. The method of claim 16, wherein the step of applying said energy absorbing  
25 material comprises staining the first or second surface of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials with said energy absorbing material.

26. The method of claim 16, which further comprises the step of applying said  
30 energy absorbing material to one of said outer surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials by doping a separate elastin layer with an energy absorbing material, and the doped separate elastin layer is



fuseable to said one of the pretreated elastin, elastin-based materials, and tropoelastin materials.

27. The method of claim 16, wherein the energy absorbing material is  
5 substantially uniformly applied to a selected one of said first and second outer surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials.

28. The method of claim 16, which further comprises the step of substantially  
10 covering the outer surface of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials with the energy absorbing material.

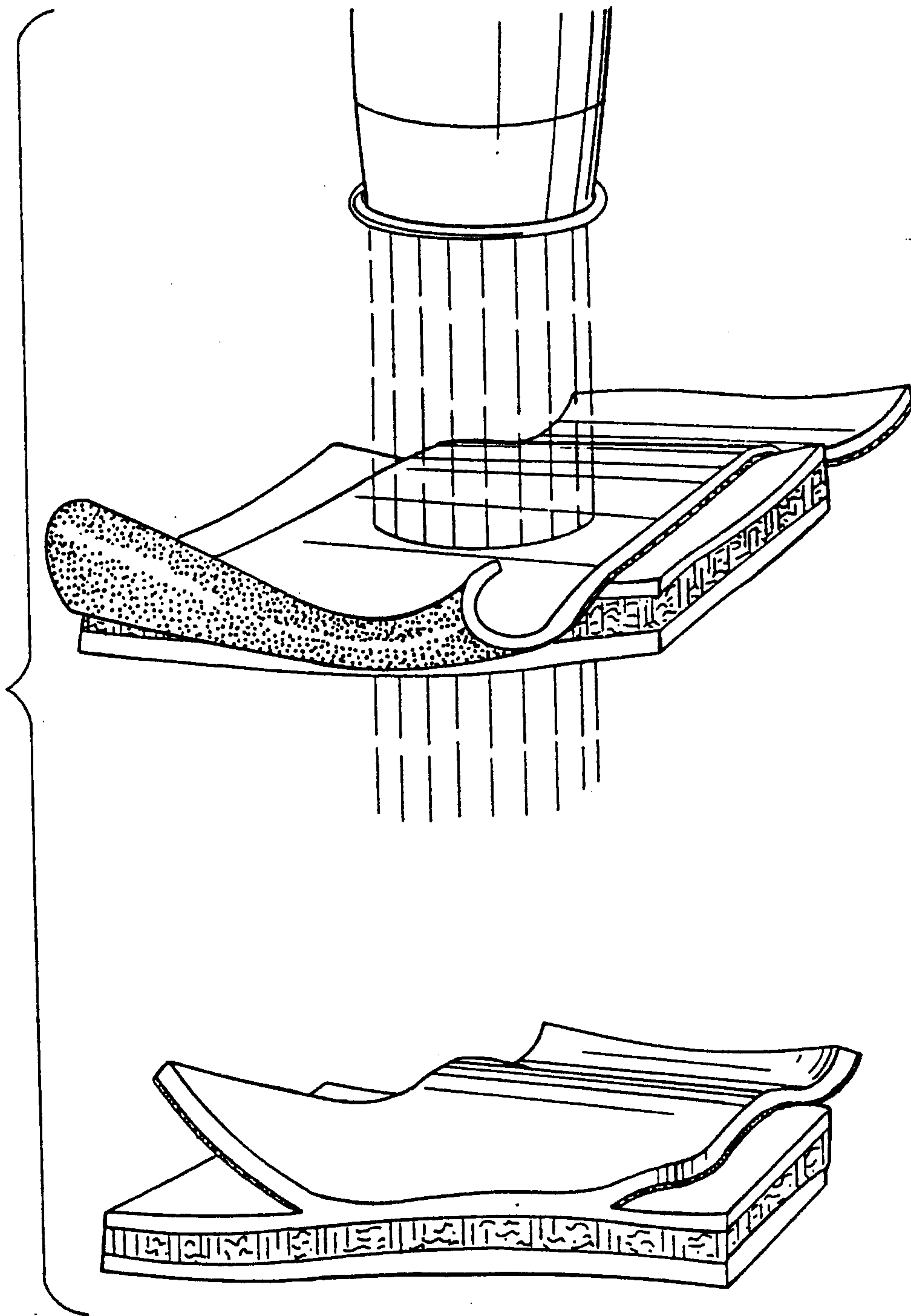
29. The method of claim 16, wherein when the energy absorbing material is  
irradiated with light energy at a localized temperature of from about 40 to 600 degrees  
15 C for a sufficient period of time, it will cause fusing together of one of said first and second outer surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and one of said first and second outer surfaces of said tissue substrate.

20 30. The method of claim 16, wherein when the energy absorbing material is irradiated with light energy resulting in a localized temperature at the interface of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and said tissue substrate is from about 50 to 100 degrees C for a sufficient duration, it will  
25 cause fusing together of one of said first and second outer surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and said tissue substrate.

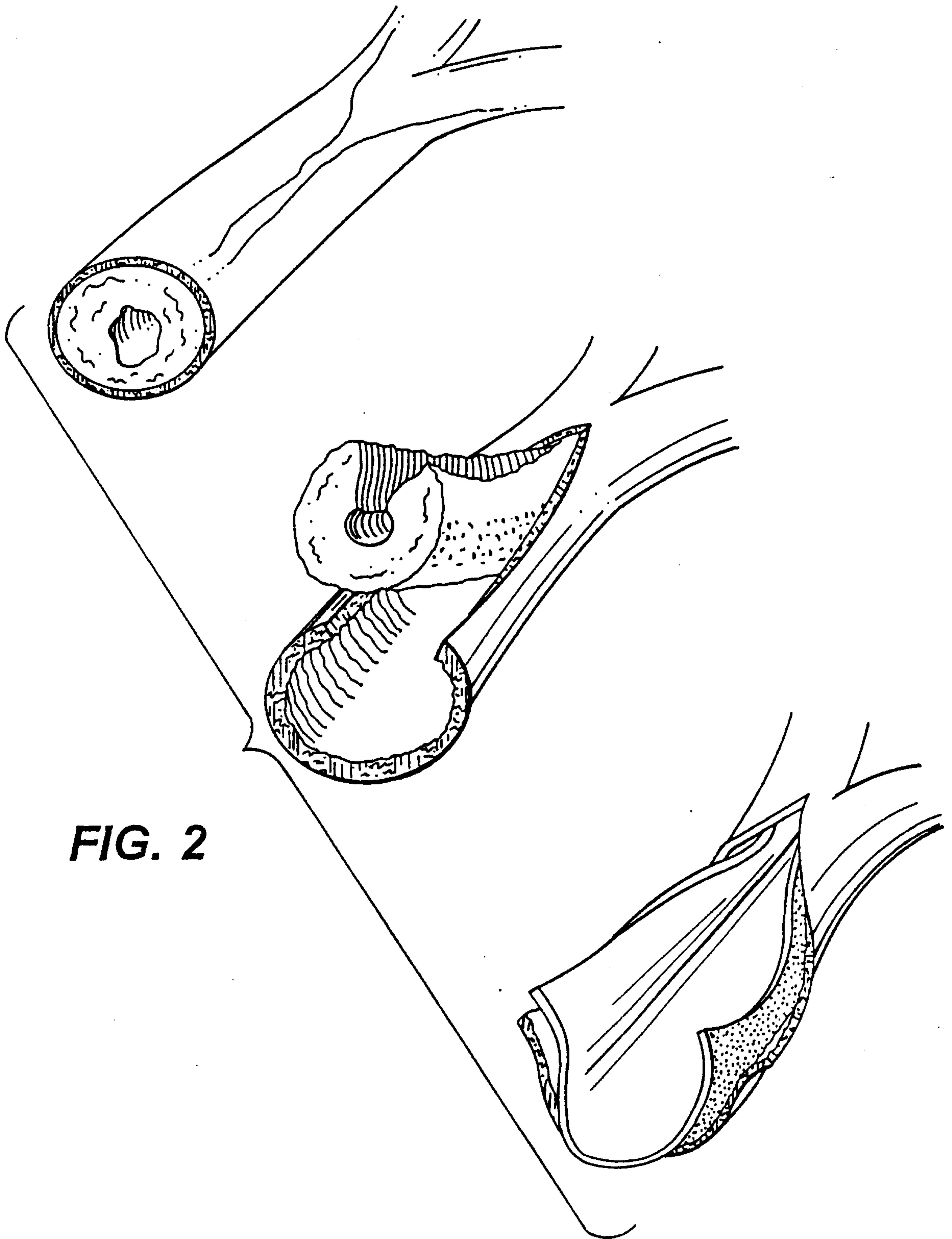
31. The method of claim 16, wherein the average thickness of the energy  
absorbing material which penetrates into the interstices of said one of the pretreated  
30 elastin, elastin-based materials, and tropoelastin materials is from about 0.5 to 300 microns.

32. The method of claim 16, wherein when the energy absorbing material is irradiated with light energy, the magnitude of the wavelength, energy level, absorption, and light intensity, and the concentration of the energy absorbing material, is such that the localized temperature at the interface of said first and second outer  
5 surfaces of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and the tissue substrate is maintained at from about 40 to 140 degrees C, to cause fusing together of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials and the tissue substrate.
- 10 33. The method of claim 16, wherein the tissue substrate is a live tissue substrate.
34. The method of claim 16, wherein the pretreated sheet of said one of the pretreated elastin, elastin-based materials, and tropoelastin materials comprises a thickness in a range between approximately 200 microns to 5 mm.

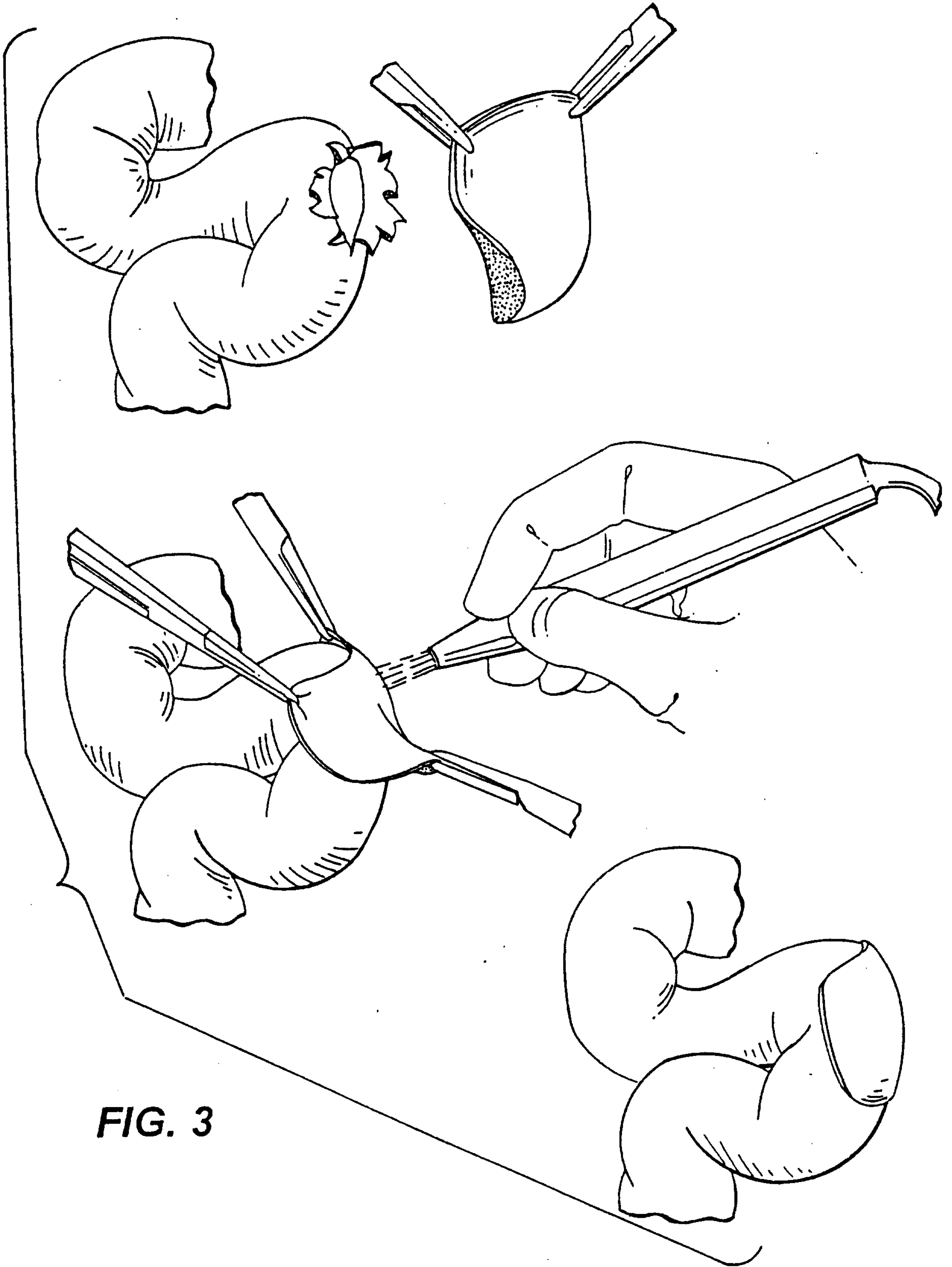




**FIG. 1**







**FIG. 3**

FOREIGN LANGUAGE DOCUMENT

RECEIVED WITH THIS APPLICATION

(DOCUMENT ON THE 10TH FLOOR ZONE 5 IN THE FILE PREPARATION SECTION)

2316908

DOCUMENT REÇU AVEC CETTE DEMANDE

DANS UNE LANGUE ÉTRANGÈRE

(DOCUMENT AU 10 IÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA  
PRÉPARATION DES DOSSIERS)