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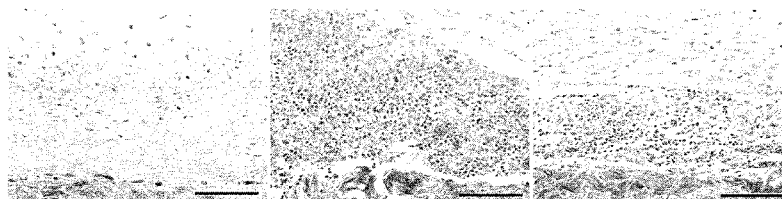
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(54) Title: METHODS FOR TISSUE PASSIVATION

FIG. 1



(57) Abstract: Methods of tissue passivation are described herein for use in preserving normal tissue architecture, reducing post-surgical inflammation, and reducing or preventing the development of pathogenic collagen bundles and adhesions following surgical procedures (e.g., grafting, implanting).

TITLE OF THE INVENTION

METHODS FOR TISSUE PASSIVATION

CROSS-REFERENCED APPLICATIONS

This application claims priority of U.S. provisional nos. 61/674,235, filed on July 20, 2012,
5 61/784,708, filed on March 14, 2013 and 61/847,794, filed July 18, 2013, the entirety of each
of which is incorporated by reference herein.

BACKGROUND OF THE INVENTION

Capsular contraction is one of the most common causes of reoperation following
implantation^{3,6}. The etiology of capsular contracture has been studied for many years³.

10 Potential etiologies include hematoma, hypertrophic scar and, microbacterial infections cause
by *Staphylococcus epidermidis*⁷⁻⁹. Regardless of the etiology, the end result is an
inflammatory response within the implant pocket and near the developing capsule^{2,10}.

Neocollagen formation and cross-linking are part of the normal human wound healing
response. In capsular contracture, these processes go awry, resulting in dense, linear bundles
15 of collagen fibers that surround the affected implant. These fibers form a firm capsule that
subsequently contracts and tightens¹¹. Histologically, this appears as an inner layer of
fibroblasts and histiocytes, which is surrounded by a thicker layer of collagen bundles
arranged in a parallel array^{2,12}. Direct pressure from a maturing capsule may deform or
rupture the implant, in addition to distorting the overlying skin and soft tissue.

20 Capsular contracture is the most common complication following augmentation
mammoplasty with prosthetic implants. Within a decade of surgery, half of the patients may
develop capsular contracture and nearly a quarter develop severe disease¹. The condition may
be painful and debilitating as well as aesthetically inferior. Despite decades of research,
effective methods of prevention of capsular contracture remain elusive³. Surgical
25 capsulotomy and/or capsulectomy are, to date, the gold standard treatments for affected
patients but neither offer protection from recurrent disease⁴⁻⁵. Therefore, methods to prevent
the initial incidence of capsular contracture as well as its recurrence would be highly
desirable.

Saphenous vein grafts (SVGs) used for coronary artery bypass graft (CABG) have
30 poor long-term patency rates compared to arterial grafts. In fact, 20% of SVGs fail within one
year of CABG and 50% fail within ten years. Of the vein grafts that remaining patent, 50%

have a significant atherosclerotic burden. Veins grafts for peripheral arterial reconstruction suffer from similar shortcomings.

The poor long-term outcomes are due to the luminal narrowing resulting from intimal hyperplasia, medial thickening and subsequent superimposed accelerated atherosclerosis.

5 Intimal hyperplasia is a consequence of the intimal injury that ensues after excessive dilation of the vein graft as it is exposed to arterial pressures.

One solution to prevent over-distention is providing external mechanical support, which has been shown to reduce the degree of intimal hyperplasia in humans. To date, every form of mechanical support involves the use of external sheaths, which are applied over the
10 vein graft prior to implantation and exposure to arterial pressure. External sheaths are cumbersome to use and can lead to technical difficulties. Furthermore, there is a risk of erosion and/or infection since it is a foreign body. A non-external mechanical support solution to reduce the degree of intimal injury caused by excessive dilation of venous graphs when they are exposed to arterial pressure would also be highly desirable.

15

SUMMARY OF THE INVENTION

In one aspect, the invention provides a method of preparing a non-adhesive tissue surface within a human subject, said method comprising the steps of: providing a photoactive agent to an intact, non-proliferative, internal tissue surface proximally located to an incision
20 in a human subject and irradiating said tissue surface at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preparing a non-adhesive tissue surface within the human subject.

In another aspect, the invention provides a method of preventing or decreasing tissue adhesion and/or contracture at a surgical site in a human subject, said method comprising the steps of: topically applying a photoactive agent to an internal tissue surface proximally
25 located to a surgical site; irradiating said tissue surface at an irradiance of less than about $1\text{W}/\text{cm}^2$; and determining that tissue adhesion and/or contracture at the surgical site is decreased compared to a reference.

In yet another aspect, the invention provides a method of preparing a non-adhesive tissue surface within a human subject, said method comprising the steps of: topically
30 applying a photoactive agent to an internal tissue surface within a cavity, wherein the surface is about 20 cm^2 , 25 cm^2 , 50 cm^2 , 100 cm^2 , 500 cm^2 to about 1000 cm^2 , and irradiating said tissue surface at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preparing a non-adhesive tissue surface within the human subject.

In one embodiment, the photoactive agent is Rose Bengal.

In another embodiment, the irradiance is provided for less than about 5 minutes.

In yet another embodiment, the tissue surface is located within a cavity of the human subject.

5 In yet another embodiment, the tissue surface is within a tissue selected from the group consisting of connective tissue, abdominal, bladder, bowel, thoracic, colorectal, rectal, intestinal, ovarian, uterine, pericardial, peritoneal, oral, endocardial, epithelial and breast tissue.

In yet another embodiment, the tissue surface comprises previously grafted tissue.

10 In yet another embodiment, the photoactive agent is applied topically to the tissue surface.

In yet another embodiment, methods of the invention further comprise providing an implant to the human subject following irradiation and closing the incision.

In yet another embodiment, methods of the invention further comprise providing an implant to the human subject containing a light source for irradiating the tissue surface.

15 In yet another aspect, the invention provides a method of preparing a tissue graft for implantation, said method comprising the steps of: providing Rose Bengal ex vivo to a surface of a tissue graft and irradiating said surface ex vivo at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preparing a tissue graft for implantation.

20 In yet another aspect, the invention provides a method of preparing a graft comprising vein for implantation, said method comprising the steps of: providing Rose Bengal ex vivo to the graft comprising vein and irradiating said graft ex vivo at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preparing the graft comprising vein for implantation.

25 In yet another aspect, the invention provides a method of preventing or reducing stenosis in a subject, said method comprising the steps of: implanting a passivated graft comprising vein into an artery, wherein said implanting of the graft replaces a/or bypasses a diseased segment of the artery, thereby preventing or reducing stenosis in a subject.

In one embodiment, the diseased segment of the artery contains a plaque.

30 In another embodiment, Rose Bengal is provided ex vivo to the exterior surface of the implanted, passivated graft comprising vein and/or the exterior surface of the artery and irradiated at an irradiance of less than about $1\text{W}/\text{cm}^2$.

In yet another aspect, the invention provides a method of preventing or reducing stenosis in a subject, said method comprising the steps of: implanting a graft comprising vein into an artery, wherein said implanting of the graft replaces and or bypasses a diseased segment of the artery, providing Rose Bengal to the exterior surface of the implanted graft

comprising vein and irradiating said graft at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preventing or reducing stenosis in a subject.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims. Thus, other aspects of the invention are described in the following disclosure and are within the ambit of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference.

Figure 1 depicts tissue harvested at two weeks, stained with mason's trichrome. Pockets that received no fibrin glue (left), fibrin glue (center), fibrin glue and pretreatment with PTP (right) are shown. (Scale Bar = $100\text{ }\mu\text{m}$).

Figure 2 depicts a graph of digestion times for fascia treated with the PTP. Error bars represent standard deviation. * indicates $p<0.005$.

Figure 3 depicts smooth muscle actin immunohistochemistry at eight weeks. Tissue from a control pocket that did not receive fibrin glue (left) and tissue from fibrin glue instilled pockets (center and right) is shown. PTP treated pocket is shown on the right. (Scale Bar = $100\mu\text{m}$).

Figure 4 depicts the results of three sub-cutaneous pockets made by surgical incision on the upper rabbit flank in a rabbit model for capsular contracture. Capsule tissue stained with mason's trichrome at eight weeks. Tissue from a control pocket that did not receive fibrin glue (left), tissue from a fibrin glue instilled pockets (center and right). PTP treated pocket on the right (Scale Bar = $500\text{ }\mu\text{m}$). In this model Fibrin Glue is used to induce capsular contracture. Histology shows a significant decrease in capsular thickness in the Fibrin Glue + Photochemical Tissue Passivation group compared to Fibrin Glue alone control. The Fibrin Glue + Photochemical Tissue Passivation group had similar capsule thickness to the healthy, normal, no Fibrin Glue control.

Figure 5 depicts a graph of capsule thickness at eight weeks in a rabbit model.

Figure 6 depicts a graph of the modulus of elasticity (Young's modulus) of untreated vein grafts, and treated vein grafts. Error bars represent standard deviation. * indicates $p=0.01$.

Figure 7 depicts a graph of collagen digestion times for untreated and treated vein grafts. * indicates $p<0.001$.

Figure 8A depicts a histologic cross-section of venous grafts four weeks following implantation. Slides were prepared with Van-Gieson elastin stain. Figure 8B depicts a graph comparing medial and intimal thickness of untreated vs treated grafts. Error bars represent standard deviation. * indicates $p=0.03$ & $p=0.04$, respectively.

5 Figure 9 depicts images of venous grafts both untreated (top) and PTP treated (bottom) after clamp removal and restoration of blood flow. Images show varying degrees of graft distention between groups.

DETAILED DESCRIPTION OF THE INVENTION

10 Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application, including definitions will control.

15 The term “passivated graft” as used herein refers to a tissue graft treated ex vivo with a photoactive agent and light.

The term “intact tissue” as used herein refers to tissue that is uninjured, continuous and/or whole (e.g., refers to tissue that has not been severed, separated or wounded). Intact tissue may be homogenous or alternatively, may comprise a mixture of native and previously grafted tissue that has become a continuous tissue as a result of grafting.

20 The term “non-proliferative tissue” as used herein refers to a state or condition of normal cell growth (e.g., refers to a state or condition that is not characterized by, for example, rapidly proliferating cell growth, such as in cancer or atherosclerosis).

25 The term “internal tissue” as used herein refers to an anatomical site located within the body (e.g., a body cavity, connective tissue or internal organ system) and not located on the external surface of the body (e.g., the term does not include external tissues, such as skin and cornea).

The term “proximally located to an incision” as used herein refers to an internal anatomical site surrounding, or adjacent to, an incision but not including the incision.

30 The term “non-adhesive tissue surface” as used herein refers to a tissue surface containing a reduced amount of pathogenic collagen bundles and/or capsular contracture compared to a reference standard.

The term “reduce” or “decrease” as used herein generally means a decrease by a statistically significant amount. However, for avoidance of doubt, “reduced” means a

decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. substantially absent or below levels of detection), or any
5 decrease between 10-100% as compared to a reference level, as that term is defined herein. As used herein, the term "standard" or "reference" can simply be a reference that defines a baseline for comparison, such as an amount or level of adhesion, capsular contracture and/or pathogenic collagen bundle formation known to occur in the absence of photochemical tissue passivation.

10 A "subject" is a vertebrate, including any member of the class mammalia, including humans, domestic and farm animals, and zoo, sports or pet animals, such as mouse, rabbit, pig, sheep, goat, cattle and higher primates.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes,"

15 "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

20 Other definitions appear in context throughout this disclosure.

Methods of the Invention

It has now been determined that treating a tissue surface with an appropriate photoactive agent and light, intra-surgically, can create a less reactive tissue surface with normal tissue architecture and reduced post-surgical inflammation, which prevents the
25 development of pathogenic collagen bundles (i.e., fibrotic response) following surgery. This approach, referred to as Tissue Passivation or Photochemical Tissue Passivation leads to reduced adhesion formation, scarring and wound contracture as a result of reducing tissue surface reactivity. Clinical applications for Tissue Passivation include, but are not limited to, scar contracture, capsular contracture around implants, inhibition of intra-abdominal
30 adhesions after abdominal, thoracic, chest, colorectal, and ovarian surgery, inhibition of adhesions following uterine fibroid resection, treatment of radiated breast tissue, pretreatment of vein to prevent stenosis, treatment of vascular graft post angioplasty to prevent stenosis, host tissue preparation for knee and hip replacements, ligament and tendon surgeries and treatment for cellulitis.

Photoactivation and Photoactive Agents

Photoactivation, as referred to herein, e.g., can be used to describe the process by which energy in the form of electromagnetic radiation is absorbed by a compound, e.g., a photoactive agent, thus “exciting” the compound, which then becomes capable of converting the energy to another form of energy, preferably chemical energy. The electromagnetic radiation can include energy, e.g., light, having a wavelength in the visible range or portion of the electromagnetic spectrum, or the ultra violet and infrared regions of the spectrum. The chemical energy can be in the form of a reactive species, e.g., a reactive oxygen species, e.g., a singlet oxygen, superoxide anion, hydroxyl radical, the excited state of the photoactive agent, free radical or substrate free radical species. The photoactivation process can involve an insubstantial transfer of the absorbed energy into heat energy.

As used herein, a photoactive agent is a chemical compound that produces a biological effect upon photoactivation or a biological precursor of a compound that produces a biological effect upon photoactivation. Exemplary photoactive agents can be those that absorb electromagnetic energy, such as light. While not wishing to be bound by theory, the photoactivated agent may act by producing an excitation state or derived species that interacts with tissue to produce effects including modification of cell signaling pathways, oxidative stress, extracellular matrix alterations, cytokine and growth factor inhibition or release.

Certain exemplary photoactive agents typically have chemical structures that include multiple conjugated rings that allow for light absorption and photoactivation. A number of photoactive agents are known to one of skill in the art, and generally include a variety of light-sensitive dyes and biological molecules. Examples include, but are not limited to, xanthenes, e.g., Rose Bengal (“RB”) and erythrosin; flavins, e.g., riboflavin; thiazines, e.g., methylene blue (MB); porphyrins and expanded porphyrins, e.g., protoporphyrin I through protoporphyrin IX, coproporphyrins, uroporphyrins, mesoporphyrins, hematoporphyrins and sapphyrins; chlorophylls, e.g., bacteriochlorophyll A, phenothiazine (e.g., Toluidine Blue), cyanine, Mono azo dye (e.g., Methyl Red), Azine mono azo dye (e.g., Janus Green B), rhodamine dye (e.g., Rhodamine B base), benzophenoxazine dye (e.g., Nile Blue A, Nile Red), oxazine (e.g., Celestine Blue), anthroquinone dye (e.g., Remazol Brilliant Blue R), riboflavin-5-phosphate (R-5-P) and N- hydroxypyridine-2-(1H)-thione (N-HTP) and photoactive derivatives thereof.

Photoactive agents include photoactive dyes, which are organic compounds that absorb visible light resulting in a photochemical reaction. Photoactive dyes include xanthenes, thiazines, porphyrins and expanded porphyrins, chlorophylls, phenothiazines,

cyanines, Mono azo dye, Azine mono azo dye, rhodamine dye, benzophenoxazine dye, oxazine, and anthroquinone dye.

In certain exemplary embodiments, a photoactive agent, e.g., RB, R-5-P, MB, or N-HTP, can be dissolved in a biocompatible buffer or solution, e.g., saline solution, and used at
5 a concentration of from about 0.1 mM to 10 mM, preferably from about 0.5 mM to 5 mM, more preferably from about 1 mM to 3 mM.

Photoactive agents can be brushed or sprayed onto, or injected into, tissue surfaces prior to the application of electromagnetic energy. The electromagnetic radiation, e.g., light, can be applied to the tissue at an appropriate wavelength, energy, and duration, to cause
10 passivation of the tissue surface. The wavelength of light can be chosen so that it corresponds to or encompasses the absorption of the photoactive agent, and reaches the area of the tissue that has been contacted with the photoactive agent, e.g., penetrates into the region where it is applied. The electromagnetic radiation, e.g., light, necessary to achieve photoactivation of the agent can have a wavelength from about 350 nm to about 800 nm, preferably from about 400
15 to 700 nm and can be within the visible, infra red or near ultra violet spectra. The energy can be delivered at an irradiance of about between 0.5 and 5 W/cm², preferably between about 1 and 3 W/cm² or less than about 1 W/cm². The duration of irradiation can be brief and sufficient to allow passivation of the tissue, e.g., of a tissue collagen.

In certain exemplary embodiments, the photoactive agent applied to a tissue is a
20 photoactive dye, e.g., RB, subject to an irradiance of less than about 1 W/cm² for less than about 5 minutes.

Suitable sources of electromagnetic energy can include, but are not limited to, commercially available lasers, lamps, light emitting diodes, or other sources of electromagnetic radiation. Light radiation can be supplied in the form of a monochromatic
25 laser beam, e.g., an argon laser beam or diode-pumped solid-state laser beam. Light can also be supplied to a non-external surface tissue through an optical fiber device, e.g., the light can be delivered by optical fibers threaded through a small gauge hypodermic needle or an arthroscope. Light can also be transmitted by percutaneous instrumentation using optical fibers or cannulated waveguides.

30 The choice of energy source can generally be made in conjunction with the choice of photoactive agent employed in the method. For example, an argon laser can be an energy source suitable for use with RB or R-5-P because these dyes are optimally excited at wavelengths corresponding to the wavelength of the radiation emitted by the argon laser.

Other suitable combinations of lasers and photosensitizers are known to those of skill in the art. Tunable dye lasers can also be used with the methods described herein.

Applications

Preparation of tissue surfaces according to passivation methods disclosed herein is useful for a variety of tissues, including but not limited to tissue surfaces within connective tissue, abdominal, bladder, bowel, thoracic, colorectal, rectal, intestinal, ovarian, uterine, pericardial, peritoneal, oral, endocardial, epithelial and breast tissue. Passivation of body cavities prior to surgery is also envisioned. For example, body cavities having a surface area of about 20 cm², 25 cm², 50 cm², 100 cm², 500 cm² to about 1000 cm² can be treated prior to, following, or prior to and following surgical procedures, including implantation of natural or synthetic materials (e.g., breast implants). In surgical procedures not including implantation, passivation methods disclosed herein are useful for reducing reactivity of tissue surfaces which may otherwise develop adhesions and/or contracture, such as uterine-abdominal adhesions that form after caesarian sections.

Specific clinical applications also include passivation of tissue grafts and/or surgical sites to prevent contracture, scar contracture, capsular contracture around implants, inhibition of intra-abdominal adhesions after abdominal, thoracic, chest, colorectal, and ovarian surgery, inhibition of adhesions following uterine fibroid resection, treatment of radiated breast tissue, host tissue preparation for knee and hip replacements, ligament and tendon surgeries, treatment for cellulitis, treatment for hernia and treatment for dilatation.

Methods of passivation disclosed here in can be adapted for any organ in the body including but not limited to, heart, lung, cornea and nerve (e.g., to prevent cardiomyopathy, bleb disease in the lung, treat corneal abrasion, treat injured nerves or nerve grafts).

Tissue grafts can be prepared for implantation according to the passivation methods disclosed herein. For example, Rose Bengal can be applied to a surface (e.g., a single surface in some cases) of a tissue graft and irradiated ex vivo at an irradiance of less than about 1W/cm². Penetration of topically applied Rose Bengal is about 100 μm or less into connective tissue. Therefore, photoactive dyes such as Rose Bengal can be topically applied to, and/or accumulated within, limited areas of a tissue graft, such as the external surface of a vein graft (i.e., the dye does not penetrate into the vessel lumen and in some cases, does not penetrate beyond the external layer of collagen fibers surrounding the vein). Passivated tissue grafts can include, but are not limited to, grafts of vein, connective tissue, bone, ligament, tendon, skin, and muscle. Passivated tissue grafts also include autologous tissue, extracellular matrix tissue (e.g., collagen or proteoglycan) and tissue substitutes. Tissue

substitutes include, but are not limited to, silicon, collagen, fibronectin, glycosaminoglycan, polyurethane, polyvinyl and nylon.

Passivated vein grafts are especially desirable, for example, to prevent or reduce stenosis. Vein grafts can be prepared for implantation by providing Rose Bengal to the graft and irradiating the graft ex vivo at an irradiance of less than about $1\text{W}/\text{cm}^2$. The passivated vein graft can then be implanted into an artery to replace or bypass a diseased segment of the artery, such as an area comprising an arterial plaque, thereby preventing or reducing stenosis by reducing intimal hyperplasia. Passivated vein grafts can be implanted according to methods known in the art, including suturing, Anastomotic Coupler and bonding. In some applications, only the exterior surface of the vein graft will be passivated.

Passivated vascular grafts are also useful to strengthen any graft including arterial grafts, for example, to prevent aneurysm. Increasing the strength of the graft prior to implantation facilitates suturing, prevents tearing and in small grafts (e.g., micro-grafts) prevents immediate thrombus.

Passivation can also be conducted during the use of interventional radiology, for example, to treat the outside of a vessel at the same time treatment to the inside of the vessel is provided, to therefore allow for more aggressive endovascular treatment.

Passivation of tissue grafts prior to implantation can be conducted according to the methods described here in to prepare improved tissue for use in grafting. For example, grafts comprising host tissue can be passivated prior to knee and hip replacements or ligament and tendon surgeries.

The present invention is additionally described by way of the following illustrative, non-limiting Examples that provide a better understanding of the present invention and of its many advantages.

EXAMPLES

Example 1: Tissue Surface Passivation in a Rabbit Model for Capsular Contraction

A pilot experiment in a rabbit model for capsular contraction was developed. Three sub-cutaneous pockets were made by surgical incision on the upper rabbit flank. One pocket was left untreated and surgically closed (No fibrin glue). Of the remaining two, one was treated with Fibrin Glue only, and one received Fibrin Glue + RB (0.1% Rose Bengal) + 532 nm light (3 min at 350 mW). Fibrin glue is known to produce a fibrotic reaction in this model. After two weeks all three pockets were surgically re-accessed and inspected. Tissue

harvested at two weeks demonstrated an aggressive inflammatory response, with a significant infiltrate within the implant pockets instilled with fibrin glue (Figure 1). The pockets treated with PTP prior to fibrin glue application, had less infiltrate. These cells also appear contained under layers of presumably, cross-linked collagen.

5 A biodegradation assay was also performed. Pockets were treated as described above at both a low (1 J/cm^2) and high (25 J/cm^2) fluence. Fluence was measured using FieldMax II laser power meter (Coherent, Santa Clara, CA). Immediately following, the skin and fascia were removed. Treated and untreated fascia were biopsied with a six mm punch biopsy, weighed and subjected to digestion in a 1% collagenase (Sigma Aldrich, St. Louis, MO)
10 solution at 37°C . Time for complete digestion (the lack of any visible fibers) was recorded for each sample and corrected by weight.

In-vitro studies confirmed that photochemical tissue passivation, under the conditions that were subsequently used in the animal study, could successfully alter the fascia of the implant pocket. The collagenase digestion assay demonstrated that PTP treatment resulted in
15 a 300% increase in degradation time, which correlates to an increase in collagen crosslinking in fascia when compared to untreated tissue (Figure 2). Increasing the power of the laser, did not increase collagen crosslinking proving adequate treatment at the lower fluence (1 J/cm^2).

Thus, RB+light leads to tissue surface passivation.

Example 2: Tissue Surface Passivation in a Pre-clinical Model of Capsular Contracture

20 Following Prosthetic Implant Placement

The effect of Photochemical Tissue Passivation in a pre-clinical model of capsular contracture following prosthetic implant placement was evaluated. Under general anesthesia, Nine New Zealand white rabbits received three, six cc smooth saline implants placed in the dorsal sub-panniculus soft tissue. Each Rabbit received one control implant and two
25 experimental implants.

Briefly, the subpanniculus carnosus plane in the dorsum region was dissected and a single customized six cc saline smooth implant with pressure port valve (Allergan Inc., CA, USA) was placed. A total of three implants were placed on the dorsum of each rabbit. The control group consisted of and implant with no Fibrin Glue and no PTP treatment. Before
30 implant placement, the pocket site of the PTP experimental group was treated with 2cc 0.1% Rose Bengal in a phosphate buffered saline, and was then exposed to green laser light at 532 nm from a continuous wave KTP laser (Laserscope Aura-I, San Jose, CA). The second experimental group consisted of an implant with Fibrin Glue only. Following implant placement, all experimental pockets were injected with five cc of fibrin glue, 500 μl of

10%CaCl, 1000 units of thrombin in one milliliter of 50 mM TrisCl as a contracture inducing agent. The incisions were closed in two layers with subdermal 4-0 Vycril and 4-0 interrupted nylon sutures. Topical fibrin glue was applied to the implant pockets in all experimental groups to induce subsequent capsular contracture.

5 Accordingly, the Experimental groups can be summarized as follows:

Group 1: Control group – “Standard Breast Augmentation” ~ One pocket per Rabbit undergoing standard implant placement with no Fibrin Glue or Passivation. One six cc smooth saline implant is placed per pocket. The incisions are closed in two layers with subdermal 4-0 Vicryl and 4-0 interrupted nylon sutures.

10 Group 2: Experimental group – “Fibrin Glue only” ~ One dorsal pocket per Rabbit is dissected. One six cc smooth saline implants will be placed per pocket. Fibrin glue is injected into the implant pocket as a capsular contracture-inducing agent. The incision is closed as above.

Group 3: Experimental group – “Pre-implant Tissue Passivation” ~ One dorsal pocket
15 per Rabbit is dissected. Rose Bengal dye is applied. The pocket is exposed to green laser light at 532 nm from a continuous wave KTP laser (Laserscope Aura-I, San Jose, CA). Following passivation, one six cc smooth saline implant is placed per pocket. Fibrin glue is injected into the implant pocket as a capsular contracture-inducing agent. The incision is closed as above.

Capsule specimens were fixed with 10% buffered formalin and embedded in paraffin.
20 Sections were stained with both Masson Trichrome, and SMA antibody and evaluated histologically and capsular thickness was measured. Smooth muscle actin immunohistochemistry revealed substantial variation in fiber thickness, orientation and number among the various groups. Untreated pockets receiving fibrin glue resulted in multiple layers of SMA within the entire capsule, hundreds of microns from the implant.
25 Treated pockets exhibited significantly less deposition of smooth muscle actin. In fact, the SMA pattern in capsules harvested from the treated groups resembled those, which had not been instilled with fibrin glue (Figure 3).

Histology shows a decrease in capsular thickness in the Fibrin Glue + Photochemical Tissue Passivation group compared to Fibrin Glue alone control. The Fibrin Glue +
30 Photochemical Tissue Passivation group had similar capsule thickness to the healthy, normal, no Fibrin Glue control (Figure 4). The passivated group had a 52% decrease in capsule thickness when compared to the controls (Figure 5). Implant capsule thickness is the number one prognostic factor for contracture development. The treated pockets also demonstrated decreased inflammation and vascularity within the capsule. PTP resulted in a less

fibrohistiocystic cells, macrophages and synovial metaplasia. Accordingly, use of Rose Bengal + Light in tissue passivation preserved normal tissue architecture and prevented the development of pathogenic collagen bundles and capsular contracture.

Example 3: Photochemical Tissue Passivation for Prevention of Vein Graft Intimal

5 Hyperplasia

Vein grafts are frequently used for Coronary Artery Bypass Grafting yet suffer poor long-term patency rates compared to arterial grafts due to accelerated atherosclerosis. This begins as intimal hyperplasia (IH), which is a consequence of intimal injury resulting from surgical trauma and excessive dilation of the vein graft as it is exposed to arterial pressures.

- 10 Limiting this stretch reduces the degree of IH. To date, this has only been achieved with external sheaths. Photochemical Tissue Passivation (PTP) was conducted to determine whether this procedure could improve the long-term patency rates of venous grafts.

Porcine jugular veins were used to evaluate the effect of PTP on the elasticity of venous tissues. Veins harvested from five pigs were divided in half. One segment served as control, the other was treated (PTP) with 0.1% Rose Bengal and a 532 nm laser (delivering 15 25 J/cm²). Veins were cut into 0.5x2cm strips (N=33; 16 control, 17 treated). Stress-strain curves were generated for each with a tensiometer and the modulus of elasticity calculated.

The modulus of elasticity (Young's modulus) of treated and untreated veins were 1008±555 and 587±228 KPa respectively (p=0.01). The modulus of elasticity of venous 20 grafts was increased 2-fold via PTP (Figure 6). The Young's modulus of arterial samples (N=12) was 1515 ± 530 KPa.

Table 1: Modulus of elasticity (Young's modulus) of untreated veins, treated veins, and artery

	Young's Modulus (KPa)	P value
Untreated porcine jugular vein (N=16)	587 ± 288	0.01
Treated porcine jugular vein - 25 J/cm ² (N=17)	1008 ± 555	
Porcine carotid artery (N=12)	1515 ± 530	

- 25 A biodegradation assay was performed to demonstrate collagen cross-linking. Collagenase digestion experiments were conducted using segments of superficial epigastric vein harvested from Sprague-Dawley rats, which was the same conduit used for our animal model. A single vein was harvested from two rats under an operating microscope using a strict "no touch" technique. Each vein was divided in four equally long segments, resulting in 30 eight total samples. Half of the samples were treated with PTP, delivering 25 J/cm² as

described above. Half served as controls. The samples were immersed in 0.1% (w/v) collagenase solution and placed in an incubator at 37°C. Time to complete digestion was measured.

Collagenase assays demonstrate significant collagen cross-linking of venous grafts after PTP (Figure 7). Collagenase digestion of controls was achieved in 66 minutes on average, while treated veins were undigested after 300 minutes.

In-vivo testing was done by placing a reversed epigastric vein interposition graft in the femoral artery of Sprague-Dawley rats. Grafts were harvested after four weeks. A total of 16 rats underwent a femoral interposition graft with reversed epigastric vein. Half of the vein grafts (N=8) were treated with PTP using 25 J/cm² of fluence. Average operative time was two hours. Additional operative time for PTP was 4-5 minutes on average. There were three instances of wound dehiscence between post-operative day one and two; one in the control group and two in the experimental group. These wounds were irrigated and resutured. None of the grafts were exposed. There were no wound infections. After four weeks, 100% of the vein grafts were patent. There were no pseudoaneurysms or aneurysmal dilatation of the grafts. Histologic cross-sections of the mid-portion of the graft were analyzed using light microscopy. Intimal thickness was measured as the distance between the internal elastic lamina and the vessel lumen. These in-vivo studies revealed neointimal thickness of 80±56 and 24±22 µm for untreated (N=8) vs treated animals respectively (N=8).

Table 2: Intimal thickness of untreated and treated vein grafts after four weeks

	Intimal Thickness (µm)	P value
Untreated vein grafts (N=8)	80 ± 56	0.03
Treated vein grafts (N=8)	24 ± 22	

There was also marked reduction in smooth muscle hypertrophy and medial thickness in the treated compared to the untreated grafts. Medial thickness in untreated grafts was 216 ± 69 µm compared to 141 ± 40 µm in treated grafts (p=0.04). Table 3: Medial thickness of untreated and treated vein grafts after four weeks

	Medial Thickness (µm)	P value
Untreated vein grafts (N=8)	216 ± 69	0.04
Treated vein grafts (N=8)	141 ± 40	

Our animal model demonstrated a 70% reduction in neointimal thickness of treated grafts (Figures 8A and 8B). Images of vein grafts after vessel clamp removal (i.e., with

restored blood flow to the graft) shows less distention in PTP treated vein grafts compared to untreated controls (Figure 9).

Therefore, PTP improves the long-term patency rates of venous grafts used for coronary revascularization and peripheral arterial disease without the use of cumbersome
5 external sheaths.

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims. The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable
10 as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

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CLAIMS

1. A method of preparing a non-adhesive tissue surface within a human subject, said method comprising the steps of: providing a photoactive agent to an intact, non-proliferative, internal tissue surface proximally located to an incision in a human subject and irradiating said tissue surface at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preparing a non-adhesive tissue surface within the human subject.

2. The method of claim 1, wherein the photoactive agent is Rose Bengal.

3. The method of claim 2, wherein the irradiance is provided for less than about 5 minutes.

4. The method of claim 1, wherein the tissue surface is located within a cavity of the human subject.

5. The method of claim 1, wherein the tissue surface is within a tissue selected from the group consisting of connective tissue, abdominal, bladder, bowel, thoracic, colorectal, rectal, intestinal, ovarian, uterine, pericardial, peritoneal, oral, endocardial, epithelial and breast tissue.

6. The method of claim 1, wherein the tissue surface comprises previously grafted tissue.

7. The method of claim 1, wherein the photoactive agent is applied topically to the tissue surface.

8. The method of claim 1, further comprising providing an implant to the human subject following irradiation and closing the incision.

9. The method of claim 1, further comprising providing an implant to the human subject containing a light source for irradiating the tissue surface.

10. The method of claim 1, wherein the tissue surface has an area of about 20 cm², 25 cm², 50 cm², 100 cm², 500 cm² to about 1000 cm².

11. A method of preventing or decreasing tissue adhesion and/or contracture at a surgical site in a human subject, said method comprising the steps of: topically applying a photoactive agent to an internal tissue surface proximally located to a surgical site; irradiating said tissue surface at an irradiance of less than about 1W/cm²; and determining that tissue adhesion and/or contracture at the surgical site is decreased compared to a reference.

12. The method of claim 11, wherein the photoactive agent is Rose Bengal.

13. The method of claim 12, wherein the irradiance is provided for less than about 5 minutes.

14. The method of claim 11, wherein the tissue surface is located within a body cavity of the human subject.

15. The method of claim 11, wherein the tissue surface is within a tissue selected from the group consisting of connective tissue, abdominal, bladder, bowel, thoracic, colorectal, rectal, intestinal, ovarian, uterine, pericardial, peritoneal, oral, endocardial, epithelial and breast tissue.

16. The method of claim 11, wherein the tissue surface comprises previously grafted tissue.

17. The method of claim 11, further comprising providing an implant to the human subject following irradiation.

18. The method of claim 17, wherein the implant comprises a tissue graft.

19. The method of claim 18, wherein the tissue graft is treated with Rose Bengal and irradiated for less than 5 minutes prior to implantation.

20. The method of claim 11, wherein the contracture comprises scar contracture or capsular contracture.

21. The method of claim 11, wherein the tissue surface has an area of about 20 cm², 25 cm², 50 cm², 100 cm², 500 cm² to about 1000 cm².

22. A method of preparing a tissue graft for implantation, said method comprising the steps of: providing Rose Bengal ex vivo to a surface of a tissue graft and irradiating said surface ex vivo at an irradiance of less than about 1W/cm², thereby preparing a tissue graft for implantation.

23. The method of claim 22, wherein the tissue graft comprises a tissue selected from the group consisting of vein, connective tissue, bone, ligament, tendon, skin, and muscle.

24. The method of claim 23, wherein the tissue graft comprises any member of the group consisting of autologous tissue, extracellular matrix tissue and tissue substitute.

25. The method of claim 24, wherein the extracellular matrix comprises collagen or proteoglycan.

26. The method of claim 24, wherein the tissue substitute comprises any member of the group consisting of silicon, collagen, fibronectin, glycosaminoglycan, polyurethane, polyvinyl and nylon.

27. The method of claim 22, wherein the Rose Bengal is provided to one surface of the tissue graft.

28. A method of preparing a non-adhesive tissue surface within a human subject, said method comprising the steps of: topically applying a photoactive agent to an internal tissue surface within a cavity, wherein the surface is about 20 cm², 25 cm², 50 cm², 100 cm², 500 cm²

to about 1000 cm^2 , and irradiating said tissue surface at an irradiance of less than about 1 W/cm^2 , thereby preparing a non-adhesive tissue surface within the human subject.

29. The method of claim 28, wherein the photoactive agent is Rose Bengal.

30. The method of claim 29, wherein the irradiance is provided for less than about 5 minutes.

31. The method of claim 28, wherein the tissue surface is within a tissue selected from the group consisting of connective tissue, abdominal, bladder, bowel, thoracic, colorectal, rectal, intestinal, ovarian, uterine, pericardial, peritoneal, oral, endocardial, epithelial and breast tissue.

32. The method of claim 28, wherein the tissue surface comprises previously grafted tissue.

33. The method of claim 1, further comprising providing an implant to the human subject following irradiation.

34. The method of claim 28, further comprising providing an implant to the human subject containing a light source for irradiating the tissue surface.

35. A method of preparing a graft comprising vein for implantation, said method comprising the steps of: providing Rose Bengal ex vivo to the graft comprising vein and irradiating said graft ex vivo at an irradiance of less than about 1 W/cm^2 , thereby preparing the graft comprising vein for implantation.

36. A method of preventing or reducing stenosis in a subject, said method comprising the steps of: implanting a passivated graft comprising vein into an artery, wherein said implanting of the graft replaces a/or bypasses a diseased segment of the artery, thereby preventing or reducing stenosis in a subject.

37. The method of claim 36, wherein the diseased segment of the artery contains a plaque.

38. The method of claim 36, wherein Rose Bengal is provided ex vivo to the exterior surface of the implanted, passivated graft comprising vein and/or the exterior surface of the artery and irradiated at an irradiance of less than about $1\text{W}/\text{cm}^2$.

39. A method of preventing or reducing stenosis in a subject, said method comprising the steps of: implanting a graft comprising vein into an artery, wherein said implanting of the graft replaces and or bypasses a diseased segment of the artery, providing Rose Bengal to the exterior surface of the implanted graft comprising vein and irradiating said graft at an irradiance of less than about $1\text{W}/\text{cm}^2$, thereby preventing or reducing stenosis in a subject.

40. The method of claim 39, wherein the diseased segment of the artery contains a plaque.

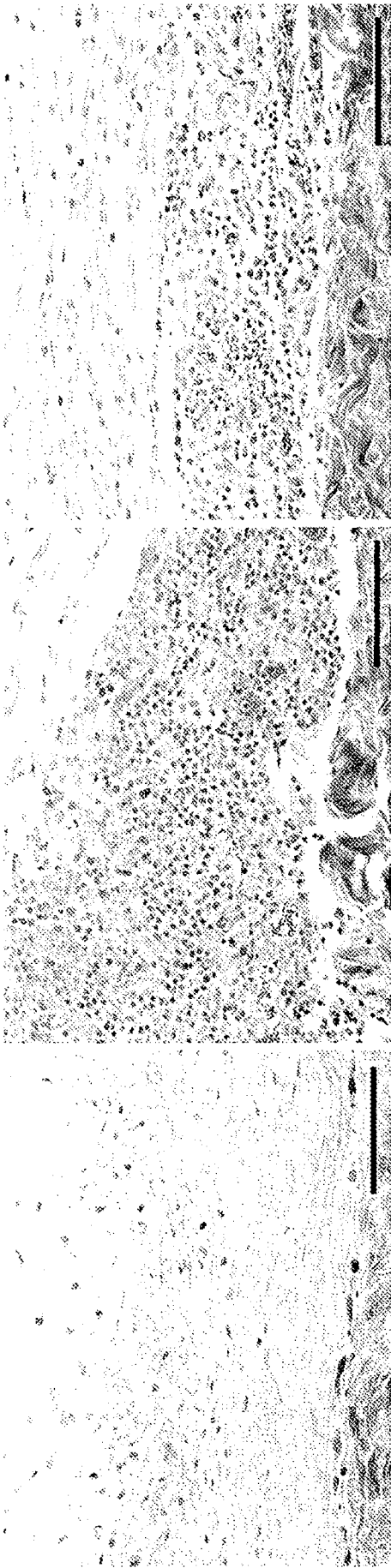


FIG. 1

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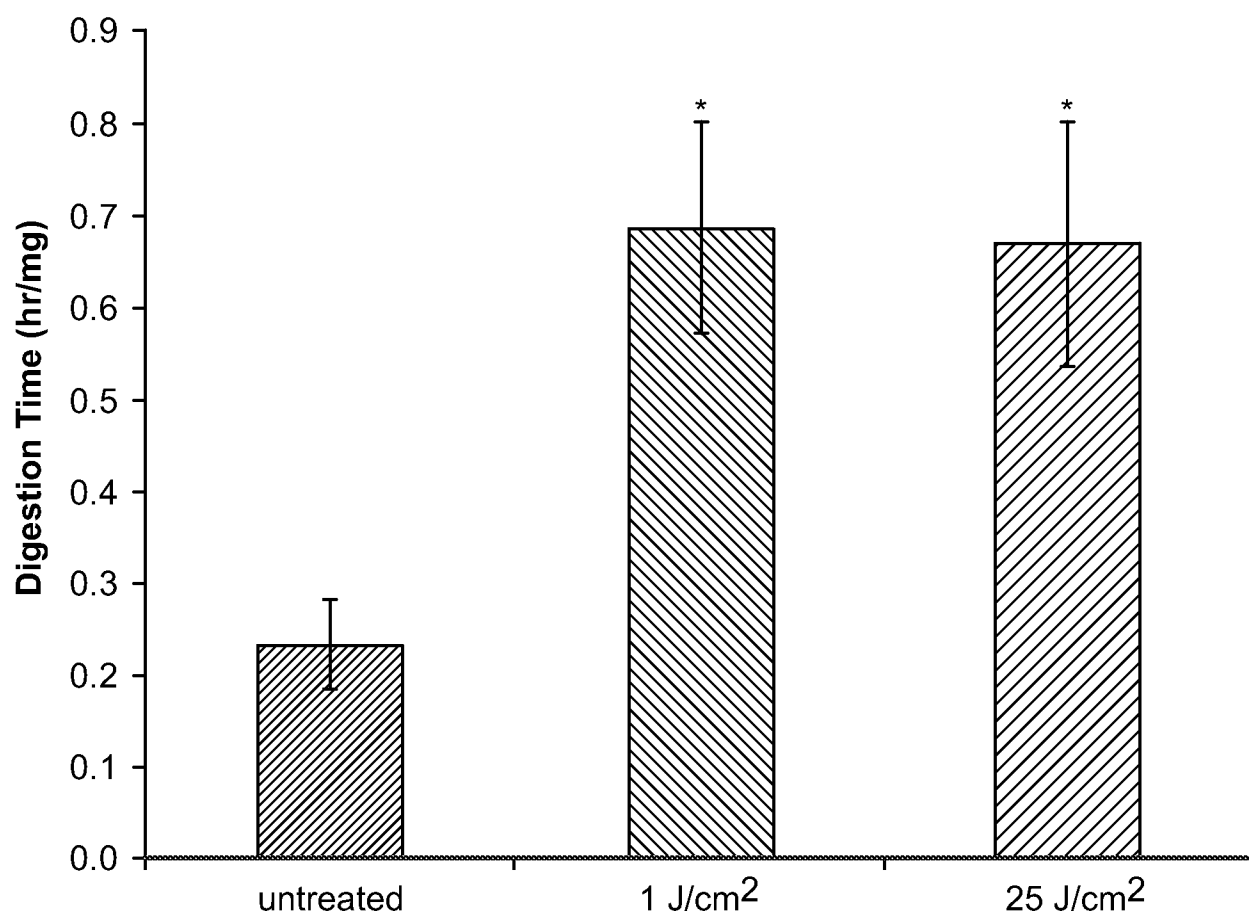
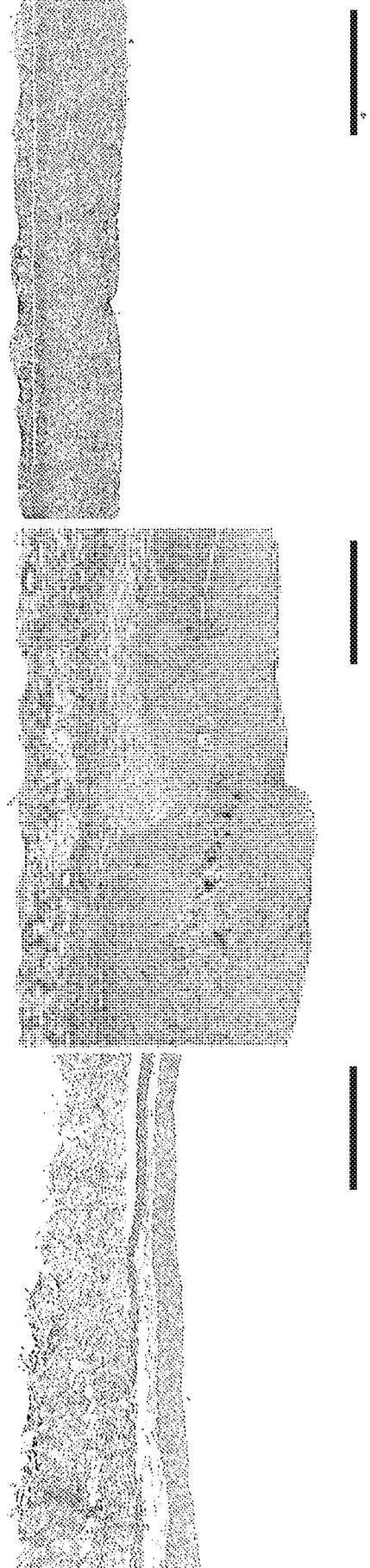


FIG. 2

FIG. 3



FIG. 4



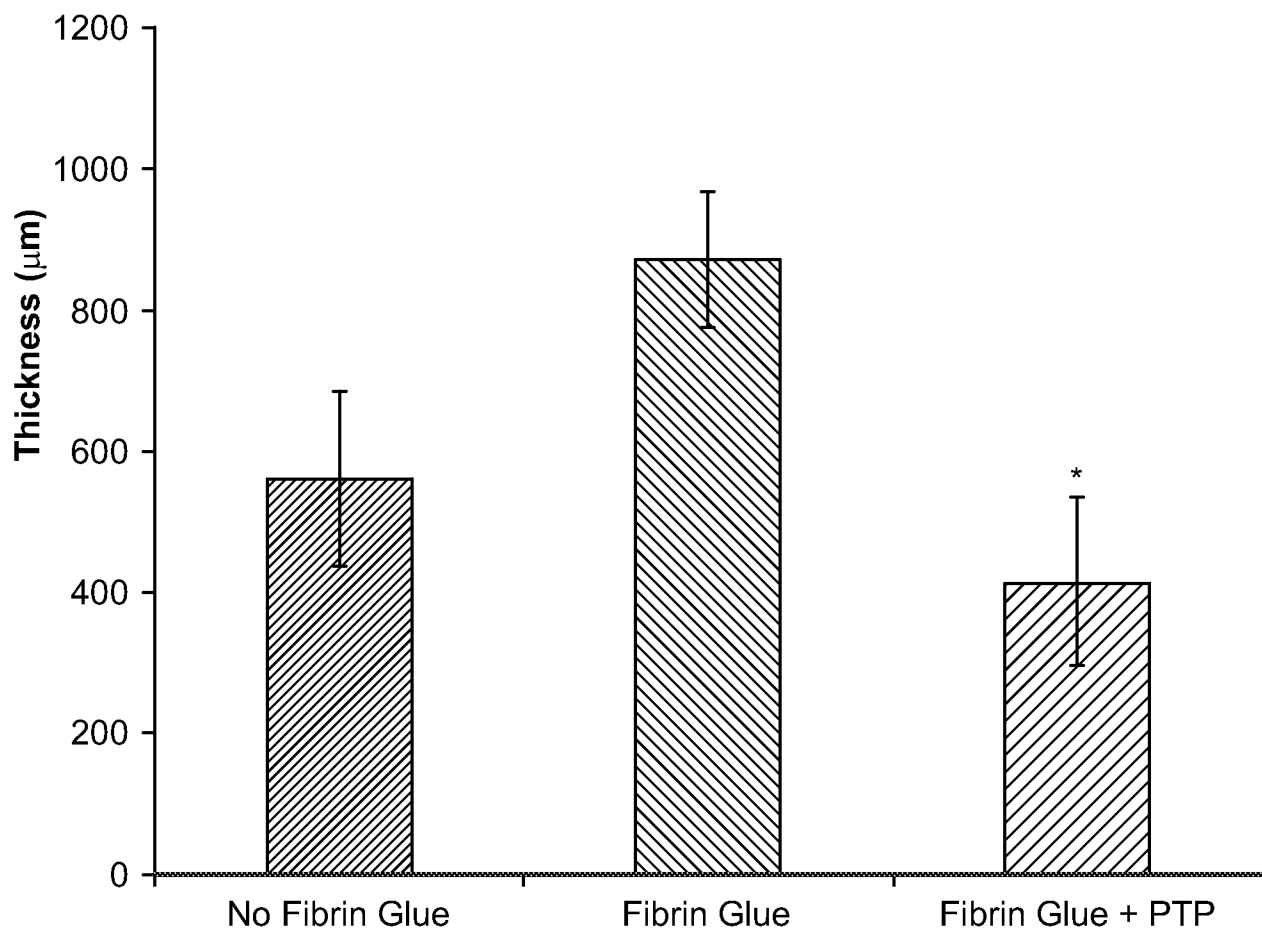


FIG. 6

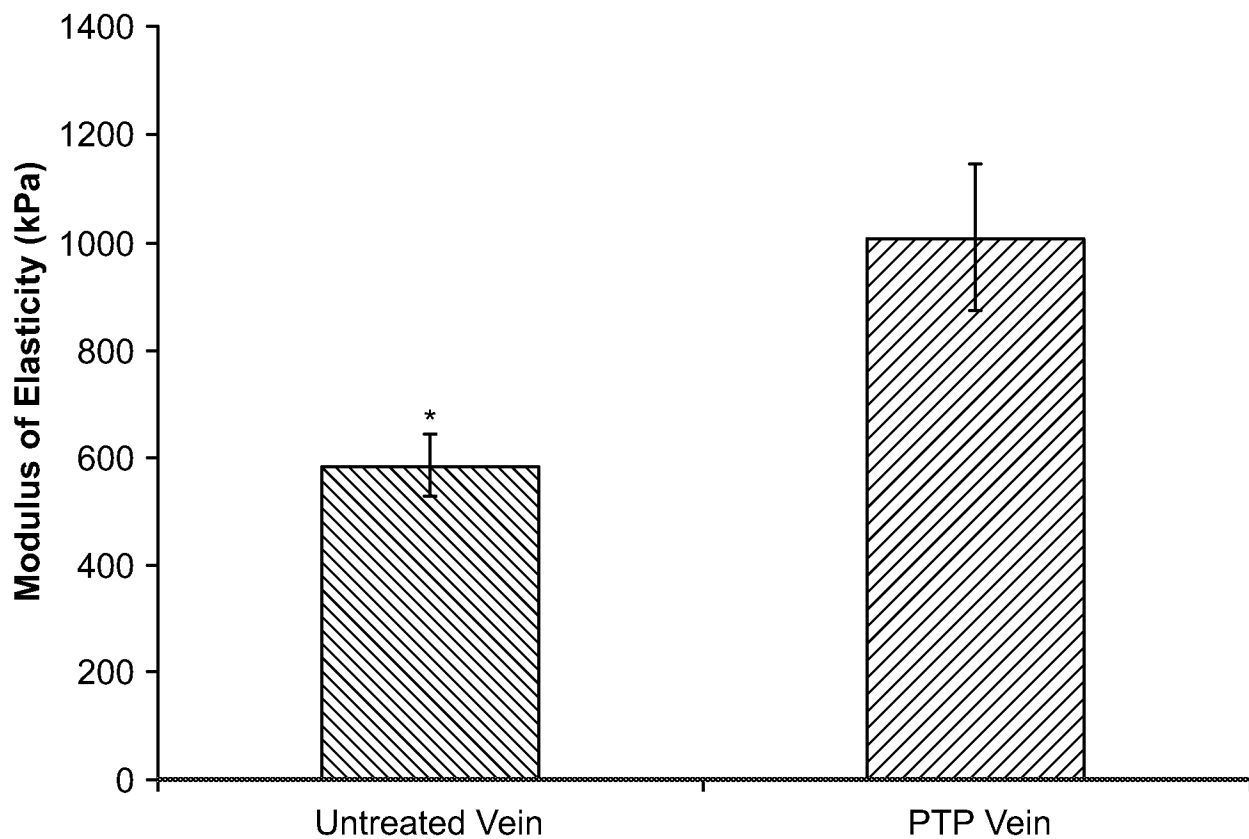


FIG. 7

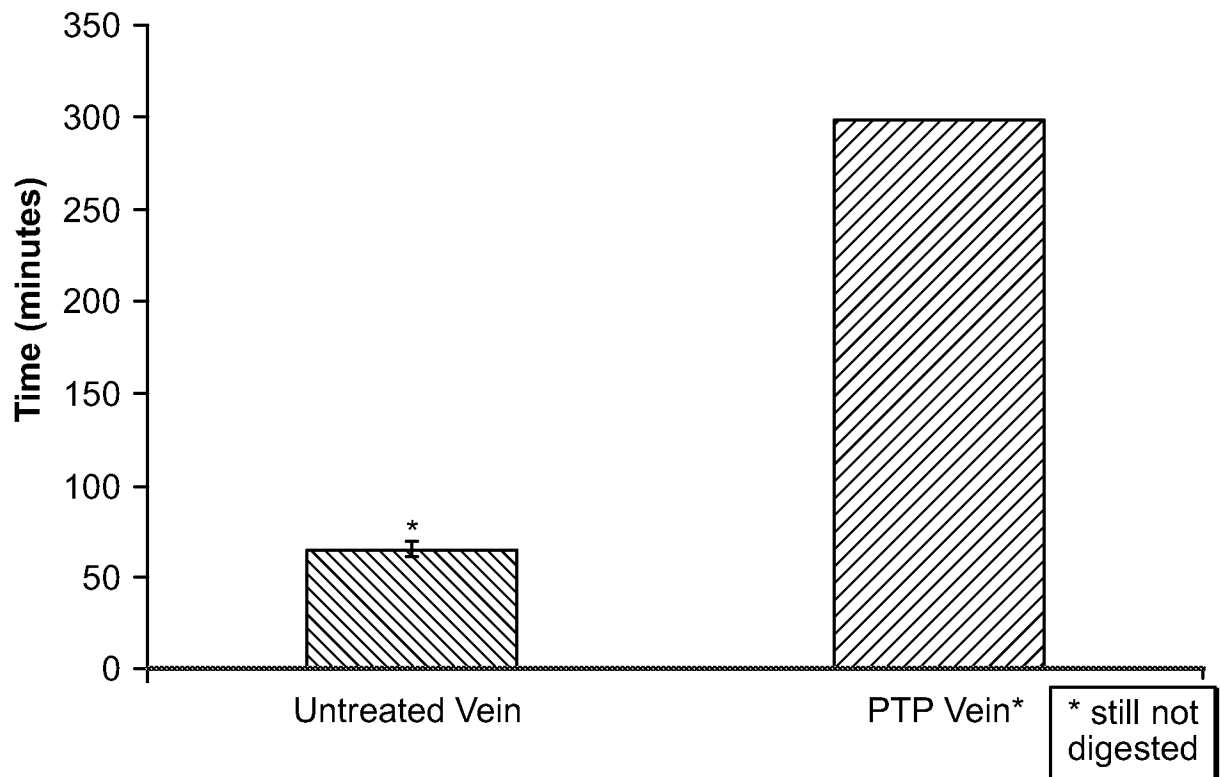
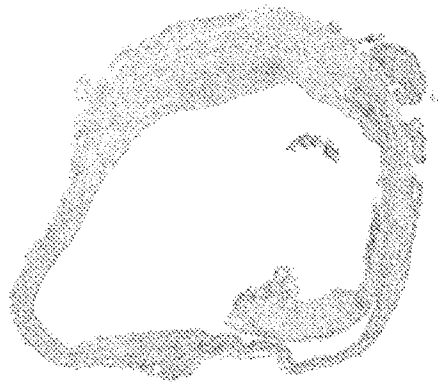


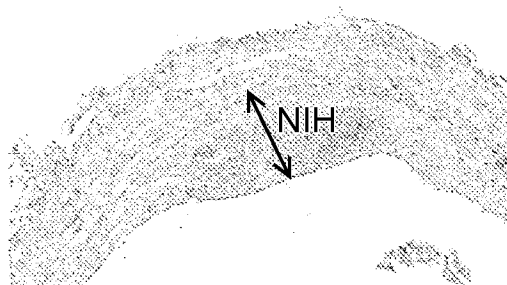
FIG. 8A

Untreated Vein

40X



100X

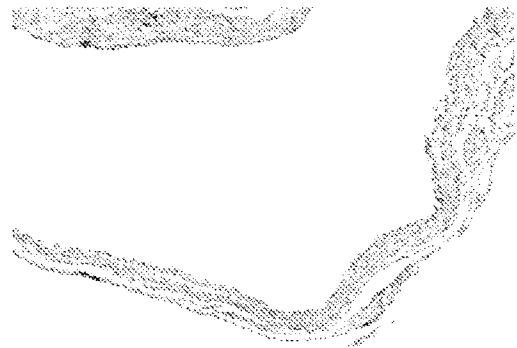


PTP Treated Vein

40X



100X



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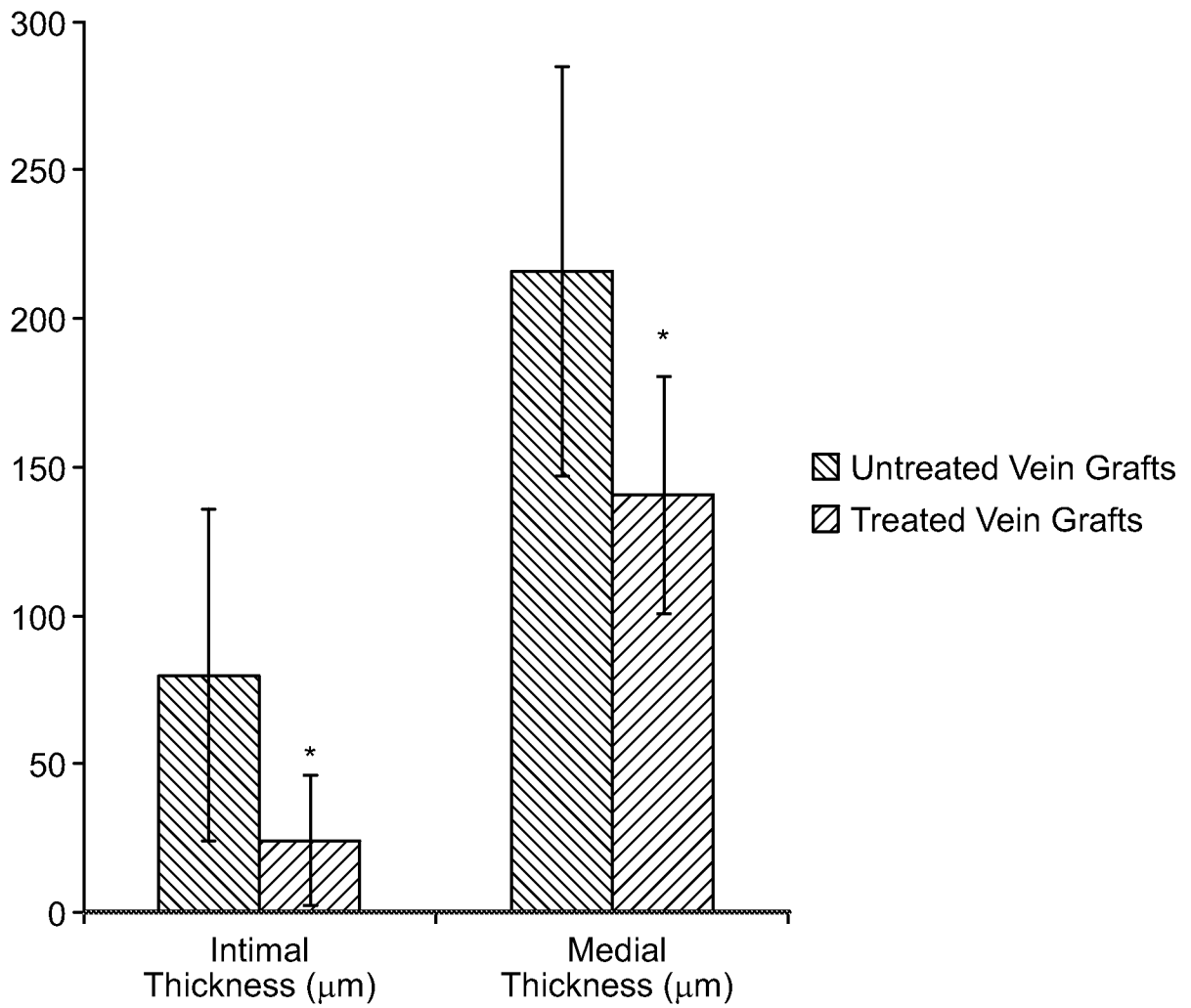


FIG. 8B

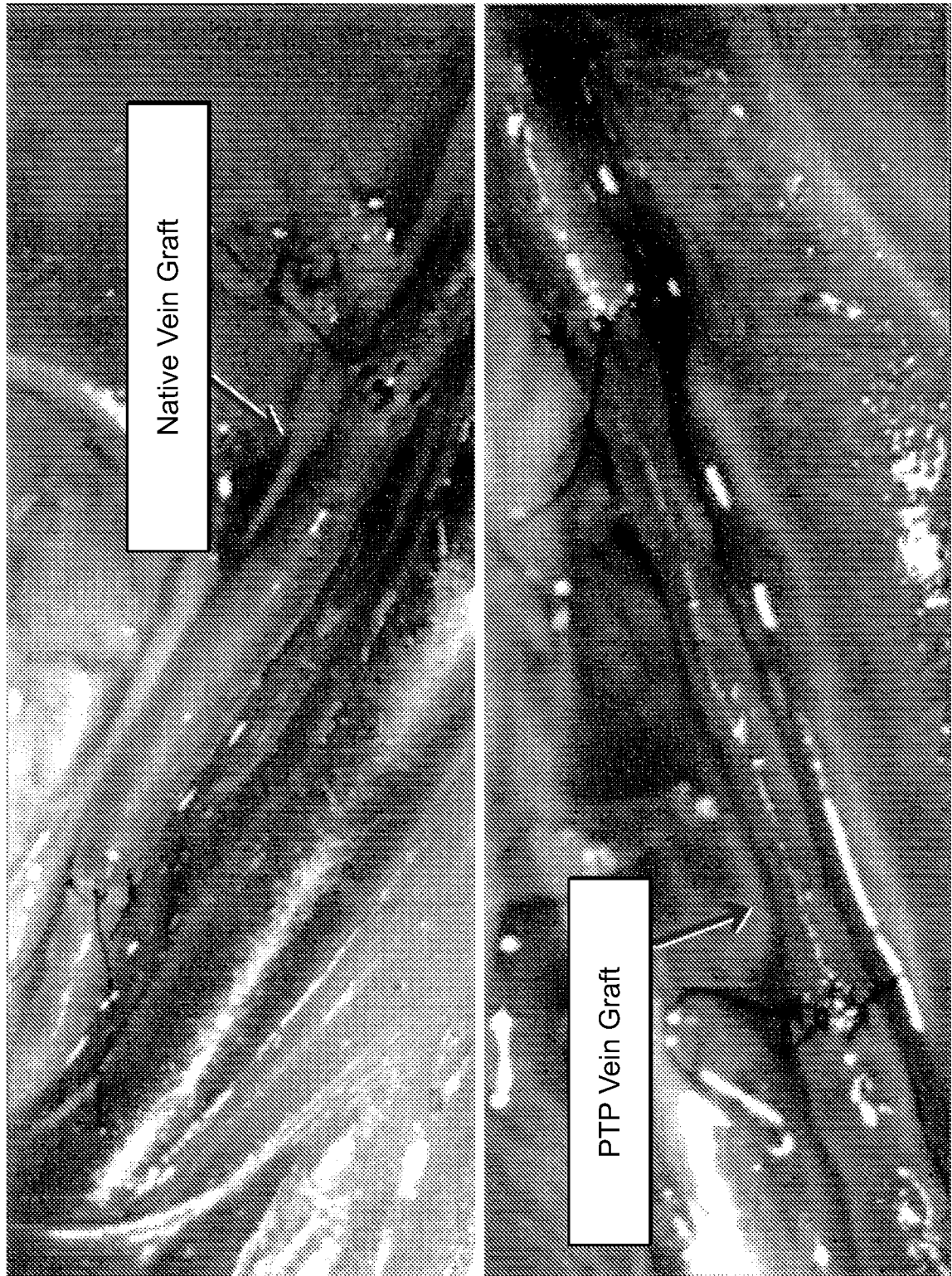


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/051333

A. CLASSIFICATION OF SUBJECT MATTER

*A61L 27/50 (2006.01)**A61L 27/14 (2006.01)**A61N 5/06 (2006.01)**A61F 2/07 (2013.01)*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61L 27/50, 27/14, A61N 5/06, A61F 2/07, 2/82, A61K 41/00, 31/34, 31/35, A61P 41/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DEPATISnet, DWPI, EAPATIS, Esp@cenet, PAJ, PCT Online, PatSearch, PubMed, RUPTO, RUSPAT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2006/0212070 A1 (ROBERT W. REDMOND et al.) 21.09.2006, paragraphs [0014]-[0016], [0018], [0054], [0066], [0070], [0177]	1-35 36-40
Y	US 2003/0100934 A1 (W. PATRICK STEPHENS et al.) 29.05.2003, claims 1, 3-4, paragraphs [0006], [0023]	36-40
A	US 7037490 B2 (GENZYME CORPORATION) 02.05.2006	1-40
A	EP 0627911 B1 (BOARD OF REGENTS THE UNIVERSITY OF TEXAS SYSTEM) 25.10.2000	1-40

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 October 2013 (01.10.2013)

Date of mailing of the international search report

07 November 2013 (07.11.2013)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/051333

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KZ 22010 A4 (THE REPUBLICAN STATE PUBLIC ENTERPRISE "KAZAKH ORDERS"BADGE OF HONOUR"SCIENTIFIC RESEARCH INSTITUTE OF EYE DISEASES" OF THE MINISTRY OF HEALTH OF THE REPUBLIC OF KAZAKHSTAN) 15.12.2009	1-40