Published in final edited form as:

Biomaterials. 2010 October ; 31(29): 7475–7484. doi:10.1016/j.biomaterials.2010.06.039.

Functional Skeletal Muscle Formation with a Biologic Scaffold

Jolene E. Valentin, PhD,

Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3130

Neill J. Turner, PhD,

Department of Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3130

Thomas W. Gilbert, PhD, and

Department of Surgery, Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3130

Stephen F. Badylak, DVM, PhD, MD [Professor of Research]*

Department of Surgery, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3130

Abstract

Biologic scaffolds composed of extracellular matrix (ECM) have been used to reinforce or replace damaged or missing musculotendinous tissues in both preclinical studies and in human clinical applications. However, most studies have focused upon morphologic endpoints and few studies have assessed the in-situ functionality of newly formed tissue; especially new skeletal muscle tissue. The objective of the present study was to determine both the in-situ tetanic contractile response and histomorphologic characteristics of skeletal muscle tissue reconstructed using one of four test articles in a rodent abdominal wall model: 1) porcine small intestinal submucosa (SIS)-ECM; 2) carbodiimide-crosslinked porcine SIS-ECM; 3) autologous tissue; or 4) polypropylene mesh. Six months after surgery, the remodeled SIS-ECM showed almost complete replacement by islands and sheets of skeletal muscle, which generated a similar maximal contractile force to native tissue but with greater resistance to fatigue. The autologous tissue graft was replaced by a mixture of collagenous connective tissue, adipose tissue with fewer islands of skeletal muscle compared to SIS-ECM and a similar fatigue resistance to native muscle. Carbodiimide-crosslinked SIS-ECM and polypropylene mesh were characterized by a chronic inflammatory response and produced little or no measureable tetanic force. The findings of this study show that non-crosslinked xenogeneic SIS scaffolds and autologous tissue are associated with the restoration of functional skeletal muscle with histomorphologic characteristics that resemble native muscle.

Introduction

Biologic scaffolds composed of extracellular matrix (ECM) have been successfully used to repair or replace a variety of damaged or diseased tissues, including cardiac,[1-3] esophageal,[4,5] dermal,[6] and musculotendinous[7-10] tissues, among others. The host response to biologic scaffolds composed of ECM can be attributed to factors such as the

* Corresponding Author: Phone (412) 235-5145, Fax (412) 235-5224, badylaks@upmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

species (human, porcine, equine, or bovine) and tissue source from which the ECM is isolated (dermis, small intestinal submucosa, or pericardium). In addition, the decellularization, disinfection and sterilization methods used during the manufacturing process, some of which may include the use of chemical crosslinking agents, the host immune response, and the post-implantation mechanical loading to which the scaffold is subjected all influence the tissue remodeling response and functional outcome. [11-19]

The biomechanical and biochemical properties of ECM scaffolds have been extensively characterized,[11,20-23] but these properties, as with any implanted material, inevitably and rapidly change in-vivo as a result of the concurrent processes of scaffold degradation, neomatrix deposition, and host cellular remodeling events.[16,19,24] Studies have shown that non-crosslinked scaffolds composed of small intestinal submucosa (SIS) are typically degraded and replaced with new host ECM and site-appropriate tissue structures within 60 – 90 days.[25,26] Arguably, one of the most important criteria of clinical success following use of an ECM scaffold for tissue reconstruction is the functionality of the newly formed tissue. The functionality of remodeled ECM has been investigated in tendinous tissues, with the results showing an initial decrease in strength after implantation as the scaffold begins to degrade, followed by an increase in strength as new host tissue deposition occurs.[9,27-29] In the context of abdominal wall repair, biomaterials are typically chosen for their strength and durability rather than their ability to restore functional tissue. As a result, the vast majority of devices for abdominal wall repair are based on synthetic materials such as polyester or polypropylene and devices that are biological in origin are typically cross-linked to resist degradation and increase structural strength. Little consideration is given to restoring the functionality of the damaged tissue. There is a paucity of data on the quantitative in-situ functional outcome of skeletal muscle that forms as a result of SIS-ECM scaffold remodeling particularly as it relates to the abdominal wall.[30]

In this present study four, clinically relevant biomaterials were investigated for their ability to promote the constructive remodeling of an abdominal wall defect in a rat model. The specific objectives of the present study were: (1) to quantify the in-situ contractility of skeletal muscle tissue that was reconstructed/repared; and (2) to characterize the histomorphologic appearance of the remodeled tissue.

Materials and Methods

Overview of Experimental Design

Twenty-six male Sprague Dawley adult rats were randomly assigned to three groups of eight and one group of two. Each rat was subjected to partial thickness excision of a 1.5 cm × 1.5 cm section of the ventral lateral abdominal wall musculature. The defect was repaired with one of four test articles: Restore™, a 10 layer configuration of an ECM biologic scaffold composed of porcine small intestinal submucosa (SIS) (n = 8); CuffPatch™, a 10 layer configuration of porcine SIS that differs from the Restore™ device by the use of carbodiimide as a crosslinking agent (n = 8); autologous body wall tissue (n = 8); or polypropylene mesh (n = 2). The animals were survived for 26 weeks after surgery. The contractile properties of both the remodeled tissue and the contralateral native tissue were determined in-situ immediately prior to euthanasia. Following euthanasia and explantation of the remodeled scaffold materials, histologic and immunolabeling methods were used to determine the morphologic characteristics of the remodeled tissue including the distribution of slow (type I) and fast (type II) skeletal muscle fibers, blood vessels, and nerves.

Test Articles

The two ECM-derived scaffold materials that were used to repair the surgically created defect were derived from porcine small intestinal submucosa (SIS) and differed mainly by the processing methods, i.e., the use of a chemical crosslinking agent. The Restore™ (DePuy Orthopaedics Inc., Warsaw, IN) device represents an SIS-ECM scaffold material that is not subjected to chemical crosslinking. The CuffPatch™ (Arthrotek, Inc., Warsaw, IN) device represents an SIS-ECM material that is crosslinked with carbodiimide. Both of the ECM materials and the polypropylene mesh (Bard Davol Inc., Warwick, RI) were purchased from the manufacturer. The handling of the materials after removal from the sterile package and prior to surgical implantation was performed according to the manufacturer's instructions as provided in the package insert. The test article in the 3rd group consisted of healthy skeletal muscle tissue (autologous tissue graft) excised from the abdominal wall surgical site of each animal.

Animal Care Compliance

All procedures were performed in accordance with the National Institute of Health (NIH) guidelines for care and use of laboratory animals, and with approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh.

Animal Source and Husbandry

Twenty-six adult male Sprague-Dawley rats weighing approximately 300 grams were purchased from Charles River Laboratory (Wilmington, MA). Each animal was housed individually in shoebox cages. The rats were fed a diet of Purina Isopro rodent chow ad libitum (LabDiet ProLab Isopro RMH 3000, PMI Nutrition International, St. Louis, MO). The housing environment was maintained at 68° to 76°C for twenty four hours a day and with a light:dark cycle of 12:12 hours.

Surgical Procedure

The partial thickness abdominal wall defect model for evaluation of muscular tissue remodeling is well established[31,32] but is briefly described herein for clarity. Each animal was anesthetized by inhalation with 2% isoflurane in oxygen, and the surgical site was prepared for sterile surgery. A ventral midline abdominal skin incision was created, and the skin and subcutaneous tissue were separated from the underlying muscle tissues on one side of the midline. A 1.5 cm × 1.5 cm section of the external and internal oblique layers of the ventral lateral abdominal wall was excised; the underlying transversalis fascia and peritoneum remained intact. The defect was repaired with a size-matched piece of the chosen test article. A single 4-0 Prolene suture was placed at each of the four corners of the test article to secure the device to the surrounding musculature, allow for mechanical loading of the test article during the normal abdominal wall activity of daily living, and to demarcate the implant for identification at the time of in-situ function testing and necropsy (Fig. 1). A subcuticular placement of 4-0 Vicryl suture was used to close the skin incision. Each animal was recovered from anesthesia and was returned to the housing unit.

Each rat received 0.02 mg Buprenex (buprenorphine hydrochloride) by subcutaneous injection the day of surgery and for two additional days. Baytril (20 mg) was given orally the day of surgery and for two additional days. The dietary habits, general health status, and the surgical site were monitored daily and recorded.

Measurement of Contractile Force In-Situ

Muscle Flap Preparation—Twenty-six weeks after the initial surgical procedure, each animal was prepared for function testing of the implant site. Twenty-six weeks was chosen

as the timepoint for analysis based upon previous studies in our laboratory which show that the remodeling process is complete within this period of time and that the amount of skeletal muscle present has been maximized. Each rat was anesthetized with 2% isoflurane in oxygen by inhalation. A midline skin incision was created, and the skin and overlying fascia was dissected from the underlying body wall at the site of test article implantation. The site of scaffold placement was identified by the preplaced Prolene sutures at the four corners of the test article. A flap of tissue containing the oblique layers of the abdominal wall was created which contained the site of scaffold placement surrounded by no more than 2 mm of adjacent native musculature. The origin and rib attachment of the external oblique muscle remained intact to maintain blood flow to the remodeled test article site. The muscle fibers on all remaining sides of the implantation site, including the insertion site at the linea alba and the underlying surface adjacent to the transversus fascia and peritoneum were dissected free of all tissue attachments to isolate the tissue flap for contractile testing (Fig. 1). The contralateral site, which did not receive a test article and was not surgically modified, was prepared in identical fashion to serve as a control for each animal. Baseline tensiometric data measurements at time 0 were attempted in native tissue that had the oblique muscle layers removed as described. No contractile forces were generated in any of the specimens that were treated as such.

The isolated tissue flap was positioned such that the direction of the muscle contraction was aligned along an axis from the costochondral arch toward the linea alba. Platinum electrodes were placed across the flap of tissue, at the proximal and distal anastomoses of the implant site, perpendicular to the direction of muscle contraction as identified by the Prolene sutures. The distal (previous insertion end) tissue was secured to a force transducer (Sensotec, Columbus, OH; model 31) with silk suture. An analog to digital data acquisition card (National Instruments, Austin, TX; PCI-6023E) received signals from the force transducer. A custom-designed program was created in LabVIEW software (National Instruments) to monitor and record the forces measured by the force transducer.

Contractile Force Testing—The isolated tissue flap was stimulated with a S88X Grass stimulator (Astro-Med, Inc., West Warwick, RI). Resting length L_R was measured, and optimum length L_O was determined as the length at which the maximum twitch force was obtained using a 2 ms pulse at 50 pulses per second (pps). The tetanic force (P_{t-MAX}) generated for the test site ($P_{t,S}$) and the contralateral native tissue ($P_{t,N}$) as a function of stimulation rate was determined by delivering 5, 10, 15, 20, 30, 40, 60, and 75 pps in 1-second trains with 2 minutes of rest between each test.[33]. The maximal contractile force for each test article was then calculated and specific force was calculated for P_{t-MAX} by normalizing with the cross-sectional area of the tissue. Thickness was determined using a digital micrometer (Mitutoyo, Japan).

Fatigue Resistance Testing—The fatigue resistance of the tissue at the test site was determined by comparing the force generated at time $t = 0$ to $t = 2$ minutes. Pulses were delivered at the minimum frequency at which the forces plateau during the tetanic force testing, in trains of 330 ms duration at a rate of 1 train per second, for a total of two minutes. The resistance to fatigue (Fatigue Index) was calculated as the ratio of the residual force at $t = 2$ to the initial force at $t = 0$.

Euthanasia and Specimen Harvest

Following completion of the function testing, each rat was euthanized with 5% isoflurane in oxygen followed by an intracardiac injection of 5 mL of potassium chloride to induce cardiac arrest. The muscle flap was excised at the rib origin, mounted on a fixed support

structure to maintain the size and shape of the tissue, and placed in 10% neutral buffered formalin (NBF).

Histologic and Immunohistochemical Analysis

The NBF-fixed specimen was sectioned perpendicular to the direction of contractile function testing at the implantation site in four separate equally spaced locations proximal to distal. The tissue was embedded in paraffin, cut into 6- μ m thick sections, and mounted for hematoxylin and eosin (H&E) or Masson's trichrome staining and immunolabeling analysis. The primary antibodies used for the immunolabeling studies were: (1) monoclonal antimyosin (skeletal, slow, clone NOQ7.5.4D; Sigma-Aldrich, St. Louis, MO) at 1:4000 dilution for identification of slow (type I) skeletal muscle fibers; (2) monoclonal antimyosin (skeletal, fast, alkaline phosphatase conjugate, clone MY-32, Sigma-Aldrich) at 1:200 dilution for identification of fast (type II) skeletal muscle fibers; (3) rabbit polyclonal von Willebrand factor (Abcam, Cambridge, MA) at 1:1000 dilution for identification of endothelial cells and blood vessels; and (4) anti-neurofilament (DakoUSA, Carpinteria, CA) at 1:100 dilution for identification of neurons. The secondary antibodies used were biotinylated anti-mouse IgG (H+L, rat absorbed) (Vector, Burlingame, CA) at 1:200 dilution, and goat anti-rabbit IgG (H+L, rat absorbed) (Vector) at 1:200 dilution.

The antibodies to the fast and slow skeletal muscle fibers were visualized with two substrate detection stains on each section. Separate tissue sections were used for the von Willebrand factor and neurofilament staining.

The following protocol was used to identify fast and slow muscle fibers. Unstained sections were deparaffinized with xylene and rehydrated through a graded ethanol series. Heat-mediated antigen retrieval was performed with 0.1 mM EDTA buffer at 95-100 °C for 25 minutes. After cooling for 15 minutes, enzyme-mediated antigen retrieval was performed with 0.1% trypsin/0.1% calcium chloride digestion at 37°C for 10 minutes. Three washes in Tris buffered saline (TBS), pH 7.6, were then performed. The slides were incubated for 10 minutes in a blocking solution containing 2% normal horse serum (Vector) and 2% normal goat serum (Vector) at room temperature to prevent non-specific antibody binding. To inhibit endogenous peroxidase activity, the slides were incubated with 3% hydrogen peroxide (Spectrum, New Brunswick, NJ) in methanol for 10 minutes at room temperature. The slow anti-myosin antibody was applied for 30 minutes at room temperature. The slides were then incubated with the secondary antibody for 30 minutes at room temperature, followed by horseradish peroxidase solution (Vector) for 30 minutes at 37°C. Diaminobenzidine (DAB) (Vector) was applied to detect positive staining cells. The blocking solution was then applied again for 10 minutes at room temperature, followed by the fast anti-myosin antibody for 60 minutes at room temperature. Red alkaline phosphatase substrate was applied to detect positive staining.

The following protocol was used to stain for von Willebrand factor and neurofilament. After deparaffinization and rehydration of the sections as described above, heat-mediated antigen retrieval was performed with 0.01M citrate buffer (Spectrum), pH 6.0, at 95-100 °C for 20 minutes. After cooling for 15 minutes, enzyme-mediated antigen retrieval was performed for the staining of von Willebrand factor using proteinase K in a buffer of 50mM Tris base and 1 mM EDTA, pH 8.0, at 60°C for 10 minutes. Three washes in phosphate buffered saline (PBS) were then performed. The slides were incubated for 30 minutes with 2% normal horse serum (Vector) at 37°C to prevent non-specific antibody binding. To inhibit endogenous peroxidase activity, the slides were incubated with 3% hydrogen peroxide (Spectrum) in methanol for 30 minutes at room temperature. The slides were incubated with the secondary antibody for 30 minutes at 37°C, followed by horseradish peroxidase solution (Vector) for

30 minutes at 37°C. Diaminobenzidine (DAB) (Vector) was applied to detect positive staining cells and the slides were counterstained with hematoxylin.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed, followed by the Tukey's *post hoc* test to determine significant differences between test articles for P_{t-MAX} , Specific Force, Fatigue Index, and the quantitative immunohistochemical analysis of blood vessels and muscle fibers. A *p* value of less than 0.05 was considered to be significant.

Results

No surgical or technical complications occurred during the study, and all animals were healthy and survived to the assigned timepoint of 26 weeks after surgery. The host/implant interface was well-integrated both by macroscopic and microscopic examination for all test articles.

Contractility Testing

Tetanic contractile responses were observed in all of the contralateral native tissue specimens and all of the tissue specimens that were harvested from site in which the Restore™ device was implanted (Fig. 2A). When normalized for cross-sectional area, the specific forces of the sites implanted with Restore™ were similar to the native tissue (Fig. 2B; Table 1). Tetanic responses were recorded in four out of eight specimens in which the CuffPatch™ device was placed and in six out of eight specimens harvested from sites containing the autologous graft was placed (Fig. 2A). None of the specimens in which the polypropylene mesh was placed showed any measurable tetanic response.

There was no significant difference ($P = 0.62$) in the fatigue of tissue repaired with the CuffPatch™ device and the Restore™ device with each retaining approximately 35% and 40% respectively of the initial contractile force following prolonged stimulation. Tissues repaired with the autologous tissue and the contralateral native tissue showed greater muscle fatigue than either the CuffPatch™ or Restore™ repaired tissue, generating approximately 20% and 15.5% of their initial contractile force respectively. There was no significant difference ($P = 0.67$) between the relative fatigue of tissue repaired with autologous tissue and the contralateral native tissue but both were significantly different to tissues repaired with the Restore™ device ($P \leq 0.05$). Tissues repaired with the CuffPatch™ device also showed a significantly greater resistance to fatigue compared to contralateral native tissue ($P \leq 0.05$) (Table 1).

Histomorphologic and Immunolabeling Findings

Native tissue—The native tissue harvested from the contralateral abdominal wall showed myofibers that were organized into muscle bundles, with clearly identifiable internal and external oblique layers and sparse amounts of interspersed collagenous connective tissue and adipose connective tissue (Fig. 3A). Mature blood vessels and capillaries (Fig. 4A) and nerve fibers (Fig. 5A) were located adjacent to the muscle bundles. The number of blood vessels and muscle types (i.e., slow type I and fast type II) were quantified by positive immunolabeling (Table 2). The native tissue showed significantly fewer blood vessels and a higher density of slow type I fibers compared to the test articles (Table 2). The majority of the myofibers were immunopositive for fast type II myosin, and the fibers that stained positive for slow type I myosin were evenly distributed between the fast fibers (Fig. 6A).

Restore™ device—The surgical site in which the Restore™ device was placed showed no histologic evidence of the originally implanted device 26 weeks after implantation. There

was no noticeable decrease in size of the implant site with the marker sutures still placed 1.5 cm apart. The implant site contained new host tissue that consisted of organized bundles of skeletal muscle cells, and partially organized collagenous connective tissue with adipose connective tissue surrounding the muscle bundles (Fig. 3B). There was no clear distinction between internal and external oblique layers and no evidence of inflammation. A moderate number of mature vessels and capillaries (Fig. 4B; Table 2) and nerve fibers (Fig. 5B) were located adjacent to the muscle bundles. Similar to the native tissue, the majority of the muscle fibers stained positive for fast type II myosin, and the muscle fibers that stained positive for slow type I myosin were dispersed uniformly among the fast fibers (Fig. 6B; Table 2).

CuffPatch™ device—At 26 weeks, the surgical site in which the CuffPatch™ device was placed showed that remnants of the original device were surrounded by mononuclear cells (Fig. 3C). There was no visible decrease in size of the implant site. The tissue surrounding the implantation site contained a robust capillary presence (Fig. 4C; Table 2) and densely organized collagenous connective tissue. Neurons were identified adjacent to the blood vessels at the host-implant interface (Fig. 5C), but no nerve fibers were identified within the implantation site. The muscle fibers were located only around the periphery of the scaffold with lower numbers of fast type II fibers compared to native tissue (Fig. 6C; Table 2).

Polypropylene mesh—The surgical site in which the polypropylene mesh was placed showed no evidence of mesh degradation. The immediate area surrounding the mesh fibers contained inflammatory cells including mononuclear cells and was composed of loosely organized collagenous connective tissue (Fig. 3D). A large number of blood vessels were located in the collagenous tissue surrounding the mesh fibers, but no neurons were identified in the area of device placement (Fig. 4D, 5D; Table 2). No muscle fibers were identified within the site of polypropylene mesh placement (Table 2).

Autologous tissue graft—The surgical site in which the autologous tissue graft was placed showed scattered islands of muscle fibers that were surrounded by a small amount of collagenous connective tissue and abundant adipose tissue (Fig. 3E). There was no evidence of inflammation and no decrease in size of the implant site. The new muscle bundles and adipose tissue contained nerve fibers and a moderate number of mature blood vessels and capillaries (Fig. 4E, 5E; Table 2). There were similar numbers of fast type II fibers when compared to native tissue, with fewer slow type I muscle fibers all of which were organized in clusters among the type II muscle fibers (Fig. 6D; Table 2).

Discussion

A number of strategies have been applied to engineer contractile skeletal muscle tissue using in-vitro or in-vivo ECM-based approaches[34-37]. Pioneering work has been conducted by Vanderburgh and colleagues[38] in which a mechanical cell stimulator device was designed to apply mechanical stimulation to “human bioartificial muscles” (i.e., skeletal muscle cells suspended in Matrigel:collagen). This approach resulted in improved myofiber diameter and alignment, increased elasticity of the muscle fibers, and improved cell-generated passive force compared to static cultures. A study by Borschel et al. showed that constructs composed of C2C12 myoblasts (satellite cells of C3H mice) seeded onto an acellular scaffold harvested from the mouse extensor digitorum longus muscle produced specific forces that were approximately 5% of that observed for the native muscle after 3 weeks of culture.[39] In a similar fashion, Christ and colleagues seeded primary human muscle precursor cells onto acellular bladder submucosa and subjected the constructs to cyclic strain for up to 4 weeks, followed by subcutaneous implantation of the cell-seeded construct onto the latissimus dorsi of mice. This study showed that specific forces of 1% of that observed

for native latissimus dorsi were produced when the constructs were preconditioned in an in-vitro bioreactor.[37] In distinct contrast, the present study showed that denovo formation of vascularized and innervated contractile skeletal muscle is possible without prior cell or mechanical preconditioning through the placement of a non-chemically crosslinked ECM scaffold.

The implant materials used in this study were chosen since they represent the spectrum of possible surgical solutions to soft tissue or musculoskeletal repair. The materials included an autologous tissue graft, decellularized ECM and synthetic non-degradable polymer scaffolds. While each scaffold material is a commercial available option for surgery, the differences in the tissue remodeling outcomes are striking. The surgical placement of polypropylene mesh and chemically-crosslinked SIS (CuffPatch™) resulted in the formation of little or no new muscle fibers and generated little to no contractile forces. These functional and morphologic results for polypropylene and CuffPatch™ are not surprising and confirm the findings of other studies.[31,40-42] Although the polypropylene group contained only 2 animals, the host response to polypropylene surgical mesh materials has been exhaustively characterized and the fibrous connective tissue response observed in the present study was consistent with the expected findings.[43,44] Autologous tissue may logically represent the ideal implant material as it most closely represents the native tissue. However, previous studies have shown that implantation of autologous tissue results in a pro-inflammatory response and necrosis of the skeletal muscle fibers within seven days, and complete resorption of the autograft muscle and replacement with connective tissue by 28 days[45]. In the present study the autologous implant showed a strong inflammatory response and the remodeled tissue generated significantly less contractile force than the uninjured muscle. While some skeletal muscle fibers were seen in the autologous graft at 6 months it is likely, given the previous data, that these are new skeletal muscle fibers that have replaced the original autograft rather than muscle fibers from the original implant. Interestingly, the non-crosslinked SIS-ECM promoted the formation of new, organized skeletal muscle that was vascularized and contractile, and which generated a maximal force similar to native tissue. Without intervention, injuries of this size would result in significant scar tissue formation and loss of function. [46,47]

Previous preclinical studies have shown that bioscaffolds derived from mammalian ECM can effect constructive remodeling of skeletal,[16] cardiac,[3,48] and smooth[18,49-51] muscle tissue. A study by Boruch et al. utilized a canine model of partial cystectomy to show that exposing ECM scaffolds to a physiologically-appropriate pattern of mechanical loading (cyclic distention) caused tissue remodeling that can be characterized by complete urothelialization along the luminal surface, vascularity, innervation, and islands of smooth muscle bundles.[18] In a study conducted by Cook and colleagues, suture repair with SIS augmentation following extensor digitorum longus laceration in a rabbit model showed that the SIS-remodeled tissue resembled native tissue and produced active contractile forces that approached normal tissue.[30] These studies in combination with the present study support the concept that the host remodeling response caused by SIS scaffold placement is associated with the site appropriate function of the newly formed muscle tissue. The mechanism by which the degradable SIS scaffold induced the formation of functional skeletal muscle tissue is unclear. However, the activation of adjacent tissue satellite cells or muscle-derived stem cells,[52] the recruitment and differentiation of circulating stem or progenitor cells to the site of injury,[52] and the role of bioactive molecules originating with the SIS scaffold material should all be considered. Furthermore, the release of signaling molecules as a result of SIS scaffold degradation may work in concert with other signals such as growth factors, to promote cell proliferation, migration and differentiation of tissue resident or circulating bone marrow-derived progenitor cells. [53-56] Beattie and colleagues investigated the chemotactic properties of ECM scaffolds harvested during the first 14 days

after repair in a murine model of Achilles tendon reconstruction.[55] The results showed that multipotential progenitor cells preferentially migrated toward the tendons repaired with urinary bladder matrix (UBM)-ECM compared to tendons repaired with autologous tissue or uninjured normal tendons. The source of cells that participated in the remodeling of new contractile skeletal tissue was not a focus of the current study, but is of interest and importance for future investigations.

Critical processes in the restoration of skeletal muscle are revascularization and reinnervation. In the present study, we observed that the skeletal muscle tissue that formed within the initially acellular CuffPatch™ and Restore™ devices contained a complex neurovascular bed adjacent to the muscle tissue; an anatomical arrangement that would provide the oxygen, nutrients and innervation required by functional skeletal muscle. Indeed, the Restore™ device and to lesser extend the autologous implant both contained neural and capillary networks akin to mature uninjured muscle. However, it is important to note that while the remodelled tissue showed positive staining for nerves, functional innervation of the remodeled tissue was not tested in this study.

The contractility testing performed in this study was achieved by direct electrical stimulation of the skeletal muscle. Propagation of this electrical impulse through the skeletal muscle membrane caused the contractility observed in this study. While nerves may have assisted in conducting the electrical impulse they did not initiate muscle depolarization. Hence, while all the implants were surrounded by at 2 mm strip of native muscle this is unlikely to have contributed significantly to the contractility results unless these muscle fibers extended through the site of implantation. The organization and morphologic characteristics of the remodeled tissue relative to the native tissue explains why no contractility was observed in the polypropylene mesh group since this material effectively acted as an insulator between the two electrodes preventing propagation of the electrical impulses.

In addition to differences in the contractility of the new tissue that formed at the different implant sites, there were also significant differences in the fatigability of the new muscle tissue. Skeletal muscle consists of a mixture of types I and II muscle fibers, and the fiber type composition and distribution can affect the speed of contraction, fatigability, and degree of force production. Slow type I fibers have slower maximum shortening velocities, lower force production, and are more resistant to fatigue when compared to fast type II fibers.[57] In the present study, muscle fiber content from sites repaired with the non-crosslinked SIS scaffolds showed similar percentages of slow type I fibers to the repair sites of the autologous tissue graft but a lower percentage of slow type I fibers compared to the native tissue. However, the noncrosslinked SIS device showed a higher fatigue resistance than the native tissue. Similarly, samples repaired with cross-linked SIS scaffolds also showed a higher fatigue resistance than the native tissue despite similar ratios of type I and II fibers. While these findings contradict the slow type I fiber data which would suggest a much lower fatigue resistance this study did not distinguish between type IIa and type IIb fibers within the muscle samples. Type IIa fibers can use both aerobic and anaerobic metabolism to produce muscle contraction. It is possible that in the CuffPatch™ and Restore™ samples the numbers of type IIa fibers compared to type IIb fibers was higher. In addition the increased vascularity of both the CuffPatch™ and Restore™ devices would likely increase both the supply of oxygen and glucose to the tissues, compared to native tissue, while increasing the removal of lactate, which may account for a delayed to onset of fatigue in these muscle samples resulting in a higher fatigue index.

An alternative explanation of the results of the present study is that the various test articles simply impeded default muscle tissue healing to different extents. For example, it has been shown that non-crosslinked SIS materials, such as Restore, are rapidly degraded following

in-vivo placement. Rapid disappearance of the scaffold may provide the least impedance to healing of skeletal muscle defects. In contrast, CuffPatch and polypropylene mesh effectively filled the defect and prevented healing at the site of surgical placement while eliciting a foreign body response. A temporal evaluation of the structural and functional remodeling events in the present study was not conducted nor was a control group included in which no test article was placed. The same animal model and the same or similar test articles have been used to investigate the morphologic changes associated with degradation of the scaffolds from 2 days to 16 weeks post-surgery[31,32,58] and the downstream histomorphologic results of the current study are consistent with those of the previous studies.

Conclusion

This study showed that biologic scaffolds composed of non-crosslinked SIS or an autologous tissue graft can support the reconstruction of functional skeletal muscle tissue in this rat model of body wall repair. This new muscle tissue was morphologically similar but not identical to native skeletal muscle, and was innervated, vascularized, and consisted of a mixture of types I and II muscle fibers in relative proportions that differed only slightly from native skeletal muscle.

Acknowledgments

Funding for this study was provided by grants from the National Institutes of Health (5R01 AR054940-03) and the Armed Forces Institute for Regenerative Medicine (W81XWH-08-2-0032).

Bibliography

1. Akhyari P, Kamiya H, Haverich A, Karck M, Lichtenberg A. Myocardial tissue engineering: the extracellular matrix. *Eur J Cardiothorac Surg* 2008;34:229–41. [PubMed: 18502661]
2. Badylak SF, Kochupura PV, Cohen IS, Doronin SV, Saltman AE, Gilbert TW, et al. The use of extracellular matrix as an inductive scaffold for the partial replacement of functional myocardium. *Cell Transplant* 2006;15 1:S29–40. [PubMed: 16826793]
3. Kochupura PV, Azeloglu EU, Kelly DJ, Doronin SV, Badylak SF, Krukenkamp IB, et al. Tissue-engineered myocardial patch derived from extracellular matrix provides regional mechanical function. *Circulation* 2005;112:1144–9. [PubMed: 16159807]
4. Nieponice A, Gilbert TW, Badylak SF. Reinforcement of esophageal anastomoses with an extracellular matrix scaffold in a canine model. *Ann Thorac Surg* 2006;82:2050–8. [PubMed: 17126109]
5. Nieponice A, McGrath K, Qureshi I, Beckman EJ, Luketich JD, Gilbert TW, et al. An extracellular matrix scaffold for esophageal stricture prevention after circumferential EMR. *Gastrointest Endosc* 2008;69:289–96. [PubMed: 18657808]
6. Brigido SA, Boc SF, Lopez RC. Effective management of major lower extremity wounds using an acellular regenerative tissue matrix: a pilot study. *Orthopedics* 2004;27:s145–9. [PubMed: 14763548]
7. Aurora A, McCarron J, Iannotti JP, Derwin K. Commercially available extracellular matrix materials for rotator cuff repairs: state of the art and future trends. *J Shoulder Elbow Surg* 2007;16:S171–8. [PubMed: 17560804]
8. De Deyne PG, Kladakis SM. Bioscaffolds in tissue engineering: a rationale for use in the reconstruction of musculoskeletal soft tissues. *Clin Podiatr Med Surg* 2005;22:521–32. v. [PubMed: 16213377]
9. Dejardin LM, Arnoczky SP, Ewers BJ, Haut RC, Clarke RB. Tissue-engineered rotator cuff tendon using porcine small intestine submucosa. Histologic and mechanical evaluation in dogs. *Am J Sports Med* 2001;29:175–84. [PubMed: 11292042]

10. Snyder SJ, Arnoczky SP, Bond JL, Dopirak R. Histologic evaluation of a biopsy specimen obtained 3 months after rotator cuff augmentation with GraftJacket Matrix. *Arthroscopy* 2009;25:329–33. [PubMed: 19245998]
11. Derwin KA, Baker AR, Spragg RK, Leigh DR, Iannotti JP. Commercial extracellular matrix scaffolds for rotator cuff tendon repair. Biomechanical, biochemical, and cellular properties. *J Bone Joint Surg Am* 2006;88:2665–72. [PubMed: 17142417]
12. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006;27:3675–83. [PubMed: 16519932]
13. Hodde J, Janis A, Ernst D, Zopf D, Sherman D, Johnson C. Effects of sterilization on an extracellular matrix scaffold: part I. Composition and matrix architecture. *J Mater Sci Mater Med* 2007;18:537–43. [PubMed: 17546412]
14. Jarman-Smith ML, Bodamyali T, Stevens C, Howell JA, Horrocks M, Chaudhuri JB. Porcine collagen crosslinking, degradation and its capability for fibroblast adhesion and proliferation. *J Mater Sci Mater Med* 2004;15:925–32. [PubMed: 15477745]
15. Liang HC, Chang Y, Hsu CK, Lee MH, Sung HW. Effects of crosslinking degree of an acellular biological tissue on its tissue regeneration pattern. *Biomaterials* 2004;25:3541–52. [PubMed: 15020128]
16. Badylak S, Kokini K, Tullius B, Whitson B. Strength over time of a resorbable bioscaffold for body wall repair in a dog model. *J Surg Res* 2001;99:282–7. [PubMed: 11469898]
17. Badylak SF, Gilbert TW. Immune response to biologic scaffold materials. *Semin Immunol* 2008;20:109–16. [PubMed: 18083531]
18. Boruch AV, Nieponice A, Qureshi IR, Gilbert TW, Badylak SF. Constructive remodeling of biologic scaffolds is dependent on early exposure to physiologic bladder filling in a canine partial cystectomy model. *J Surg Res* 2009;161:217–25. [PubMed: 19577253]
19. Liang R, Woo SL, Takakura Y, Moon DK, Jia F, Abramowitch SD. Long-term effects of porcine small intestine submucosa on the healing of medial collateral ligament: a functional tissue engineering study. *J Orthop Res* 2006;24:811–9. [PubMed: 16514641]
20. Sacks MS, Gloeckner DC. Quantification of the fiber architecture and biaxial mechanical behavior of porcine intestinal submucosa. *J Biomed Mater Res* 1999;46:1–10. [PubMed: 10357130]
21. Freytes DO, Stoner RM, Badylak SF. Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds. *J Biomed Mater Res B Appl Biomater* 2008;84:408–14. [PubMed: 17618508]
22. Freytes DO, Tullius RS, Valentin JE, Stewart-Akers AM, Badylak SF. Hydrated versus lyophilized forms of porcine extracellular matrix derived from the urinary bladder. *J Biomed Mater Res A* 2008;87:862–72. [PubMed: 18228251]
23. Gilbert TW, Wognum S, Joyce EM, Freytes DO, Sacks MS, Badylak SF. Collagen fiber alignment and biaxial mechanical behavior of porcine urinary bladder derived extracellular matrix. *Biomaterials* 2008;29:4775–82. [PubMed: 18801572]
24. Ko R, Kazacos EA, Snyder S, Ernst DM, Lantz GC. Tensile strength comparison of small intestinal submucosa body wall repair. *J Surg Res* 2006;135:9–17. [PubMed: 16650864]
25. Record RD, Hillegonds D, Simmons C, Tullius R, Rickey FA, Elmore D, et al. In vivo degradation of ¹⁴C-labeled small intestinal submucosa (SIS) when used for urinary bladder repair. *Biomaterials* 2001;22:2653–9. [PubMed: 11519785]
26. Gilbert TW, Stewart-Akers AM, Simmons-Byrd A, Badylak SF. Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *J Bone Joint Surg Am* 2007;89:621–30. [PubMed: 17332112]
27. Badylak S, Arnoczky S, Plouhar P, Haut R, Mendenhall V, Clarke R, et al. Naturally occurring extracellular matrix as a scaffold for musculoskeletal repair. *Clin Orthop Relat Res* 1999;367:S333–43. [PubMed: 10546657]
28. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomater* 2009;5:1–13. [PubMed: 18938117]
29. Badylak SF, Tullius R, Kokini K, Shelbourne KD, Klootwyk T, Voytik SL, et al. The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. *J Biomed Mater Res* 1995;29:977–85. [PubMed: 7593041]

30. Crow BD, Haltom JD, Carson WL, Greene WB, Cook JL. Evaluation of a novel biomaterial for intrasubstance muscle laceration repair. *J Orthop Res* 2007;25:396–403. [PubMed: 17106884]
31. Valentin JE, Badylak JS, McCabe GP, Badylak SF. Extracellular matrix bioscaffolds for orthopaedic applications. A comparative histologic study. *J Bone Joint Surg Am* 2006;88:2673–86. [PubMed: 17142418]
32. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials* 2008;30:1482–91. [PubMed: 19121538]
33. Watchko JF, Daood MJ, Vazquez RL, Brozanski BS, LaFramboise WA, Guthrie RD, et al. Postnatal expression of myosin isoforms in an expiratory muscle--external abdominal oblique. *J Appl Physiol* 1992;73:1860–6. [PubMed: 1474062]
34. Khodabukus A, Baar K. Regulating fibrinolysis to engineer skeletal muscle from the C2C12 cell line. *Tissue Eng Part C Methods* 2009;15:501–11. [PubMed: 19191517]
35. Huang YC, Dennis RG, Larkin L, Baar K. Rapid formation of functional muscle in vitro using fibrin gels. *J Appl Physiol* 2005;98:706–13. [PubMed: 15475606]
36. Dhawan V, Lytle IF, Dow DE, Huang YC, Brown DL. Neurotization improves contractile forces of tissue-engineered skeletal muscle. *Tissue Eng* 2007;13:2813–21. [PubMed: 17822360]
37. Moon du G, Christ G, Stitzel JD, Atala A, Yoo JJ. Cyclic mechanical preconditioning improves engineered muscle contraction. *Tissue Eng Part A* 2008;14:473–82. [PubMed: 18399787]
38. Powell CA, Smiley BL, Mills J, Vandeburgh HH. Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am J Physiol Cell Physiol* 2002;283:C1557–65. [PubMed: 12372817]
39. Borschel GH, Dennis RG, Kuzon WM Jr. Contractile skeletal muscle tissue-engineered on an acellular scaffold. *Plast Reconstr Surg* 2004;113:595–602. discussion 3-4. [PubMed: 14758222]
40. Druecke D, Lamme EN, Hermann S, Pieper J, May PS, Steinau HU, et al. Modulation of scar tissue formation using different dermal regeneration templates in the treatment of experimental full-thickness wounds. *Wound Repair Regen* 2004;12:518–27. [PubMed: 15453834]
41. Konstantinovic ML, Lagae P, Zheng F, Verbeken EK, De Ridder D, Deprest JA. Comparison of host response to polypropylene and non-cross-linked porcine small intestine serosal-derived collagen implants in a rat model. *BJOG* 2005;112:1554–60. [PubMed: 16225578]
42. Macleod TM, Williams G, Sanders R, Green CJ. Histological evaluation of Permacol as a subcutaneous implant over a 20-week period in the rat model. *Br J Plast Surg* 2005;58:518–32. [PubMed: 15897038]
43. Krause HG, Galloway SJ, Khoo SK, Lourie R, Goh JT. Biocompatible properties of surgical mesh using an animal model. *Aust N Z J Obstet Gynaecol* 2006;46:42–5. [PubMed: 16441692]
44. Bellon JM, Rodriguez M, Garcia-Honduvilla N, Gomez-Gil V, Pascual G, Bujan J. Comparing the behavior of different polypropylene meshes (heavy and lightweight) in an experimental model of ventral hernia repair. *J Biomed Mater Res B Appl Biomater* 2009;89B:448–55. [PubMed: 18837449]
45. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials* 2008;30:1482–91. [PubMed: 19121538]
46. Jarvinen M. Healing of a crush injury in rat striated muscle. 2. a histological study of the effect of early mobilization and immobilization on the repair processes. *Acta Pathol Microbiol Scand [A]* 1975;83:269–82.
47. Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. *Am J Sports Med* 2005;33:745–64. [PubMed: 15851777]
48. Ota T, Gilbert TW, Badylak SF, Schwartzman D, Zenati MA. Electromechanical characterization of a tissue-engineered myocardial patch derived from extracellular matrix. *J Thorac Cardiovasc Surg* 2007;133:979–85. [PubMed: 17382638]
49. Kropp BP, Sawyer BD, Shannon HE, Rippey MK, Badylak SF, Adams MC, et al. Characterization of small intestinal submucosa regenerated canine detrusor: assessment of reinnervation, in vitro compliance and contractility. *J Urol* 1996;156:599–607. [PubMed: 8683741]

50. Badylak SF, Vorp DA, Spievack AR, Simmons-Byrd A, Hanke J, Freytes DO, et al. Esophageal reconstruction with ECM and muscle tissue in a dog model. *J Surg Res* 2005;128:87–97. [PubMed: 15922361]
51. Lopes MF, Cabrita A, Ilharco J, Pessa P, Paiva-Carvalho J, Pires A, et al. Esophageal replacement in rat using porcine intestinal submucosa as a patch or a tube-shaped graft. *Dis Esophagus* 2006;19:254–9. [PubMed: 16866856]
52. Ehrhardt J, Morgan J. Regenerative capacity of skeletal muscle. *Curr Opin Neurol* 2005;18:548–53. [PubMed: 16155438]
53. Reing JE, Zhang L, Myers-Irvin J, Cordero KE, Freytes DO, Heber-Katz E, et al. Degradation products of extracellular matrix affect cell migration and proliferation. *Tissue Eng Part A* 2008;15:605–14. [PubMed: 18652541]
54. Li F, Li W, Johnson S, Ingram D, Yoder M, Badylak S. Low-molecular-weight peptides derived from extracellular matrix as chemoattractants for primary endothelial cells. *Endothelium* 2004;11:199–206. [PubMed: 15370297]
55. Beattie AJ, Gilbert TW, Guyot JP, Yates AJ, Badylak SF. Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds. *Tissue Eng Part A* 2008;15:1119–25. [PubMed: 18837648]
56. Brennan EP, Tang XH, Stewart-Akers AM, Gudas LJ, Badylak SF. Chemoattractant activity of degradation products of fetal and adult skin extracellular matrix for keratinocyte progenitor cells. *J Tissue Eng Regen Med* 2008;2:491–8. [PubMed: 18956412]
57. Koning M, Harmsen MC, van Luyn MJ, Werker PM. Current opportunities and challenges in skeletal muscle tissue engineering. *J Tissue Eng Regen Med* 2009;3:407–15. [PubMed: 19575392]
58. Badylak S, Kokini K, Tullius B, Simmons-Byrd A, Morff R. Morphologic study of small intestinal submucosa as a body wall repair device. *J Surg Res* 2002;103:190–202. [PubMed: 11922734]

Figure 1.

Top: the musculoskeletal defect was created by excising the external and internal oblique layers of the abdominal wall, leaving the transversus fascia intact. Middle: the test article was implanted in the defect site and secured with Prolene sutures at each of the four corners. Bottom: twenty-six weeks post-implantation, a flap of tissue was created which contained the site of test article placement, identified by the preplaced Prolene sutures. The tissue flap maintained the integrity of the muscular arteries and the thoracic spinal nerve branches that supplied the site of tissue remodeling. The dense connective tissue at the insertion site at the linea alba was connected to the force transducer with silk suture, and positioned such that the direction of the contractile function testing was aligned parallel to the rib origin.

Platinum electrodes were placed across the flap proximal and distal to the scaffold placement site.

Figure 2.

Tetanic forces of remodeled implants. In-situ muscle contraction studies were performed on muscle flaps from implants at 26 weeks post-surgery and compared to contralateral, native muscle. (A) The tetanic forces (N) generated by remodeled and native tissue following electrical stimulation between 5 and 75 pps. (B) Contractile forces (N/cm²) of tissue, which peaked at 40 pps for the native tissue, and 50 pps for the remodeled tissue. Data expressed as mean $\pm$ standard error.

Figure 3.

Histologic images of the native muscle tissue and treated remodeled tissue at 26 weeks post surgery. The native tissue (A) was composed mainly of muscle cells organized into well-defined bundles with adjacent mature blood vessels (open arrows) and nerves (closed arrows). The site in which the Restore device was implanted (B) showed new host tissue comprised of bundles of muscle fibers surrounded by vascularized, organized collagenous connective tissue. The site of CuffPatch device implantation (C) showed identifiable remnants of the originally implanted device (asterisks), which were surrounded by dense collagenous tissue, numerous blood vessels, and inflammatory cells. The site in which the polypropylene mesh material was implanted (D) showed highly vascularized, loosely organized connective tissue surrounded the mesh (polymer was displaced from tissue sections due to histologic processing). The site implanted with the excised autologous tissue (E) showed randomly dispersed muscle bundles surrounded by fibrous connective tissue and adipose connective tissue. Tissue sections were stained with Masson's trichrome. Scale bars = 300 μ m.

Figure 4.

Blood vessels distribution of native and remodeled tissue. Tissue sections at 6 months post surgery were immunolabeled for Von Willibrand Factor to identify endothelial cells associated with the lumen of blood vessels in the native and remodeled tissue sections. Mature blood vessels (A) were located adjacent to the muscle bundles for the native tissue. The tissue repaired with the Restore device (B), and the tissue repaired with the autologous graft (E) showed that larger blood vessels were identified throughout the implantation site. The remnants of the original devices (indicated by *) for CuffPatch (C) and polypropylene mesh (D) were surrounded by a substantial amount of capillaries and larger vessels. Scale bars = 300 μm .

Figure 5.

Innervation of native and remodeled tissue. Tissue sections at 6 months post surgery were immunolabeled for anti-neurofilament to identify nerve structures in the native and remodeled tissue sections. Nerves (A) were located adjacent to the muscle bundles for the native tissue. The tissue repaired with the Restore device (B), and the tissue repaired with the autologous graft (E) showed nerves adjacent to the new muscle cells. Individual neurons were located in close proximity to new blood vessels that surrounded the remnants of the CuffPatch device (indicated by *) (C), but no neurons were detected near the polypropylene mesh (indicated by *) (D). Scale bars = 300 μm .

Figure 6.

Distribution of slow and fast skeletal muscle fibers in native tissue and remodeled tissue at 26 weeks post surgery. Tissue sections were double-labeled for antimyosin slow (type I) skeletal muscle (brown stain) and antimyosin fast (type II) skeletal muscle (red stain). The native tissue (A) and the tissue repaired with the Restore device (B) showed that the slow muscle fibers were uniformly distributed between the fast muscle fibers. Skeletal muscle fibers surrounded the proximal edges of the CuffPatch device (C) but were not located within the graft site. (D) In the autologous tissue graft, the slow muscle fibers were distributed in an irregular pattern among the fast muscle fibers in the remodeling site, in that the slow fibers were sporadically distributed in some areas and arranged in clusters for other areas. The polypropylene mesh did not show staining for fast or slow skeletal muscle (figure not shown). Scale bars = 300 μ m.

Table 1

The contractile properties for each scaffold group and the contralateral native tissue

Scaffold	$\frac{L_o - L_R}{L_o}$	Cross-sectional area (cm ²)	Maximum Tetanic Force (N)	Specific Force (N/cm ²)	Fatigue Index
Restore™	0.248 ± 0.056	0.181 ± 0.218	0.418 ± 0.184 ^a	5.663 ± 4.892 ^{a,b}	0.407 ± 0.181 ^{a,b}
CuffPatch™	0.218 ± 0.044	0.166 ± 0.052	0.142 ± 0.176 ^a	0.888 ± 1.081 ^{a,c}	0.349 ± 0.228 ^c
Polypropylene Mesh	$L_o = L_R$	0.192 ± 0.001	0	0	0
Autologous	0.227 ± 0.053	0.181 ± 0.040	0.342 ± 0.332	2.020 ± 2.064 ^{b,d}	0.201 ± 0.229 ^b
Native	0.219 ± 0.044	0.151 ± 0.104	0.662 ± 0.310 [*]	5.876 ± 2.993 ^{c,d}	0.155 ± 0.095 ^{a,c}

Values reported as mean ± standard deviation. Specimens that showed no contractile forces were assigned a value of zero. Superscripts a, b, c, or d show significance between implant pairs, and * show significance compared to all scaffolds (p<0.05).

Table 2

Quantitative immunohistochemical analysis for each scaffold group and the contralateral native tissue

Scaffold	# Blood Vessels per FOV	Slow Fibers per FOV	Fast Fibers per FOV	% Slow Fibers
Restore™	25.3 ± 20.8	24.9 ± 16.9	98.4 ± 53.9*	20.0 ± 6.9
CuffPatch™	56.4 ± 23.2†	23.1 ± 50.9	52.1 ± 68.8*	26.4 ± 21.5*
Polypropylene Mesh	48.23 ± 12.0†	0	0	0
Autologous	27.7 ± 18.8	34.1 ± 24.2	147.8 ± 69.2	19.2 ± 10.5
Native	11.0 ± 4.9*	62.2 ± 10.5*	143.1 ± 30.9	30.7 ± 5.4*

Immunopositive blood vessels and slow (Type I) and fast (Type II) muscle fibers were counted per 100× field of view (FOV), 6 fields per graft site, examined at the implantation site. % slow fibers were compared to total number of muscle fibers (slow + fast) in same tissue section. Values reported as mean ± standard deviation. Superscripts *, † show significance ($p < 0.05$).