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Extracellular Matrix for Myocardial Repair

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ABSTRACT

Objective: To evaluate the remodeling characteristics of an extracellular matrix (ECM) scaffold when used as a template for myocardial repair.

Background: Xenogeneic ECM has been shown to be an effective scaffold for the repair and reconstitution of several tissues, including lower urinary tract structures, dura mater, the esophagus, musculotendinous tissues, and blood vessels. These ECM scaffolds are completely degraded in vivo and induce a host cellular response that supports constructive remodeling rather than scar tissue formation.

Methods: Full-thickness circular defects measuring approximately 2.5 cm in diameter were created in the right ventricular anterior walls of 6 adult Yucatán pigs and 4 adult mongrel dogs. The defects were repaired with an ECM sheet 80 μm thick that was derived from either the porcine small intestinal submucosa or the porcine urinary bladder matrix. The animals lived for periods of 6 to 24 weeks before sacrifice.

Results: There was a complete replacement of the acellular scaffolds by a mixture of tissue types, including well-vascularized fibrous connective tissue, cartilage, adipose connective tissue, and myocardial tissue. The remodeled scaffold tissue showed spontaneous contractility and peak contractile force equivalent to 70% of the contractile force of the adjacent native myocardium.

Conclusions: We conclude that porcine ECM scaffolds alter the typical scar tissue healing response in myocardial tissue and instead support vascularization and the local development of multiple tissue types, including contractile myocardium.

INTRODUCTION

Surgical methods for repairing and replacing congenital or acquired myocardial defects consist mainly of implanting synthetic materials such as polyethylene terephthalate fiber (Dacron), polytetrafluoroethylene, or treated biologic material such as glutaraldehyde–cross-linked pericardium. Although these materials patch tissue effectively and prevent abnormal blood flow patterns, they have inherent problems, such as mineralization, fibrosis, risk of infection, and, importantly, an inability to become viable functioning components of the heart. Surgical attempts to restore functional myocardium have included patch ventriculoplasty or ventricular reduction operations [Birdi 1997, Dor 1997, Buckberg 1999, Dowling 1999, Ohara 2000, Franco-Cereceda 2001]. Skeletal muscle augmentation with conditioned muscle flaps have been proposed as methods of functional cardiac assistance [Magovern 2001, Moreira 2001]. Although these procedures have been associated with occasional benefit, none have been widely adopted.

More recently, strategies for replacing damaged or missing myocardium have involved the targeted placement of multipotential or unipotential progenitor cells, the administration of molecules with specific biologic activity (eg, growth factors/cytokines), or the targeted expression of selected genes that produce such biologically active molecules [Chiu 1995, Klug 1996, Li 1996, Li 1998, Taylor 1998, Tomita 1999, Bittira 2002, Leyh 2002, Li 2002]. A current approach for replacing noncardiac tissues has shown utility and efficacy in both preclinical animal studies and human clinical applications. Instead of supplying exogenous progenitor cells or specific proteins, it has been shown that constructive remodeling of irreversibly damaged, terminally differentiated tissues can be induced by providing a niche microenvironment that consists of a combination of structural and functional proteins derived from a biologic source. Decellularized extracellular matrix (ECM) attracts circulating, bone marrow–derived progenitor cells to sites of tissue repair [Badylak 2001] and also strongly supports vascularization through a combination of angiogenic growth factors (eg, vascular endothelial cell growth factor and basic fibroblast growth factor) [Voytik-Harbin 1997, Hodde 2001] and endothelial cell–friendly ligands [Badylak 1999, Hodde 2001, Hodde 2002].

Xenogeneic ECM has been used successfully as a constructive scaffold for the repair and functional replacement of numerous tissues and organs in both preclinical animal studies and human clinical applications. The ECM derived from the porcine small intestinal submucosa (SIS) and urinary bladder submucosa (UBS) has been used in preclinical studies as a vascular graft [Lantz 1990, Lantz 1992, Badylak 1989], a lower urinary tract repair device [Knapp 1994, Kropp 1995, Kropp 1996, Kropp 1998], a dura mater substitute [Cobb 1996, Cobb 1999], a body wall repair device [Clarke 1996, Badylak 2001], an esophageal graft [Badylak 2000], a musculotendinous scaffold [Badylak 1995, Hodde 1997], and a

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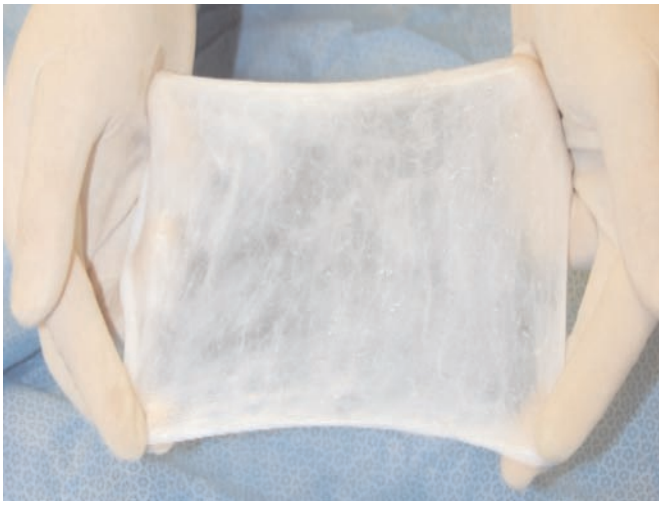

Figure 1. Macroscopic view of the urinary bladder matrix–xenogeneic extracellular matrix scaffold. Note the semitransparent lucency of this 80-μm thick material.

dermal substitute. Porcine-derived SIS-ECM is currently being used in humans as a scaffold for musculotendinous reinforcement and replacement (Restore; DePuy, Warsaw, IN, USA), as a vaginal sling for the surgical treatment of stress incontinence (StrataSIS; Cook Biotech, West Lafayette, IN, USA), and as a graft for lower urinary tract reconstruction, body wall repair, and dura mater replacement. Allogeneic cadaveric UBS-ECM has been used successfully in humans as a scaffold for augmentation cystoplasty and urethral reconstruction in humans [Probst 1997, Dahms 1998, Dahms 1998, Piechota 1998, Chen 1999, Calvano 2000, Merguerian 2000].

The purpose of the present study was to determine the morphologic changes that occur in these porcine-derived ECM scaffold materials when they are used as replacement scaffolds for full-thickness myocardial defects in 2 separate animal models, the dog and the pig. We also determined the contractile properties of the remodeled scaffold material at the time of sacrifice.

MATERIALS AND METHODS

Overview of Study Design

A full-thickness defect was created in the free wall of the right ventricle (RV) of 6 adult Yucatán pigs and 4 adult female mongrel dogs. In addition to the RV wall defects, interatrial septal defects were created in the 4 dogs. The animals lived from 6 weeks to 6 months before sacrifice. The measured end points of the study included macroscopic and microscopic assessment of the repair site (all animals) and in vitro contractility assessments of the excised tissue (2 dogs).

Preparation of Extracellular Matrix from Porcine Small Intestine

Preparation of the porcine SIS-ECM has been previously described but is briefly described here for the sake of clarity [Badylak 1998, Badylak 2001]. Sections of freshly harvested small intestine were delaminated of the superficial layers of the tunica mucosa and the entire tunica muscularis externa. The remaining tunica submucosa with basilar layers of the tunica mucosa consisted of extracellular matrix with a small number of intact cells. Subsequent treatment with dilute (0.1%) peracetic acid and rinsing in phosphate-buffered saline (pH 7.0) and deionized water rendered the material acellular. The remaining ECM consisted of a complex mixture of structural proteins left in their native 3-dimensional microarchitecture, including collagen types I, III, IV, V, and VI, proteoglycans, glycoproteins, glycosaminoglycans, and growth factors [Hodde 1996, McPherson 1998].

Preparation of Extracellular Matrix from Porcine Urinary Bladder

The method for producing urinary bladder matrix (UBM) was fundamentally the same as that described for the SIS-ECM. Substantially all of the muscularis externa (smooth muscle cell layers and serosa) and the tunica submucosa were mechanically delaminated from the more superficial tunica mucosa and tunica propria. The transitional epithelium was removed by exposure to 1.0 N saline, and this step left the basement membrane and the extracellular matrix of the tunica propria. This matrix was rendered acellular by methods identical to those described for the SIS-ECM. Figure 1 shows the appearance of the prepared UBM scaffold material.

Surgical Procedure

The pigs were anesthetized with an intramuscular injection of ketamine hydrochloride (15 mg/kg), followed by intubation and the maintenance of a surgical plane of anesthesia with 2.5% isoflurane. The dogs were anesthetized with an intravenous injection of thiopental sodium (Pentothal), followed by intubation and the maintenance of a surgical plane of anesthesia with 2.5% isoflurane. Following routine preparation of the surgical field, a right lateral thoracotomy was performed. The pericardium was divided and retracted, a procedure that exposed the RV. Following the administration of 50 mg lidocaine intravenously, we placed a partial occlusion clamp on the RV anterior wall and created a full-thickness circular defect between 2 cm and 3 cm in diameter. The defect site was repaired with a single-layer sheet consisting of the SIS-ECM scaffold in the 4 dogs and the UBM-ECM scaffold in the 6 pigs. The ECM scaffolds were sutured to the myocardium with 5-0 Prolene suture material (Ethicon, Somerville, NJ, USA). Because the ECM scaffold was approximately 80 μm thick, there remained a significant mismatch between the thickness of the native RV free wall and the scaffold material. Vascular tape was placed at the edges of the ECM graft in the 6 pigs to serve as a marker for the boundary of the resorbable graft placement. Prolene suture was placed in the 4 dogs to mark the graft placement site.

In addition to the RV anterior wall defects, we also created a defect 1.5 cm in diameter in the interatrial septum of the 4 dogs, and this defect was repaired with the SIS-ECM and 5-0 Prolene sutures. The chest was closed with absorbable suture, and a chest tube was placed for drainage.

Postoperative Care

All animals were given antibiotics perioperatively (cefazolium sodium [Kefzol], 1 g intravenously) and for 7 days postoperatively (cephalexin [Keflex], 500 mg orally 3 times a day). Postoperative anticoagulation therapy consisted of 1 aspirin (325 mg) per day. All animals were monitored daily for appetite, fever, heart rate, and respiratory pattern. The dogs were sacrificed at 12 weeks (n = 2) and 20 weeks (n = 2). One pig was sacrificed at each of the following times: 6, 8, 10, 14, 20, and 24 weeks.

In Vitro Contractility Testing

In vitro contractility testing was conducted on freshly excised tissue from the scaffold placement site of the 2 dogs sacrificed at 20 weeks.

Tissue Bath Apparatus

A 50-mL bath (Radnoti Glass, Monrovia, CA, USA) was used to maintain homeostatic physiologic conditions of the excised remodeled scaffold tissue. Force transducers were attached and adjusted to the desired resting tension and measured the force of contraction. The tissue baths were filled with 50 mL of modified Krebs solution and were maintained at 37°C. The resistivity of the Krebs solution was measured with a conductivity bridge (Model 31; Yellow Springs Instrument, Yellow Springs, OH, USA) and was 53 Ω cm at 37°C.

Tissue Harvesting and Mounting

Immediately following euthanasia, approximately 50% of the scaffold site was excised and placed in an ice-chilled bath of continuously oxygenated Krebs solution. No surrounding native tissue was included in the excised tissue specimens. Care was taken to prevent direct contact between the cardiac tissue and the ice. While still immersed in the chilled, oxygenated solution, the site of ECM scaffold placement was excised and attached to the force transducer with No. 4 silk suture. The tissue was immersed in the Radnoti tissue bath. A 1-g force resting tension was applied to the tissue specimen. Stainless steel plate electrodes, 2.5 cm wide by 5 cm long, were placed on each side of the bath with the specimen suspended between the electrodes. The excised tissue specimen never contacted the electrodes. The electrodes were parallel and spaced 2 cm apart.

Tissue Stimulation and Data Collection

The force transducer was connected to a graphic recorder to determine the force of contraction versus time and the presence of any spontaneous contraction. An event marker was connected to the stimulator so that an evoked contraction could be linked with each stimulus. A digital storage oscilloscope (Model 2230; Tektronix, Beaverton, OR, USA) displayed the current applied to the tissue bath electrodes. A rectangular pulse stimulator (Model S44; Grass Instruments, Quincy, MA, USA) was used to deliver single, 2-millisecond stimuli in a beaker filled with 50 mL of Krebs solution.

Pulse duration and pulse intensity were adjusted on the Grass stimulator until a tissue contraction response was obtained for each stimulus. A series 10-Ω resistor was used to measure the stimulating current. The digital storage oscilloscope connected across either of these 2 resistors displayed the voltage drop from which the current was calculated and the pulse duration was measured.

The strength-duration curve for the excised tissue specimen was determined after the threshold stimulus for each pulse stimulus duration was established. The intensity-versus-duration data were then fit to the hyperbolic form of the strength-duration curve. The normalized sum of the least squares error was minimized to determine the chronaxie and rheobase.

Histopathologic Examination

Immediately following the euthanasia of the 6 pigs and 2 of the 4 dogs, the scaffold placement site and approximately 1 cm of surrounding normal tissue was explanted and divided into 2 equal parts. One part was placed in either 10% buffered formaldehyde or quick-frozen in liquid nitrogen. For the remaining 2 dogs, the excised tissue was similarly fixed after in vitro contractility testing. After a minimum of 48 hours fixation time, the specimens were trimmed, sectioned, embedded in paraffin, and stained with both hematoxylin and eosin and Masson trichrome. Sections of the frozen myocardium were subjected to immunostaining for von Willebrand factor (Dako, Carpinteria, CA, USA) and cardiac muscle actin (Sigma Chemical Company, St. Louis, MO, USA). Avidin-biotin horseradish peroxidase was used as a secondary marker for both immunohistochemical procedures.

All experiments were performed in accordance with US National Institutes of Health guidelines (National Institutes of Health 1996). In addition, the Purdue University Animal Care and Use Committee approved this study.

RESULTS

Macroscopic Examination

The macroscopic appearance of the remodeled ECM scaffold was similar in all animals, and the progressive changes that appeared were dependent on the time allotted for remodeling. The outer surface was a brownish red color similar to that of the adjacent normal myocardium and had scattered areas of paler-appearing tissue. There was a noticeable absence of scar tissue demarcating the line between native myocardium and graft material. In the animals (pigs) in which vascular tape was placed to define the graft site, the size of the defect had decreased by approximately 20%. However, the remodeled tissue within the graft site was similar to the remodeled tissue at the graft site in the animals in which no vascular tape had been placed (Figure 2).

The endocardial surface showed a smooth, glistening lining, no evidence of thrombus accumulation, and the formation of endocardial folds and trabeculae similar to the adjacent native tissue. The cut surface of the remodeled graft had a thickness that was equal to that of the adjacent native myocardium and had a mottled brown and white color. The consistency of the remodeled tissue was slightly firmer than normal RV myocardial tissue.

The macroscopic appearance of the remodeled graft material at the interatrial defect site showed a white, glistening

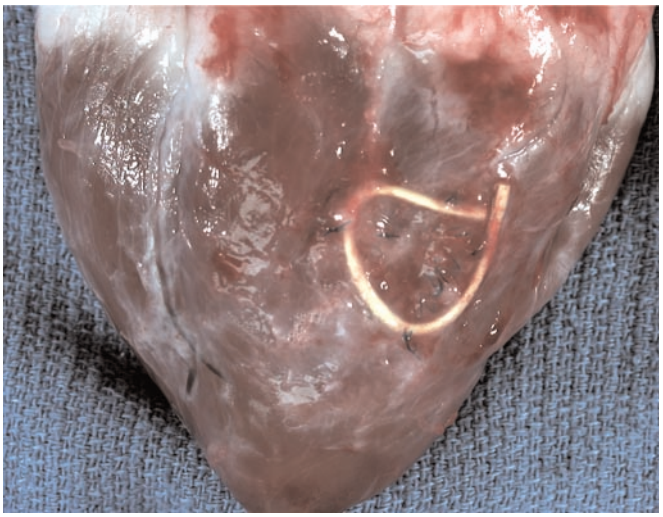

Figure 2. Macroscopic view of the external surface of the right ventricle in a pig 2 months after surgery. Vascular tape demarcates the limits of the originally resected myocardial tissue. The remodeled extracellular matrix scaffold (within the boundaries of the tape) has a color that is virtually identical to the adjacent myocardium.

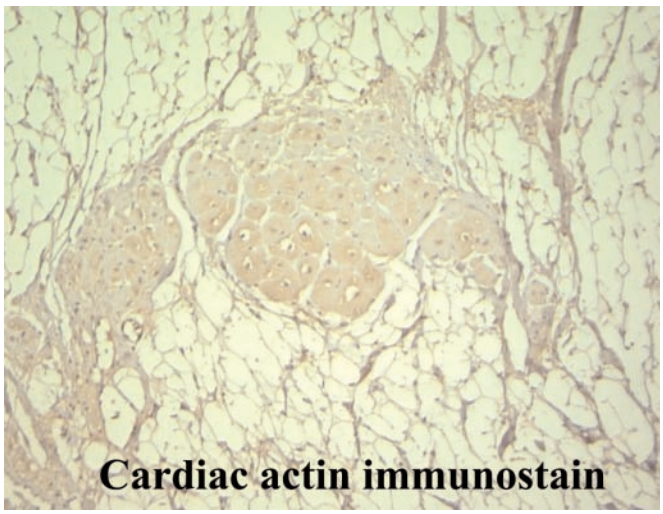

Figure 4. Immunohistochemical-stained section of remodeled urinary bladder matrix–extracellular matrix scaffold 3 months after surgery. Note the islands of cardiomyocytes that stain positively for cardiac muscle actin and that are surrounded by sheets of adipose connective tissue (original magnification ×225).

surface. There was no evidence of thrombus accumulation, and the consistency of the graft site was firm.

Microscopic Examination

Histologic examination of the remodeled tissue in the RV free wall showed a mixture of tissue types. Islands of striated cells that had central nuclei and stained positively for cardiac actin were scattered throughout the graft site (Figures 3-5). These striated cells were surrounded by adipose connective tissue and collagenous connective tissue. The amount of myocardial tissue ranged from approximately 20% of the graft site in the animals sacrificed after 2 months to approximately 50% of the graft site in the animals sacrificed after 6 months. There was no evidence of the ECM scaffold material in any of the animals sacrificed after 12 weeks. The graft sites were well vascularized with blood vessels of capillary size and arteriolar size coursing throughout the tissue (Figure 6). The vascularization was more abundant in the early remodeling period (ie, 6 weeks to 3 months) than in the later remodeling period.

The endocardial surface of the grafts was completely covered by cells staining positively for von Willebrand factor, a

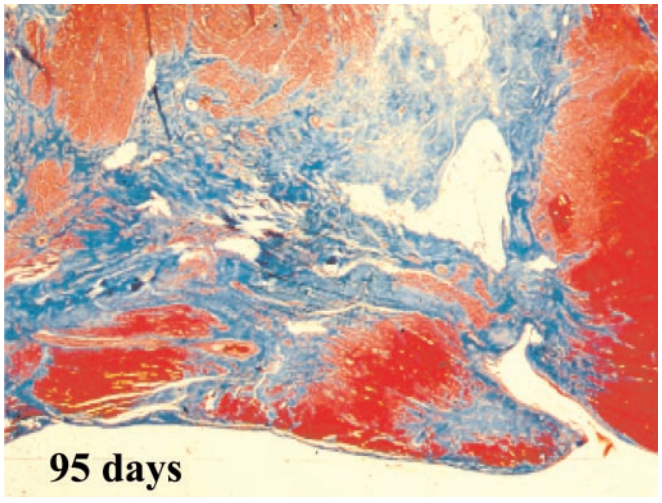

Figure 3. Masson trichrome staining of section of remodeled scaffold 95 days following surgery. Note the islands of red-staining muscle tissue surrounded by blue-staining collagenous tissue. Islands of adipose tissue are present in the upper right portion of the photograph.

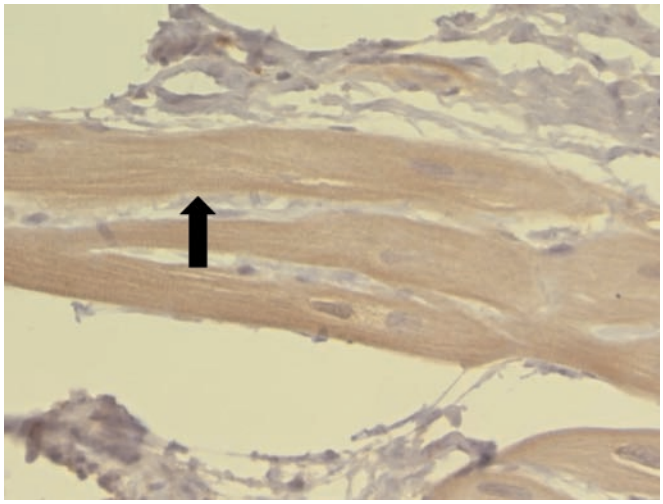

Figure 5. Photomicrograph of an island of cardiomyocytes that stain positive for cardiac actin. These cells have populated the urinary bladder matrix–extracellular matrix scaffold. The cells contain central nuclei and show striations (original magnification ×450).

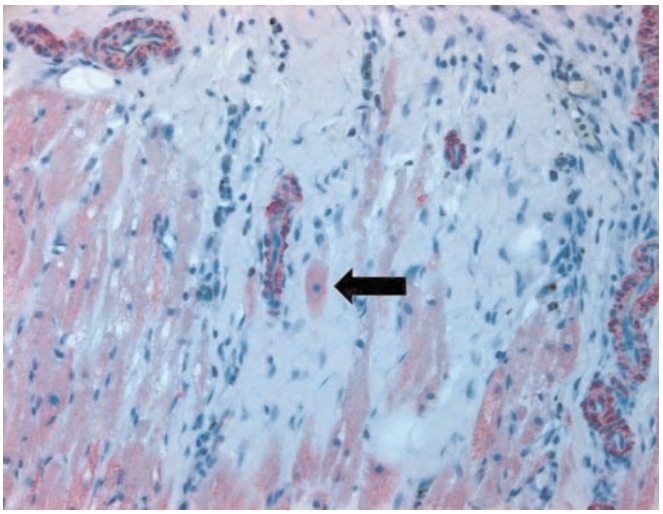

Figure 6. Immunostain for smooth muscle actin in the center of a remodeled small intestinal sub mucosa–extracellular matrix scaffold 5 months after surgery. Note the positive staining of smooth muscle cells surrounding arterials that are part of the vascularization process. The section is counterstained with Masson trichrome stain that also shows muscle tissue (arrow) and blue staining collagenous connective tissue (original magnification ×250).

finding consistent with a morphologically normal endothelium. There was no evidence of microscopic thrombus formation.

The interatrial septal defect sites were replaced by a similar accumulation of connective tissues. However, there were only widely scattered cardiomyocytes present with much more collagenous connective tissue than was found in the RV free wall graft sites. In addition, there were accumulations of adipose connective tissue and scattered islands of cartilaginous tissue. The endocardial surface was covered with normal endothelial cells identical to those found in the RV free wall graft site.

In Vitro Contractility Testing

The excised RV graft site tissue from the 2 dogs sacrificed at 20 weeks showed spontaneous contractility in vitro and spontaneous electrical activity. The controlled studies of strength of contraction showed peak contractile forces that were equivalent to up to 70% of the contractile force of the adjacent native myocardial tissue (Figure 7). The excised atrial graft site tissue in these 2 dogs did not show spontaneous contractility and responded weakly to electrical stimulation.

DISCUSSION

The results of this study showed that both the UBM and SIS-ECM scaffolds were totally resorbed following surgical implantation and were replaced by a mixture of connective tissues including cardiac muscle, fibrous connective tissue, adipose connective tissue, and cartilaginous connective tissue. Vascularization of the remodeled tissue was abundant and more prominent in the early scaffold-remodeling period.

The presence of collagenous connective tissue in the remodeled ECM scaffolds was not surprising, because it is well accepted that damaged or missing adult myocardial tissue heals by scar tissue formation [Carlson 1983, Farza 1995]. However, the simultaneous presence of other mesenchymal tissue types that included adipose connective tissue, cartilage, and especially contractile myocardial tissue was unexpected. The ECM scaffolds are associated with changes of the default scar tissue healing response of adult myocardial tissue in these dogs and pigs.

The UBM and SIS scaffold materials were acellular at the time of implantation. The source of the cells that contributed to scaffold remodeling is not certain. An induction of mitosis would contradict the conventional thought that terminally differentiated cardiomyocytes are unable to undergo further cell division [Carlson 1983, Farza 1995]. It has recently been shown that the cells that repopulate the

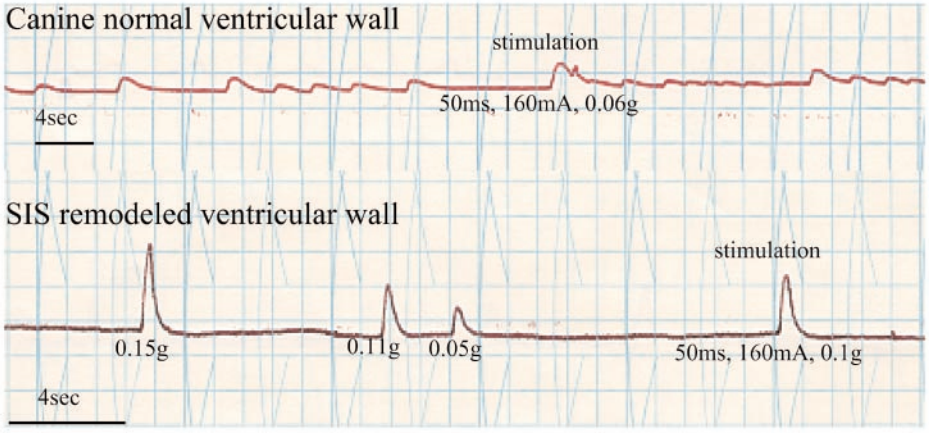

Figure 7. Myograph tracing of small intestinal submucosa–remodeled right ventricular defect (bottom tracing) and adjacent normal right ventricular free wall. The tracing of normal tissue shows spontaneous electrical activity and a contractile response following electrical stimulation. The lower tracing of remodeled acellular scaffold also shows spontaneous electrical activity, although not as frequent as that of the normal ventricular wall, and the contractile response to externally applied electrical stimulation.

ECM scaffolds are derived from a mixture of local reserve cells and circulating, bone marrow–derived progenitor cells [Badylak 2001]. The variety of cell types that tend to replace these scaffolds following surgical implantation suggests a process that is different from classic wound healing and scar tissue formation. The use of stem cells to replenish damaged myocardium has received considerable attention in recent years. It appears that native or injected progenitor cells can remain viable and eventually function in recipient animals [Chiu 1995, Klug 1996, Li 1996, Li 1998, Taylor 1998, Tomita 1999, Badylak 2001, Hodde 2001]. Both adjacent cardiac satellite cells and circulating progenitor cells [Badylak 2001, Bittira 2002, Leyh 2002, Li 2002] should be considered as potential sources of the new cardiomyocytes observed in the present study. The major difference between this proposed mechanism and that described in previous studies is the in vivo recruitment in the present study of naturally occurring host progenitor cells.

The in vitro contractility testing and automaticity noted in the remodeled scaffold sites showed that the new cardiomyocytes have functional capability. Whether or not the function of this new cardiac tissue was, or would have been, synchronized with adjacent myocardium is unknown. It is also not known if this functional capacity could be augmented by other therapeutic methods, such as preseeding the scaffolds with autologous stem cells or injecting stem cells at various times during the remodeling process.

Strategies for myocardial repair and replacement involve both surgical and cell-based approaches. Cell-based therapy with viable skeletal muscle cells and progenitor cells has been attempted in both preclinical animal studies and in human patients. These cell-based methods have been attempted both with and without collagenous scaffolds and have shown promise as methods for replacing damaged myocardial tissue to improve ventricular function [Taylor 1998, Tomita 1999]. However, questions remain regarding long-term cell viability, immune-mediated rejection, and failure to integrate effectively with the surrounding native myocardium [Chiu 1995, Klug 1996, Li 1996, Li 1998, Leyh 2002].

Biologically active molecules (ie, growth factors) have been evaluated for their ability to promote cell division and induce angiogenesis in damaged myocardium. However, it has been difficult to achieve optimal controlled release of these compounds from carrier materials or the site of injection, and this difficulty has caused inconsistent and unpredictable outcomes.

In summary, the present study showed in 2 animal models that porcine-derived ECM scaffolds are associated with the constructive remodeling of adult myocardial tissue. The default scar tissue response following injury is replaced by the deposition of well-vascularized multiple tissue types, including functional myocardium. The source of the cells that remodel these scaffolds is still open to speculation, but preliminary evidence suggests that host-derived, circulating multipotential cells participate in the healing response. Naturally occurring biologic scaffolds may provide an attractive adjunct in the treatment of absent or damaged myocardial tissue.

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