Extracellular Matrix Scaffold for Cardiac Repair

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Background—Heart failure remains a significant problem. Tissue-engineered cardiac patches offer potential to treat severe heart failure. We studied an extracellular matrix scaffold for repairing the infarcted left ventricle.

Methods and Results—Pigs (n=42) underwent left ventricular (LV) infarction. At 6 to 8 weeks, either 4-layer multilaminate urinary bladder-derived extracellular matrix or expanded polytetrafluoroethlyene (ePTFE) was implanted as full-thickness LV wall patch replacement. At 1-week, 1-month, or 3-month intervals, pigs were terminated. After macroscopic examination, samples of tissue were prepared for histology, immunocytochemistry, and analysis of cell proportions by flow cytometry. One-week and 1-month patches were intact with thrombus and inflammation; at 1 month, there was also tissue with spindle-shaped cells in proteoglycan-rich and collagenous matrix. More α-smooth muscle actin-positive cells were present in urinary bladder matrix (UBM) than in ePTFE (22.2±3.3% versus 8.4±2.7%; P=0.04). At 3 months, UBM was bioresorbed, and a collagen-rich vascularized tissue with numerous myofibroblasts was present. Isolated regions of α-sarcomeric actin-positive, intensely α-smooth muscle actin-immunopositive, and striated cells were observed. ePTFE at 3 months had foreign-body response with necrosis and calcification. Flow cytometry showed similarities of cells from UBM to normal myocardium, whereas ePTFE had limited cardiomyocyte markers.

Conclusions—Appearance of a fibrocellular tissue that included contractile cells accompanied biodegradation of UBM when implanted as an LV-free wall infarction patch. UBM appears superior to synthetic material for cardiac patching and trends toward myocardial replacement at 3 months. (Circulation. 2005;112[suppl I]:I-135–I-143.)

Key Words: heart failure ▪ surgery ▪ tissue engineering

Chronic congestive heart failure plagues an increasing population of cardiac patients worldwide and is primarily a manifestation of dilated cardiomyopathy from ischemic coronary artery disease.1 Surgical ventricular restoration (SVR) has been shown effective for treatment of ischemic cardiomyopathy.2 In its most prevalent form, SVR involves the Dor procedure for ventricular reduction, which includes placement of an endocardial patch.3 Cardiac patch surgery can also be performed to correct congenital defects and repair traumatic ventricular perforations and aneurysms.4–6 Materials currently available for correction of cardiac patients include synthetics, such as woven nylon (Dacron) and expanded polytetrafluoroethlyene (ePTFE), as well as glutaraldehyde-cross-linked biological membranes, like bovine pericardium. Although such materials perform adequately to fill tissue voids or reinforce weaknesses, they have no capacity for bioresorption and do not become viable, functioning components of the recipient organ. Such patches become incorporated by fibrotic encapsulation and, thus, cannot restore regional tissue functionality.

Acellular xenogeneic extracellular matrix (ECM) has been used successfully as a construct for the repair and replacement of numerous tissues and organs in both preclinical studies and human clinical applications. ECM derived from porcine small intestinal submucosa and urinary bladder matrix (UBM) has been tested preclinically as a vascular graft,2.8 lower urinary tract repair device,9 dura mater substitute,10 body wall repair device,11 musculotendinous scaffold,12,13 articular cartilage replacement,14,15 and cardiac valve replacement.16 Porcine-derived small intestinal submucosa-ECM is currently being used in humans as a scaffold for musculotendinous reinforcement and replacement (Restore, DePuy, Inc.), as a vaginal sling for the surgical treatment of stress incontinence (Stratisis, Cook Biotech, Inc.), and as a graft for lower urinary tract reconstruction, body wall repair, and dura mater replacement. These investigations have shown that decellularized and sterilized but not chemically cross-linked ECM scaffold material has desirable characteristics as a tissue replacement in diverse anatomic sites. Preliminary studies showed the feasibility of UBM implant in the cardiac right ventricular outflow tract17,18 and supported investigation as a myocardial patch in the left ventricle.19

In this study, we report for the first time that a form of UBM can be used to patch the infarcted left ventricular (LV)
Our results indicate that the material in this form has suitable surgical handling characteristics and performance and, during bioresorption, fosters cardiac tissue repair that resembles a regenerative process.

**Methods**

**Animal Model**
Castrated male and female Yorkshire hybrid domestic pigs (*Sus scrofa domestica*, n=42) were used. Animal experimentation was consistent with federal and American Heart Association guidelines and was approved by the Institutional Animal Care and Use Committee of the American Cardiovascular Research Institute. Infarction of the anterolateral LV free wall was performed by catheter embolization with collagen or gelspheres of the first or second diagonal branch of the left anterior descending coronary artery from a femoral artery approach (Figure 1). Pigs were sedated with IM telazol (2 to 4 mg/kg) and then intubated. Anesthesia was induced and maintained using inhalant isoflurane (1% to 3% in O₂ or O₂/N₂O).

**Patch Material**
Four-ply vacuum-pressed UBM was provided by ACell Vet, Inc. The UBM is prepared by mechanical removal of epithelium, serosa, muscularis externa, and the tunica submucosa, followed by hypotonic cell lysis, disinfection by peracetic acid (0.1%), and rinsing in phosphate-buffered saline solution. The end product consists of the basement membrane of the tunica mucosa and the subjacent tunica propria. Final preparation included lyophilization, vacuum pressing of 4 UBM sheets, and sterile packaging. Sterile ePTFE was used as a control.

**Cardiac Patch Surgery**
At 6 to 8 weeks after infarction, pigs were anesthetized as above. A pressure transducer-tipped wire was advanced into the left ventricle via the carotid artery, and hemodynamic measurements were taken. A midsternal thoracotomy was performed and partial cardiopulmonary bypass was initiated. With the heart decompressed and beating, a circular transmural defect ~2 to 3 cm in diameter of the infarcted region was made by excision (Figure 2). Using a parachute technique, an endocardial UBM or ePTFE patch was secured with 4 to 0 polypropylene sutures and ePTFE pledgets (Figure 2). Bypass was discontinued, and a second epicardial patch was placed to prevent pericardial effusion and tamponade, using UBM or surgical latex. Bleeding was controlled using ligatures when needed, the pericardium and chest were closed, and the animals were recovered.

**Terminal Restudy**
At 1 week, 1 month, or 3 months, the pigs were anesthetized as above. The pigs were terminated using intravenous KCl, and the hearts were harvested. The LV endocardium was exposed, and photographs were taken before and after transection of the patched region. The apical half of the patch was taken for flow cytometry, and the basal half was split into 2 pieces. One was fixed in 10% formalin, and the other was freeze-embedded in glycerol-based medium.

**Histology and Immunocytochemistry**
Formalin-fixed tissue was dehydrated in graded ethanol series, exchanged in xylene, and embedded in paraffin. Five-micrometer sections were cut on a rotary microtome, collected on glass slides, and deparaffinized. Adjacent or near-adjacent sections were stained with hematoxlin-eosin, Verheoff-Masson elastic-trichrome, and Movat pentachrome, and by indirect immunocytochemistry for α-smooth muscle actin (SMA), sarcomeric actin, von Willebrand factor (for endothelial cells), and proliferating cell nuclear antigen (PCNA). Frozen sections were cut on a cryotome and immunostained for CD45 (leukocytes) and CD25 (activated macrophages and T cells).

**Flow Cytometry**
Patch LV regions and posterior wall normal myocardium samples were minced thoroughly (tissue was removed from ePTFE by thorough scraping with a scalpel blade), and cells were disaggregated by incubation in collagenase solution (1 mg/mL in DMEM) for 30 minutes at 37°C. The resulting suspension was passed through cheesecloth and immunostained for cell surface (unfixed cells) and intracellular (fixed, permeabilized cells) antigens. Primary antibodies against CD45 (leukocytes), CD31 (endothelium), SMA (smooth muscle cells), myosin heavy-chain (striated myocytes), vimentin, and PCNA (proliferating cells) were added to individual cell aliquots. Secondary antibodies were fluorescently tagged with fluorescein isothiocyanate or phycoerythrin, and nuclear DNA was stained with 7-amino-actinomycin D. Aliquots of selected 3-month samples underwent additional characterization for the striated myocyte-specific markers sarcomeric actin (double-staining with SMA),...
troponin, tropomyosin, and the cardiac gap junction protein connexin 43. Immunostained cell samples, along with isotype controls, were analyzed in a Becton Dickinson FACSCalibur flow cytometer, and gated regions were quantified for cell-specific markers.

**Statistical Analysis**
Continuous variables are expressed as mean ± SE and were compared between groups using unpaired 2-tailed Student t tests. Rates of mortality were compared between groups using \( \chi^2 \) tests with Yates correction factor for calculations. Critical values of \( P<0.05 \) were considered to indicate significant between-group differences.

**Results**

**Mortality and Morbidity**
Thirty-seven of 42 pigs survived myocardial infarction and underwent patch implant; of these, 31 survived to terminal restudy. Three of 16 pigs implanted with UBM died within 3 days after surgery, and 3 of 15 ePTFE-patched pigs died within 4 days. Mortality was not different between groups (3 of 16 for UBM versus 3 of 15 for ePTFE; \( P=0.71 \)).

**Macroscopy**
Representative macroscopic images of ventricular patches are shown in Figure 3. At 1 week postimplant, patched regions showed central red coloration with a pearly white corona on the endocardial aspect (data not shown). Thrombus filled the area between endocardial and epicardial layers.

At 1 month, central red regions were present in UBM and ePTFE; pearly white coronas were also present in both (Figure 3a through 3c). Thrombi extending from the patched region into the left atrium were seen in 2 of 4 ePTFE pigs (Figure 3c), but none were detected in 6 UBM patches (Figure 3a). Transection of UBM patches showed red-brown material filling the interpatch space (Figure 3b).

Three months after implant, UBM patch layers were not macroscopically apparent. There was partial, punctate red coloration of the endocardial aspect of the UBM patch, whereas ePTFE showed mostly red color (Figure 3d and 3f). Transmural bisection of the UBM-patched region showed a mixture of white and pearly white tissue with regions of
brown and red color at the borders (Figure 3e). Bisection of ePTFE revealed thick regions of red coloration on the endocardial aspect and occasional pockets of gelatinous and caseous material, as well as hard nodules resistant to cutting (Figure 3g).

**Histology and Immunocytochemistry**

Representative histology images of sections from UBM-patched and ePTFE-patched regions are shown in Figures 4 through 6. At 1 week in both groups there was stratified thrombus consisting primarily of erythrocyte masses and fibrinoid material with scattered leukocytes (data not shown). At 1 month, inspissated thrombus was present in the regions of both UBM and ePTFE patching, and there was fibrocellular tissue that extended from the edges of the infarct excision and covered the endocardial aspect of the graft (Figure 4a). Numerous α-SMA, CD45+, and CD25+ cells were observed adjacent to patch material (Figure 4b through 4e); α-SMA+ cells appeared more prevalent in UBM, whereas inflammatory phenotypes were higher in ePTFE.

At 3 months, UBM material remnants were difficult to detect (Figure 5a). In the patched region, there was fibrocellular tissue with widespread expression of α-SMA-positive cells (Figure 5b). There were also isolated regions of nonvascular, intensely α-SMA-positive cells (Figure 5c). Additionally, sarcomeric actin-positive cells were present in a similar pattern (Figure 5d and 5e). ePTFE-grafted regions showed a fibrotic encapsulation with abscess, calcification, necrosis, and foreign-body giant cell reaction (Figure 6).

**Flow Cytometry**

Disaggregation and flow-cytometric analysis of cells from cardiac-patched regions yielded a mixed population at 1-month and 3-month intervals (Figure 7). At 1 month, both patches displayed a higher proportion of CD45+ infiltrating inflammatory cells than normal myocardium (6.9±1.7% normal versus 37.3±8.8% UBM, \(P=0.017\); versus 66.1±8.8% ePTFE, \(P<0.001\), and inflammation tended to be higher in ePTFE than in UBM (\(P=0.11\)). The proportion of α-SMA-positive cells was higher in UBM-patched than in ePTFE-patched regions (22.2±3.3% versus 8.4±2.7%; \(P=0.04\)). Myosin-HC+ cells were less in both patches, more so in ePTFE (62.3±3.7% normal versus 43.3±5.9% UBM, \(P=0.01\); versus 38.8±2.5% ePTFE, \(P<0.001\). Endothelial cells identified by CD31 immunostaining were less in both patch types with no difference between groups. Total cell proliferation (PCNA immunostaining) tended to be higher in UBM than in ePTFE (36.4±7.5% versus 14.6±8.6%; \(P=0.10\)).

At 3 months, the total cells harvested from UBM patched regions was higher than ePTFE (24.3±6.5 \(\times\) 10\(^3\) cells/mg tissue for UBM versus 4.7±2.2 \(\times\) 10\(^3\) cells/mg tissue ePTFE;
cell proportions tended to remain elevated in both patched regions relative to normal myocardium (3.8±1.1% normal versus 26.0±10.2% UBM, *P*=0.10; versus 43.1±12.2% ePTFE, *P*=0.08). Vimentin-positive cells were higher in patched regions than in normal myocardium (25.5±5.6% normal versus 54.0±6.8% UBM, *P*=0.01; versus 48.5±7.4% ePTFE, *P*=0.08). The α-SMA-positive cell population of UBM-patched regions was higher than ePTFE (27.5±4.8% versus 9.9±3.0%; *P*=0.04). Myosin-HC+ cells were less than in normal myocardium with no difference between patch groups. Endothelial cells were less numerous in UBM than normal myocardium and tended to be decreased in ePTFE. PCNA+ cells tended to be higher in UBM than in ePTFE (37.3±6.8% versus 26.0±2.3%; *P*=0.10).

A substudy of aliquots from two 3-month UBM and one ePTFE patch showed tropomyosin and connexin 43-positive cell proportions similar between normal myocardium and UBM with minimal cells expressing these markers in ePTFE (data not shown). Dot-plots of cell aliquots from 2 samples double-stained for α-SM actin and sarcomeric actin showed a population of cells coexpressing the 2 filament types in patches of both types at 3 months, whereas only UBM showed cells uniquely expressing sarcomeric actin (Figure 8).

Cardiac repair by surgical patching is currently limited by the inability to foster local tissue replacement with available materials. In this study, for the first time, we have demonstrated the utility of a decellularized biological ECM scaffold to repair infarcted LV myocardium when implanted as a full-thickness LV free wall patch. ECM scaffolds, unlike glutaraldehyde-cross-linked materials of biological origin, have been shown to retain matrix elements, including matrix-bound growth factors that are thought to support cell growth and differentiation.20–23 Our results endorse this concept and provide evidence for the repair of myocardial tissue with ECM scaffold through a process involving cell recruitment and tissue-specific differentiation.

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Figure 4. Histology of 1-month UBM. (a) endocardial patch layer is clearly discerned (*); broken lines indicates approximate edges of infarct excision. Fibrocellular tissue ingrowth in UBM (b) shows numerous α-SMA+ myofibroblasts (red), which are less prevalent in ePTFE (c). CD25+ inflammatory cells (red) are seen in UBM (d) but are more numerous in ePTFE (e); nuclei are counterstained with hematoxylin (blue). bars = 50 μm (b–d); bar = 25 μm (e).
10% of the label present at the site of implant in grafted dog urinary bladders at 3 months. Replacement tissue demonstrated characteristics of both healing scar (extensive myofibroblast cell population, abundant collagen and proteoglycan matrix, neovascularization, and leukocyte infiltration), as well as normal myocardium (cells expressing α-sarcomeric actin, myosin-HC, tropomyosin, and connexin 43, and cells showing sarcomeric structure). Furthermore, ePTFE patches showed an unfavorable histologic reaction with foreign-body giant cells, necrosis, and calcification. Altogether, these results indicate that 4-ply UBM is a superior material for use as a cardiac patch.

A unique pattern of myocardial repair resembling a regenerative process was observed ongoing at 3 months after UBM patch implant. In addition to α-SMA-positive myofibroblasts widespread throughout the newly formed tissue, there were isolated regions or “islands” of tightly grouped cells intensely positive for α-SMA, as well as those expressing the cardiomyocyte-specific filament protein α-sarcomeric actin. Of the latter, immunocytochemistry showed cells of various morphologies, including spindle-shaped and stellate, as well as rhomboidal shapes typical of cardiomyocytes. Flow cytometry confirmed and quantified these findings and additionally identified a cell population uniquely coexpressing these 2 markers. Similar cell types expressing both smooth muscle and cardiac or skeletal muscle proteins are present in the developing heart and in models of heart failure induced by pressure overload or chronic pacing. The significance of this finding in regions implanted with the ECM scaffold is presently unclear, but we speculate that the cells may represent a transitional or dedifferentiated form capable of full differentiation into a syncytial cardiomyocyte phenotype. The additional evidence of isolated regions of cells with cytoplasmic cross-striated filament bundles (ie, sarcomeric structure) lends additional support to this notion.
The islands of myocytes appeared spatially distinct from normal myocardium, remote from the apparent edge of the excised portion of the LV free wall, and within the center of the patched region. Because of this separation and because fully differentiated cardiomyocytes are nonmigratory, it seems unlikely that mature myocytes in normal cardiac tissue adjacent to the patch could represent their source. Whether they originated from cardiac stem cells, cardiac fibroblasts, vascular cells, or blood-borne progenitors is an unanswered question that is being addressed in our current research.

Previous studies showed that bone marrow-derived cells populated SC ECM implants and that fractionated components of ECM are chemoattractant for endothelial cells, lending credence to the concept that both circulating and adjacent tissue cells populate the scaffolds. Because the healing process of UBM at 3 months appears incomplete, we are also pursuing long-term experiments to evaluate the consequences of UBM scaffold implant at 6 months in adult miniature pigs. The material we implanted is devoid of cells; it seems likely that histologic and functional recovery of myocardium could be accelerated and rendered more complete by seeding the patches with cardiomyocytes before implant.

Flow-cytometric findings provided detailed quantitative comparisons of the temporal cell recruitment processes and were well correlated to the histologic findings. Summarily, UBM promoted myofibroblast recruitment, had decreased inflammation, and supported increased cell proliferation. Curiously, myosin-HC expression was found in a substantial proportion of cells from both UBM and ePTFE at both 1-month and 3-month time points; yet, histology showed few striated cells in UBM and none in ePTFE. Because fewer total cells were obtained from 3-month ePTFE than from UBM, it may be that small amounts of adherent adjacent myocardium may have inadvertently been included in the samples, which would have skewed the results. Alternatively, myosin may be expressed by cells surrounding the ePTFE patches without overt myocyte differentiation pattern, including coexpression with CD45. It is also of interest that, apparently, different patterns of cell marker expression were seen in normal myocardium at the 2 time points; this may reflect ongoing ventricular remodeling processes.

Although we measured ventricular function in some animals, technical difficulties precluded sufficient numbers of

![Figure 6. Histology of 3-month ePTFE. (a) patch (*) is encircled by collagen (blue) and shows abscess with mineralized rim (arrow). (b) Movat stain shows proteoglycan (turquoise) at endocardial aspect of patch. (c) border and interstices of graft (*) show numerous multinucleated foreign-body giant cells (arrows); bar = 20 \( \mu \)m.

Figure 6](http://circ.ahajournals.org/)

![Figure 7. Flow-cytometric analysis of cells disaggregated from normal myocardium (NM) and UBM-patched and ePTFE-patched regions. (a) 1 month; (b) 3 months.](http://circ.ahajournals.org/)
hemodynamic and sonomicrometry data points for statistical analysis from continuous variables. Measurement of functional LV recovery after patch implant was not the primary aim of this study; instead, we focused on testing the feasibility of cardiac patching with UBM and assessing the tissue and cellular responses to this novel tissue-engineered biomaterial. Our 6-month study will include global and regional LV function analysis and, therefore, will determine whether UBM can aid functional recovery in this model.

In addition to a potential use for LV infarct repair and treatment of heart failure, UBM and similar ECM scaffolds may be applicable for treatment of traumatic ventricular perforation, ventricular aneurysm, and closure or patching of congenital cardiac defects. In the latter condition, it seems likely that because of the nature of the response we have observed, and unlike synthetic patches, the bioresorbable ECM scaffolds will accommodate growth and reduce or eliminate the need for repeat surgeries. It also seems likely that certain modifications to the ECM material might accelerate the functional recovery of viable tissue. Such variations might include preattachment or seeding with autologous differentiated muscle cells, autologous multipotent cells, or some other form of primitive stem cells that might be coaxed into a cardiomyocyte phenotype. Alternatively, the provision of chemotactic and cell differentiation factors by ionic or covalent attachment to the ECM scaffold could enhance the recruitment and favorable phenotypic differentiation of endogenous recipient cells.

In conclusion, the ECM scaffold was a suitable biologically derived material for cardiac patch surgery and was implanted without adverse consequences. A reparative process suggestive of tissue regeneration was observed over 3 months. Additional research is ongoing to elucidate long-term effects, evaluate the consequences of ECM scaffold infarction patching on LV function, and optimize the cardiac repair process.

References

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