The effect of polymer degradation time on functional outcomes of temporary elastic patch support in ischemic cardiomyopathy

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Abstract

Biodegradable polyurethane patches have been applied as temporary mechanical supports to positively alter the remodeling and functional loss following myocardial infarction. How long such materials need to remain in place is unclear. Our objective was to compare the efficacy of porous onlay support patches made from one of three types of biodegradable polyurethane with relatively fast (poly(ester urethane) urea; PEUU), moderate (poly(ester carbonate urethane)urea; PECUU), and slow (poly(carbonate urethane) urea; PCUU) degradation rates in a rat model of ischemic cardiomyopathy. Microporous PEUU, PECUU or PCUU (n = 10 each) patches were implanted over left ventricular lesions 2 wk following myocardial infarction in rat hearts. Infarcted rats without patching and age-matched healthy rats (n = 10 each) were controls. Echocardiography was performed every 4 wk up to 16 wk, at which time hemodynamic and histological assessments were performed. The end-diastolic area for the PEUU group at 12 and 16 wk was significantly larger than for the PECUU or PCUU groups. Histological analysis demonstrated greater vascular density in the infarct region for the PECUU or PCUU versus PEUU group at 16 wk. Improved left ventricular contractility and diastolic performance in the PECUU group was observed at 16 wk compared to infarction controls. The results indicate that the degradation rate of an applied elastic patch influences the functional benefits associated patch placement, with a moderately slow degrading PECUU patch providing improved outcomes.

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Appendix A. Supplementary data
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Keywords
Polyurethane; Polycarbonate; Biodegradation; Heart; Elastomer; Scaffold

1. Introduction
The impairment in cardiac function following myocardial infarction (MI) is typically accompanied by left ventricular (LV) remodeling; a process that includes left ventricular enlargement and changes in chamber geometry [1]. Late post-infarction remodeling involves the LV globally and includes compensatory LV chamber dilatation with time and alterations in LV architecture to distribute the increased wall stresses more evenly [2]. Clinically, it has been reported that survival rate after MI is inversely correlated with severity of LV dilatation [3]. Moreover, LV dilatation can give rise to mitral valve regurgitation by the tethering of chorda tendinea. Thus, therapies designed to attenuate post infarction LV dilatation have been considered to alleviate morbidity and mortality in these patients. Indeed, therapeutic agents, including beta-blockers and angiotensin converting enzyme (ACE) inhibitors, have been reported to act through their effect on remodeling [2,4].

To directly reduce LV dilatation following MI, surgical ventricular restoration can be applied as a means to reshape the ventricle using a non-elastic, non-degradable endocardial patch (e.g. expanded poly(tetrafluoroethylene)) such as in the Dor or septal anterior ventricular exclusion (SAVE) procedures [5,6]. Recently, however, the Surgical Treatment for Ischemic Heart failure (STICH) trial demonstrated no benefit in clinical outcome by adding SVR to coronary bypass surgery. This negative outcome has been considered to be attributable to a reduction in diastolic distensibility, thereby impeding LV filling response [1]. Conceptually, an epicardial onlay patch placed onto the infarct lesion has advantages over endocardial patching in that extracorporeal circulation is not required during the procedure, an elastic patch could prevent mechanical compliance mismatch, and such a patch would have the potential to be loaded with cells or bioactive agents should these be deemed necessary. Furthermore, torsion, rotational movement during the cardiac cycle, is greater in the endocardium than the epicardium [7]. Several studies have examined epicardial patch implantation onto the infarcted heart with non-degradable [8,9] or biodegradable materials [10–13].

The potential benefits of employing biodegradable materials for an epicardial patch include less risk for infection, host tissue ingrowth, and less adhesion formation. Previously, we have demonstrated temporary mechanical supports with biodegradable polyurethane patches positively alter the remodeling and functional loss following MI in a rat [14] and porcine model [15]. At this time, however, no study has explored how long such materials need to remain in place. In an effort to address the question of patch degradation rate, our objective was to compare the efficacy of porous onlay support patches made from one of three types of biodegradable polyurethane with 1) quicker (poly(ester urethane)urea; PEUU), 2) medium (poly(ester carbonate urethane)urea; PECUU), and 3) slower (poly(ester carbonate) urea; PCUU) degradation rates in a rat model of ischemic cardiomyopathy.

2. Materials and methods

2.1. Animal study
Adult female syngeneic Lewis rats (Harlan Sprague Dawley Inc.) 10–12 wk old, weighing 160–210 g were used for this study. The research protocol followed the National Institutes of Health guidelines for animal care and was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh (#0903312A-3).
2.2. Polymer synthesis and scaffold fabrication

PEUU and PCUU were synthesized from soft segments of polycaprolactone (PCL, MW = 2000, Sigma) or poly(hexamethylene carbonate) (PHC, MW = 2000, Sigma) diols respectively, and diisocyanantobutane (BDI, Sigma) hard segment with chain extension by putrescine (Sigma) according to a previous report [16], while PECUU was synthesized from a soft segment 50/50 (molar ratio) blend of PCL and PHC diol, also with BDI and putrescine. Detailed polymer characteristics, including in vitro and in vivo degradation, mechanical properties and cytocompatibility, have been reported previously [16]. The soft segment:hard segment:chain extender molar ratio was set as 1:2:1. For scaffold fabrication, polymer samples were completely dissolved in hexafluoroisopropanol (HFIP) to obtain a 40% (w/v) solution. This solution (1 mL) was blended uniformly with 5 g salt particles (NaCl, Sigma), which had particle sizes of 75–100 µm obtained by serial treatment with American standard sieves. The polymer/salt mixture was poured into a 1 cm diameter cylindrical glass mold. After complete solvent evaporation, the mixture was immersed in an excess of 30% ethanol solution to remove the salt particles from the scaffold with frequent solution changes over 2 d of immersion. The scaffold was then placed in pure deionized water to exchange the ethanol solution for 3 h, and then frozen at −80 °C, followed by lyophilization for 2 d to obtain a porous scaffold for implantation [16]. The material was sized to circular patches 6 mm in diameter and 300 µm in thickness. The patches were immersed in 70% ethanol for 30 min, followed by washing in phosphate-buffered saline and exposure to the ultraviolet light source for 1 h before implantation. Scaffold morphology was observed with scanning electron microscopy (SEM) after sputter coating. Tensile mechanical properties of the scaffolds were measured on an MTS Tytron 250 MicroForce Testing Workstation at 25 mm/min according to ASTM D638-98. Four samples were tested for each scaffold. The scaffold porosity was determined using an ethanol displacement method [16].

2.3. Chronic left ventricular infarction model

The detailed procedure for creating the rat MI model has been described previously [17]. Briefly, rats were anesthetized with 3.0% isoflurane inhalation with 100% oxygen followed by intubation and respiratory support with a rodent volume-controlled mechanical ventilator (683 Ventilator, Harvard Apparatus, Holliston, MA) at a tidal volume of 3 mL and 80 breaths/min. Rats were placed in the right decubitus position, and the chest was shaved and prepared with povidone-iodine solution. Procedures were performed in a sterile environment on a heating blanket. The heart was exposed through a 4th left thoracotomy, monitoring electrocardiogram. The proximal left anterior descending (LAD) coronary artery was ligated with 7-0 polypropylene. Myocardial ischemia was confirmed by decreased movement in the left ventricle (LV) free wall, regional cyanosis and ST-segment elevation. The incision was closed in layers with 5-0 polypropylene continuous sutures. The animals were allowed to recover from anesthesia and returned to their cages. For prophylaxis of lethal ventricular arrhythmia, 10 mg/kg of lidocaine was administered intramuscularly once prior to surgery. For postoperative analgesic treatment, 0.1 mg/kg of buprenorphine was administered subcutaneously 3 times daily for 3 d after surgery. For prophylaxis of surgical site infection, 100 mg/kg of cefuroxime was administered intramuscularly twice daily for 3 d after surgery.

2.4. Patch implantation

Two weeks after coronary artery ligation, animals were anesthetized and examined echocardiographically for infarct size as estimated by the percentage of scar area (akineti c or dyskinetic regions) to LV free wall area [10]. A total of 52 rats with infarcts greater than 25% of the LV free wall were randomly divided into 4 groups: 1) PEUU patch repair, 2) PECUU patch repair, 3) PCUU patch repair, and 4) sham repair (infarction control group). Through a 5th left thoracotomy, the infarcted anterior wall was exposed. Before affixing the
patch, the surface of the infarcted area (less than 0.1 mm thickness), including the remnant epicardium and some of the integrated fibrous tissue, was scraped and removed at the patch implant site. Subsequently, the anterior infarcted myocardium was covered with a patch, using 7-0 polypropylene with over-and-over peripheral continuous sutures. For the infarction control group, a thoracotomy was performed 2 weeks after coronary ligation, but no scraping or patch placement was performed. Ten age-matched rats without coronary ligation or surgical intervention served as a healthy control group.

2.5. Echocardiography

Echocardiography was performed immediately prior to patch implantation (pre-implantation time point, which was 2 wk post-infarction), as well as 4, 8, 12 and 16 wk after patch implantation. Rats were anesthetized with 1.25–1.5% isoflurane inhalation with 100% oxygen. Standard transthoracic echocardiography was performed using the Acuson Sequoia C256 system with 13-MHz linear ultrasonic transducer (15L8; Acuson Corporation, Mountain View, CA) in a phased array format. B-mode measurements on the LV short axis view (papillary muscle level) were performed. The end-diastolic (EDA) and end-systolic (ESA) LV internal cavity areas were measured by tracing the endocardial border. M-mode tracing images were also recorded from the same short axis view. The LV fractional area change (%FAC) was estimated as, %FAC = [(EDA – ESA)/EDA] × 100%.

Myocardial performance index (MPI) was calculated using Doppler pulsed-wave traces of mitral inflow and aortic outflow measured at the level of the LV outflow tract from the apical view at 16 wk endpoint for all groups. Ejection time and the isovolumetric contraction and relaxation times were averaged from three consecutive cardiac cycles. MPI was calculated as the sum of the isovolumetric contraction and relaxation times divided by the ejection time [18]. Sphericity index, determined as the ratio of long axis to short axis diameters both at the end-diastolic and end-systolic phase [19], and apical axis diameter, defined as a diameter of the sphere that best fits the apex [20], were measured to assess LV geometry. The maximum diameters of the left atrium for all groups at the 16 wk time point were also measured from the longitudinal axis view. All measurements were performed using OsiriX image processing application v.3.7.1.

2.6. Hemodynamic catheterization

At the endpoint of 16 wk, prior to euthanasia, rats were anesthetized with 1.25–1.5% isoflurane inhalation with 100% oxygen and intubated for cardiac catheterization procedures [21]. Briefly, animals were ventilated and a 2F micromanometer-tipped catheter (Model SPR-838 Millar Instruments, Houston, TX) was inserted via the right common carotid artery and advanced into the left ventricle to obtain LV pressure and conductance. All signals were digitized at a sampling rate of 200 Hz and were acquired to a data acquisition system (PowerLab 4/30, ADInstruments, Colorado Springs, CO) at steady state with the ventilator temporarily turned off. LabChart Pro v.7 software with PV-loop module (ADInstruments) was utilized for subsequent assessment of LV function including heart rate and LV mean pressure. Systolic function was quantified by dP/dt max contractility, and end-systolic pressure–volume relationship (ESPVR). Diastolic function was assessed by measuring the dP/dt min relaxation, and LV time constant of isovolumic relaxation (Tau; using the Weiss method [22]). Hemodynamic parameters including ESPVR and Emax (determined as the slope of ESPVR), were automatically calculated value by LabChart software. After hemodynamic measurements under anesthesia, rats were euthanized with cardiac arrest by apical injection of 2 mL of a hypothermic arresting solution containing (in mmol/L) 68 NaCl, 60 KCl, 36 NaHCO₃, 2.0 MgCl₂, 1.4 Na₂SO₄, 11 dextrose, 30 butanedione monoxime, and 10,000 U/L of heparin.
2.7. Histology and immunohistochemistry

The heart (n = 6 per each group) was explanted and fixed in 2% paraformaldehyde for 2 h at 4 °C and then embedded with optimal cutting temperature compound (Tissue-Tek, Torrance, CA) followed by freezing at −80 °C. Embedded, frozen LV tissues were serially sectioned at 8 µm in the LV transverse direction at the center of patched area and mounted on microscopic glass slides and stained with Masson’s trichrome. Other sections of each heart were fixed in 4% paraformaldehyde, blocked with staining buffer for 1 h (2% goat serum in PBS) at room temperature, and incubated with mouse monoclonal antibody against alpha-smooth muscle actin (αSMA; 1:200, Abcam) or rabbit polyclonal antibody against elastin (1:100 Abcam) and mouse monoclonal antibody against CD68 (1:100, AbD Serotec) as a pan-macrophage marker. Mouse monoclonal antibody against CD163 (1:100, AbD Serotec) was used to identify polarized macrophage phenotype M2. Sections were also reacted with primary antibodies against collagen type I (monoclonal 1:100, Abcam), and collagen type III (monoclonal 1:400, Abcam). Nuclei were stained with 2′-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole trihydrochloride trihydrate (Hoechst 33342, 1:10,000, Invitrogen). Sections that were stained with only the secondary antibody were used as negative controls. Slides were examined with an Olympus IX51 microscope and images captured using DP2-BSW software (Olympus America Inc.). For each retrieved sample, 10 different microscopic fields at 200× magnification were photographed for αSMA or CD163 positive structures. To determine quantity of vessels or arterioles, the number of αSMA-positive structures was measured using a digital image analyzer (ImageJ v.1.41, National Institutes of Health, Bethesda, Maryland) at 200× magnification. Vessels were identified as tubular structures positively stained for αSMA [23]. Arterioles were defined as αSMA-positive structures, having visible lumen, and more than 10 µm in diameter [24]. Non-vascular αSMA-positive area was measured within patched scar area and this parameter included not only clustered regions of αSMA-positive tissue but also endocardial αSMA-positive area. All measurements and assessments were performed using a digital image analyzer (ImageJ). Values are reported as the area (µm²) per 200× magnification of high-powered filed (HPF, approximately 0.581mm²) for non-vascular αSMA and as numbers per HPF for αSMA-positive vessels and arterioles, and CD68- and CD163-positive structures. The number of structures positive for a specific antibody was counted for vessel, arteriole, and CD163 evaluation, while the area expressed in pixels was measured for the evaluation of non-vascular αSMA, CD68, elastin, collagen type I, and collagen type III.

2.8. Determination of infarction size, scar area, and LV anterior wall thickening

The cross-sectional surface during sectioning was digitally photographed at the level of the center of patches. Infarction size was defined as a percentage of the sum of the epicardial and endocardial infarct circumference divided by the sum of the total LV epicardial and endocardial circumferences [25]. Scar area was measured as an infarction scar area using computer-based planimetry. LV anterior wall thickness was expressed as follows: scar area/[(epicardial circumference + endocardial circumference)/2]. Measurement of each parameter (n = 6 per each group) was performed using ImageJ analysis software on Masson’s trichrome stained sections.

2.9. Elastin and collagen assays for infarcted LV wall

Elastin levels in retrieved infarcted LV walls were measured using the Fastin elastin assay kit (Biocolor Ltd, UK), as previously described [26]. Briefly, the hearts were retrieved at 16 w after patch implantation, and the infarcted scar lesions were carefully dissected by surgical scissors without apron border zone myocardial tissue. The dissected scar tissue was weighed and cut into pieces with fine scissors and processed according to the instructions provided with the assay kit. Results were expressed as mg elastin per total scar lesion of each sample.
Collagen levels in retrieved patches were measured using the Sircol collagen assay kit (Biocolor Ltd, UK), as described previously [27]. The approximately 15 mg (dry weight) samples of the infarcted wall without apron tissue were weighed and processed according to the instructions provided with the assay kit. Results were normalized as mg collagen/g wet tissue.

2.10. Magnetic resonance imaging

Cardiac MRI was performed with FLASH-cine mode protocol (TE:2.5 ms, TR:8.0 ms, 256 x 256 pixels) and FLASH-cine tagging (TE:2.5 ms, TR: 15 ms, 1.5 mm tagging grids, 256 x 256 pixels) using a Bruker Biospec 7T/30 system at 16 wk under anesthesia with 1.25–1.5% isoflurane inhalation with 100% oxygen (n = 2 each group).

2.11. Statistical analyses

Statistical evaluations were performed using Prism version 4.0c (GraphPad Software Inc.). Results are listed as mean ± standard error of the mean. The Komolgorov–Smirnov test for normality was performed for each data set to determine the appropriate statistical testing. One-way ANOVA followed by Bonferroni multiple comparison testing was applied where multiple comparisons were made at the same time point. For the temporal analysis of echocardiography including EDA and %FAC, two-way repeated measures analysis of variance (ANOVA) was performed using the Bonferroni correction. Differences were considered to be statistically significant at p < 0.05.

3. Results

3.1. Material characteristics

All of the fabricated scaffolds were white in color and exhibited a foam-like structure with pore sizes ranging from 75 to 100 µm and a cubic pore shape reflective of the salt crystals used in the processing (Fig. 1). As shown in Table 1, all scaffolds had high distensibility (>100% peak strain) and porosity (>80%), but PEUU scaffolds had significantly greater tensile strength and initial modulus than PECUU and PCUU (n = 4 each scaffold). PECUU and PCUU did not differ in tensile strength and initial modulus.

3.2. Postoperative course and gross observations

A total of 65 animals were infarcted with an operative mortality rate of 9.2% (n = 6). Two weeks after infarction, 7 animals (11.9%) were excluded from the study because of small infarction size (<25%) based on echocardiographic assessment. The intraproductive mortality rate for the patch implantation procedure was 7.7% overall, distributed across the patch groups: PEUU (n = 2), PECUU (n = 1), and PCUU (n = 1). There was neither late mortality nor morbidity after patch placement for all treatment groups, nor was there late mortality for the infarction control group. A total of 48 rats, 12 each in the PEUU, PECUU, PCUU, and infarction control groups, reached the 16 wk endpoint. There was no difference between the groups in terms of body weight (228.8 ± 2.6 g) and tibial length (49.7 ± 0.1 mm) at 16 wk. At the time of sacrifice, a vaguely delineated edge was observed for PEUU patches, whereas for the PECUU and PCUU groups the remnant patch was more distinct (Fig. 2A). One case of massive mitral and tricuspid regurgitation was observed in the infarction control group but not in any of the patched groups (Fig. 2B).

3.3. Infarction size, scar area, and LV anterior wall thickening

The ventricular wall thickness of patched infarction risk areas was greater than that observed for the infarction control group (Fig. 2C) (n = 6 per group). While no significant difference was found in scar area between groups including the infarction control (Fig. 2D), a
significant decrease in infarction size (% ventricular circumference) was observed in the PECUU and PCUU, but not in the PEUU group compared with the infarction control group (Fig. 2E).

3.4. Masson’s trichrome staining
Masson’s trichrome staining of the hearts 16 wk after the patch implantation revealed that the majority of the PEUU scaffold was degraded, with loose connective tissue occupying the implant area, and remnant material sporadically present. For the PECUU scaffolds, more remnant material was seen, however fragmentation of the remnant was observed. Qualitatively, thicker tissue was found beneath PECUU and PCUU scaffolds versus PEUU. For the PCUU scaffolds, largely continuous areas of remnant scaffold were found, with a relatively thicker cell-infiltrated scaffold present (Fig. 3).

3.5. EDA and %FAC by echocardiography
Echocardiography showed a greater EDA and lower %FAC in all infarcted rats, including the patched and infarction control group, compared with healthy controls at each time point tested (p < 0.001) (n = 10 per group). There were no significant differences in EDA and %FAC between infarcted groups 2 wk after LAD ligation (at the time of patch implantation). The EDA in the patch groups was significantly decreased versus the infarction control group (PECUU and PCUU from 4 wk onward, PEUU from 8 wk onward). There were no significant differences between the PECUU and PCUU groups in both EDA and %FAC, whereas the EDA with PEUU patching significantly increased versus PECUU after 8 wk and versus PCUU after 12 wk. The %FAC in PECUU and PCUU groups was significant higher than for the infarction control group after 4 wk, while PEUU achieved significance only at 16 wk compared with the infarction control group. The %FAC of the PECUU and PCUU was significantly increased versus PEUU at 16 wk (Fig. 4A–D).

3.6. MPI and left atrial diameter by echocardiography
Combined assessment of both systolic and diastolic function using myocardial performance index (MPI, also denoted as the Tei index) at 16 wk showed patch implantation improved MPI for all patched groups (Supplemental Fig. 1) (n = 10 per group). Assessment of the left atrial diameter at 16 wk demonstrated that patched groups had significantly smaller left atria than infarction controls, and were not statistically different from healthy controls (p > 0.05) (Fig. 4D). No differences were detected between the three patched groups for MPI and left atrial diameter.

3.7. Geometrical analysis by echocardiography
Geometrical analysis of the left ventricle demonstrated no effect on the sphericity index by any patch implantation, while apical diameter analysis showed that PECUU and PCUU patch implantation had a significant beneficial effect over the infarction group at 16 wk (Supplemental Fig. 2).

3.8. Hemodynamic catheterization
No statistical differences were found between all infarcted groups and the healthy control group in terms of the mean LV pressure (58.9 ± 1.8 mmHg) and heart rate (362 ± 7 beats per min) at 16 wk (n = 10 per group). Hemodynamic analysis 16 wk after patch implantation is presented in Fig. 5. Cardiac output was improved for PECUU and PCUU groups relative to infarction controls (Fig. 5A). For systolic functional assessment, the dP/dt max and stroke work (SW) showed significant improvement in the PECUU group compared with the infarction control (Fig. 5B and C). For diastolic functional assessment, the dP/dt min was improved with PECUU (Fig. 5D) and Tau showed improvement for all patched groups (Fig.
5E) compared to infarction controls. Representative pressure-volume loops (PV-loop) for each group are shown Fig. 5F. $E_{\text{max}}$, another measure of systolic function, calculation revealed improvement in the PECUU and PCUU compared with the infarction control (Fig. 5G).

### 3.9. Elastin and collagen assays

Collagen and elastin protein content in the infarcted LV wall (risk zone) were measured for the infarction control group and all patched groups at 16 wk ($n = 4$ per group). The collagen assay revealed no significant differences between the assessed groups (Fig. 6A), whereas PECUU and PCUU patched LV walls had higher elastin levels compared with the infarction control and PEUU patched walls (Fig. 6B). Patch type also did not affect the type of collagen elaborated as determined histologically. No significant differences were observed in type I and type III collagen with immuno-histochemical assay (Supplemental Fig. 3), which was consistent with the results of the collagen protein content measurement (Fig. 6A).

### 3.10. Immunohistochemistry for $\alpha$SMA

The ventricular walls to which PEUU and PECUU patches were applied contained greater $\alpha$SMA positive cellular areas than for those patched with PCUU (Fig. 7A–B) ($n = 6$ per group). The $\alpha$SMA regions were found under the patch and did not appear to be associated with vascular structures. (Fig. 7A).

### 3.11. Neovascularization

The density of $\alpha$SMA–positive vascular structures was significantly increased 16 wk after patch implantation for the PECUU and PCUU versus PEUU patched animals (Fig. 7C). Arteriole formation in the PECUU group was also increased versus the PEUU group (Fig. 7D).

### 3.12. Immunohistochemistry for macrophages

The CD68 (pan-macrophage marker)-positive area was greater with PECUU and PCUU patching at 16 wk relative to PEUU (Fig. 7E–F). The CD163-positive (M2 macrophage marker) structures in the PECUU group were greater in number than for the PEUU or PCUU groups (Fig. 7G), as seen in representative images for CD163 staining of the patched groups in Fig. 7H. Also, the CD163/CD68 ratio in the PECUU group was significantly greater than that found for the PEUU group (Fig. 7I).

Considering the elastin-staining presented in Fig. 7E and quantified in Fig. 7J, PECUU and PCUU patching was associated with greater labeling at 16 wk relative to PEUU, which was consistent with elastin protein content measurement (Fig. 6B).

### 3.13. MRI analysis

MRI showed that systolic and diastolic LV cavities with PECUU and PCUU patch implantation appeared to be smaller than with PEUU patching or for the infarction control at 16 wk ($n = 2$ per group) (Supplemental Fig. 4 and Supplemental Movies 1–4). MRI tagging imaging, in which the strain of six ventricular segments in short axis view was traced, indicated that regional circumferential strain with PECUU patch implantation appeared to be qualitatively more coordinated than for the other groups. Specifically there appeared to be less dyssynchronous LV movement, although this result is limited to being qualitative in nature due to the low number of observations.
4. Discussion

Adverse remodeling of the LV is a compensatory mechanism of chronic ischemic cardiomyopathy, characterized by wall lengthening and thinning, overall ventricular dilatation and geometrical sphericity to maintain cardiac output by increasing stroke volume [28]. This compensatory LV deformation in turn precipitates maladaptive changes in LV structure and function and produces a cycle in which the wall thinning increases end-systolic circumferential and longitudinal wall stresses by LaPlace’s law, leading to further wall thinning and chamber dilatation which ultimately leads to decompensated congestive heart failure even in the absence of recurrent ischemic events [29]. We previously reported that microporous, elastic, and biodegradable polyurethane patches (PEUU) act as temporary mechanical supports to positively alter the LV remodeling and functional loss following myocardial infarction [14,15]. However, how long such materials need to remain in place is unclear. Recently, we have also reported a family of biodegradable polyurethane elastomers (PECUU and PCUU) where partial substitution of polyester segments with polycarbonate segments in the polymer backbone leads to slower degradation behavior [16]. The data in the current report demonstrated that implantation of slower degrading PECUU or PCUU patches resulted in a greater benefit in treating chronic ischemic cardiomyopathy in terms of cardiac function and histology compared with faster degrading PEUU patches.

Biodegradable material implantation induces an inflammatory response. It follows that the period over which a degradable epicardial patch remains discernible by host cells would be associated with elevated inflammatory activity in the region of the patch. The magnitude of inflammatory response would, of course, also be dependent upon material chemistry and other physical parameters. As the primary cell type of the post-acute foreign body response, macrophages produce a spectrum of enzymes and cytokines that facilitate tissue remodeling in terms of matrix degradation, cell recruitment, proliferation and extracellular matrix formation for new tissue regeneration. The infarction and remodeling processes are associated with macrophage activity as the ventricular wall remodells, with hypothesized positive and negative benefits being associated with this inflammatory activity. Indeed, studies with a macrophage depletion animal model have shown that wound healing is severely impaired, leading to LV wall thinning and aggravated LV adverse remodeling, when macrophages are ablated [30–32]. Furthermore, macrophages facilitate angiogenesis through the delivery of angiogenic factors such as basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) [33]. If macrophage activity is viewed in a positive light, would extending this activity be beneficial? In a similar model of ischemic cardiomyopathy in the rat (without patch placement), it was demonstrated that early stage inhibition of cell apoptosis in granulation tissue improved cardiac remodeling and reduced dysfunction at the chronic stage [34]. From a similar perspective, a slower degrading material placed in the region of an infarct may have an advantage in terms of extending the presence of macrophages toward longer time points. The significantly greater vessel numbers and elastin elaboration in the slower degrading polymer groups (PECUU and PCUU) may be attributed to an extended macrophage presence.

It may be too simplistic to assume that extending the period over which macrophages persist in the region of an infarct would provide positive benefit since macrophages may exert both detrimental [35] and beneficial effects [32,36–38] towards LV tissue repair following ischemic events. The growing body of literature describing differing macrophage phenotypes and their associated functions has significantly improved our understanding of tissue healing and raised the potential for strategies targeting a specific macrophage subset. The primary dichotomy of macrophages into M1 (cytotoxic, classically activated, pro-inflammatory), and M2 cells (pro-healing, angiogenic) [39–44] suggests that latter stage M2 activity may be desired to facilitate a better healing outcome in the ischemic ventricular
The data demonstrated that M2 immuno-reactivity was higher in the PECUU group followed by PCUU and PEUU groups using an anti-CD163 antibody, which is one surface marker representative of M2 macrophages [45]. Furthermore, the ratio of CD163 to CD68, the latter being a pan macrophage marker [45,46], was also greater in the PECUU group. M2 macrophages have been reported to produce TGF-β [47,48], which has been described as a multipotent cytokine with healing potential in various tissues by promoting cell growth and matrix accumulation, thus facilitating the ability of tissue to withstand stress without expanding [49]. Although the segregation of macrophages into two distinct phenotypes is a simplified framework of the in vivo reality, our findings may suggest that epicardial implantation of a patch with a moderate degradation rate has beneficial impact on infarct tissue healing through M2 polarization.

In addition to the findings regarding the presence and phenotype of macrophages in the patched ventricular walls, it was also observed that patch type appeared to have an effect on elastin elaboration. Elastin fibers are major components of insoluble extracellular matrix assemblies that impart elastic properties to tissue and provide not only extensile and resilient properties, but maintain the architecture in the face of repeated extension and recoil cycles [50]. The lack of such elastic recoil can lead to the thinning and extension of the infarcted ventricular wall, which gradually progresses after a myocardial infarction and eventually results in cardiac failure [51,52]. Given this background, several reports have examined the efficacy of promoting elastin synthesis or inhibiting elastin degradation in cardiovascular degenerative diseases, including cardiac dilatation after infarction [53–56] and aortic aneurysm [26,57,58]. The detection of putatively newly synthesized elastin networks with patching, particularly in the materials with medium to longer degradation rates, could in theory contribute to the cardiac systolic and diastolic functions and to the inhibition of adverse LV remodeling by adding recoil to the infarct scar. Although further long-term studies are needed, the induced elastic fibers may potentially have a prolonged influence on cardiac function since elastin molecules may have an extended life [59], beyond the point where the synthetic elastomer has effectively degraded.

Several limitations of the present report should be mentioned. First, while one might hypothesize that the longer degrading patches were associated with softer, more elastic walls (possibly due to the elastin elaboration), mechanical studies were not performed on the hearts at the end of the study period. Earlier work with PEUU patches showed that the patched walls were significantly softer than the infarct controls at 8 wk [14]. Of note, the PEUU patches used in that study were made with a thermally induced phase separation technique, whereas the current study utilized salt-leaching to generate the applied scaffolds. Second, further studies with longer endpoints would provide better insight into how all animal groups, in particular PECUU and PCUU, performed after complete patch degradation. At 16 wk PECUU and PCUU material was still present as part of the ventricular wall. While this material may not have provided substantial mechanical support by this time, it would still likely be a source of ongoing macrophage polymer phagocytic activity, potentially leading to a lasting M2 macrophage activity. Indeed, as several advantages of PECUU over PCUU in terms of histology have been presented in the study, a longer study endpoint may allow us to detect significant functional differences. An extended endpoint, possibly towards the latter stages of the rat lifespan may yield insight into how well the patch material influences cardiac function after the material has completely degraded. A better approach would be to address the longer term function questions in a large animal model, which would better capture the scale for the human physiology. Finally, it is important to note that it was not possible to independently separate patch degradation time and patch mechanics. While the stiffness of the PEUU patches used in the study were generally of the same order of magnitude as the PECUU and PCUU patches prior to implantation, the impact of this mechanical difference may not be excluded and the softer
carbonate containing patches may have provided more appropriate support for the remodeling process and the relatively stiffer PEUU material was less beneficial. Some evidence against this argument is that in an earlier report PEUU patches were applied in the same rat model at shorter time points, but with thermally induced phase separation as opposed to salt-leaching to introduce porosity [14]. The benefit of the phase separation patches appeared to be qualitatively better or at least similar to the salt-leached patches over an 8 wk period if the studies are compared, even though the stiffness of the phase separation patches was greater.

5. Conclusions

The efficacy of porous onlay support patches made from one of three types of biodegradable polyurethane with 1) quicker (poly(-ester urethane)urea; PEUU), 2) medium (poly(ester carbonate urethane)urea; PECUU), and 3) slower (poly(ester carbonate)urea; PCUU) degradation rates was compared in a rat model of ischemic cardiomyopathy. The results indicate that the slower degrading patches, and in particular the PECUU polyurethane patch, provided greater benefit in treating ischemic cardiomyopathy than morphologically similar PEUU patches in the rat model. This conclusion was supported by both functional and histological assessment which showed that PECUU patch prevented further functional deterioration while being associated with more desirable extracellular matrix components and markers of positive remodeling. The results support a tuned biomaterial approach to positively interrupting the negative remodeling process that occurs in ischemic cardiomyopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


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Fig. 1.
Electron micrographs of scaffold cross-sections generated from PEUU, PECUU, and PCUU using the technique of salt leaching. Scale bars: 200 µm.
Fig. 2.
Representative macroscopic views of rat heart implant sites for PEUU, PECUU and PCUU scaffolds and their trans-sectional views after 16 wk, including the healthy and infarction control (A). Scale bars: 5 mm (white) and 10 mm (black). The yellow arrows indicate estimated edge of the remnant implanted material. Severe ischemic mitral and tricuspid regurgitation developed in the infarction control group as evidenced in this color Doppler image (B). RV: right ventricle, RA right atrium, LV: left ventricle, LA: left atrium. Wall thickness of patched infarction risk area was greater than for the infarction control group (C). There was no significant difference in the cross-sectional scar area between groups including the infarction control (D). A significant decrease in infarction size was observed with PECUU and PCUU patches, but not in the PEUU group compared with the infarction control group (E). *p < 0.05 versus infarction control. IC: infarction control, LV; left ventricle, and RV: right ventricle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Fig. 3.
Representative composite views of Masson’s trichrome stained cross-sections of the hearts at 16 wk for PEUU, PECUU and PCUU scaffolds, and the infarction control demonstrating the variable resorption behavior between scaffold types. Black boxes indicate higher magnification area shown in right panels. Red arrows indicate suture lines which were placed along the scaffold edge at the time of implantation. Black arrows indicate regions with remnant scaffold material. Scale bars: 2.0 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Fig. 4.
Temporal echocardiographic assessment for end-diastolic area (EDA) and fractional area change (%FAC) after patch implantation (A and B). *p < 0.05, †p < 0.01, ‡p < 0.001, §p < 0.05 compared with both PECUU and PCUU, and ¶; p < 0.05 compared with PEUU at the same time points. Representative M-mode echocardiographic images of the left ventricle for each group (C). Scale bar: 1.0 cm. Representative longitudinal echocardiographic view for each group to assess the left atrial diameter (right panel), and the quantification showing significantly decreased left atrial diameter by patch implantation (left panel) (D). Scale bar: 5.0 mm *p < 0.05 compared with infarction control.
Fig. 5.
Hemodynamic analysis 16 wk after patch implantation with controls. Cardiac output in each group was obtained for overall evaluation for cardiac function (A). The $dP/dt$ max (B) and stroke work (SW) (C) were measures of systolic function, whereas $dP/dt$ min (D) and Tau (Weiss) (E) were measures of diastolic function. Representative pressure-volume loops (PV-loop) for each group are shown in (F), these were used to generate another measure of systolic function, $E_{\text{max}}$ (G). *$p < 0.05$ versus infarction control and †$p < 0.05$ versus healthy control. IC; infarction control, HC; healthy control. Note that the PECUU has beneficial profiles not only for contractile but also for diastolic function.
Fig. 6.
Protein content measurement 16 wk after patch implantation with infarction control. Collagen protein content in whole risk area did not vary across all groups (A), whereas elastin protein content in whole risk area was significantly decreased in the infarction control (IC) and PEUU groups compared with PECUU and PCUU (n = 4 per group) (B).
Fig. 7.
Representative immunostained micrographs for alpha-smooth muscle actin in the patched groups (A). The white and red arrowheads indicate non-vascular αSMA positive structures and vascular αSMA, respectively. The white arrows indicate αSMA-positive membrane found along the endocardial surface of the infarcted risk zone. The broken lines indicate the approximate implanted material area. The non-vascular αSMA-positive area was increased in the PEUU and PECUU compared with PCUU groups (B). The quantification for αSMA-positive vessels (C) and arterioles (D) revealed increased vessel numbers for PECUU and PCUU versus PEUU patched walls. Representative images stained for elastin and CD68 (pan-macrophage marker) of the patched groups (E). The number of CD68-positive structures in the PEUU was significantly decreased relative to the PECUU and PCUU groups (F). The CD163-positive (M2 macrophage marker) structures in the PECUU group were greater in number than for the PEUU or PCUU groups (G), as seen in representative images for CD163 staining of the patched groups in H. Representative images for CD163 (M2) of the patched group (H). The CD163/CD68 ratio in the PECUU was significantly greater than that found with the PEUU (I). The elastin-positive area was significantly decreased in the PEUU versus PECUU and PCUU groups (J). (n = 6 per group) Scale bar: 200 µm epi: epicardial side, end: endocardial side. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
## Table 1

### Scaffold characterization.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak tensile strength (kPa)</th>
<th>Peak strain (%)</th>
<th>Initial modulus (kPa)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEU</td>
<td>337 ± 22(^a)</td>
<td>124 ± 11(^a)</td>
<td>513 ± 40(^a)</td>
<td>81 ± 4(^a)</td>
</tr>
<tr>
<td>PECU</td>
<td>232 ± 42(^b)</td>
<td>195 ± 11(^b)</td>
<td>164 ± 24(^b)</td>
<td>86 ± 2(^b)</td>
</tr>
<tr>
<td>PCU</td>
<td>156 ± 23(^b)</td>
<td>152 ± 7(^a)</td>
<td>107 ± 24(^b)</td>
<td>84 ± 4(^a)</td>
</tr>
</tbody>
</table>

\(^a\) and \(^b\) superscripts represent significantly different groups.

\(N = 4\) for each sample type.