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# 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.



## 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

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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 diisocynantobutane (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 post-operative 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 (akinetic 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, $\%FAC = [(EDA - ESA)/EDA] \times 100\%$.

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