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Placement of an elastic, biodegradable cardiac patch on a sub-acute infarcted heart leads to cellularization with early developmental cardiomyocyte characteristics

Kazuro L. Fujimoto, MD, PhD^{1,2}, Kimimasa Tobita, MD^{2,3,4}, Jianjun Guan, PhD^{2,5}, Ryotaro Hashizume, MD, PhD^{1,2}, Keisuke Takanari, MD, PhD^{1,2}, Christina M. Alfieri, BS⁶, Katherine E. Yutzey, PhD⁶, and William R. Wagner, PhD^{1,2,4}

¹Department of Surgery, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15213

²McGowan Institute for Regenerative Medicine, University of Pittsburgh, 100 Technology Drive Suite 200, Pittsburgh, PA 15219

³Department of Developmental Biology, University of Pittsburgh, Rangos Research Center, One Children's Hospital Dr. 4401 Penn Ave. Pittsburgh, PA 15224

⁴Departments of Bioengineering and Chemical Engineering, University of Pittsburgh, 300 Technology Dr, Pittsburgh, PA 15219

⁶Division of Molecular Cardiovascular Biology, Cincinnati Children's Medical Center, 240 Albert Sabin Way, Cincinnati, OH 45229

Abstract

Background—Placement of an elastic and biodegradable patch onto a sub-acute myocardial infarct (MI) provides temporary elastic support that may act to effectively alter adverse left ventricular (LV) remodeling processes.

Method—Two weeks after permanent left coronary ligation in Lewis rats, the infarcted anterior wall was covered with poly(ester urethane)urea PEUU (MI+PEUU, n=15) or expanded polytetrafluoroethylene (MI+ePTFE, n=15) patches, or had no implantation (MI+sham, n=12). Eight weeks after surgery, cardiac function and histology were assessed.

Results—The ventricular wall in the MI+ePTFE and MI+sham groups was comprised of fibrous tissue, while PEUU implantation induced α -smooth muscle actin positive muscle bundles co-expressing sarcomeric α -actinin and cardiac specific troponin-T. This pattern of co-localization was also found in developing embryonic myocardium. Cardiac transcription factors Nkx-2.5 and GATA-4 were strongly expressed in the muscle bundles. In the MI+sham group end-diastolic LV cavity area (EDA) increased and the %fractional area change (%FAC) decreased. For ePTFE

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Address for correspondence: William R. Wagner, PhD, McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Dr., Suite 300, Pittsburgh, PA 15219, Phone: (412) 624-5324, Fax: (412) 624-5228, wagnerwr@upmc.edu.

⁵Current address: Department of Materials Science and Engineering, The Ohio State University, 477 Watts Hall 2041 College Rd, Columbus, OH 43210

Disclosure

The University of Pittsburgh has filed a patent application on the results described in this work. Co-authors WRW, KF, KT, JG are inventors on this patent application.

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patched animals, both EDA and %FAC decreased. In contrast, with MI+PEUU patching %FAC increased while EDA was maintained. With dobutamine stress echocardiography MI+PEUU patched LVs possessed contractile reserve significantly larger than the MI+sham group.

Conclusion—MI+PEUU patch implantation onto sub-acute infarcted myocardium induced muscle cellularization with characteristics of early developmental cardiomyocytes as well as providing a functional reserve.

Keywords

infarction; remodeling; patch implantation; cellularization

Introduction

Unlike skin, liver, skeletal muscle, and other organs, it had been thought that the adult mammalian heart could not regenerate in a significant manner because of the questionable proliferative potential possessed by adult cardiomyocytes.^{1,2} Recently several reports have described the existence of stem cell populations in human and rodent hearts that proliferate and may give rise to cardiomyocytes, vascular smooth muscle cells and endothelial cells.³⁻⁵ However, the regenerative capacity of the heart remains limited for practical purposes. The most promising approaches may involve the stimulation of host cell populations to alter the default healing response in a manner that would ameliorate heart damage.⁶

Previously, we reported that implantation of an elastic and biodegradable polyester urethane urea (PEUU) patch regionally onto a chronic myocardial infarct prevented LV dilatation, altered LV wall thickness and compliance toward normal levels at the patch site, and improved global and regional contractile function relative to non-treated animals.⁷ A particularly interesting finding was the development of alpha-smooth muscle actin (α -SMA) positive muscle bundles cellularized in the infarcted wall beneath the implanted patch. While mature cardiomyocytes do not normally express α -SMA,⁸ early developmental cardiomyocytes do transiently expressed α -SMA which disappears over the 1–7 days following birth.^{9,10} The α -SMA positive muscle bundles induced by PEUU cardiac patch placement were shown to possess markers consistent with contractile smooth muscle cells, but the presence of cardiac markers on these cells was not explored. In this report we hypothesized that such markers may in fact be present, and have performed such analysis, comparing the results of cells found in PEUU patched hearts to those from embryonic rat hearts. An intriguing possibility is that these cells might possess protein and transcription factors that would indicate a potential to assume a cardiomyocyte lineage, such as would be found in the developing embryonic myocardium.

Ventricular restraint is a form of therapy that has been explored to prevent further dilatation in advanced heart failure patients. Most extensively investigated have been a woven textile wrap (the CorCapTM device¹¹) and a nitinol mesh (Paracor HeartNetTM)¹². Both of these approaches involve wrapping the entire heart with non-biodegradable material, which carries the concern of fibrous encapsulation of the epicardium over time as the foreign body response to the implant matures.¹³ The permanent nature of both the implanted material and the resulting capsular formation may be detrimental to positive remodeling phenomena that might be possible, and are not attractive for subsequent surgical access to the myocardial field. By utilizing an elastic, biodegradable patch applied regionally on top of the infarcted epicardial surface, the PEUU patch approach avoids encapsulating the entire myocardium and provides temporary elastic support that mechanically may alter the wall stress experienced by the infarcted region of the LV in the remodeling period. Altering this experienced wall stress may affect the remodeling course pursued by the tissue towards a more desirable end. The degradable nature of the patch also avoids the existence of a

permanent foreign body on the myocardial surface. As a control material for regional patch placement, we evaluated expanded-polytetrafluoroethylene (ePTFE), which has been used for reconstructive cardiovascular procedures.¹⁴ Both patch types were investigated for their functional and histological effect, together with MI+sham surgical controls. Given the finding of improved functional and structural parameters with PEUU patching, we further investigated the hypothesis that hearts treated in this manner possessed a contractile functional reserve under dobutamine stress echocardiography.

Methods

Experimental animals

Lewis adult female rats (200–250g), gestational day 14 rat embryos, and neonate day 3 rats were used (Harlan Sprague Dawley, Indianapolis, IN). The research protocol followed the National Institutes of Health guidelines for animal care and was approved by the University of Pittsburgh's Institutional Animal Care and Use Committee and Children's Hospital of Pittsburgh Animal Research Care Committee.

Cardiac patch

Circular micro-porous patches made of polyester urethane urea (PEUU, 6 mm diameter $\times$ 300 μ m thickness), whose synthesis,¹⁵ processing,¹⁶ mechanical properties, and sterilization^{7,17} were described previously, were used in this study. Also, for control purposes, similarly sized ePTFE patches (6 mm diameter $\times$ 400 μ m, Impra-Bard, Tempe, AZ) were utilized. The mechanical properties and average pore size of PEUU and ePTFE were shown in Table 1. The PEUU patch microstructure, prepared using thermally induced phase separation technique is seen in Fig. 1a,⁷ as is the ePTFE patch.

Sub-acute infarction model

Adult female rats were anesthetized with isoflurane inhalation. Through a left thoracotomy, the proximal left anterior descending coronary artery was ligated with 7-0 polypropylene. After two weeks, animals were examined echocardiographically. Rats with infarcts greater than 25% of the LV free wall¹⁸ at the baseline evaluation were selected for patch placement or MI+sham surgery.

Cardiac patch implantation

The anterior infarcted myocardium was covered with a PEUU (MI+PEUU group, n=15) or ePTFE patch (MI+ePTFE group, n=15), using 7-0 polypropylene with over-and-over running peripheral sutures, after lightly scraping the surface of the infarcted area. In the myocardial infarction control group (MI+sham group, n=12), a thoracotomy was performed two weeks after coronary ligation with no scraping or patch placement.⁷

Histological assessment

The hearts were harvested from 12 rats each in the MI+PEUU and MI+sham groups, and 9 rats in the MI+ePTFE group, 8 weeks after the patch or sham surgery. Hearts were also harvested at 16 weeks after patch surgery for 3 rats in the MI+PEUU group. In addition, embryonic hearts (n=3), and neonatal hearts (n=3) were harvested. The embedded frozen LV tissues were serially sectioned in the LV transverse direction at a thickness of 8 μ m. Hematoxylin and eosin (H&E) staining and immunohistochemical staining were performed on each sample, as described previously.⁷ Sections for immunohistochemistry were fixed with 2% paraformaldehyde for 5 min and reacted with an antibody against, alpha-smooth muscle actin (α -SMA, Sigma, St Louis, MO), sarcomeric alpha-actinin (α -actinin, Sigma), cardiac troponin-T (cTnT, Abcam, Cambridge, MA), connexin 43 (Cx-43, Abcam),

Nkx-2.5, and GATA-4 (Santa Cruz Biotechnology, Santa Cruz, CA). Subsequently the relevant antigen was visualized fluorescently (Alexa 488, and Alexa 594, Molecular Probes, Eugene, OR) together with 4',6-diamidino-2-phenylindole (DAPI, Sigma) nuclear staining, or stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) metal enhanced substrate, following an avidin-biotin-peroxidase complex method (Pierce/Thermo Fisher Scientific, Rockford, IL), counterstained with hematoxylin. To confirm co-localization 3D projection images were reconstructed from stacks of z-axis optical scans using a standard laser confocal microscopy system (FV1000, Olympus, Tokyo, Japan) and Scion Image software (Scion Corp, MD, USA).¹⁹ For vascular density measurement, from each LV sample 10 different fields at 200x magnification were quantified for vascular density. Vessels were recognized as tubular structures positively stained for CD31 as previously described.⁷

In situ hybridization

A digoxigenin-labeled mouse Nkx-2.5 antisense RNA probe was generated,²⁰ and in situ hybridization of histological sections obtained 8 weeks following patch placement was performed as previously described.²¹

Assessment of cardiac function

Standard echocardiography—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.²² Eight weeks following patch implantation (10 weeks after myocardial infarction) standard echocardiography was performed under 1.5% isoflurane with 100% oxygen inhalation in all experimental animals. The end-diastolic (EDA) and end-systolic (ESA) LV internal cavity area were measured by tracing the endocardial border from a standard LV short-axis view. The LV fractional area change (%FAC) was estimated as, $\%FAC = [(LVEDA - LVESA)/LVEDA] \times 100$ (MI+PEUU group, n=12, MI+ePTFE group, n=9, and myocardial infarction control, MI+sham group, n=12).

Dobutamine stress echocardiography—Six animals from the MI+PEUU, MI+sham, and MI+ePTFE groups were utilized in the dobutamine study. Following standard echocardiography, the right jugular vein was dissected and cannulated for intravenous dobutamine infusion under anesthesia with 1.5 % isoflurane inhalation. Baseline measurements were obtained before beginning dobutamine infusion, and results were compiled 5 min after infusing the drug at 15 µg/kg/min. Six age-matched Lewis adult female rats without coronary ligation or surgical intervention served as normal controls.

Statistical analysis

All data are expressed as the mean ± standard deviation. Longitudinal echocardiography data were compared using 2-way repeated measures analysis of variance (ANOVA) with Tukey post-hoc testing (factor-1: MI+sham, MI+ePTFE, and MI+PEUU; factor-2: 0 weeks and 8 weeks after patch implantation). Dobutamine stress echocardiography data were similarly compared using 2-way ANOVA with Tukey post-hoc with factor-2 being pre and post dobutamine administration. Statistical significance was defined at $P < .05$. All calculations were performed using SigmaStat (Systat Software Inc, Point Richmond, CA).

Results

Mortality and infarct size

The infarction procedure was performed in a total of 57 rats. Within 36 hours of infarction 10 rats died for a mortality rate of 17.5%. Among the surviving 47 rats, 5 rats with less than a 25% functional infarct were excluded from the study. There were no additional deaths during the observation period for all groups.

Histological characterization

Infarcted anterior ventricular wall—In the MI+sham group, the infarcted ventricular wall was generally replaced with thin fibrous tissue with the exception of some regions of residual myocardial tissue (Fig. 1b). For the MI+ePTFE group, the ventricular wall consisted of the ePTFE material with loose fibrous tissue beneath the patch material and some residual myocardium near the endocardial surface (Fig. 1c). For the MI+PEUU group, the majority of the PEUU patch material was absorbed with putative macrophages and fibroblasts infiltrating the area of the remnant patch material. Underneath the remnant patch muscle-like tissue was apparent (Fig. 1d). Immunohistochemical analysis revealed that α -SMA positive muscle bundles were not observed in the MI+sham group (Fig. 1e) or the MI+ePTFE group (Fig. 1f). On the other hand, abundant α -SMA positive muscle bundles consistently were identified beneath the PEUU patch area (Fig. 1g). These findings in the MI+sham and MI+PEUU groups were in agreement with our previous report.⁷ Further immunohistochemical assessment showed that the α -SMA positive cells within the muscle bundles co-expressed α -actinin, while residual myocardium was completely negative for α -SMA labeling (Fig. 2a–c). Higher magnification of the α -SMA positive cells clearly revealed co-localization with α -actinin (Fig. 2d–f), and also with cTnT (Fig. 2g–i). In addition, Cx-43 was expressed within the α -SMA positive muscle bundle area beneath the PEUU patch (Fig. 2j–l). The Cx-43 expression did not appear to form gap junctions with native cardiomyocytes and the expression was limited to the α -SMA positive bundles beneath the patch. These bundles generally did not merge continuously into the peripheral healthy myocardial tissue. At 16 weeks following patch implantation, histological sections showed that α -actinin, cTnT, and Cx43 were positively stained and co-localized with α -SMA positive cells (Fig. 3a–i), however the intensity of this staining was notably reduced.

Expression pattern of α -SMA with α -actinin and cTnT in embryonic and neonatal myocardium—To confirm that α -SMA expression patterns co-localized with cardiac specific proteins in early developmental stage rat myocardium, gestational day 14 embryonic myocardium was shown to express both α -SMA and α -actinin (Fig. 4a–c), whereas neonatal day 3 myocardium did not express α -SMA (data not shown). It was noted that the developing dorsal aortic wall expressed only α -SMA and did not express α -actinin, confirming the lack of false positive staining for α -actinin in the embryonic heart (Fig. 4d–f). Higher magnification images of the embryonic heart revealed that α -SMA co-localized with α -actinin and cTnT (Fig. 4g–l).

Cardiac transcription factor expression—Cardiac transcription factors, Nkx-2.5 and GATA-4, were found to be expressed in the α -SMA positive myocardium from gestational day 14 embryonic rat hearts (Fig. 5a–c). Both of these transcription factors were also found to be expressed in the α -SMA positive muscle bundles in adult rat hearts implanted with PEUU patches (Fig. 5d–f). Observations from several angles with confocal fluorescence immunohistochemistry further showed that these transcription factors were expressed in intra- or peri-nuclear sites of the α -SMA positive muscle cells beneath the PEUU patch material (Fig. 6a–f). For further verification of the phenomenon, in situ hybridization revealed that Nkx-2.5 mRNA was upregulated in the α -SMA positive muscle bundles (Fig.

7a, b). In contrast, in the infarcted LV wall for MI+sham control animals, Nkx-2.5 mRNA was only weakly positive in limited areas (Fig. 7c, d). The vascular density of the PEUU patch group was significantly increased compared to the infarction control (312 ± 43 vs. $98 \pm 19/\text{mm}^2$, $P < .05$, Fig. 8). Staining for GATA-4 at 16 weeks following PEUU patch placement did not show positive staining for GATA-4 in the α -SMA positive regions (Fig. 3j–l).

Cardiac functional assessment

Standard echocardiography—Eight weeks after surgery rats in the surgical MI+sham group experienced significant increases in the EDA and decreases in the %FAC from the baseline time point (2 weeks after infarction). In the MI+ePTFE group, both EDA and %FAC significantly decreased. For the MI+PEUU group a different response was found with an increase in the %FAC with no EDA increase. ($P < .05$, Table 2).

Dobutamine stress echocardiography—Following dobutamine administration LV EDA decreased significantly in all groups. While LV contraction (%FAC) of the infarction control group did not respond with dobutamine and trended negatively, the %FAC for the MI+PEUU patch group was significantly increased as was the normal control group ($P < .05$ vs. pre-infusion) indicating that the MI+PEUU patched group LVs had a functional reserve (Fig. 9).

Discussion

Our results demonstrated that implantation of an elastic, biodegradable PEUU patch onto a rat chronic LV infarct induced muscle cellularization, forming tissue that positively expressed proteins characteristic of both smooth and cardiac muscle as well as cardiac transcription factors. We found analogous co-expression of α -SMA and α -actinin, cTnT in the embryonic myocardium of the same rat species. These histological results in the patched, infarcted LV were associated with preserved cardiac function with contractile reserve.

Differential responses were noted between rats implanted with relatively stiff, non-degradable ePTFE patches versus degradable PEUU patches which had a lower initial tensile modulus. Both patch types prevented chamber dilation, an effect that might serve to lessen the impact of the infarct on wall stress in noninfarcted myocardium. The functional benefit associated with implantation of a PEUU patch was consistent with previous findings, however, hearts where ePTFE patches were applied in a similar fashion experienced decreased contractility despite having their ventricular volume reduced. This result might be explained by the application of a mechanically stiff material that is unable to provide a reduction in wall stress in tandem with allowance of myofiber stretch during diastole. Earlier work by Dang et al. generally investigated the mechanical impact of applying patches to surgically remodeled myocardial surfaces using a finite element model and noted the limits of applying stiff materials in terms of detrimental effects on cardiac function.²³ In addition to the stiff mechanical properties attributed to the ePTFE material, there was no evidence of regenerated muscle beneath the patch material, but rather a loose layer of fibrous tissue. Quite different results were observed with PEUU patch placement. Even though the original mechanical properties of the applied PEUU patch will degrade with the material over time, the induced muscle bundle generated below the patch serves to increase the wall thickness (thus reducing wall stress). PEUU patch placement results in a ventricular wall that is mechanically softer under tensile loading than the unpatched infarcted wall.⁷

In healthy adult human ventricular tissue there is no evidence of α -SMA expression.⁸ While in embryonic myocardium α -SMA is transiently expressed and disappears over the 1–7 days following birth.^{9,10} Smooth muscle proteins (α -SMA and calponin) are markers of early

stage cardiomyocyte development and may contribute to the slow shortening speed that is characteristic of the embryonic myocardium.²⁴ We confirmed α -SMA expression in the same species, Lewis rat, gestational day 14 embryonic myocardium and also examined its co-expression with α -actinin and cTnT. In our previous study, we reported substantial α -SMA positive muscle bundles beneath the implanted PEUU patch and considered these to be simply contractile smooth muscle cells. However, further characterization in this study revealed that this cellularized muscle tissue co-expressed α -SMA and α -actinin as well as cTnT, with positive Cx-43 expression, which has not been previously reported. Cardiac transcription factors, Nkx 2.5 and GATA-4, are expressed predominantly in the heart²⁵ and members of the GATA-4, -5, and -6 subfamily of transcription factors are co-expressed with the homeoprotein Nkx 2.5 in the precardiac mesoderm during the earliest stages of its specification and are known to be important determinants of cardiac gene expression.²⁶ In the region of regenerated muscle below the PEUU patch, the immunohistochemical expression of Nkx-2.5 and GATA-4 transcription factors and Nkx-2.5 mRNA upregulation provided further evidence that the developed muscle cells were in the myocardial lineage at 8 weeks after PEUU patch placement.

At the 16 week point after PEUU patch placement, as at 8 weeks, an LV wall tissue region under the PEUU patch was found to be α SMA-positive and to also positively stain with anti- α -actinin, cTnT, and Cx43 antibodies, but not with an anti-GATA4 antibody. For the positively stained regions, the trend appeared to be for less extensive staining for these markers relative to that observed at 8 weeks. There was thus no evidence of maturation towards an adult cardiomyocyte lineage over these additional 8 weeks, although the cells of interest were persisting. The question remains as to whether these cells might be receptive to strategies designed to push them towards a cardiomyocyte lineage. It is also notable that the PEUU patch material had mostly degraded at this 16 week time point. One could speculate that the patch degradation rate may be too fast to allow these cells to develop under a protected mechanical environment. It has been shown in other work that appropriate mechanical stimulation is essential for proper myocardial tissue formation and contractile function in developing embryonic myocardium during cardiac morphogenesis.^{27,28} The degradation rate of the patch material is hence of great interest if one speculates that an extended period of mechanical support might be beneficial.

Dobutamine stress echocardiography is commonly used in clinical practice to investigate patients with a wide variety of cardiovascular diseases to detect myocardial ischemia, evaluate valvular diseases, measure myocardial viability and assess myocardial contractile reserve.²⁹⁻³¹ Even in the rat model, dobutamine echocardiography has proven its usefulness to assess myocardial contractile reserve.³² In the normal response, a segment is normokinetic at rest, and normal-hyperkinetic during stress, which was the result for the normal control group in this study. In the necrotic response, a segment with resting dysfunction is not improved during stress. This result was observed with our MI+sham control group. On the other hand, a segment with resting dysfunction can improve during stress, which is considered to be “the viability response”.³¹ We found this viability response in the MI+PEUU patch group, but not in the ePTFE patch group. Even though we have not elucidated the specific relationship between the generated muscle and the viability response, this LV contractility reserve could have positive implications for both the prognosis and quality of life for patients.

We have demonstrated the unique character of the regenerated muscle tissue and cardiac functional reserve induced by PEUU patch implantation, both confirming and extending our initial report on this therapeutic approach, yet a new set of questions arises from these findings. While an expression pattern is seen that is similar to the embryonic developing cardiomyocyte, the origin of the multiple muscle protein expressing cells that populate the

patched LV wall has not been defined. Recently it has been shown that the adult mammalian heart has myocardial regenerative potential attributed to resident cardiac stem cells.^{3–5} Other researchers have suggested the possibility that circulating stem cells from bone marrow are recruited into the injured heart for myocardial repair.^{33,34} On the other hand, it has been reported that rat adult cardiomyocytes re-expressed smooth muscle proteins in culture, which assumes that adult cardiomyocytes can dedifferentiate back to a developmental state similar to embryonic cardiomyocytes.⁸ Furthermore, *in vivo* re-expression of α -SMA in cardiomyocytes is reported in certain circumstances, such as hypertrophy,³⁵ stunned and hibernating myocardium.³⁶ Some growth factors have been suggested to regulate the fetal SMA gene trigger (fetal gene reprogramming) such as acidic and basic FGF (fibroblast growth factor) and TGF-beta (transforming growth factor beta), which selectively induce an ensemble of fetal cardiac genes in cultured cardiac myocytes, including alpha-SMA.³⁷ In contrast, SRF (Serum response factor) could play an important role in stressed cardiomyocytes to de-repress the fetal SMA gene.³⁸ In our previous work, we found regions of basic FGF and VEGF staining that were within the regions of α -SMA positive staining beneath the PEUU patch. Further exploration of growth factors in this setting as well as investigating the presence of cell proliferation (BrdU or KI67) might be of great value in exploring these phenomena.

To evaluate the various hypotheses for cellular origin, specific studies would need to be pursued that could involve the use of chimeric animals, to evaluate bone marrow cell sources, or the use of more extensive immunohistopathology to evaluate cellular surface markers consistent with resident precursor cells. In addition, the epicardial scraping in PEUU cardiac patching might affect the healing and cellularization process. However, in e-PTFE patch studies, which employed identical scraping, no induction of similar regenerated muscle tissue was observed. Further studies could be performed in the future to evaluate a variety of other degradable synthetic polymer patches with different characteristic degradation rates and mechanical properties. Especially, in light of decreased immun-expression for cardiac specific proteins including cTnT and Cn43 at 16 week time point, a material with longer degradation rate should be tested, because the implanted PEUU were mostly degraded at this time point.

In conclusion, this report demonstrates that the application of an elastic, biodegradable cardiac patch to the infarcted LV induced muscle cellularization that expressed markers consistent with both smooth muscle and embryonic myocardium. Infarcted hearts patched with a biodegradable elastic material displayed a functional cardiac reserve not found in infarcted control animals. These findings provide a new strategy to stimulate and facilitate more functional healing of the injured heart and suggest an intriguing pathway that may exist by which the infarcted LV wall could be driven towards viable myocardial tissue.

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Abbreviations

LV	left ventricular
EDA	end-diastolic LV cavity area
ESA	end-systolic LV cavity area

%FAC	%fractional area change, PEUU, polyester urethane urea
ePTFE	expanded polytetrafluoroethylene
α-SMA	alpha-smooth muscle actin
DAPI	4',6-diamidino-2-phenylindole
α-actinin	sarcomeric alpha-actinin
cTnT	cardiac troponin-T
DAB	3,3'-diaminobenzidine tetrahydrochloride

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Figure 1.

Electron micrographs of PEUU and ePTFE patch materials. Please note the different magnifications used (a). Representative H&E stained sections of the LV wall 8 weeks after patch implantation surgery (MI+sham (b), MI+ePTFE (c), MI+PEUU (d)). Representative α-SMA distribution in immunohistochemistry of the infarcted anterior wall (MI+sham (e), MI+ePTFE (f), MI+PEUU (g)). Yellow dotted lines trace the implanted material area (MI+ePTFE in (c, f), MI+PEUU in (d, g)). The PEUU implanted area is infiltrated with putative macrophages and remnants of the polymeric material. The regenerated muscle tissue is indicated with blue arrows and dotted line tracing in the H&E section of (d) and by white arrows in the immunohistochemistry section of (g). α-SMA staining appears green and nuclear staining appears blue with immunohistochemistry. Scale bars: 100 μm (a), 200 μm in (b, -d), 500 μm in (e-g). The PEUU micrograph in (a) appears in ref.#7.

Figure 2.

Immunohistochemical staining of the endocardial side of the regenerated muscle area under the PEUU patch (a–c). α -SMA staining appears green and α -actinin staining appears red. White arrows indicate the regenerated muscle tissue, and yellow arrows indicate residual myocardium. Only the regenerated muscle expressed both α -SMA and α -actinin (c). Higher magnification of the regenerated muscle area below the PEUU patch (d–l). Blue; nuclear. Green; α -SMA. Red in (d–f); α -actinin. Red in (g–i); cTnT. Red in (j–l); Cx-43. Scale bars: 200 μ m in (a–c), 100 μ m in (d–l).

Figure 3.

Representative immuno-histochemical images of the PEUU patched infarct LV wall 16 weeks after patch placement. α SMA-positive structures were evident below the patch, and positive staining was observed for α -actinin (a–c), cTnT (d–f), and Cx43 (g–i), but not for GATA4 (j–l). The extent of staining for the positive markers generally appeared to be less than that seen at 8 weeks post patch placement. White arrows indicate the positively stained structures in the targeted region, and white arrow heads point to cardiomyocyte remnant. Scale bars: 200 μ m.

Figure 4. Immunohistochemical staining of gestational day 14 rat myocardium. Blue; nuclear. Green; α -SMA. Red; α -actinin (a–f). The whole heart was included for (a–c), and a part of the heart and descending aorta was included in (d–f). White arrows indicate the heart, and yellow arrows indicate the descending aorta. Only the myocardium expressed both α -SMA and α -actinin. Higher magnification of the embryonic myocardium (g–l). Blue; nuclear. Green; α -SMA. Red in (g–i); α -actinin. Red in (j–l); cTnT. Scale bars: 200 μ m in (a–f), 50 μ m in (g–l).

Figure 5.

Light microscopic immunohistochemical staining with DAB (brown) and counter stained with hematoxylin of gestational day 14 rat myocardium (a–c) and the regenerated muscle area beneath the PEUU patch (d–f), utilizing three serial sections from each. DAB showed α -SMA in (a, d) and blue arrows and dots indicate the regenerated muscle area in (d). GATA-4 (b, e), and Nkx-2.5 (c, f) was stained with DAB in higher magnification images of the white rectangle areas.

Figure 6.

Confocal fluorescence immunohistochemical staining of the regenerated muscle area. α -SMA staining appears green, GATA-4 (a–c) and Nkx-2.5 (d–f) staining appears red, and nuclear staining appears blue. Several angles of observation were employed: -60° , 0° , and $+60^\circ$. White arrows indicate typical cases where the transcription factors were expressed in intra- or peri-nuclear sites of the α -SMA positive muscle cells beneath the PEUU patch material. Scale bars: 100 μ m

Figure 7. Immunohistochemical staining and in situ hybridization of the regenerated muscle area in the MI+PEUU group and unpatched infarcted LV wall in the MI+sham surgery group. Using the serial sections from the regenerated muscle area (a, b) or unpatched infarcted wall (c, d), α -SMA was immunohistochemically stained with DAB (brown) in (a, c), and Nkx-2.5 in situ hybridization stained with NBT/BCIP (blue) in (b, d). Black arrows indicate the regenerated muscle area. In the α -SMA positive area of the regenerated muscle, Nkx-2.5 mRNA was upregulated. In the unpatched infarcted wall, α -SMA was sporadically expressed and primarily associated with vascular structures. Nkx-2.5 mRNA was weakly positive in limited areas and not associated with α -SMA. Scale bars: 100 μ m

Figure 8.

The vascular density at 8 weeks of MI+sham and MI+PEUU groups. MI+PEUU patch group was significantly increased compared to MI+sham group (312 ± 43 vs. $98 \pm 19/\text{mm}^2$, $P < .05$).

Dobutamine stress echocardiography

Figure 9.

Dobutamine stress echocardiographic assessment of MI+PEUU, MI+ePTFE patched and MI+sham surgery control hearts at 8 weeks together with age-matched normal controls. EDA (end-diastolic area) is shown to the left, and %FAC (fractional area change) to the right. EDA and %FAC in the normal control group were significantly different compared to the MI+PEUU, MI+ePTFE, or MI+sham surgery groups ($P < .05$) both pre- and post-application of dobutamine stress. EDA in all groups tended to decrease and %FAC in the normal and MI+PEUU groups significantly increased from pre to post-dobutamine stress. †: $P < .05$ and ‡: $P < .01$ vs. pre-dobutamine stress. %FAC in the MI+ePTFE and MI+sham surgery group did not significantly change. Post-dobutamine stress, the MI+PEUU group had significantly higher %FAC than MI+sham surgical controls. *: $P < .05$ between groups, ns; not significant.

Table 1

The mechanical properties and average pore size of PEUU and ePTFE

	PEUU	ePTFE
Initial modulus	1.57 MPa	51.5 MPa
Tensile strength	0.78 MPa	1.1 MPa
Average pore size	91 μm *	9.5 μm

* Ref.#7. J Am Coll Cardiol. 2007 49(23):2292-300

Table 2

Echocardiography data

	EDA (cm ²)		FAC (%)	
	0 week	8 weeks	0 week	8 weeks
MI+Sham	0.50 ± 0.07	0.68 ± 0.10 *	23 ± 6.0	18 ± 7.7 *
MI+ePTFE	0.54 ± 0.16	0.40 ± 0.07 * [†]	24 ± 8.4	19 ± 6.0 *
MI+PEUU	0.51 ± 0.05	0.52 ± 0.10 [†]	21 ± 5.4	26 ± 6.7 * [†]
Normal **	0.32 ± 0.10	0.31 ± 0.12	61 ± 8.8	60 ± 9.7

* $P < .05$ vs. 0 week within a group,

[†] $P < .05$ vs. MI+sham surgery group at same time-point.

** Data from ref.#7.