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Evaluation of a polyurethane-reinforced hydrogel patch in a rat right ventricle wall replacement model

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Abstract

Congenital heart defects affect about 1% births in the United States. Many of the defects are treated with surgically implanted patches made from inactive materials or fixed pericardium that do not grow with the patients, leading to an increased risk of arrhythmia, sudden cardiac death, and heart failure. This study investigated an angiogenic poly(ethylene glycol) fibrin-based hydrogel reinforced with an electrospun biodegradable poly(ether ester urethane) urea (BPUR) mesh layer that was designed to encourage cell invasion, angiogenesis, and regenerative remodeling in the repair of an artificial defect created onto the rat right ventricle wall. Electrocardiogram signals were analyzed, heart function was measured, and fibrosis, macrophage infiltration, muscularization, vascularization, and defect size were evaluated at 4- and 8-weeks post-surgery. Compared with rats with fixed pericardium patches, rats with BPUR-reinforced hydrogel patches had fewer arrhythmias and greater right ventricular ejection fraction and cardiac output, as well as greater left ventricular ejection fraction, fractional shorting, stroke work and cardiac output. Histology and immunofluorescence staining showed less fibrosis and less patch material remaining in rats with BPUR-reinforced hydrogel patches at 4- and 8-weeks. Rats with BPUR-reinforced hydrogel patches also had a greater volume of granular tissue, a greater volume of muscularized tissue, more blood vessels, and a greater number of leukocytes, pan-macrophages, and M2 macrophages at 8 weeks. Overall, this study demonstrated that the engineered BPUR-reinforced hydrogel patch initiated greater regenerative vascular and muscular remodeling with a limited fibrotic response, resulting in fewer incidences of arrhythmia and improved heart function compared with fixed pericardium patches when applied to heal the defects created on the rat right ventricle wall.

Statement of significance—The study tested a polyurethane-reinforced hydrogel patch in a rat right ventricle wall replacement model. Compared with fixed pericardium patches, these reinforced hydrogel patches initiated greater regenerative vascular and muscular remodeling with a

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Declaration of Competing Interest
None.

Supplementary materials

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reduced fibrotic response, resulting in fewer incidences of arrhythmia and improved heart function at 4- and 8-weeks post surgery. Overall, the new BPUR-reinforced hydrogel patches resulted in better heart function when replacing contractile myocardium than fixed pericardium patches.

Keywords

Congenital heart defects; Surgical correction; Cardiac tissue engineering; Poly(ether ester urethane) urea; Poly(ethylene glycol); Fibrin gel

1. Introduction

Congenital heart defects (CHD) affect 1 of every 111–125 births in the United States. An estimated 40,000 infants are affected by CHD each year; of these, about 25% require invasive treatment in the first year of life [1]. Surgical repair of CHD often requires the use of a polymer or fixed tissue patch to close septal defects or enlarge stenosed structures. Approximately 50% of Tetralogy of Fallot repairs include a patch in the right ventricular (RV) outflow tract [2]. Currently, surgeons use synthetic or biological materials, including knitted polyethylene terephthalate (most commonly Dacron® [3,4]), expanded polytetrafluoroethylene (such as Gore-Tex® [5,6]), and glutaraldehyde-fixed bovine pericardium (such as SJ Medical [7] and CardioCel® [8,9]). These materials do not grow with the pediatric patients, are not electromechanically integrated, have mismatched mechanical properties compared with the surrounding tissue, and often become fibrotic [10], leading to an increased risk of malicious arrhythmia, sudden cardiac death, and heart failure [11–13]. About 25% of patch-implanted patients require a second surgery [14].

Previous research from our laboratory indicated that RV wall muscle replaced with a multi-layered patch composed of a chitosan-gelatin-heart matrix hydrogel reinforced with a polycaprolactone (PCL) membrane resulted in higher RV ejection fractions compared with fixed bovine pericardium at 8 weeks post-surgery. However, the multi-layered patch induced significant fibrosis in the RV wall and relatively poor vascularization [15]. In order to minimize immune response and increase vascularization, a gel composed of fibrin covalently decorated with poly(ethylene glycol) (PEG) was developed. Fibrin could be produced autologously from patient's blood and it plays critical roles in blood clotting, cell-matrix interaction, inflammation, and wound healing [16]. Fibrin gel is widely used as biomaterial for engineered adipose, dermal and cardiovascular tissues [17,18]; but the main disadvantages of using fibrin gel as a scaffold are low mechanical stiffness and rapid degradation [19,20]. Incorporation of PEG into fibrin dramatically increased scaffold stiffness and stability. After culture for 14 days, the weight retentions of fibrin-alone hydrogels were completely degraded, while PEG-fibrin gels remained intact. No significant difference was seen in amniotic fluid stem cells attachment or viability after 7 days when comparing fibrin-alone, PEG-fibrin or Matrigel [21]. PEG-fibrin hydrogel could well support vascular network formation when analyzed at 2 weeks in vitro culture after seeding amniotic fluid stem cell-derived endothelial cells or human umbilical vascular endothelial cells; the vascular network formation was even better when perivascular cells such as amniotic fluid stem cells or mesenchymal stem cells were cocultured [21]. Our group has

tested PEG-fibrin scaffolds in a subcutaneous rat and -subcutaneous mouse models and the results showed that there was a rapid gel vascularization 2 weeks post implantation [22,23].

Biodegradable polyurethane formations have been used in cardiac tissues [24], fibrocartilage repair [25], nerve guidance channels [26], wound dressings [27], and bone grafts [28] owing to the excellent biocompatibility and hemocompatibility known today; mechanical properties such as durability, elasticity, elastomer-like character, fatigue resistance, compliance or tolerance in the body during the healing that can be mediated by modifying the chemical structure [29]. Biodegradable polyurethanes with hydrolytically or enzymatically cleavable moieties [30,31] were selected as an alternative to PCL to reduce fibrosis because PCL takes 2–3 years to resorb in vivo, which might be the major reason for a long term of foreign body response [32]. Kishan et al. [30] studied biodegradation and cytocompatibility of electrospun biodegradable poly(ether ester urethane) urea (BPUR) mesh. Gravimetric analysis of mass loss was performed after treatment in accelerated hydrolytic and enzymatic solutions for a period of 20 weeks. Base-accelerated hydrolytic degradation of the BPUR with 10% hard segment (10HS) resulted in significantly greater mass loss at 20 weeks than the phosphate-buffered saline (PBS) control. Degradation kinetics in lipase solution were similar to base-accelerated hydrolysis. Initial cytocompatibility of electrospun BPUR meshes was assessed using a standard Live/Dead assay to confirm the ability of these scaffolds to support adequate cell attachment and retention for use as a tissue engineering scaffold [30]. Puperi et al. [31] also reported that valve interstitial cells were viable and active through 28 days of exposure to BPUR meshes.

In this study, we fabricated a myocardial replacement patch of PEG-fibrin reinforced with an electrospun BPUR mesh layer. This engineered cardiac patch was tested in an artificial defect created on the adult rat RV wall and compared with a sham surgery control and a clinical control of glutaraldehyde-fixed pericardium. Heart function was measured at 4- and 8-weeks post-surgery, and histologic sections were evaluated for fibrosis, macrophage infiltration, vascularization, muscularization, and defect size. Collectively, this study tests the hypothesis that this engineered cardiac patch will induce muscular and vascular ingrowth with a limited foreign body response, resulting in improved heart function over a current clinical control of fixed pericardial patches.

2. Methods and materials

2.1. Patch fabrication

A BPUR with 10HS was synthesized as previously described [18]. Briefly, a poly(ether ester) triblock was synthesized by reacting PEG diisocyanate (PEG-DI) and PCL (MW=530 Da). PCL with stannous octoate (0.1 wt% with respect to the polymer) was added dropwise into a flask containing PEG-DI to a final PEG-DI:PCL molar ratio of 1:2 under nitrogen with stirring at 80 °C for 7 h. BPUR was then synthesized in a two-step process from this triblock diol and hexane diisocyanate (HDI) using ethylene diamine (ED) as a chain extender at a molar ratio of 1:2:1 triblock diol:HDI:ED. A 10 wt% solution of the triblock diol in N,N-dimethylformamide (DMF) containing 0.1 wt% stannous octoate was first added dropwise to a flask containing a 10 wt% solution of HDI in DMF under nitrogen. The reaction proceeded at 80 °C under a nitrogen blanket with constant stirring until no change

in the hydroxyl stretch was observed via transmission Fourier transform infrared (FTIR) spectroscopy (~5 h) and then cooled to room temperature. Chain extension was then performed by adding a 10 wt% solution of ED in DMF dropwise to the prepolymer solution under vigorous stirring. The BPUR chemical structure was confirmed using transmission FTIR spectroscopy. Neat BPUR films were cast onto KBr pellets from 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma, St. Louis, MO) solutions (5 wt%) and placed under vacuum for 1 h under ambient conditions to remove the solvent. Spectra were recorded using a Nicolet iS10 (Thermo Scientific) FTIR spectrometer at a resolution of 2 cm^{-1} for 64 scans.

A 10 wt% solution of BPUR in HFIP was used to electrospin fibrous meshes. BPUR solutions were dispensed using a syringe pump at a constant rate of 0.3 ml/h. A positive voltage of 7.5 kV was applied at the needle tip, which was placed 17 cm from a -5-kV charged copper plate. All the electrospinning runs were performed at ambient conditions (25 °C, 45–55% relative humidity). The fabricated meshes were vacuum dried for a minimum of 12 h prior to characterization. The fiber morphology was characterized using scanning electron microscopy (SEM) (Phenom Pro, NanoScience Instruments, Phoenix, AZ) at 10 kV accelerating voltage. Specimens were cut from the center of each fiber mesh to avoid edge effects. Prior to SEM imaging, the specimens were coated with 4 nm of gold using a sputter coater (Sputter Coater 108, Cressington Scientific Instruments, Hertfordshire, UK). Finally, tensile testing of electrospun BPUR meshes were performed on dogbone specimens cut in accordance with ASTM D1708 and strained to failure at a rate of 100%/min using an Instron 3345 uniaxial tensile tester equipped with a 100 N load cell and pneumatic side action grips (Instron 2712–019).

BPUR support layers (7 mm in diameter) were cut from the electrospun BPUR mesh. To enhance gel integration with the mesh, 5 evenly spaced holes were made with a 22-gauge needles at 2mm away from the edge, BPUR meshes were sterilized by UV in a culture hood for 1 h before use. PEGylated human fibrinogen (Sigma, St. Louis, MO) was prepared as previously described [21]. Briefly, Human fibrinogen (F3879; Sigma-Aldrich, St Louis, MO) was solubilized in PBS at a concentration of 40 mg/ml. After 1 h of incubation at 37 °C and brief vortexing, the solution was sterilized using a 0.20- μm filter. Succinimidyl glutarate-modified bifunctional PEG (3.4 kDa SG-PEG-SG; NOF America Corporation, White Plains, NY) was dissolved in PBS at 4 mg/ml and syringe filtered. To make PEGylated fibrinogen, fibrinogen and PEG solutions were combined in a 1:1 vol ratio, mixed thoroughly, and incubated at 37 °C for 1 h. Patches were fabricated in a sterile Teflon mold 7-mm in diameter and 2-mm deep. The PEG-fibrin gel was made with 30 μl sterile PBS containing 20 mg/ml of PEGylated human fibrinogen, adding 30 μl sterile ice-cold 40 mM CaCl_2 containing 20 U/ml human thrombin (Sigma, St. Louis, MO) and mixing well to promote gel formation.

The storage and loss moduli of the PEG-fibrin gels were measured using a parallel-plate rheometer (Discovery Hybrid 2; TA Instruments). PEG-Fibrin gels (80 μl were formed directly between the plates at a gap of 1400 μm . Samples were allowed to gel for 5 min at 37 °C before being compressed 20% (gap of 1120 μm). Five replicates were subjected to shear at 1% strain through a dynamic angular frequency range of 0.1 to 100 rad/s. The elastic modulus was calculated from the linear region of the storage modulus using Hooke's

law. Poisson's ratio was assumed to be 0.5, corresponding to an incompressible material [33]. For implantable patch fabrication, a BPUR mesh was laid on the top of fibrin gel immediately after gelation and another 10 μ l of PEGylated fibrinogen solution was added on the top of the BPUR layer to enhance integration. Molds were incubated for 30 min at 37 °C and 5% CO₂ before patches were detached and implanted onto heart defects. To show the integration between the PEG-fibrin gel and the BPUR mesh, samples were prepared for SEM imaging by fixing for 1 h in 2.5% glutaraldehyde and then mounted on a SEM stub with double-sided carbon tape. Imaging was performed using a JSM-6010LA field emission SEM (JEOL LTD, Tokyo, Japan) operated at 20 kV.

Bovine pericardium patches (7 mm in diameter, 270 μ m in thickness) were directly cut from commercially available glutaraldehyde-fixed bovine pericardium (St. Jude Medical, Saint Paul, MN).

2.2. Patch implantation

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Colorado Anschutz Medical Campus, in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication Nos. 85–23, Revised 2011). Sixty-two male Sprague-Dawley rats weighing 400 ± 10 g (Envigo, Cambridgeshire, UK) were randomly assigned to a 4-week sham group (Sham, $n = 10$), a 4-week glutaraldehyde-fixed bovine pericardium group (Pericardium, $n = 10$), a 4-week BPUR-reinforced hydrogel patch group (BPUR PEG-fibrin, $n = 10$), an 8-week sham group (Sham, $n = 11$), an 8-week pericardium group (Pericardium, $n = 10$), or an 8-week BPUR PEG-fibrin group (BPUR PEG-fibrin, $n = 11$). Rats were anesthetized using 5% isoflurane inhalation with 100% oxygen followed by intubation and respiratory support with a rodent mechanical ventilator (Harvard Apparatus, Holliston, MA) at a peak inspiratory pressure of 11 cmH₂O and 75 beats/min. Surgery procedures were performed in a sterile environment on a controlled heating pad. An Animal Bio Amp (FE 136, ADInstruments) and an Animal Oximeter Pod (ML325, ADInstruments) that attached to a PowerLab 4/30 system (ADInstruments, Spring, CO) were used to monitor electrocardiogram and SpO₂. Anesthesia was maintained with 2% isoflurane inhalation with 100% oxygen. The rat heart was exposed via a 3-cm incision through a 4th left thoracotomy as previously described [34]. As shown in Fig. 1A1–4, a purse string suture, 4 mm in diameter, was created on the RV free wall with a 6–0 polypropylene suture (Ethicon, US). Both ends of the stitch suture were passed through a 22-gauge plastic vascular cannula (VWR International, Radnor, PA) that served as a tourniquet to secure the purse string. Three quarters of the bulging part of the purse string was excised to create a 2–3 mm full-thickness defect contacting blood (Fig. 1B1 and B2). The patch was stitched over the defect with 7–0 polypropylene suture (Ethicon, US), first fixed by 4 stitches at positions of 90°, 180°, 270° and 360°, then continuously sutured fully around the patch. Animals in the sham group experienced the same chest opening and pericardium tearing, but no defect was created, and no patch was sutured onto the RV free wall. The muscle layers of the chest and the skin were closed with a 4–0 polyglactin absorbable suture (AD Surgical, Sunnyvale, CA, USA). Isoflurane supply was stopped immediately after the skin layer was closed. Before animals regained consciousness, Meloxicam (5 mg/ml, MWI Animal Health, Grand Prairie, TX, USA) 0.5 mg/kg was

administered subcutaneously once to reduce post-surgery pain. One animal in the 4-week pericardium group and 1 animal in the 8-week BPUR PEG-fibrin group died from massive bleeding during surgery, and 1 animal in the 4-week BPUR PEG-fibrin group died from acute RV myocardial infarction during surgery.

2.3. Echocardiography

To assess left ventricular (LV) function, echocardiography was performed at the end of the 8-week timepoint. Animals were anesthetized and placed on a controlled heating pad, and anesthesia was maintained with 2% isoflurane inhalation with 100% oxygen. Standard transthoracic echocardiography was performed using the GE Vivid 7 system (GE Vingmed Ultrasound AS, N-3190 Horten, Norway) fitted with an GE S10 transducer. LV parameters were obtained from two dimensional images and M-mode interrogation in parasternal short-axis and long-axis view as previously described [34], and then LV end-diastolic dimension (LVEDd), ejection fraction (LVEF) and fractional shortening (LVFS), and end-diastolic area (LVEDA) were calculated. All echocardiographic measurements were averaged from at least five cardiac cycles.

2.4. Electrocardiogram (ECG)

ECG signals were recorded at 4- and 8-week endpoints. Animals were anesthetized using 5% isoflurane inhalation with 100% oxygen followed by intubation and respiratory support, placed on a controlled heating pad, and then anesthesia was maintained with 2% isoflurane inhalation with 100% oxygen. An ECG signal was recorded for 30 min with an Animal Bio Amp that attached to a PowerLab 4/30 system, by inserting a needle anode (MLA1213, ADInstruments) into the left front leg of the animal, a needle cathode into the right front leg, and using the testis skin as ground. LabChart (ADInstruments) was used for analysis of malicious arrhythmia, categorized as frequent atrial premature beats (APBs), atrial tachycardia (AT), atrial fibrillation, frequent ventricular premature beats (VPBs), ventricular tachycardia, and ventricular fibrillation.

2.5. Hemodynamic catheterization

After recording ECG signals, the heart was exposed through a 5th left thoracotomy and a 2F micromanometer tipped catheter (SPR-869 Millar Instruments, Houston, TX) was inserted into the LV apex, and advanced into the LV to obtain LV pressure and conductance. After stabilization for 15 min, the signals were digitized at a sampling rate of 1 kHz/s using MPVS-300 (Millar Instruments) and were acquired with a PowerLab 4/30 system at steady state. LabChart Pro-v.8.10 software with the pressure-volume (PV) loop module (ADInstruments) was utilized for subsequent assessment of LV hemodynamic parameters. Heart rate (HR), LV systolic pressure (LVSP), LV end-diastolic pressure, maximal slopes of systolic pressure increment ($LV\ dp/dt_{max}$) and diastolic pressure decrement ($LV\ dp/dt_{min}$), ejection fraction (LVEF), stroke volume (SV), end-diastolic volume (EDV), cardiac output (CO), and stroke work (SW) were computed using the cardiac PV-loop module. After completion of the hemodynamic assessment of the LV, the catheter was inserted into the RV apex and advanced into the RV to acquire RV hemodynamics including systolic pressure (RVSP), RV end-diastolic pressure (RVEDP), maximal slopes of systolic pressure increment ($RV\ dp/dt_{max}$), and diastolic pressure decrement ($RV\ dp/dt_{min}$). A 20-gauge IV catheter

(VWR International, Radnor, PA) was inserted into the right jugular vein. After a stable signal was recorded from either LV or RV, 20 μ l hypertonic saline (30%) bolus injection were performed at least 2–3 times for both ventricles to obtain a value for Vp for the saline calibration. After hemodynamic measurements under anesthesia, animals were euthanized with cardiac arrest by apical injection of 1 ml of 10% KCl. Hearts were excised, weighed, placed in a peel-away disposable embedding mold (VWR International, Radnor, PA), frozen in liquid N₂, and then immediately immersed in Tissue Tek OCT compound (VWR International, Radnor, PA) and placed in a –80 °C freezer.

2.6. Histology and immunohistochemistry

Each heart sample was sliced using a cryostat (Cryotome E, Thermo Shandon). Whole heart longitudinal sections, directly through the middle of the defect, from the base to apex of the heart were cut at a thickness of 10 μ m. The sections were placed on VWR Microslides for preparation of morphological and immunofluorescence examinations. For measurements of patch implantation-induced fibrosis and defect thickness, whole heart sections were stained with Masson's trichrome reagents (Sigma) according to the manufacturer's protocol. Section images (200x magnification) were taken under Zeiss 2.1 microscope (Germany), and the images of whole heart sections were stitched together using the Series feature within the Zeiss microscopy software. The whole scar area (mm²) and the patch material remaining area (mm²) were measured by tracing the edge of the scar and the edge of the remaining patch materials in each patch area. The patch implantation-induced fibrosis area was calculated as the whole defect area minus the patch material remaining area. The size of the external scar was measured and expressed as the external curve length (mm) in each sample and averaged; the size of the internal defect was measured between the internal muscle breaks and expressed as the internal curve length (mm) in each sample and averaged.

For immunofluorescence staining, whole heart sections of 10 μ m thickness directly through the middle of the defect were fixed in 4% paraformaldehyde at 4 °C for 20 min; nonspecific epitope antigens were blocked with 10% goat serum (Sigma) at room temperature for 45 min. Sections were incubated with specific mouse anti- α -actinin antibody (1:200, Sigma, A7811), goat anti- α -actinin (1:75, Santa Cruz, sc7453), mouse anti-cardiac troponin T (cTnT, 1:200, Invitrogen, MA5–12,960), mouse anti-vimentin (1:200, Sigma, C9080), rabbit anti-von Willebrand factor (vWF; 1:750, Abcam, ab6994), mouse anti- α -smooth muscle actin (α -SMA; 1:200, Sigma, C6198), rabbit anti-CD45 (1:200, Abcam, ab10558), mouse anti-CD68 (1:200, Invitrogen, MA5–16,654), rabbit anti-CD206 (1:200, Abcam, ab64693), and anti-F-actin phalloidins (1:40, ThermoFisher, Alexa Fluor 488, Alexa Fluor 546, and Alexa Fluor 647) at room temperature for 1 h. Subsequently, sections were treated with goat anti-mouse or goat anti-rabbit secondary antibodies (1:400, Invitrogen, Alexa Fluor 488, Alexa Fluor 546, and Alexa Fluor 647), or donkey anti-goat (1:400, Invitrogen, Alexa Fluor 647) at room temperature for 1 h. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 2.5 μ g/ml) for 5 min at room temperature. Fluorescent images were obtained with a Zeiss 2.1 microscope. For determination of granular-like tissue, the volume of vimentin positive signals (staining fibroblasts and endothelial cells) were measured by whole defect area $\times$ intensity mean value in the section. For evaluation of muscularization, the volume of α -actinin positive signal was measured from 3 random 200 $\times$ magnification

patch material-centered ocular fields by area $\times$ intensity mean value in each section. For evaluation of blood vessels, the total number of vWF positive signals was counted from 5 random $400 \times$ magnification patch material-centered ocular fields in the section. For evaluation of acute inflammation, the volume of CD45, CD68 and CD206 positive signals (staining leukocytes, pan-macrophages and M2 macrophages respectively) were measured with the average of 3 random $200 \times$ magnification patch material-centered ocular fields calculated by area $\times$ intensity mean value in each section.

2.7. Statistics

Results are presented as mean $\pm$ standard deviation. ECG arrhythmia was analyzed by Chi-square (and Fisher's exact) test. Comparisons between two groups were made using the independent-samples *t*-test, and comparisons among three groups were made using a one-way analysis of variance followed by a Tukey post hoc comparison test. In all tests, differences were considered statistically significant at a value of $p < 0.05$.

3. Results

3.1. Patch fabrication

Full reaction of the BPUR was confirmed by absence of the isocyanate peak at 2267 cm^{-1} (Fig. 2A). Peaks at 3333 cm^{-1} (N-H stretch) and 1630 cm^{-1} (C=O stretch of ordered C=O $\cdots$ H-N in the urea group) indicated successful chain extension. Whole BPUR spectrum peak assignments was shown in Supplementary Table 1. These results were consistent with previous infrared spectral analysis [30]. The average thickness of BPUR meshes was $80 \pm 10 \text{ }\mu\text{m}$ ($n = 5$) (Fig. 2B). BPUR fiber meshes displayed an average fiber diameter of $1.5 \pm 0.8 \text{ }\mu\text{m}$ ($n = 5$) (Fig. 2C). BPUR meshes had an average tensile modulus of $2.9 \pm 0.4 \text{ MPa}$ ($n = 5$).

The PEG-fibrin gel syntheses are the following:

A secant modulus based on 2% strain was calculated for the elastic modulus of PEG-fibrin gel (Fig. 2D) from the resultant engineering stress-strain plots ($n = 5$) (Fig. 2E). Young's modulus of PEG-fibrin was $893 \pm 193 \text{ Pa}$ ($n = 5$). The driven force to integrate the mesh layer and the PEG-fibrin gel came from two components. One was the hydrophilic affinity of the BPUR and the other was the five holes penetrated with the 22-gauge needle which allowed hydrogel to go through and enhanced the stickiness. The SEM characterization of the cross-section of PEG-fibrin gel and the BPUR mesh is shown in Supplementary Fig. 1.

3.2. Animal survival

One animal in the 8-week pericardium group died from cardiac arrest the second day post-surgery, and 1 animal in the 8-week BPUR PEG-fibrin group became paraplegic and was euthanatized the second day post-surgery. All other animals surviving the surgery survived to the endpoint (57/62 total rats).

3.3. ECG arrhythmia

During a 30-min ECG recording, no animals with sham surgery had arrhythmia at either endpoint. At the 4-week endpoint, 2 animals (2/9) in the pericardium group had arrhythmia, 1 with frequent APBs and 1 with frequent VPBs; 3 animals (3/9) in the BPUR PEG-fibrin group had arrhythmia, 1 with frequent APBs and 2 with frequent VPBs. Fisher's exact test showed no significant difference ($p = 1.000$). At the 8-week endpoint, 3 animals (3/9) in the pericardium group had arrhythmia, 2 with frequent VPBs and 1 with frequent APBs plus AT; no arrhythmia was found in any rats in the BPUR PEG-fibrin group. Fisher's exact test showed no significant difference ($p = 0.2059$) (Fig. 3).

3.4. Echocardiography

Echocardiography was performed on animals at 8 weeks post-surgery. As shown in Fig. 4, implantation of a fixed pericardium patch at 8 weeks post-surgery resulted in a significant decrease in LVEDA ($67.0 \pm 9.7 \text{ mm}^2$, $n = 6$) and LVEDd ($5.63 \pm 0.46 \text{ mm}$, $n = 6$) when compared with LVEDA ($83.8 \pm 10.6 \text{ mm}^2$, $n = 6$, $p < 0.05$) and LVEDd ($6.37 \pm 0.56 \text{ mm}$, $n = 6$, $p < 0.05$) in the sham group; implantation of BPUR PEG-fibrin patch slightly decreased LVEDA ($71.0 \pm 7.7 \text{ mm}^2$, $n = 6$) and LVEDd ($5.70 \pm 0.32 \text{ mm}$, $n = 6$), but there was no significant difference when compared with either the sham group or the fixed pericardium group. Implantation of a fixed pericardium patch at 8 weeks post-surgery significantly decreased LVEF ($69.2 \pm 5.3\%$, $n = 6$) and LVFS ($36.0 \pm 3.5\%$, $n = 6$) when compared with LVEF ($82.0 \pm 2.3\%$, $n = 6$) and LVFS ($45.7 \pm 2.7\%$, $n = 6$) in the sham group ($p < 0.01$); however, the LVEF ($80.2 \pm 5.7\%$, $n = 6$) and LVFS ($41.5 \pm 3.3\%$) were significantly larger ($p < 0.05$) in the BPUR PEG-fibrin patch group compared with the pericardium group.

3.5. Hemodynamics

Body weight (BW), heart weight (HW), HW/BW, and heart rate (HR) are shown in Supplementary Table 2. Four weeks after surgery, BW, HW, HW/BW, and HR were not significantly different between any experimental groups. Eight weeks after surgery, BW was significantly decreased ($p < 0.05$) and HW/BW ratio was significantly increased ($p < 0.05$) in both the pericardium and the BPUR PEG-fibrin groups compared with the sham group.

An RV PV loop was measured at 4 weeks (Fig. 5A1–3) and 8 weeks (Fig. 5B1–3) after surgery. At both 4 weeks (Fig. 5A2 and B2) and 8 weeks (Fig. 5A3 and B3), the RV PV loop was shifted to the left compared with the sham group (Fig. 5A1 and B1). RVSP, RVEDV and RVEF were significantly lower in the pericardium group at 4- and 8-weeks, and RVSP, RVEDV and RVEF were significantly lower in the BPUR PEG-fibrin group at 4 weeks and RVEDV was significantly lower at 8 weeks post-surgery compared with the sham group ($p < 0.05$; $p < 0.01$). However, RVEF was significantly higher in the BPUR PEG-fibrin group at 8 weeks post-surgery compared with the pericardium group ($p < 0.05$). RV SW and RV CO

were both significantly lower in the pericardium group at both 4- and 8-weeks post-surgery compared with the sham group ($p<0.05$; $p<0.01$); however, RV CO was significantly higher in the BPUR PEG-fibrin group at 8 weeks post-surgery ($p<0.05$) compared with the pericardium group. At 8 weeks post-surgery, RV dP/dt_{max} and RV dP/dt_{min} were significantly lower in the pericardium group compared with the sham control ($P<0.01$); in contrast, RV dP/dt_{max} and RV dP/dt_{min} in the BPUR PEG-fibrin group at 8 weeks post-surgery were not significantly different from the sham group, but were significantly higher compared with the pericardium group ($p<0.05$; $p<0.01$) (Supplementary Table 2).

As shown in Supplementary Table 2, LVSP, LVEDV, LVEF, LV SW and LV CO at both 4- and 8-weeks were dramatically decreased in the pericardium group, and LVSP and LVEF at 4 weeks, LVEF at 8 weeks were dramatically decreased in the BPUR PEG-fibrin group compared with the sham control ($p<0.05$; $p<0.01$). LVEF was higher at 8 weeks, and LV SW and LV CO were higher at both 4-and 8-weeks post-surgery in the BPUR PEG-fibrin group compared with the pericardium group ($p<0.05$).

3.6. Histology

Macroscopic images of the patch on the RV wall are shown in Fig. 6A1–A5. At 4- and 8-weeks post-surgery, one third of patch-implanted hearts exhibited minimal thoracic adhesions. Neither group showed any dehiscence or aneurysm formation at the site of the implanted patch. In the BPUR PEG-fibrin group, the BPUR support layer degraded to an apparent loss of structure integrity and was replaced with native-like tissue at 4 weeks (Fig. 6A4) and further at 8 weeks (Fig. 6A5); however, the pericardium group showed no degradation or native-like tissue replacement at both 4 weeks (Fig. 6A2) and 8 weeks (Fig. 6A3) post-surgery.

3.7. Fibrosis and patch material

Masson's trichrome staining was used to evaluate fibrosis, patch material remaining and wall thickness. As shown in Fig. 6B1–5, the pericardium group had significantly higher fibrotic area at both 4- and 8-weeks ($10.93 \pm 1.97 \text{ mm}^2$, $n = 7$ and $8.43 \pm 1.67 \text{ mm}^2$, $n = 7$) compared with the BPUR PEG-fibrin group ($5.52 \pm 1.06 \text{ mm}^2$, $n = 7$ and $6.23 \pm 1.39 \text{ mm}^2$, $n = 7$) ($p<0.01$; $p<0.05$), but the fibrotic area was significantly smaller at 8 weeks compared with at 4 weeks post-surgery in the pericardium group ($p<0.05$) (Fig. 5E). The areas of remaining patch material were measured to quantify degradation. As shown in Fig. 6C1–5, the patch material area in the pericardium group at 4 weeks ($2.01 \pm 0.60 \text{ mm}^2$, $n = 7$) and 8 weeks ($1.99 \pm 0.49 \text{ mm}^2$, $n = 7$) was greater than in the BPUR PEG-fibrin group ($0.82 \pm 0.26 \text{ mm}^2$, $n = 7$ and $0.84 \pm 0.17 \text{ mm}^2$, $n = 7$) ($p<0.01$) even though before implantation (at 0 weeks) the cross-section area in the pericardium ($2.03 \pm 0.38 \text{ mm}^2$, $n = 7$) was much smaller than the BPUR PEG-fibrin group ($6.89 \pm 0.39 \text{ mm}^2$, $n = 7$) ($p<0.01$). Compared with at 0 weeks in the BPUR PEG-fibrin group, the patch area dramatically decreased both at 4- and 8-weeks ($p<0.01$), but that did not happen in the pericardium group ($p>0.05$) (Fig. 6F). The wall thickness in the pericardium group at 4 weeks ($1.29 \pm 0.33 \text{ mm}$, $n = 7$) was greater than the BPUR PEG-fibrin group ($0.69 \pm 0.13 \text{ mm}$, $n = 7$) ($p<0.01$). The wall thickness in the pericardium group at 8 weeks ($0.95 \pm 0.21 \text{ mm}$, $n = 7$) was smaller than at 4 weeks ($p<0.05$). There was no significant difference between wall thicknesses in the pericardium

group and the BPUR PEG-fibrin group (0.75 ± 0.11 mm, $n = 7$) at 8 weeks post surgery. Compared with the normal thickness of the RV wall at 4 weeks (1.42 ± 0.12 mm, $n = 6$) and 8 weeks (1.43 ± 0.11 mm, $n = 6$), the wall thickness in the BPUR PEG-fibrin group at 4- and 8-weeks and the wall thicknesses in the pericardium group at 8 weeks were much smaller ($p < 0.05$; $p < 0.01$) (Fig. 6G).

3.8. Vimentin expression, muscularization and vascularization

Immunofluorescence staining of vimentin in the center of the patched area at 4- and 8-weeks post-surgery was qualified and used as a measure of granulation tissue (Fig. 6D1–5). There were fewer vimentin-positive cells in the patch area in the pericardium group (Fig. 6D2 and D3) compared with the BPUR PEG-fibrin group (Fig. 6D4 and D5). When compared with vimentin expression volume at 8 weeks, there was a significant difference between the pericardium group (5.13 ± 1.29 , $n = 6$) and the BPUR PEG-fibrin group (9.58 ± 2.49 , $n = 6$) ($p < 0.01$). The volume of vimentin-positive cells in the pericardium group at 8 weeks was significantly lower compared with 4 weeks (8.98 ± 2.31 , $n = 7$) ($p < 0.01$) (Fig. 6H). The muscularization was evaluated using the immunofluorescence staining of α -actinin in the patch material-centered area at 4- and 8-weeks post-surgery (Fig. 7B1–5). The amount of α -actinin expression volume were bigger in the BPUR PEG-fibrin group than the pericardium group both at 4 weeks (2.50 ± 0.30 , $n = 6$ vs 0.55 ± 0.08 , $n = 6$) ($p < 0.01$) and 8 weeks (4.00 ± 0.75 , $n = 6$ vs 1.12 ± 0.17 , $n = 6$) ($p < 0.01$). Compared with 4 weeks, both patch implantation groups significantly increased α -actinin expression volume at 8 weeks ($p < 0.01$). However, when compared with the sham group at 4 weeks (7.79 ± 0.24 , $n = 6$) and 8 weeks (8.05 ± 0.21 , $n = 6$), the amount of α -actinin expression volume were much smaller in both patch implantation groups ($p < 0.01$) (Fig. 7D). The number of blood vessels was counted using the immunofluorescence staining of vWF in the patch material-centered area at 4- and 8-weeks post-surgery (Fig. 7C1–5). There was little ingrowth of blood vessels in the pericardium group at 4 weeks (Fig. 7C2) and 8 weeks (Fig. 7C3) compared with the BPUR PEG-fibrin group at 4 weeks (Fig. 7C4) and 8 weeks (Fig. 7C5). The number of blood vessels in the BPUR PEG-fibrin group at 4 weeks (142 ± 18 , $n = 7$) and 8 weeks (162 ± 15 , $n = 7$) was greater than in the pericardium group at 4 weeks (59 ± 20 , $n = 7$) and 8 weeks (59 ± 8 , $n = 7$) post surgery ($p < 0.01$); the number of blood vessels in the BPUR PEG-fibrin group at 8 weeks was greater than at 4 weeks ($p < 0.05$). However, the number of blood vessels in the sham group at 4 weeks (365 ± 36 , $n = 7$) and 8 weeks (361 ± 38 , $n = 7$) was much greater than in the two patch implantation groups ($p < 0.01$) (Fig. 7E).

3.9. Infiltration of leukocytes and M2 macrophages

Immunofluorescence staining of CD45, CD68 and CD206 was used to evaluate the infiltration of leukocytes, especially neutrophils and monocytes, pan-macrophages, and M2 macrophages (Fig. 8B1–5, C1–5 and D1–5) respectively at 4- and 8-weeks post-surgery. There was a significant difference in CD45 expression between the pericardium group (1.40 ± 0.54 , $n = 7$) and the BPUR PEG-fibrin group (2.34 ± 0.47 , $n = 7$) at 8 weeks ($p < 0.01$), and the CD45 expression significantly decreased between 4 weeks (2.77 ± 0.75 , $n = 7$) and 8 weeks in the pericardium group ($p < 0.01$) (Fig. 8E). There was a significant difference in CD68 expression between the pericardium group (1.28 ± 0.32 , $n = 7$) and the BPUR PEG-fibrin group (2.35 ± 0.61 , $n = 7$) at 8 weeks ($p < 0.01$), and the CD68 expression significantly

decreased between 4 weeks (2.10 ± 0.26 , $n = 7$) and 8 weeks in the pericardium group ($P < 0.01$) (Fig. 8F). The CD206 expression was greater in the pericardium group (1.15 ± 0.06 , $n = 7$) than in the BPUR PEG-fibrin group (0.94 ± 0.02 , $n = 7$) at 4 weeks ($p < 0.05$); but at 8 weeks, the CD206 expression was greater in the BPUR PEG-fibrin group (1.08 ± 0.29 , $n = 7$) than in the pericardium group (0.63 ± 0.08 , $n = 7$) ($p < 0.01$); furthermore, the CD206 expression in the pericardium group significantly decreased between 4- and 8-weeks ($p < 0.01$) (Fig. 8G). The percentage of CD206/CD68 was also calculated, and no significant difference was found between the pericardium group and the BPUR PEG-fibrin group at either 4 weeks ($53.9 \pm 6.9\%$, $n = 7$ vs $47.8 \pm 10.5\%$, $n = 7$) or 8 weeks ($53.1 \pm 11.6\%$, $n = 7$ vs $46.5 \pm 8.3\%$, $n = 7$) ($p > 0.05$) post-surgery (Fig. 8H).

3.10. External scar size and the internal defect size

Fig. 9 shows the external scar size and the internal defect size at 4- and 8-weeks post-surgery. The curve length of the external scar was not significantly different between the pericardium and the BPUR PEG-fibrin groups at 4 weeks (10.09 ± 1.09 mm, $n = 7$ vs 9.09 ± 1.73 mm, $n = 7$) and 8 weeks (10.45 ± 0.79 mm, $n = 7$ vs 9.51 ± 1.49 mm, $n = 7$); however, the curve length of the external scar was slightly higher in the two patch implantation groups at 4 weeks compared with at 8 weeks ($p > 0.05$) (Fig. 9D). At 4 weeks, there was not a significant difference in the curve length of the internal defect between the pericardium group (4.97 ± 1.14 mm, $n = 7$) and the BPUR PEG-fibrin group (4.82 ± 0.91 mm, $n = 7$). At 8 weeks, the internal defect in the pericardium group grew dramatically larger (6.56 ± 1.08 mm, $n = 7$) compared with that at 4 weeks ($p < 0.05$) and was also much larger than the BPUR PEG-fibrin group (5.14 ± 1.01 mm, $n = 7$) ($p < 0.05$) (Fig. 9E).

4. Discussion

In this study, we found that a cardiac patch comprised of PEG-fibrin reinforced by a BPUR mesh induced greater muscular and vascular ingrowth with a limited foreign body response compared to a commercial glutaraldehyde-crosslinked pericardium patch, resulting in improved heart function in an adult rat RV wall replacement model. At 8 weeks post-surgery, rat hearts patched with BPUR PEG-fibrin had less fibrosis, a decreased patch material size, and increased infiltration of endothelial cells, leukocytes, pan-macrophages, and M2 macrophages compared to hearts patched with fixed pericardium. These regeneratively remodeled patches at 8 weeks resulted in fewer incidences of arrhythmia, greater RV function as shown by RVEF and RV CO, and greater LV function as shown by LVEF, LVFS, LV SW and LV CO compared with fixed pericardium. However, all patched hearts exhibited arrhythmias, decreased RV and LV function, and enlarged defect sizes compared with sham controls.

This study additionally found that the multilayer cardiac patch provided mechanical support of the full thickness defect and continued to support the ventricular wall as the material degraded and invading cells and secreted extracellular matrix replaced the implanted materials, as evidenced by the lack of dehiscence or aneurysm formation at the site of the implanted patch at both 4- and 8-weeks post-surgery. The inner PEG-fibrin gel had an average tensile modulus of 893 ± 193 Pa and the outer BPUR mesh had an average tensile

modulus of 2.9 ± 0.4 MPa. Their combined elastic strength was compatible to the patch remodeling process as was shown in the 4- and 8- observations. The tensile stress of the current BPUR mesh could be enhanced by increasing the percentage of its hard segment [30]; thus, this BPUR mesh itself might be used for the replacement of native heart valves [35] and BPUR reinforced PEG-fibrin patch might be used for the replacement of native left ventricle heart muscles. Furthermore, this study shows that both the formation of granular tissue, indicated by vimentin-positive cell staining, and the infiltration of neutrophils, monocytes, and M2 macrophages were higher in rat hearts with BPUR-reinforced hydrogel patches than with pericardium patches at 8 weeks post-surgery. Tissue regeneration and repair proceed in a cascade fashion beginning with a coagulation and inflammatory phase, followed by granulation tissue formation, which is characterized by proliferation of fibroblasts and new thin-walled, delicate capillaries, as well as infiltrated inflammatory cells in a loose extracellular matrix [36,37]. Within the first days after scaffold implantation, disruption of the tissue structure and subsequent cell damage initiates an acute inflammatory response with a rapid influx of innate immune cells, predominantly neutrophils, mast cells, and monocytes [38,39]. Neutrophils and monocytes are of hematopoietic origin and are involved in phagocytosis and pathogen clearance. Upon activation, resident tissue macrophages are supplemented by an active recruitment of blood monocytes, which then differentiate into macrophages and dendritic cells in the scaffold. Depending on the scaffold properties, this is followed by an M1/T_H1 cell dominated pro-inflammatory response or an M2/T_H2 cell dominated pro-regenerative response [39–41]. The former is characterized by the prolonged presence of M1 macrophages, and recruited fibroblasts typically acquire an activated phenotype, producing fibrous scar tissue. In contrast, the pro-regenerative process is dominated by M2 macrophages under influence of T_H2 cell secreted cytokines [42–44].

This study also found that the BPUR-reinforced patch was more rapidly resorbed than the glutaraldehyde-fixed pericardium patch. This could be because M2 macrophages, which mediate regenerative remodeling, were more populous in the BPUR PEG-fibrin group than the pericardium group at 8 weeks. Additionally, faster degradation and more M2 macrophages coincided with a better muscularization evidenced with α -actinin expression volume both at 4- and 8-weeks and a smaller defect size at 8 weeks post-surgery in the BPUR PEG-fibrin group compared with the pericardium group, which might be responsible for less or no arrhythmia at 8 weeks in the BPUR PEG-fibrin group. Macrophages have been shown to play a pivotal role in material degradation, via the production of enzymes and reactive oxygen species that can accelerate degradation [45,46]. A study by Wu et al. [47] also found that faster-degrading elastomers enable rapid remodeling of a cell-free synthetic graft into a neoartery. A regenerative remodeling response to the BPUR-reinforced hydrogel patch is thought to have paved the way for better action potential conduction, resulting in the absence of arrhythmia at 8 weeks post-surgery, and improved mechanical performance in the patched area.

The defect sizes in this study, confirmed from images at postmortem, are 2–3 mm in diameter; however, defects will be bigger in a heart under pressure and beating. In both patch materials, the defects grew larger both between 4- and 8-weeks post-surgery, and wall thickness were thinner than the normal RV wall in the sham group, especially at 8 weeks post-surgery.

5. Conclusions

In the correction of a heart defect, the main requirements for a successful cardiac construct are mechanical integrity, biocompatibility and regenerative tissue remodeling. This study found that a BPUR-reinforced hydrogel patch maintains integrity as cells and secreted extracellular matrix replace the degrading patch and initiates greater regenerative vascular and muscular remodeling with a limited fibrotic response, resulting in fewer incidences of arrhythmia and greater heart function compared with a commercially available, glutaraldehyde-crosslinked bovine pericardium patch.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Vi-rani SS, Willey JZ, Woo D, Yeh RW, Turner MB, C. American heart association statistics, S. stroke statistics, Heart disease and stroke statistics-2015 update: a report from the American heart association, *Circulation* 131 (4) (2015) e29–322, [PubMed: 25520374]
- [2]. Murphy JG, Gersh BJ, Mair DD, Fuster V, McGoon MD, Ilstrup DM, McGoon DC, Kirklin JW, Danielson GK, Long-term outcome in patients undergoing surgical repair of tetralogy of Fallot, *N. Engl. J. Med* 329 (9) (1993) 593–599, [PubMed: 7688102]
- [3]. Chou D, Tulloch A, Cossman DV, Cohen JL, Rao R, Barmparas G, Mirocha J, Wagner W, The influence of collagen impregnation of a knitted dacron patch used in carotid endarterectomy, *Ann. Vasc. Surg* 39 (2017) 209–215, [PubMed: 27666808]
- [4]. Al Meslmani BM, Mahmoud GF, Sommer FO, Lohoff MD, Bakowsky U, Multifunctional network-structured film coating for woven and knitted polyethylene terephthalate against cardiovascular graft-associated infections, *Int. J. Pharm* 485 (1–2) (2015) 270–276 . [PubMed: 25796119]
- [5]. Gammie JS, Bartus K, Gackowski A, D'Ambra MN, Szymanski P, Bilewska A, Kusmierczyk M, Kapelak B, Rzucidlo-Resil J, Moat N, Duncan A, Yadev R, Livesey S, Diprose P, Gerosa G, D'Onofrio A, Pitterello D, Denti P, La Canna G, De Bonis M, Alfieri O, Hung J, Kolsut P, Beating-Heart mitral valve repair using a novel ePTFE cordal implantation device: a prospective trial, *J. Am. Coll. Cardiol* 71 (1) (2018) 25–36. [PubMed: 29102688]
- [6]. Penford G, Quandt D, Mehta C, Bhole V, Dhillon R, Seale A, Stumper O, Stenting and overdilating small gore-tex vascular grafts in complex congenital heart disease, *Catheter. Cardiovasc. Interv* 91 (1) (2018) 71–80. [PubMed: 29266703]
- [7]. Hopkins RA, Bert AA, Buchholz B, Guarino K, Meyers M, Surgical patch closure of atrial septal defects, *Ann. Thorac. Surg* 77 (6) (2004) 2144–2149 author reply 2149–50. [PubMed: 15172284]
- [8]. Bell D, Prabhu S, Betts K, Justo R, Venugopal P, Karl TR, Alphonso N, Durability of tissue-engineered bovine pericardium (CardioCel®) for a minimum of 24 months when used for the repair of congenital heart defects, *Interact. Cardiovasc. Thorac. Surg* 28 (2) (2019) 284–290. [PubMed: 30101317]

- [9]. Pavy C, Michielon G, Robertus JL, Lacour-Gayet F, Ghez O, Initial 2-year results of cardiocel® patch implantation in children, *Interact. Cardiovasc. Thorac. Surg* 26 (3) (2018) 448–453. [PubMed: 29069409]
- [10]. Endo S, Saito N, Misawa Y, Sohara Y. Late pericarditis secondary to pericardial patch implantation 25 years prior, *Eur. J. Cardiothorac. Surg* 20 (5) (2001) 1059–1060. [PubMed: 11675208]
- [11]. Diller GP, Breithardt G, Baumgartner H, Congenital heart defects in adulthood, *Dtsch. Arztebl. Int* 108 (26) (2011) 452–459. [PubMed: 21776319]
- [12]. Verheugt CL, Uiterwaal CS, Grobbee DE, Mulder BJ, Long-term prognosis of congenital heart defects: a systematic review, *Int. J. Cardiol* 131 (1) (2008) 25–32. [PubMed: 18687485]
- [13]. Pok S, Jacot JG, Biomaterials advances in patches for congenital heart defect repair, *J. Cardiovasc. Transl. Res* 4 (5) (2011) 646–654. [PubMed: 21647794]
- [14]. Solomon NA, Pranav SK, Jain KA, Kumar M, Kulkarni CB, Akbari J, In search of a pediatric cardiac surgeon's 'Holy grail': the ideal pulmonary conduit, *Expert Rev. Cardiovasc. Ther* 4 (6) (2006) 861–870. [PubMed: 17173502]
- [15]. Pok S, Stupin IV, Tsao C, Pautler RG, Gao Y, Nieto RM, Tao ZW, Fraser CD Jr., Annapragada AV, Jacot JG, Full-thickness heart repair with an engineered multilayered myocardial patch in rat model, *Adv. Healthc. Mater* 6 (5) (2017) 1600549.
- [16]. van Hinsbergh VW, Collen A, Koolwijk P, Role of fibrin matrix in angiogenesis, *Ann. N. Y. Acad. Sci* 936 (2001) 426–437. [PubMed: 11460496]
- [17]. Klar AS, Guven S, Biedermann T, Luginbuhl J, Bottcher-Haberzeth S, Meuli-Simmen C, Meuli M, Martin I, Scherberich A, Reichmann E, Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells, *Biomaterials* 35 (19) (2014) 5065–5078. [PubMed: 24680190]
- [18]. Atluri P, Miller JS, Emery RJ, Hung G, Trubelja A, Cohen JE, Lloyd K, Han J, Gaffey AC, MacArthur JW, Chen CS, Woo YJ, Tissue-engineered, hydrogel-based endothelial progenitor cell therapy robustly revascularizes ischemic myocardium and preserves ventricular function, *J. Thorac. Cardiovasc. Surg* 148 (3) (2014) 1090–1097 discussion 1097–8, [PubMed: 25129603]
- [19]. Shaikh FM, Callanan A, Kavanagh EG, Burke PE, Grace PA, McGloughlin TM, Fibrin: a natural biodegradable scaffold in vascular tissue engineering, *Cells Tissues Organs* 188 (4) (2008) 333–346. [PubMed: 18552484]
- [20]. Ye Q, Zund G, Benedikt P, Jockenhoevel S, Hoerstrup SP, Sakyama S, Hubbell JA, Turina M, Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering, *Eur. J. Cardiothorac. Surg* 17 (5) (2000) 587–591. [PubMed: 10814924]
- [21]. Benavides OM, Quinn JP, Pok S, Petsche Connell J, Ruano R, Jacot JG, Capillary-like network formation by human amniotic fluid-derived stem cells within fibrin/poly(ethylene glycol) hydrogels, *Tissue Eng. Part A* 21 (7–8) (2015) 1185–94. [PubMed: 25517426]
- [22]. Jiang B, Waller TM, Larson JC, Appel AA, Brey EM, Fibrin-loaded porous poly(ethylene glycol) hydrogels as scaffold materials for vascularized tissue formation, *Tissue Eng. Part A* 19 (1–2) (2013) 224–234. [PubMed: 23003671]
- [23]. Benavides OM, Brooks AR, Cho SK, Petsche Connell J, Ruano R, Jacot JG, In situ vascularization of injectable fibrin/poly(ethylene glycol) hydrogels by human amniotic fluid-derived stem cells, *J. Biomed. Mater. Res. A* 103 (8) (2015) 2645–2653. [PubMed: 25631778]
- [24]. Baheiraei N, Yeganeh H, Ai J, Gharibi R, Azami M, Faghihi F, Synthesis, characterization and antioxidant activity of a novel electroactive and biodegradable polyurethane for cardiac tissue engineering application, *Mater. Sci. Eng. C Mater. Biol. Appl* 44 (2014) 24–37. [PubMed: 25280676]
- [25]. Kon E, Filardo G, Zaffagnini S, Di Martino A, Di Matteo B, Marchegiani Muccioli GM, Busacca M, Marcacci M, Biodegradable polyurethane meniscal scaffold for isolated partial lesions or as combined procedure for knees with multiple comorbidities: clinical results at 2 years, *Knee Surg. Sports Traumatol. Arthrosc* 22 (1) (2014) 128–134. [PubMed: 23223879]
- [26]. Hsu SH, Chang WC, Yen CT, Novel flexible nerve conduits made of water-based biodegradable polyurethane for peripheral nerve regeneration, *J. Biomed. Mater. Res. A* 105 (5) (2017) 1383–1392. [PubMed: 28152586]

- [27]. Wagstaff MJ, Schmitt BJ, Coghlan P, Finkemeyer JP, Caplash Y, Greenwood JE, A biodegradable polyurethane dermal matrix in reconstruction of free flap donor sites: a pilot study, *Eplasty* 15 (2015) e13, [PubMed: 25987938]
- [28]. Lavrador C, Mascarenhas R, Coelho P, Brites C, Pereira A, Gogolewski S, Elastomeric enriched biodegradable polyurethane sponges for critical bone defects: a successful case study reducing donor site morbidity, *J. Mater. Sci. Mater. Med* 27 (3) (2016) 61, [PubMed: 26800692]
- [29]. Shin EJ, Choi SM, Advances in waterborne polyurethane-based biomaterials for biomedical applications, *Adv. Exp. Med. Biol* 1077 (2018) 251–283, [PubMed: 30357693]
- [30]. Kishan AP, Wilems T, Mohiuddin S, Cosgriff-Hernandez EM, Synthesis and characterization of plug-and-play polyurethane urea elastomers as biodegradable matrixes for tissue engineering applications, *ACS Biomater. Sci. Eng* 3 (12) (2017) 3493–3502.
- [31]. Puperi DS, Kishan A, Punske ZE, Wu Y, Cosgriff-Hernandez E, West JL, Grande-Allen KJ, Electrospun polyurethane and hydrogel composite scaffolds as biomechanical mimics for aortic valve tissue engineering, *ACS Biomater. Sci. Eng* 2 (9) (2016) 1546–1558.
- [32]. Hiob MA, Crouch GW, Weiss AS, Elastomers in vascular tissue engineering, *Curr. Opin. Biotechnol* 40 (2016) 149–154. [PubMed: 27149017]
- [33]. Wufsus AR, Rana K, Brown A, Dorgan JR, Liberatore MW, Neeves KB, Elastic behavior and platelet retraction in low- and high-density fibrin gels, *Bio-phys. J* 108 (1) (2015) 173–183.
- [34]. Tao ZW, Li LG, Geng ZH, Dang T, Zhu SJ, Growth factors induce the improved cardiac remodeling in autologous mesenchymal stem cell-implanted failing rat hearts, *J. Zhejiang Univ. Sci. B* 11 (4) (2010) 238–248, [PubMed: 20349520]
- [35]. Hasan A, Ragaert K, Swieszkowski W, Selimovic S, Paul A, Camci-Unal G, Mofrad MR, Khademhosseini A, Biomechanical properties of native and tissue engineered heart valve constructs, *J. Biomech* 47 (9) (2014) 1949–1963. [PubMed: 24290137]
- [36]. Kessides MC, Khachemoune A, A review of epidermal maturation arrest: a unique entity or another description of persistent granulation tissue? *J. Clin. Aesthet. Dermatol* 7 (12) (2014) 46–50.
- [37]. Hirotsu K, Kannan S, Brian Jiang SI, Treatment of hypertrophic granulation tissue: a literature review, *Dermatol. Surg* (2019), doi:10.1097/DSS.0000000000002059. [Epub ahead of print].
- [38]. Franz S, Rammelt S, Scharnweber D, Simon JC, Immune responses to implants - a review of the implications for the design of immunomodulatory biomaterials, *Biomaterials* 32 (28) (2011) 6692–6709. [PubMed: 21715002]
- [39]. Anderson JM, Rodriguez A, Chang DT, Foreign body reaction to biomaterials, *Semin. Immunol* 20 (2) (2008) 86–100. [PubMed: 18162407]
- [40]. Martinez FO, Gordon S, The M1 and M2 paradigm of macrophage activation: time for reassessment, *F1000Prime Rep* 6 (2014) 13,
- [41]. Wynn TA, Chawla A, Pollard JW, Macrophage biology in development, homeostasis and disease, *Nature* 496 (7446) (2013) 445–455. [PubMed: 23619691]
- [42]. McBane JE, Cai K, Labow RS, Santerre JP, Co-culturing monocytes with smooth muscle cells improves cell distribution within a degradable polyurethane scaffold and reduces inflammatory cytokines, *Acta Biomater* 8(2) (2012) 488–501. [PubMed: 21971418]
- [43]. Song E, Ouyang N, Horbelt M, Antus B, Wang M, Exton MS, Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts, *Cell Immunol* 204 (1) (2000) 19–28. [PubMed: 11006014]
- [44]. Davies LC, Jenkins SJ, Allen JE, Taylor PR, Tissue-resident macrophages, *Nat. Immunol* 14 (10) (2013) 986–995. [PubMed: 24048120]
- [45]. Forbes SJ, Rosenthal N, Preparing the ground for tissue regeneration: from mechanism to therapy, *Nat. Med* 20 (8) (2014) 857–869. [PubMed: 25100531]
- [46]. Zhang H, Zhou L, Zhang W, Control of scaffold degradation in tissue engineering: a review, *Tissue Eng. Part B Rev* 20 (5) (2014) 492–502. [PubMed: 24547761]
- [47]. Wu W, Allen RA, Wang Y, Fast-degrading elastomer enables rapid remodeling of a cell-free synthetic graft into a neoartery, *Nat. Med* 18 (7) (2012) 1148–1153. [PubMed: 22729285]

Fig. 1.

Patch implantation. (A1) A purse string suture (diameter = 4 mm) was created on the RV wall and secured with a tourniquet. (A2) The distend portion was excised to create a fullthickness defect. (A3) The patch was sutured over the defect. (A4) The purse string suture was released. (B1) A representative dissection and (B2) an inset, show that the excision of purse string forms a 2.3 mm full thickness defect, contacting blood.

Fig. 2. Fabrication of biodegradable poly(ether ester urethane) urea (BPUR)-reinforced hydrogel patches. (A) Fourier transform infrared (FTIR) spectrum of BPUR. The peak at 1260 cm⁻¹ corresponds to the ester group in PEG, while the peak at 1730 cm⁻¹ corresponds to the carbonyl in urethanes and esters. The peaks at 3333 and 1630 represent N-H stretching and carbonyls in ureas, respectively. The absence of a peak at 2267 cm⁻¹ suggests negligible unreacted NCO. (B) BPUR reinforcement layers (7 mm in diameter, 80 μ m in thickness) cut from an electrospun mesh. (C) Scanning electron microscopy image of an electrospun BPUR mesh. (D) A BPUR- reinforced fibrin gel patch (7 mm in diameter, 1 mm in thickness) before implantation. and (E) Frequency spectrum of PEG-fibrin gel (n=5).

Fig. 3.

ECG arrhythmia recorded for 30 min at 4- and 8-weeks post surgery. There were no arrhythmias in any animals in the sham groups. At 4 weeks, 2 animals (2/9) in the pericardium group suffered from arrhythmia, 1 with frequent atrial premature beats (APBs) and 1 with frequent ventricular premature beats (VPBs); 3 animals (3/9) had arrhythmia in the BPUR PEG- fibrin group, 1 with frequent APBs and 2 with frequent VPBs. At 8 weeks, 3 animals (3/9) in the pericardium group had arrhythmia, 2 with frequent VPBs and 1 with frequent APBs plus atrial tachycardia (AT); no arrhythmia was found in the BPUR PEG-fibrin group.

Fig. 4.

Changes in function of left ventricle (LV) at 8 weeks post surgery. (A1), (B1) and (C1) M-mode images from the parasternal short-axis view. (A2), (B2) and (C2) B-mode images from the parasternal long-axis view indicating the LV end-diastolic area (LVEDA). (A3), (B3) and (C3) B-mode images from the parasternal long-axis view indicating the LV end-systolic area. LVEDd, LV enddiastolic diameter; LVEF, LV ejection fraction; LVFS, LV fractional shortening. Values are mean ± standard deviation. *p < 0.05, **p < 0.01.

Fig. 5.

Changes of RV pressure-volume (PV) loop at 4- and 8-weeks post surgery. (A1–3) RV PV loop at 4 weeks post surgery. (B1–3) RV PV loop at 8 weeks post surgery. (C–G) RV hemodynamics. RVSP, right ventricular systolic pressure; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RV SW, right ventricular stroke work; RV CO, right ventricular cardiac output. Values are mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$.

Fig. 6.

Implantation-induced fibrosis, patch degradation, wall thickness and vimentin-positive cell volume at 4- and 8-weeks post surgery. (A1–5) Patch area on the RV wall. (B1–5) Masson's trichrome staining show patch implantation-induced fibrosis on sections directly through the center of the defect. (C1–5) Insets show the degradation of patch materials and defect thickness. (D1–5) Immunofluorescence staining show filament actin (F-actin positive, red), cardiac fibroblasts and endothelial cells (vimentin positive, green), and nuclei (DAPI, blue). (E–H) Graphs show changes of patch implantation-induced fibrosis, patch material remaining, wall thickness and vimentin-positive cell volume. Values are mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$.

Fig. 7.

Muscularization and vascularization at 4- and 8-weeks post surgery. (A1–5) A view (200× magnification images stitched together) of a patched area stained for α -actinin, α SMA, vWF and DAPI in the whole heart section. (B1–5) vWF staining indicates α -actinin positive signals. (C1–5) vWF staining indicates blood vessels. (D) Amount of α -actinin indicating muscularization. (E) Number of blood vessels. Values are mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$.

Fig. 8.

Leukocyte and macrophage infiltration at 4- and 8-weeks post surgery. (A1–5) A view (200 × magnification images stitched together) of a patched area stained for α-actinin, αSMA, vWF and DAPI. (B1–5) CD45 staining indicates leukocytes. (C1–5) CD68 staining indicates pan- macrophages. (D1–5) CD206 staining indicates M2 macrophages. (E) Amount of CD45 expression indicating leukocytes. (F) Amount of CD68 expression indicating pan-macrophages. (G) Amount of CD208 expression indicating M2 macrophages. (H) Percentage of M2 over Pan- macrophages. Values are mean ± standard deviation. * $p < 0.05$, ** $p < 0.01$.

Fig. 9. The external scar size and the internal defect size at 4- and 8-weeks post surgery. (A1-C1) At 4 weeks. (A2-C2) At 8 weeks. (D) External scar size. (E) Internal defect size. Values are mean $\pm$ standard deviation. *p < 0.05, **p < 0.01.