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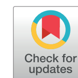
Engineered cardiac tissue patch maintains structural and electrical properties after epicardial implantation



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ABSTRACT

Functional cardiac tissue engineering holds promise as a candidate therapy for myocardial infarction and heart failure. Generation of "strong-contracting and fast-conducting" cardiac tissue patches capable of electromechanical coupling with host myocardium could allow efficient improvement of heart function without increased arrhythmogenic risks. Towards that goal, we engineered highly functional 1 cm × 1 cm cardiac tissue patches made of neonatal rat ventricular cells which after 2 weeks of culture exhibited force of contraction of 18.0 ± 1.4 mN, conduction velocity (CV) of 32.3 ± 1.8 cm/s, and sustained chronic activation when paced at rates as high as 8.7 ± 0.8 Hz. Patches transduced with genetically-encoded calcium indicator (GCaMP6) were implanted onto adult rat ventricles and after 4–6 weeks assessed for action potential conduction and electrical integration by two-camera optical mapping of GCaMP6-reported Ca^{2+} transients in the patch and RH237-reported action potentials in the recipient heart. Of the 13 implanted patches, 11 (85%) engrafted, maintained structural integrity, and conducted action potentials with average CVs and Ca^{2+} transient durations comparable to those before implantation. Despite preserved graft electrical properties, no anterograde or retrograde conduction could be induced between the patch and host cardiomyocytes, indicating lack of electrical integration. Electrical properties of the underlying myocardium were not changed by the engrafted patch. From immunostaining analyses, implanted patches were highly vascularized and expressed abundant electromechanical junctions, but remained separated from the epicardium by a non-myocyte layer. In summary, our studies demonstrate generation of highly functional cardiac tissue patches that can robustly engraft on the epicardial surface, vascularize, and maintain electrical function, but do not couple with host tissue. The lack of graft-host electrical integration is therefore a critical obstacle to development of efficient tissue engineering therapies for heart repair.

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1. Introduction

Coronary artery disease often results in myocardial infarction (MI), one of the leading causes of mortality worldwide [1]. Patients suffering from MI can progress to heart failure as the injured ventricles dilate and remodel. These patients are also at increased risk of fatal arrhythmias due to the presence of electrically inactive scar tissue [2,3]. While current therapies for MI are based on interventional and surgical revascularization techniques to prevent further ischemic injury, the irrecoverable loss of functional myocardium typically leads to progressive pathological remodeling and loss of pumping function [4–7]. In terminal situations, mechanical assist devices and heart transplantation provide therapeutic options, however, available donor hearts are limited and these interventions are not without complications [8]. Consequently, significant effort is being devoted to developing methods for repair and/or regeneration of damaged myocardium to prevent or improve the physical and electrical sequela of MI.

Previous studies have described the implantation of engineered cardiac tissues in injured animal hearts, with notable results including partial preservation of ventricular function [9–13], reduction in fibrosis and scar size [10,11], and improved vasculature

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in the infarct region [9,10,14,15]. Expression of genetically encoded calcium sensors within stem cell-derived cardiomyocytes (CM) prior to intramyocardial injection has indicated that these cells can survive long-term, exhibit functional calcium transients, and electrically couple with host tissue [16–19]. Interestingly, results in rodent models have reported suppression of arrhythmias after implantation of human CMs [16,17], while CM implantation in non-human primate models resulted in significant induction of arrhythmias [18,20]. Although it has been speculated that these conflicting observations can be attributed to the differences in animal sizes and heart rates [18], the ability to precisely monitor electrical activity at the host-graft interface is critical to understanding the mechanism of any observed anti- or pro-arrhythmic effects of cardiac cell therapy. Thus, in this study we developed an optical mapping technique to simultaneously visualize action potential propagation in both the implant and the recipient myocardium.

Mesoscopic hydrogel molding methods have been previously utilized for engineering of functional cardiac tissue network patches [21–24]. More recently, dynamic free-floating culture conditions were reported to enhance structural and functional properties of miniature and large engineered heart tissues termed "cardiobundles" [25,26] and "cardiopatches" [27]. In the present study, we combined a simple hydrogel molding fabrication procedure and dynamic culture to produce $1 \times 1\ cm^2$ engineered cardiac tissue patches with high contractile forces and fast action potential propagation. These patches were implanted onto nude rat hearts, and 4–6 weeks later a dual-camera optical mapping system was used to simultaneously and distinctly record action potential propagation in the implanted patch and the recipient heart, thus enabling the precise evaluation of their electrical interactions and ability to undergo functional coupling. While no electrical integration was observed between the patch and the heart, the patch remained structurally intact, maintained electrical properties comparable to those before implantation, and did not alter electrophysiological function of the underlying myocardium. These results demonstrate generation of highly functional cardiac tissue patches capable of long-term preservation of structural and functional properties on the epicardial surface of the heart, and warrant future studies to improve functional coupling of graft and host cardiomyocytes.

2. Materials and methods

2.1. Isolation of neonatal rat ventricular cells

Cardiac cells were isolated from the ventricles of 2-day old neonatal rat hearts according to previously described methods [25,28]. Animals were sacrificed using humane standards approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University. Ventricles were harvested, minced, and enzymatically dissociated by serial digestion with trypsin and collagenase. Isolated cells were resuspended in DMEM/F-12 with 10% Fetal Bovine Serum and 10% Horse Serum, and pre-plated for 45 min at 37 °C to increase the fraction of cardiomyocytes in the cell suspension [28,29]. To assess cardiomyocyte purity, pre-plated cells were fixed and permeabilized according to the manufacturer instructions (BD Biosciences # 554714), then stained with cardiac troponin T (cTnT) primary antibody (Abcam ab45932) and Alexa Fluor-conjugated secondary antibody. To assess fraction of endothelial cells, pre-plated cells were washed with PBS and stained with PE-conjugated CD31 antibody (BD Biosciences #555027) and APC-conjugated CD90 antibody (BD Biosciences #561409). Stained cells were analyzed in Beckman Astrios Sorter and BD FACSCanto A analyzer at the Duke Cancer Institute's Flow Cytometry Shared Resource. Endothelial cells and fibroblasts were identified as $CD31^+$ and $CD90^+/CD31^-$ [30] cells, respectively.

2.2. Patch fabrication and culture

Cellular preparations obtained from the neonatal rat hearts were utilized for fabrication of cardiac tissue patches by adapting previously described methods [22,23,31]. Reusable polydimethysiloxane (PDMS, Dow Corning) tissue molds were created by curing the polymer in a custom PTFE template. The tissue mold consisted of an 18 mm × 18 mm × 2 mm volume with rectangular posts arranged near the outer boundary of the mold (Fig. 1A). After coating the tissue mold with 0.2% pluronic F-127 for 1 h, a porous "frame" (inner dimensions 15 mm × 15 mm) laser-cut from spunbound nylon fabric (PBN-II, Cerex Advanced Fabrics) was inserted around the rectangular posts. The frame served to anchor the tissue patch during culture and facilitate functional measurements *in vitro* and implantation *in vivo*. A cell/hydrogel mixture was prepared with a final composition of 2 mg/mL fibrinogen, 1 U/mL thrombin, 10% matrigel, and 10×10^6 cells/mL [25]. To allow for tracking of cells and measurement of electrical propagation after implantation, a lentivirus was prepared to induce expression of a genetically-encoded calcium indicator under a myocyte-specific promoter (pRRL-MHCK7-GCaMP6 [32], full plasmid information available on AddGene). The lentivirus and polybrene (8 μg/mL) were mixed with the suspension of heart cells immediately prior to preparation of the hydrogel for patch fabrication. The cell/hydrogel mixture was pipetted into each tissue mold (600 μL per mold) and allowed to polymerize for 45 min at 37 °C. The tissue molds with polymerized cell/hydrogel mixture were then submerged in culture media consisting of low-glucose DMEM, 8% horse serum, 1% chick embryo extract, 1 mg/mL aminocaproic acid, 50 μg/mL ascorbic acid, and 5 U/mL penicillin. Immediately after submerging the polymerized gel in culture media, the frame with patch was carefully removed from the tissue mold (Fig. 1B) and cultured in a 6 well plate under free-floating dynamic culture conditions, as previously described [25]. Media was initially changed 24 h after patch fabrication and every 48 h thereafter. Engineered patches were cultured for 12–14 days prior to functional assessment, immunohistology, or implantation.

2.3. Patch histology

In vitro cultured tissue patches were fixed, permeabilized, and blocked as previously described [25]. Primary antibodies against sarcomeric alpha-actinin (Sigma A7811, 1:200 dilution), vimentin (Abcam ab92547, 1:500), collagen 1 (Abcam ab34710, 1:500), GFP (ThermoFisher A11122/A11120, 1:500/1:100), CD31 (Abcam ab28364, 1:50), N-cadherin (Abcam ab12221, 1:500), and connexin-43 (Abcam ab11370, 1:200) were diluted in blocking solution and incubated overnight at 4 °C. Alexa Fluor-conjugated secondary antibodies (ThermoFisher, 1:500 dilution) and nuclear stain (DAPI or Hoechst) were applied for 2 h at room temperature, then samples were imaged with a confocal microscope (Zeiss LSM 510).

2.4. Patch electrical and mechanical function

Action potential propagation in the cardiac patch was measured following 12–14 days of culture using a voltage-sensitive membrane dye, as previously described [22,23,33]. Patches were stained with 10 μM di-4 ANEPPS, incubated in 37 °C Tyrode's solution with the excitation-contraction uncoupler blebbistatin (10 μM), and stimulated at the patch periphery by a platinum point electrode connected to a Grass SD9 stimulator. Optical signals were recorded by a photo diode array connected to 504 hexagonally arranged, 750 μm optical fibers. Data were acquired in 2–5 s episodes after

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