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Functional engineered human cardiac patches prepared from nature's platform improve heart function after acute myocardial infarction

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ABSTRACT

With the advent of induced pluripotent stem cells and directed differentiation techniques, it is now feasible to derive individual-specific cardiac cells for human heart tissue engineering. Here we report the generation of functional engineered human cardiac patches using human induced pluripotent stem cells-derived cardiac cells and decellularized natural heart ECM as scaffolds. The engineered human cardiac patches can be tailored to any desired size and shape and exhibited normal contractile and electrical physiology *in vitro*. Further, when patching on the infarct area, these patches improved heart function of rats with acute myocardial infarction *in vivo*. These engineered human cardiac patches can be of great value for normal and disease-specific heart tissue engineering, drug screening, and meet the demands for individual-specific heart tissues for personalized regenerative therapy of myocardial damages in the future.

1. Introduction

Cardiovascular disease is now a leading cause of death worldwide [1]. Since postnatal cardiomyocytes exhibit nearly no regenerative capacity, myocardium damages often develop into scar tissues and progressively lead to heart dilatation and failure [2]. Except for heart transplantation, there are no satisfactory treatments for heart failure to date. However, this practice is limited by the scarce number of donor organs as well as the lifelong immunosuppression for patients.

Engineered heart tissues (EHTs) represent a promising alternative therapy for myocardium damage. There have been many studies using cardiomyocytes from non-human animals especially from rodents for preparation of bioartificial heart tissues [3]. However, the difference in species and electrophysiological properties of animal cardiomyocytes with human ones making them not an ideal cell source for future clinical applications. With the advent of induced pluripotent stem cells (iPSCs), it is now possible to generate individual-specific human cardiomyocytes for personalized human heart tissue engineering [4–7]. Another important component for heart tissue engineering is the scaffold for cell attachment and growth. A number of scaffolds have been fabricated using synthetic polymers, natural biological materials including collagen and alginate, as well as decellularized heart matrix [8,9]. In 2008, Ott et al. showed for the first time that the rat heart can be decellularized by perfusion using detergents, which generated natural heart extracellular matrix (ECM) [10]. Compared to the synthetic polymers and biological materials, the decellularized heart matrix represents a better scaffold because it preserved

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the architecture and ECM protein components of the natural heart.

Using similar methods, a recent study by Lu et al. decellularized the mouse whole heart and recellularized it with human pluripotent stem cells-derived multipotent cardiovascular progenitors (MCPs) [11]. This pioneering work demonstrated the possibility of combining human iPSC-derived cardiovascular cells and decellularized heart matrix for preparation of human EHTs. However, the repopulated whole heart was not uniform in cell distribution and still left giant gaps of uncoupled areas. Whether engineered tissues from such recellularized mouse heart are effective in treating myocardial damages remains unknown. It is also worth to note that normal heart tissues are composed of ~40% cardiomyocytes and ~60% cardiac fibroblasts [12]. Using a single population of iPSC-derived MCPs as the "seed" cells to repopulate the whole heart is difficult to control the precise optimal ratio of cardiomyocytes and non-cardiomyocytes for the EHTs. During preparation of this manuscript, Guyette et al., reported recellularization of decellularized human cadaveric heart matrix with hiPSC-derived cardiomyocytes [13]. However, the above mentioned problems were still not solved.

In this study, we combined fixed ratio of human iPSCs-derived cardiomyocytes and fibroblasts with pieces of decellularized natural rat heart ECM for preparation of engineered human cardiac patches. This method allowed us to generate human cardiac patches of any desired shape and size with well distributed cells. Further, decellularized natural heart ECM improved maturation of human iPSCs-derived cardiomyocytes. The decellularized natural heart ECM-based human cardiac patches exhibited beating activity and electrophysiology similar with human normal heart muscles and responded well to pharmaceutical agents affecting cardiomyocyte physiology. We further patched such engineered human cardiac patches on the infarct area of the heart of rat MI models. We show that engineered human cardiac patches effectively helped reduce the infarct size and improved the heart function after acute MI.

Our data indicated that, using decellularized natural heart ECM and human iPSCs-derived cardiac cells, it is feasible to generate individual-specific human cardiac patches of different sizes and shapes. These human cardiac patches can be of great value for drug screening and study of inherited heart diseases, as well as meeting the demands for individual-specific (size and shape) EHTs for personalized regenerative therapy of myocardial infarctions.

2. Materials and methods

2.1. Animals

Sprague-Dawley (SD) rats were obtained from the Laboratory Animal Care Facility of Shanghai Medical College at Fudan University. All the protocols in this study were approved by the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication No. 85-23, Revised) and was carried out under the supervision of the Fudan University Institutional Animal Care and Use Committee. All rats received cyclosporine (10 mg/kg/day, supplemented with food) to suppress immune rejection after xenotransplantation.

2.2. Decellularization and characterization of rat cadaveric hearts

For preparation of decellularized natural heart extracellular matrix (DC-ECM), we used 10-12-weeks-old SD rat cadaveric heart and decellularized it with methods modified from the previously published protocol [11]. Briefly, hearts were obtained immediately after euthanasia of adult rats and kept frozen at −80 °C. Before decellularization, the hearts were thawed in deionized water at room temperature. A blunted 20-gauge needle was cannulated into the ascending aorta to allow retrograde coronary perfusion. Sterile deionized water was perfused for 15–30 min at 2.0 ml/min, followed by perfusion with sterile phosphate-buffered saline (PBS). The heart was then perfused with 1% sodium dodecyl sulphate (SDS) for 2 h, 1% Triton X-100 with 0.5% EDTA (PH 8.0) for another 30 min at room temperature, followed by washing with antibiotic-containing deionized water and PBS (100 U/ml penicillin (Life Technologies, USA), 100 μg/ml streptomycin (Life Technologies) and 1.25 μg/ml amphotericin B (Sigma Aldrich, USA)) for 2 h.

2.3. Derivation, culture and characterization of hiPSCs

The protocol for derivation of human skin fibroblasts in this study was approved by the Institutional Ethical Review Board of Fudan University for Human Subjects. Human iPSC lines were generated as previously described [14] using the CytoTune®-iPS 2.0 Sendai Reprogramming Kits (Life Technology) following manufacturer's instruction. The cells were assayed for the expression of pluripotency markers by immunofluorescence and alkaline phosphatase staining. Derived hiPSCs were maintained on tissue culture dishes coated with Matrigel (growth factor reduced; BD Biosciences) using mTeSR-1 medium (Stemcell Technology) for subsequent analyses and differentiation.

2.4. Teratoma formation

To form teratomas, approximately 2 million undifferentiated human skin fibroblast derived-hiPSCs were harvested, mixed with Matrigel, and injected subcutaneously to immunodeficient mice. After 6–8 weeks, teratomas were dissected, fixed with 10% formaldehyde in PBS, embedded in paraffin wax, sectioned and stained with H&E.

2.5. Differentiation of hiPSCs

Differentiation into cardiac lineage was carried out with the protocol described by Sean P Palecek et al. [15]. The hiPSCs were initially cultured in mTeSR1 medium on Matrigel-coated plates until they were ~90% confluent. Medium was changed to RPMI/B-27 without insulin, consisting of RPMI 1640 (Corning) and B-27 minus insulin (Life technologies). On day 0–1, medium was supplemented with 12 μm CHIR-99021 (Selleck) in RPMI/B-27 without insulin. After 24 h, IWR-1 (5 μm, Sigma) was added into the fresh RPMI/B-27. On day 5–6, medium was changed to RPMI/B-27 minus insulin. On day 7 of differentiation and every 2 day thereafter, aspirated the medium and added RPMI/B-27 medium. Cultures were maintained in a 37 °C, 5% CO_2 environment. Contracting cells should be observed from day 7 post differentiation.

Spontaneous differentiation of hiPSCs were carried out for obtaining $CD90^+$ cells. Briefly, hiPSCs were digested from culture plates and floating embryiod bodies (EBs) were formed using ultralow attachment plates (Corning). EBs were then cultured with DMEM high glucose (GIBCO) supplemented with 20% FBS under a 37 °C, 5% CO_2 environment for 14 days with medium change every 2 days. The spontaneously differentiated EBs were then dissociated and subjected to fluorescence activated cell sorting (FACS) using anti-CD90 antibodies (Supplementary Table 1).

2.6. Flow cytometry

Sheets of cells were dissociated as previously reported [16]. Briefly, cells were digested with Collagenase I (1 mg/ml, Sigma) with DNase I (40 Unit/ml, Calbiochem) in PBS for 20 min, and followed by 0.25% trypsin/EDTA treatment for 5 min at 37 °C. For