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Spring-like fibers for cardiac tissue engineering

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ABSTRACT

Recapitulation of the cellular microenvironment of the heart, which promotes cell contraction, remains a key challenge in cardiac tissue engineering. We report here on our work, where for the first time, a 3-dimensional (3D) spring-like fiber scaffold was fabricated, successfully mimicking the coiled perimysial fibers of the heart. We hypothesized that since *in vivo* straightening and re-coiling of these fibers allow stretching and contraction of the myocardium in the direction of the cardiomyocytes, such a scaffold can support the assembly of a functional cardiac tissue capable of generating a strong contraction force. In this study, the mechanical properties of both spring-like single fibers and 3D scaffolds composed of them were investigated. The measurements showed that they have increased elasticity and extensibility compared to corresponding straight fibers and straight fiber scaffolds. We have also shown that cardiac cells cultivated on single spring-like fibers formed cell–fiber interactions that induced fiber stretching in the direction of contraction. Moreover, cardiac cells engineered within 3D thick spring-like fiber scaffolds formed a functional tissue exhibiting significantly improved function, including stronger contraction force ($p = 0.002$), higher beating rate ($p < 0.0001$) and lower excitation threshold ($p = 0.02$), compared to straight fiber scaffolds. Collectively, our results suggest that spring-like fibers can play a key role in contributing to the *ex vivo* formation of a contracting cardiac muscle tissue. We envision that cardiac tissues engineered within these spring-like fiber scaffolds can be used to improve heart function after infarction.

1. Introduction

According to the 2013 American Heart Association report, cardiovascular diseases account for more than 32% of all deaths in the United States with costs in excess of $312 billion and this is expected to exceed $1.5 trillion by 2030 [1]. Myocardial infarction (MI; heart attack) captures a significant segment of this population and is associated with sudden death as well as significant morbidity and mortality. MI results from the blockage of one of the coronary arteries which supply the cardiac tissue leading to ischemia (i.e. oxygen deprivation) of a segment of the heart. This process eventually leads to the death of contractile cells and the formation of scar tissue. Since cardiomyocytes (cardiac muscle cells) cannot proliferate and the number of stem cells in the heart is limited, the heart is unable to regenerate, leading to chronic cardiac dysfunction [2]. Currently the only cure for end-stage heart failure is cardiac transplantation. As cardiac donors are scarce, there is an urgent need to develop new strategies to repopulate the scar tissue formed on the heart with contracting cells to regain function. Direct cell injection therapies are limited due to inefficient cell accumulation in the infarct zone and cell death due to apoptosis before they form cell–cell or cell–matrix interactions [3]. These shortfalls motivated the development of the tissue engineering concept where 3D biomaterials serve as extracellular matrix (ECM)-like scaffolds to the cells, fostering their assembly into a functional cardiac tissue that could restore the infarcted heart function [4–6]. A prerequisite for such scaffolds is elasticity for providing support of tissue contraction *in vitro*. Furthermore, elastic biomaterials are essential for maintaining tissue structure and patch integrity at high loads post-transplantation on the infarcted heart [7].

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The natural cardiac tissue is composed of a 3D network of hierarchically organized fibrous matrix that provides both structural support and micro- and nano-scale cues for various physiological processes, including morphogenesis [8,9]. The matrix contains three distinct fiber types: supporting single cells, cell bundles, or the entire myocardium (endomysial, perimysial, and epimysial, respectively) [10]. The natural ECM topography and 3D structure have been recapitulated by using various types of polymeric nanofibers made by electrospinning [11]. The potential of cardiac cells to assemble into a functioning tissue when cultivated on electrospun fibers with diameters ranging between several micrometers and a few hundreds of nanometers was investigated [12,13]. In other studies, researchers seeded cardiac cells on aligned fibers, patterned hydrogels, or accordion-like honeycombs to induce the formation of elongated and aligned tissues [12,14–17]. Focus was given to the mechanical properties of the biomaterials with an effort to fabricate matrices meeting the needs of a viscoelastic tissue such as the heart, required to generate strong contraction forces [17–22].

In attempt to synthetically recreate the natural fibrous matrix and investigate the effect of the various fiber morphologies, topographies, and mechanical properties on cardiac tissue assembly, a unique subpopulation of the perimysial fibers was neglected. These relatively large fibers ranging between 1 and several μm in diameter have a coiled shape which contributes to the elastic properties of the cardiac muscle [23]. Robinson and colleagues have shown that in the heart these fibers are arranged parallel to the sarcomeres in a relative abundance of one fiber to several cardiomyocytes, revealing tortuous pathways in-between the cells [23]. It was found that straightening and re-coiling of these fibers allow stretching and contraction of the cardiac muscle in the direction of the cardiomyocytes [24].

Recognizing the importance of these coiled, extensible fibers for physiological myocardium contraction (Fig. 1), we were able to fabricate for the first time 3D scaffolds for cardiac tissue engineering composed of spring-like fibers. We report here on our investigation of the mechanical properties of single spring-like fibers and 3D stretchable matrices composed of them. In addition, we examined the potential of the spring-like scaffolds for engineering cardiac tissues.

2. Materials and methods

2.1. Decellularization

Hearts were decellularized as previously described [25]. Briefly, left ventricular myocardium was excised from adult Sprague–Dawley rat hearts and sectioned into specimens with dimensions of about 3 mm × 1 mm × 1 mm. Specimens were immediately washed in phosphate buffered saline (PBS). The specimens were then treated with 10 mM Tris buffer and 0.1% wt/vol EDTA for 2 days and then with 0.5% SDS for 5 days for decellularization. Decelluarized specimens were extensively washed to remove residual SDS. To remove nucleic remnants the specimens were treated with 40 U/ml benzonase (Novagen, Madison, WI) in 50 mM Tris, 1 mM $MgCl_2$, 0.01% BSA, pH 8.0 at 37° for 1 day. Following, specimens were extensively washed with Hank's balanced salt solution (HBSS). All steps were performed under continuous orbital mixing.

2.2. Fabrication of spring-like and straight fibers

Poly(ε-caprolactone) (PCL) (Mn 80,000, Aldrich) was dissolved in dichloromethane (DCM) and dimethylformamide (DMF) in ratios of 3:1, 2:1, 1:1 and 1:2. Varying concentrations of PCL, ranging from 10% to 17.5% w/v% were used. A syringe pump (Harvard apparatus, Holliston, MA) was used to deliver the polymer solution through a stainless steel 20G capillary at a rate of 0.5 mL/h for spring-like fibers and 7 mL/h for straight fibers. A high voltage power supply (Glassman High Voltage Inc.) was used to apply a 17.5 kV potential between the capillary tip and the grounded aluminum collector placed 15 cm away. The obtained fibers were examined under a light microscope to verify spring-like morphology. Prior to seeding PCL scaffolds were cut to final dimensions (8 mm diameter) and incubated in a solution of 10 μg/ml of fibronectin (Biological Industries) in PBS for 24 h in order to improve cell adhesion to the scaffolds.

2.3. Single fiber mechanical properties

The mechanical properties of single fibers were investigated as previously described [26]. Briefly, before the tensile test, the spring constants of AFM cantilevers were determined by calibration on a scanning probe microscope (SPM/DI) by the thermal noise method [27]. An AFM tip was brought toward a drop of liquid epoxy glue using a manipulator (Kleindiek Nanotechnik, NanoControl NC-2-3). The tip was then attached to a single fiber, electrospun on an aluminum foil with parallel gaps, by contacting with the drop of glue. After the glue solidified (within 40 min), the fiber attached to the tip was easily removed from the cover slip. Subsequently, the free end of the fiber was plunged into a large drop of liquid epoxy glue spread on a stub attached to the manipulator. The epoxy drop solidified within 40 min as before. A tensile test was then performed by inducing tension in the fiber by pulling it away from the AFM tip by the manipulator. The deformation speed was 80 μm/min. Experiments were video-recorded and subsequently analyzed by ImageJ as previously described [26].

2.4. Mechanical properties of the scaffolds

Scaffolds were cut into a rectangular shape (gauge length: 20 mm, and width: 4 mm) and tested using an Instron tensile testing instrument (model 4502) with a 10 N load cell at a rate of 5 mm/min.

2.5. Cardiac cell isolation, seeding and cultivation

Cardiac cells were isolated as previously described [28]. Briefly, left ventricles of 0–3 day old neonatal Sprague–Dawley rats were harvested and cells were isolated using 6 cycles (30 min each) of enzyme digestion with collagenase type II (95 U/ml; Worthington, Lakewood, NJ) and pancreatin (0.6 mg/ml; Sigma, St Louis, MO) in Dulbecco's modified Eagle Medium (DMEM, $CaCl_2 \cdot 2H_2O$ (1.8 mM), KCl (5.36 mM), $MgSO_4 \cdot 7H_2O$ (0.81 mM), NaCl (0.1 M), $NaHCO_3$ (0.44 mM), NaH_2PO_4 (0.9 mM)). After each round of digestion cells were centrifuged (600G, 5 min) and re-suspended in culture medium composed of M-199 supplemented with 0.6 mM $CuSO_4 \cdot 5H_2O$, 0.5 mM $ZnSO_4 \cdot 7H_2O$, 1.5 mM vitamin B12, 500 U/ml penicillin and 100 mg/ml streptomycin, and 0.5% (v/v) fetal bovine serum (FBS). To enrich the cardiomyocytes population, cells were suspended in culture medium with 5% FBS and pre-plated twice (30 min). Cell number and viability was determined by hemocytometer and trypan blue exclusion assay.

5×10^5 cardiac cells were seeded onto the scaffolds by adding 10 μl of the suspended cells followed by 1 h incubation (37 °C, 5% CO_2). Following, cell constructs were supplemented with culture medium (5% FBS) and incubated for 7 days.

2.6. Immunostaining

Immunostaining was performed as previously described [29]. Cardiac cell constructs were fixed and permeabilized in 100% cold methanol for 10 min, washed three times in DMEM-based buffer and then blocked for 1 h, at room temperature, in

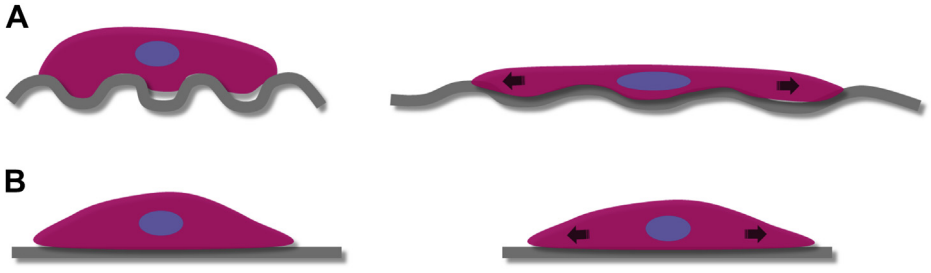

Fig. 1. Schematic illustration of a cardiomyocyte contraction and relaxation on an ECM fiber. (A) Spring-like fibers support cell stretching by providing physical support without resistance. (B) Straight fibers resist cardiomyocyte stretching, limiting their contraction potential.