Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Cardiac extracellular matrix–fibrin hybrid scaffolds with tunable properties for cardiovascular tissue engineering

Corin Williams [a], Erica Budina [a,1], Whitney L. Stoppel [a], Kelly E. Sullivan [a], Sirisha Emani [b], Sitaram M. Emani [b], Lauren D. Black III [a,c,*]

[a] Department of Biomedical Engineering, Tufts University, 4 Colby St, Medford, MA 02155, USA
[b] Department of Cardiac Surgery, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115, USA
[c] Cellular, Molecular and Developmental Biology Program, Sackler School for Graduate Biomedical Sciences, Tufts University School of Medicine, 145 Harrison Ave, Boston, MA 02111, USA

ARTICLE INFO

Article history:
Received 24 May 2014
Received in revised form 15 November 2014
Accepted 18 November 2014
Available online xxxx

Keywords:
Biomimetic material
Cardiac tissue engineering
Extracellular matrix
Fibrin
Mechanical properties

ABSTRACT

Solubilized cardiac extracellular matrix (ECM) is being developed as an injectable therapeutic that offers promise for promoting cardiac repair. However, the ECM alone forms a hydrogel that is very soft compared to the native myocardium. As both the stiffness and composition of the ECM are important in regulating cell behavior and can have complex synergistic effects, we sought to develop an ECM-based scaffold with tunable biochemical and mechanical properties. We used solubilized rat cardiac ECM from two developmental stages (neonatal, adult) combined with fibrin hydrogels that were cross-linked with transglutaminase. We show that ECM was retained within the gels and that the Young's modulus could be tuned to span the range of the developing and mature heart. C-kit+ cardiovascular progenitor cells from pediatric patients with congenital heart defects were seeded into the hybrid gels. Both the elastic modulus and composition of the scaffolds impacted the expression of endothelial and smooth muscle cell genes. Furthermore, we demonstrate that the hybrid gels are injectable, and thus have potential for minimally invasive therapies. ECM–fibrin hybrid scaffolds offer new opportunities for exploiting the effects of both composition and mechanical properties in directing cell behavior for tissue engineering.

1. Introduction

Eight in every 1000 infants are born with a congenital cardiovascular disorder [1]. These include various cardiomyopathies, congenital heart defects (CHDs) or arrhythmias that can lead to heart failure at a young age [2]. Currently, the majority of available therapies are geared toward slowing down the progression of heart failure and not toward restoring the proper contractile function of the myocardium [3]. For patients with end-stage heart failure, heart transplantation is the only successful long-term option. However, there is a shortage of available donor organs for pediatric patients and mortality on the waiting list is high [4,5]. Furthermore, patients who receive a heart transplant are subject to potentially serious post-surgery side-effects and lifelong immune suppression therapies [4]. Thus, there is a great need for the development of alternative strategies to treat CHDs and heart failure in the young.

Recently, c-kit+ cardiovascular progenitor cells (CPCs) have gained much attention for their potential to improve heart function after myocardial infarction in adults [6]. CPCs can also be isolated from young patients with various CHDs and differentiated into the three cardiovascular lineages of the heart: endothelial cells, vascular smooth muscle cells and cardiomyocytes [7]. In addition, the regenerative potential of CPCs from infants is greater than adult CPCs [8], suggesting that c-kit+ CPCs may be a promising cell source for cardiovascular tissue engineering or regenerative medicine strategies to treat pediatric patients. However, the efficiency of directed differentiation of CPCs with established protocols is low (~1% or less for all three cell types) [7]. A recent study suggests that although c-kit+ cells do not contribute significantly to the cardiomyocyte population in vivo, they are highly capable of generating cardiac endothelial cells [9]. Promoting vascularization of damaged tissue and endothelial cell–cardiomyocyte communication are key factors in cardiac repair [10]. The development of biomaterials that promote cardiovascular differentiation of CPCs would greatly enhance their regenerative potential for future therapies.

* Corresponding author at: Department of Biomedical Engineering, Tufts University, 4 Colby St, Medford, MA 02155, USA. Fax: +1 617 627 3231.
E-mail address: lauren.black@tufts.edu (L.D. Black II.
[1] Current address: School of Engineering and Applied Sciences, 29 Oxford St, Cambridge, MA 02138, USA.

http://dx.doi.org/10.1016/j.actbio.2014.11.035
1742-7061/

Recent studies point to a critical role of the extracellular matrix (ECM) in stem cell differentiation. In particular, the composition and mechanical properties of the ECM can influence stem cell fate. For example, human pluripotent stem cells showed robust cardiac differentiation when established growth-factor-based protocols were used in combination with culture on Matrigel [11]. Adult rat c-kit+ CPCs had higher expression of cardiac genes when cultured on adult porcine cardiac ECM compared to collagen I [12]. The stiffness of the substrate alone was found to drive mesenchymal stem cells towards neurogenic, myogenic or osteogenic fates [13], and chick embryonic cardiac cells showed increased maturation when cultured on hydrogels that stiffened over time in culture [14]. The above studies have been carried out in two dimensions; however, the complex functionality of cells can be lost or altered when cultured on planar surfaces [15]. Furthermore, the effect of substrate stiffness and composition on the cardiovascular fate of c-kit+ cells (i.e., endothelial and smooth muscle cell lineages) has not been investigated.

Solubilized cardiac ECM shows promise as an injectable biomaterial for the repair of myocardial infarction [16–18]. Solubilized ECM can reform a three-dimensional (3-D) nanofibrous hydrogel at physiological pH and temperature [16]. However, ECM hydrogels have a limited range of mechanical properties that do not match the native myocardium, and since they are very soft and rapidly degraded, pure ECM hydrogels cannot be used for 3-D cell encapsulation [19]. Thus, the aim of this work was to create cardiac ECM–fibrin hybrid scaffolds with tunable composition and elasticity in order to mimic properties of the native myocardium during development and maturation. Fibrin is commonly used for tissue engineering [20–23] and it is also highly angiogenic [24–26]; thus, by combining cardiac ECM and fibrin, our long-term goal is to promote both vascular and cardiac differentiation within the same scaffold, and further modulate cell response by tuning scaffold stiffness. Solubilized cardiac ECM from neonatal or adult rat hearts was incorporated into fibrin gels and scaffold stiffness was altered via cross-linking with transglutaminase (TG). CPCs from pediatric patients had good viability within the scaffolds and interacted with and remodeled the ECM over time in culture. Gene expression at 21 days showed that endothelial and smooth muscle markers were influenced by ECM developmental age and scaffold elastic modulus. Furthermore, these scaffolds are injectable through a 25G needle and thus may be beneficial for minimally invasive cell delivery in future applications.

2. Materials and methods

2.1. ECM isolation and solubilization

All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee at Tufts University and the NIH Guide for the Care and Use of Laboratory Animals. ECM was isolated and solubilized according to previously published methods [27]. Briefly, hearts were harvested from euthanized P2-P3 neonatal pups and adult female Sprague–Dawley rats (Charles River Laboratories). The ventricular tissue was minced and decellularized using 0.5% or 1% (w/v) sodium dodecyl sulfate (SDS) in deionized water (neonatal or adult hearts, respectively) with gentle shaking for 1–2 days until decellularization was complete. The ECM was transferred to a 0.5% or 1% TritonX-100 (v/v) solution for ~4–8 h to remove SDS and then washed with deionized water at least three times for 12–24 h per wash. The ECM was frozen at −20 °C, lyophilized for 24 h and solubilized at a concentration of 10 mg ml^{-1} in 1 mg ml^{-1} pepsin dissolved in 0.1 M HCl (4–6 h or 24–48 h for neonatal or adult hearts, respectively). Immediately prior to use, the cardiac ECM solution was neutralized to pH 7 with 1 N NaOH.

2.2. Preparation of transglutaminase stock solutions

Two separate stock solutions of transglutaminase (TG; 60 μg ml^{-1} and 600 μg ml^{-1}) were prepared fresh for each experiment. First, a 5 mg ml^{-1} solution was prepared by dissolving TG (Ajinomoto, Chicago, IL) in 20 mM HEPES buffer. The 5 mg ml^{-1} solution was then further diluted in 20 mM HEPES to create the 60 μg ml^{-1} stock solution (used for final concentrations of 1.2 and 12 μg ml^{-1} TG in the gels) and the 600 μg ml^{-1} stock solution (used for a final concentration of 120 μg ml^{-1} TG in the gels).

2.3. Preparation of ECM-only hydrogels

ECM hydrogels were prepared using various concentrations of TG (0, 1.2, 12 and 120 μg ml^{-1}). Decellularized ECM solution was neutralized and diluted to 6 mg ml^{-1} [16] before adding TG, vortexed briefly and then pipetted into 15 × 15 × 5 mm Tissue-Tek cryomolds (Ted Pella, Inc.). Samples were incubated at 37 °C for ~30 min. Collagen I (BD Biosciences) at the same concentration was used as a control.

2.4. Preparation of ECM–fibrin hydrogels cross-linked by transglutaminase

ECM–fibrin hybrid scaffolds were prepared using the various concentrations of TG described above. The gel solution was composed of fibrinogen (33 mg ml^{-1} stock, Sigma) at a final concentration of 3.3 mg ml^{-1}, 20 mM HEPES buffer, Dulbecco's modified Eagle medium (DMEM) (Invitrogen), thrombin (25 U ml^{-1} stock, Sigma) at a final concentration of 0.425 U ml^{-1}, calcium (1 M stock, Sigma) at a final concentration of 1.3 mM and cardiac ECM (2 mg ml^{-1} stock) at a final concentration of 340 μg ml^{-1}. In preliminary experiments, we found that higher concentrations of ECM inhibited the polymerization of fibrin (data not shown). The gel solution was mixed by gentle inversion, pipetted into molds or well plates and allowed to gel at 37 °C prior to carrying out the experiments described below.

2.5. Uniaxial mechanical testing

ECM–fibrin hydrogels were prepared as described above and pipetted into 15 × 15 × 5 mm cryomolds (300 μl per mold, six molds per condition) which were pre-coated in 5% pluronics solution to facilitate removal of the gels from the molds. The samples were allowed to gel for 30 min at 37 °C, removed from the molds and incubated in phosphate buffered saline (PBS) at 37 °C overnight. For uniaxial testing, the hydrogels were removed from the six-well plate and the excess PBS was gently removed using a Kim wipe™. Custom-made aluminum clamps were attached to each end of the hydrogel using superglue. An image of the sample's thickness was acquired using a custom-built device. The sample was then transferred to a PBS bath and attached to the custom-built uniaxial stretcher, as previously described [28,29]. Prior to stretching, the sample's initial length and width were determined using a digital caliper (Fisher Scientific). A custom-written LabVIEW program then applied a triangle waveform to stretch the sample to 25% strain. Each sample was pre-conditioned for at least 5 cycles before recording the force and length data. A stress–strain curve was generated for each sample, and the Young's modulus was determined by calculating the slope in the linear region of the curve between 15 and 20% strain.

2.6. Swelling test

Samples were prepared as described above for uniaxial stretching. After incubating in PBS overnight, excess fluid was carefully

Please cite this article in press as: Williams C et al. Cardiac extracellular matrix–fibrin hybrid scaffolds with tunable properties for cardiovascular tissue engineering. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.11.035