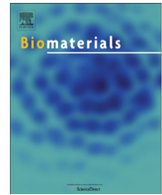
Biomaterials 35 (2014) 5462–5471

Contents lists available at ScienceDirect

Biomaterials

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



Micromolded gelatin hydrogels for extended culture of engineered cardiac tissues

Megan L. McCain [1], Ashutosh Agarwal [2], Haley W. Nesmith, Alexander P. Nesmith, Kevin Kit Parker*

Disease Biophysics Group, Wyss Institute for Biologically Inspired Engineering, School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 21 January 2014
Accepted 21 March 2014
Available online 14 April 2014

Keywords:
Tissue engineering
Organs on chips
Metabolism
Mechanotransduction

ABSTRACT

Defining the chronic cardiotoxic effects of drugs during preclinical screening is hindered by the relatively short lifetime of functional cardiac tissues *in vitro*, which are traditionally cultured on synthetic materials that do not recapitulate the cardiac microenvironment. Because collagen is the primary extracellular matrix protein in the heart, we hypothesized that micromolded gelatin hydrogel substrates tuned to mimic the elastic modulus of the heart would extend the lifetime of engineered cardiac tissues by better matching the native chemical and mechanical microenvironment. To measure tissue stress, we used tape casting, micromolding, and laser engraving to fabricate gelatin hydrogel muscular thin film cantilevers. Neonatal rat cardiac myocytes adhered to gelatin hydrogels and formed aligned tissues as defined by the microgrooves. Cardiac tissues could be cultured for over three weeks without declines in contractile stress. Myocytes on gelatin had higher spare respiratory capacity compared to those on fibronectin-coated PDMS, suggesting that improved metabolic function could be contributing to extended culture lifetime. Lastly, human induced pluripotent stem cell-derived cardiac myocytes adhered to micromolded gelatin surfaces and formed aligned tissues that remained functional for four weeks, highlighting their potential for human-relevant chronic studies.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Cardiotoxicity is a leading cause of market withdrawal for drugs in the United States [1,2]. Many drugs demonstrate cardiotoxicity due to chronic exposure, especially the anthracycline class of cytotoxic cancer drugs, such as doxorubicin [3,4]. Chronic cardiotoxicity is usually identified in animal models, such as mice [5,6] or dogs [7], that are exposed to the drug for at least two months. To reduce costs and improve throughput, cardiotoxicity is screened *in vitro* with neonatal rat ventricular myocyte cultures, but these studies typically do not extend beyond ten days [8,9], limiting their relevance as models for chronic exposure. Traditional *in vitro* systems also do not recapitulate the native tissue architecture or extracellular microenvironment of the heart, both of which are known to regulate myocyte phenotype [10–13]. Another limitation of many pre-clinical assays is a focus on toxicity [14] or ion channel activity [15], which are not direct indicators of cardiac output. Furthermore, studies with animals and animal cells are not always relevant to humans due to species-dependent differences [16], indicating that it is important to develop *in vitro* systems that are compatible with human-derived cardiac myocytes [17]. Due to the limitations of current *in vitro* systems, there is a need for biomaterials that can support long-term culture of engineered animal and human cardiac tissues and facilitate readouts of contractility to better predict adverse or functional effects of drugs on the heart.

Our group has previously described several *in vitro* platforms for engineering neonatal rat cardiac tissues and quantifying contractile function in response to variables such as tissue architecture [10,18], mechanical stretch [19], or acute drug exposure [20]. These "Heart on a Chip" platforms utilized microcontact printing [18,20] or micromolding [21] to engineer anisotropic cardiac tissues that mimicked the aligned, laminar structure of ventricular

* Corresponding author. Harvard School of Engineering and Applied Sciences, 29 Oxford St, Pierce Hall 321, Cambridge, MA 02138, USA. Tel.: +1 617 495 2850; fax: +1 617 496 1793.

E-mail address: kkparker@seas.harvard.edu (K.K. Parker).

[1] Present address: Department of Biomedical Engineering, Viterbi School of Engineering, University of Southern California, Los Angeles, CA 90089, USA.

[2] Present address: Department of Biomedical Engineering, Department of Pathology, DJTMF Biomedical Nanotechnology Institute, University of Miami, Coral Gables, FL 33146, USA.

http://dx.doi.org/10.1016/j.biomaterials.2014.03.052
0142-9612/© 2014 Elsevier Ltd. All rights reserved.

myocardium. Stress generation was quantified by culturing engineered tissues on polydimethylsiloxane (PDMS) polymer [18,20] or alginate hydrogel [21] cantilevers, forming muscular thin films (MTFs) that curled as tissues contracted. These two substrates each have advantages and disadvantages, from both biological and practical perspectives. PDMS can be spin-coated and laser-cut [20], which simplifies and standardizes the fabrication process, but PDMS is much stiffer than native myocardium, limiting its physiological relevance. Alginate hydrogels better match the elastic modulus of the heart, but alginate is a non-fouling biomaterial that is not native to the heart and requires additional fabrication steps for fibronectin adhesion [21]. Our previous method for fabricating alginate hydrogel MTFs also lacked control over thickness and cantilever shape [21], which compromises the throughput and reproducibility of the assay. To date, these *in vitro* tools have been used to quantify contractile stresses after three to five days in culture.

The extracellular matrix in many organs, including the heart [22], consists mostly of collagen. Gelatin is a derivative of collagen and thus forms hydrogels that are naturally non-toxic and amenable to cell adhesion [23], as shown for endothelial cells [24] and chondrocytes [25]. Gelatin hydrogels also have tunable elastic moduli and can be made thermostable by cross-linking with microbial transglutaminase [26–27], improving their robustness for cell culture. Because of these desirable properties, our objective for this study is to develop gelatin hydrogels as MTF substrates and use them to measure contractile stresses generated by both neonatal rat and human induced pluripotent stem (iPS) cell-derived engineered cardiac tissues. We will also determine if gelatin hydrogels improve the health and culture lifetime of engineered cardiac tissues compared to PDMS micropatterned with fibronectin, a commonly used culture substrate.

2. Materials and methods

2.1. Elastic modulus measurements

Solutions of 5%, 10%, and 20% w/v gelatin from porcine skin (175 Bloom, Type A, Sigma–Aldrich, St. Louis, MO) were prepared with 1%, 2%, and 4% microbial transglutaminase (MTG, Ajinomoto, Fort Lee, NJ), for a total of nine solutions, similar to previously published protocols [24,25]. Gels were cured overnight in 35 mm Petri dishes (6 mL/dish) in triplicate. After curing, a 6 mm diameter biopsy punch was used to remove a cylinder of gel for each dish. The diameter of the gel cylinder was measured and stress–strain curves were derived using an Instron 3342 (Norwood, MA) in uniaxial unconfirmed compression mode. The compressive Young's modulus was taken as the slope in the linear region where deformation was first detected. For each gel solution, the Young's modulus values were averaged for the three technical replicates. Three independent sets of gel solutions (biological replicates) were fabricated, measured, and averaged together to determine average Young's modulus values.

2.2. Soft lithography and microcontact printing

Elastomeric stamps were fabricated from polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) using previously published protocols [10,28,29]. Briefly, custom photomasks were used to shield wafers spin-coated with SU-8 2002 photoresist (MicroChem, Newton, MA) during UV exposure with a mask aligner. After exposure, wafers were rinsed in propylene glycol monomethyl ether acetate to dissolve un-exposed regions, dried, and silanized. PDMS was poured onto the wafer, cured at 65 °C for at least 4 h, carefully peeled from the wafer, and cut into stamps. For micromolding, stamps with 10 μm lines separated by 10 μm gaps were used. For microcontact printing, stamps with 15 μm lines separated by 2 μm gaps were incubated with fibronectin (50 μg/mL, BD Biosciences, San Jose, CA) for 1 h. Glass coverslips were spin-coated with PDMS and exposed to UV-ozone for 8 min prior to being stamped with fibronectin, as previously described [10,18].

2.3. Gelatin muscular thin film (MTF) substrate fabrication

Glass coverslips (22 mm × 22 mm) were covered with low-adhesive tape (3M, St. Paul, MN), which was cut using a laser engraving system (Epilog Laser, Golden, CO). The tape was selectively peeled from each coverslip such that the outer border and two inner rectangles remained covered with tape. Coverslips were then immersed in 0.1 M NaOH for 5 min, followed by 0.5% APTES in 95% ethanol for 5 min and 0.5% glutaraldehyde for 30 min, as previously described [30]. Coverslips were rigorously rinsed and dried. The inner two rectangles were then peeled, leaving a region of selectively-activated glass exposed.

To make gelatin solutions, 20% w/v gelatin (Sigma–Aldrich, St. Louis, MO) was warmed to 65 °C until all powder was dissolved. MTG (Ajinomoto, Fort Lee, NJ) at 8% w/v was warmed to 37 °C. Equal parts of the gelatin and MTG solutions were then mixed to produce a final solution of 10% w/v gelatin and 4% w/v MTG. The solution was quickly pipetted onto the exposed regions of glass coverslips. PDMS stamps with 10 μm × 10 μm line features were then inverted on top of the gelatin drop and gentle pressure was applied so that the bottom edges of the stamp were touching the taped edges. Gelatin was then left to cure overnight at room temperature with the stamp in place.

After gelatin was cured, the coverslip and stamp were immersed in distilled water to re-hydrate the gelatin. The stamp was then carefully peeled off the gelatin. Gel rehydration minimized damage to the gelatin during peeling. Coverslips with molded gelatin were then dried in a tissue culture hood for 2–3 h.

Cantilevers (1 mm wide × 2 mm long) were laser engraved into the dehydrated micromolded gelatin using an Epilog laser engraving system (Golden, CO) with the following settings: Power 3%, Speed 6, Frequency 2500. Laser cuts were centered over the un-activated regions of the coverslips. Diagonal cuts through the corners of the tape and a square cut just inside the tape border were also made to ease tape removal. After cutting, the outer border of the tape was carefully removed without disrupting the gelatin cantilevers. Gelatin chips were they re-hydrated in PBS and stored at 4 °C until cell seeding.

To measure gelatin thickness, gelatin solutions were doped with AlexaFluor 488 200 nm fluorescent beads (Invitrogen, Carlsbad, CA) at a concentration of 1:1000 prior to fabricating gelatin MTFs. *Z*-stacks of hydrated substrates were imaged on a Zeiss LSM 5 LIVE confocal microscope (Oberkochen, Germany) at six locations on each coverslip using a 10× objective. Three coverslips were imaged for each of three independent batches of gelatin. *Z*-stacks were re-sliced in ImageJ (NIH, Bethesda, MD) to acquire side views and the height of each z-stack was manually measured.

2.4. Cell culture

Neonatal rat ventricular myocytes were isolated according to previously published protocols approved by the Harvard University Animal Care and Use Committee [18,31]. Briefly, ventricles were extracted from two day old Sprague–Dawley rats and incubated in Trypsin solution (1 mg/mL) overnight at 4 °C. Ventricles were then subjected to four collagenase (1 mg/mL, Worthington Biochemical Corp., Lakewood, NJ) digestions for 1–2 min at 37 °C followed by manual pipette agitation. Cell solutions were strained, re-suspended in M199 culture media supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES, 0.1 mM MEM nonessential amino acids, 20 mM glucose, 2 mM L-glutamine, 1.5 μM vitamin B-12, and 50 U/mL penicillin, and pre-plated twice to reduce non-myocyte cell populations. One million cardiac myocytes were seeded for each well of a 6-well plate. Fetal bovine serum concentration was reduced to 2% after two days in culture.

2.5. Immunostaining and image analysis

Engineered cardiac tissues were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) and 0.5% Triton-X (Sigma–Aldrich, St. Louis, MO) for 5 min. Tissues were then incubated with primary antibodies against sarcomere α-actinin (Sigma–Aldrich, St. Louis, MO) for 90 min at room temperature followed by secondary antibodies against mouse IgG conjugated to Alexa Fluor 546 (Invitrogen, Carlsbad, CA) and DAPI (Invitrogen, Carlsbad, CA) for 60 min at room temperature. For each coverslip, ten fields of view were imaged using a Leica DMI 6000B inverted fluorescent microscope with a 40× objective (Wetzler, Germany).

For each image, the orientation angles of α-actinin immunosignals were calculated, as previously described [10]. The orientational order parameter (OOP) [18] was then calculated for all orientation angles consolidated from all images taken on each coverslip. The OOPs for each condition were averaged and compared using student's *t*-test.

2.6. MTF experiments and analysis

Gelatin MTF substrates with engineered cardiac tissues were transferred to a 35 mm Petri dish and soaked in Tyrode's solution (1.8 mM $CaCl_2$, 5 mM glucose, 5 mM HEPES, 1 mM MgCl, 5.4 mM KCl, 135 mM NaCl, 0.33 mM of NaH_2PO_4, pH 7.4). The dish was placed on the stage of a Leica MZ9.5 stereomicroscope (Wetzlar, Germany). Using fine forceps, the excess gelatin and tissue surrounding the MTFs was removed and discarded and each MTF was gently peeled from the glass coverslip. The dish was then placed in a heating block to restore 37 °C within the bath and field stimulation electrodes were inserted into the top of the dish. Rows of contracting MTFs were recorded at 100 frames per second using a Basler A601f-2 camera (Exton, PA) while pacing from 1 to 6 Hz at 5–7 V using a MyoPacer Cell Stimulator (IonOptix, Milton, MA).

To convert movies to stress measurements, movies were thresholded and the radius of curvature for each MTF was calculated using the *x*-projection and original length [18]. The radius of curvature, thickness, and elastic modulus of each MTF was used to calculate stress using modified Stoney's equation [31]. For each MTF, the

Download English Version:

https://daneshyari.com/en/article/6065

Download Persian Version:

https://daneshyari.com/article/6065

Daneshyari.com