Journal of Pharmacological and Toxicological Methods 65 (2012) 126–135

Contents lists available at SciVerse ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Original article

Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle

Anna Grosberg, Alexander P. Nesmith, Josue A. Goss, Mark D. Brigham, Megan L. McCain, Kevin Kit Parker *

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

ARTICLE INFO

Article history:
Received 10 December 2011
Accepted 3 April 2012

Keywords:
Assay
Contractility
In vitro
Micropatterning
Muscular micro-devices
Tissue engineering

ABSTRACT

Introduction: To evaluate the viability of a muscle tissue, it is essential to measure the tissue's contractile performance as well as to control its structure. Accurate contractility data can aid in development of more effective and safer drugs. This can be accomplished with a robust *in vitro* contractility assay applicable to various types of muscle tissue. **Methods:** The devices developed in this work were based on the muscular thin film (MTF) technology, in which an elastic film is manufactured with a 2D engineered muscle tissue on one side. The tissue template is made by patterning extracellular matrix with microcontact printing. When muscle cells are seeded on the film, they self-organize with respect to the geometric cues in the matrix to form a tissue. **Results:** Several assays based on the "MTF on a chip" technology are demonstrated. One such assay incorporates the contractility assay with striated muscle into a fluidic channel. Another assay platform incorporates the MTFs in a multi-well plate, which is compatible with automated data collection and analysis. Finally, we demonstrate the possibility of analyzing contractility of both striated and smooth muscle simultaneously on the same chip. **Discussion:** In this work, we assembled an ensemble of contractility assays for striated and smooth muscle based on muscular thin films. Our results suggest an improvement over current methods and an alternative to isolated tissue preparations. Our technology is amenable to both primary harvests cells and cell lines, as well as both human and animal tissues.

1. Introduction

High-throughput methods for measuring contractility of smooth and striated muscle are currently an unmet need for drug discovery and safety (Bass, Kinter, & Williams, 2004). This is especially difficult because it is important to recapitulate the *in vivo* microenvironments of muscular organs (Heeckt, Halfter, Schraut, Lee, & Bauer, 1993; Streeter, Spotnitz, Patel, Ross, & Sonnenblick, 1969). One of the key features of the microenvironment in muscular organs is the hierarchical organization of the muscle tissue. While it is desirable to recreate the tissue architecture to replicate physiological function, to date, *in vitro* methods have not supported this effort.

There have been two primary methods for *in vitro* testing of muscle contractility. A common tissue scale method involves excising a muscle strip from the heart wall, the papillary muscle, vessel ring, or trachea wall and suspending it in a bath with a force transducer attached, which allows for direct measurement of developed force (Effron, Bhatnagar, Spurgeon, Ruano-Arroyo, & Lakatta, 1987; Lakatta, Gerstenblith, Angell, Shock, & Weisfeldt, 1975; Uehata et al., 1997). The second approach is to measure single cell contractility using a range of methods, such as cell shortening or traction force microscopy (Jacot, McCulloch, & Omens, 2008). To automate testing of muscle contractility, organs or isolated cells in microfluidic systems, "lab on a chip" assays have been developed (Addae-Mensah & Wikswo, 2008). However, most "lab on a chip" contractility assays are based on single cells within microfluidic channels (Cheng, Klauke, Sedgwick, Smith, & Cooper, 2006; Cheng, Klauke, Smith, & Cooper, 2010; Tan et al., 2003; Werdich et al., 2004; Zhao, Lim, Sawyer, Liao, & Zhang, 2007), which do not always reproduce multi-cellular pharmacological responses (Kaneko, Kojima, & Yasuda, 2007). Various tissue contractility assays (Kim et al., 2008; Linder et al., 2010; Park et al., 2005) are difficult to translate to higher throughput systems with controlled cellular microenvironments. Impedance measurement of contracting myocyte monolayers within multi-well plates is another *in vitro* approach (Guo et al., 2011). Unfortunately, impedance measurements are an artificial index, which cannot be correlated to contractile force, stress, or strain.

In this report, we show that muscular thin films (MTF) technology can be adapted for various assays for both smooth and striated muscle types. MTFs consist of engineered monolayers of muscle cells on an elastic film, and have been used to measure contractile properties, including peak systolic and diastolic stresses, of multiple muscle types

Abbreviations: CM, cardiomyocytes; cMTF, cardiac muscular thin film; ET-1, Endothelin-1; FN, fibronectin; MTF, muscular thin films; PBS, Phosphate Buffered Saline; PDMS, polydimethylsiloxane; PIPAAm, Poly(N-isopropylacrylamide); ROCK, Rho associated protein kinase; vMTF, vascular smooth muscle thin film; VSMC, vascular smooth muscle cell.

* Corresponding author at: Disease Biophysics Group, 29 Oxford St, Pierce Hall Rm 321, Harvard University, Cambridge, MA 02138, USA. Tel.: +1 617 495 2850; fax: +1 617 495 9837.
E-mail address: kkparker@seas.harvard.edu (K.K. Parker).

1056-8719/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2012.04.001

(Alford, Feinberg, Sheehy, & Parker, 2010; Feinberg et al., 2007). In this work, we show that the more efficient "heart on a chip" MTF assay (Grosberg, Alford, McCain, & Parker, 2011) can be adapted to smooth muscle, and we illustrate the use of the MTF technology adapted to both a higher throughput multi-well format and a fluidic device. Additionally, we demonstrate the use of MTFs to construct multi-tissue chips for simultaneous contractility measurements in both vascular smooth muscle cell (VSMC) and cardiomyocyte tissues.

2. Methods

2.1. Substrate fabrication

The simple MTF chip substrates were made via a multi-step fabrication process using large sections of #1 glass (Porvair, Ltd., Norfolk, UK). The glass was cleaned by sonicating in 50% ethanol for 30 min, and then air dried inside a sterile culture hood. After the protective film (Static Cling Film, McMaster-Carr, Robbinsville, NJ) was attached to both sides of the glass, parallel strips (5–8 mm) were removed using a razor blade. An excess of Poly(N-isopropylacrylamide) (PIPAAm, Polysciences, Inc., Warrington, PA) dissolved in 99.4% 1-butanol (10% w/v) was deposited onto the exposed glass, and spin coated on the surface at 6000 rpm for 1 min. Sylgard 184 (Dow Corning, Midland, MI) polydimethylsiloxane (PDMS) elastomer was mixed with the curing agent with 10:1 ratio, and the mixture was pre-cured at room temperature for 1–6 h depending on the desired thickness of the MTFs (Feinberg et al., 2007). Before the PDMS was spin coated onto the glass section (ramp up to 4000 rpm for 2 min), the remaining strips of the protective film were removed from the top of the glass. After the PDMS was cured for 8–12 h at 65 °C, the bottom protective film was removed. Individual rectangular cover slips (12 × 15 mm) were made by scoring the exposed glass with a diamond glass cutter.

Substrates for multi-well plates were fabricated with the same procedure as simple MTF chips, but instead of cutting out strips from the protective film, half-well shapes were cut out. Similarly, the procedure for manufacturing substrates for an MTF in a fluidic channel was the same except round 25 mm cover slips were used instead of glass sections, and scotch tape was used as the protective film. The two-tissue chips were manufactured the same way as the simple MTF chip substrates. Extra substrates were prepared for each type of system and used to measure the thickness of the PDMS with a profilometer (Dektak 6 M, Veeco Instruments Inc., Plainview, NY). The thickness ranged 13–20 μm for different samples.

2.2. Micro-patterning of fibronectin (FN)

To provide guidance cues for organizing smooth and striated muscle cells in the dish, fibronectin (FN, BD Biosciences, Sparks, MD), an extracellular matrix protein, was microcontact printed on the substrate (Bray, Sheehy, & Parker, 2008; Feinberg et al., 2007; Geisse, Sheehy, & Parker, 2009). Small PDMS stamps (~1 cm^2) with 15 μm lines and 4 μm spacing were used for all VSMCs samples. Large format stamps (~7 cm × 5 cm) with 20 μm lines and 20 μm spacing were used to pattern the multi-well plate substrates. For all other cardiomyocytes samples, small stamps with a "brick-wall" pattern (each brick had dimensions of 20 × 100 μm, with each short edge terminating in 5 μm long interlocking "saw-teeth") were used. The stamps were sterilized in 50% ethanol by sonicating for 30 min and then air-dried under sterile conditions in a culture-hood. The patterned surface of each stamp was completely covered with excess FN (50 μg/mL) and incubated for at least 1 h. The PDMS surface of the substrates was sterilized and functionalized for 8 min of UV ozone treatment (Model No. 342, Jetlight Company, Inc, Phoenex, AZ). In a sterile culture hood, the stamps were dried with compressed air, and used to transfer the FN pattern to the functionalized substrates. The substrates were then treated for 5 min with 1% Pluronics F127 (BASF Group, Parsippany, NJ) in deionized water and washed three times with Phosphate Buffered Saline (PBS). As in previously established protocols (Alford et al., 2011, 2010; Feinberg et al., 2007), the substrates were stored dry at 4 °C for no more than three days prior to cell seeding.

2.3. MTF muti-well plate assembly

To assemble the MTF multi-well plate, the substrate with the FN patterning was attached to a skeleton of a 24-well plate top (Porvair, Ltd., Norfolk, UK). By spin coating a layer of PDMS onto a glass section (2000 rpm for 2 min) and within 20 min temporarily pressing it to the skeleton plate top, the spaces between the wells were covered with an even, thin layer of PDMS. Immediately after removing the transfer glass, the functionalized substrate was pressed onto the multi-well skeleton top. The edges were sealed with a clear nail polish, and the whole plate was left at 37 °C for 24 h before being moved to 4 °C until cell seeding.

2.4. Fluidic channel chip assembly

Fluidic channels, designed using vector-based drawing software (Corel), were cut from 1.5 mm Poly(methylmethacrylate) sheets (McMaster-Carr, Robbinsville, NJ). A 25 mm round glass cover slip was used as an optically clear chamber top. Both materials were laser machined using a Versalaser VLS 3.50 (Universal Laser Systems, Scottsdale, AZ). Fluidic wells were constructed using 1000 μL pipet tips (VWR, Radnor, PA). The parts were assembled by adhering with 5-minute epoxy (Devcon, Danvers, MA).

2.5. Vascular smooth muscle cell culture

Human vascular smooth muscle cells (VSMCs) were cultured using the same protocol previously described (Alford et al., 2011, 2010). Briefly, human umbilical artery VSMCs purchased from Lonza at passage 3 and were cultured until passage 5–7 in growth medium–M199 culture medium (GIBCO, Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen), 10 mM HEPES (GIBCO, Invitrogen, Carlsbad, CA), 3.5 g/L glucose, 2 mg/L vitamin B-12, 50 U/mL penicillin, and 50 U/mL streptomycin (GIBCO, Invitrogen, Carlsbad, CA). To create a confluent tissue the VSMCs were seeded at 250 cells/mm^2, and cultured in growth medium for 24 h. To induce the contractile phenotype, growth medium was exchanged for serum free medium for 24 h prior to contractility experiments (Han, Wen, Zheng, Cheng, & Zhang, 2006).

2.6. Cardiomyocyte harvest, seeding, and culture

Neonatal rat ventricular myocytes were harvested from two day old Sprague–Dawley rats (Charles River Labarotories, Wilmington, MA) (Adams et al., 2007). The extracted ventricles were washed in Hanks balanced salt solution and then homogenized by incubating in trypsin (1 mg/mL) for approximately 12 h at 4 °C. Thereafter, collagenase (1 mg/mL, Worthington Biochemical Corp., Lakewood, NJ) at 37 °C was used to digest the tissue and release the cardiomyocytes into solution. M199 culture medium (GIBCO, Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen), 10 mM HEPES (GIBCO, Invitrogen, Carlsbad, CA), 0.1 mM MEM non-essential amino acids, 3.5 g/L glucose, 2 mM L-glutamine, 2 mg/L vitamin B-12, and 50 U/mL penicillin was used to re-suspend the cardiomyocytes. After two 45 min pre-plating steps in a culture flask to increase cell purity, the cardiomyocytes were seeded on the substrates at a density of approximately 1000 cells/mm^2. During incubation, the cells were kept in 10% FBS media at 37 °C and 5% CO_2 for the first 48 h. Thereafter, the cardiomyocytes were maintained in 2% FBS media. All laboratory animal use in this study followed the guidelines of Institutional Animal Care and Use Committee of Harvard