Journal of Pharmacological and Toxicological Methods xxx (2012) xxx–xxx

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
Available online xxxx

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

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

University (Animal Experimentation Protocol permit number 24–01). This protocol meets the guidelines for the use of vertebrate animals in research and teaching of the Faculty of Arts and Sciences of Harvard University. It also follows recommendations included in the NIH Guide for the care and use of laboratory animals and is in accordance with existing Federal (9 CFR Parts 1, 2 and 3), state and city laws and regulations governing the use of animals in research and teaching.

2.7. VSMCs MTF on a chip contractility experiments

After 24 h in serum free medium, the substrate with VSMCs was transferred to a normal Tyrode's solution (1.192 g of HEPES, 0.901 g of glucose, 0.265 g of $CaCl_2$, 0.203 g of $MgCl_2$, 0.403 g of KCl, 7.889 g of NaCl, and 0.040 g of NaH_2PO_4 per liter of deionized water; reagents from Sigma, St. Louis, MO) at 37 °C, and the films were cut using a straight-blade razor. The first two cuts were approximately 0.2 mm apart in the center of the chip perpendicular to the tissue direction, and the resultant thin strip of film was peeled away separating the two sides of the MTF. The subsequent cuts were parallel to the tissue, and their spacing controlled the widths of the films (1–2 mm) and the distance between films (0.5–1.5 mm). Every-other strip was peeled away and discarded leaving 4–6 distinct MTFs on the chip, and a picture of this configuration was taken for each chip through a stereomicroscope (Model MZ6 with darkfield base, Leica Microsystems, Inc., Wetzlar, Germany) using a Basler camera (A602f Basler Inc, Exton, PA) controlled by LabView (National Instruments, Austin, TX). During the cutting process the bath equilibrated with room temperature, and PIPAAm dissolved releasing the rest of the films. The MTFs were peeled up gently by the corner using a pair of tweezers. These VSMC MTFs (vMTFs) were then attached to the chip by one edge only, and bent up off the substrate due to the contraction in VSMCs. The chip was moved to a temperature controlled bath, and all subsequent experiments were done at 34–37 °C. During the experiment the films were imaged through the stereomicroscope every 30 s. After recording approximately 4 min of baseline film configuration, Endothelin-1 (ET-1, Sigma-Aldrich, St. Louis, MO) at a concentration of 50 nM was used to stimulate the VSMCs, and after approximately 12 min, a Rho associated protein kinase (ROCK) Inhibitor (0.1 mM HA-1077, Sigma-Aldrich, St. Louis, MO) was used to completely relax the VSMCs (Alford et al., 2011). The MTF chip was imaged every 30 s until the films were completely flat on the substrate.

2.8. Cardiomyocyte MTF in a multi-well plate contractility experiments

During the *Cardiomyocyte MTF* (cMTF) -in-a-plate contractility experiments the culture medium in the plate was exchanged with a normal Tyrode's solution, and the plate was moved to the stereomicroscope (Model MZ6 with darkfield base, Leica Microsystems, Inc., Wetzlar, Germany). A single film was cut inside each well, and the unwanted MTF pieces were peeled away and discarded. A Basler camera (A602f Basler Inc, Exton, PA) was used to record the cMTF's length while it was still fully attached to the bottom of the plate. Once the PIPAAm dissolved, the cMTF was attached to the plate by one edge only, and it would bend up from the plate bottom as the cardiomyocytes contracted. The film position was recorded from the top at 100–120 fps using the Basler camera as the cardiomyocytes spontaneously contracted.

2.9. cMTF in a fluidic channel contractility experiment

To combine the cMTF chip with the fluidic top, the films were cut co-localized with the channels in the fluidic top. To cut the cMTF, the chip was moved to a 60 mm Petri dish with the normal Tyrode's solution. When all the cuts were completed there was a single film left per channel, with each film attached to the substrate by one edge only.

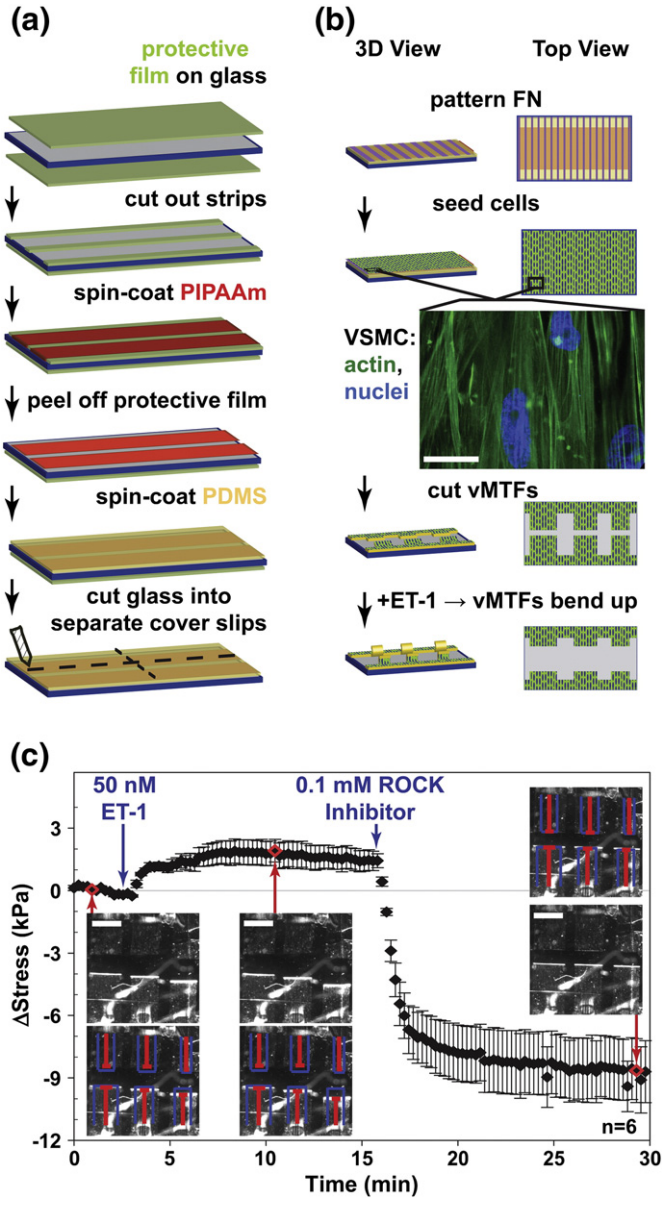

Fig. 1. (a) Schematic of an MTF on a chip substrate fabrication; (b) Schematic illustrating the FN microcontact printed onto the substrate to form confluent tissue—VSMC fluorescent image shows anisotropic organization of actin within a confluent monolayer, scale bar = 30 μm; (c) The change of stress generated by VSMCs with the time points where 50 nM ET-1 and 0.1 mM ROCK Inhibitor were added indicated in blue; brightfield images of the vMTF chip shown at three data points indicated with red diamonds, scale bar = 2 mm; the projection of the films is shown with red bars, and the blue outline indicates the original length of the film.

The fluidic channel top was covered in a thin layer of vacuum grease and lowered directly onto the cMTF chip. After the seal was made between the cMTF chip and the fluidic channel top, the bath was emptied, and only the Tyrode's in the channel and fluidic wells remained. The cardiomyocytes were paced by placing platinum electrodes in the inlet and outlet wells. The cMTFs were imaged (120 fps) through the transparent fluidic channel top with a Basler camera (A602f Basler Inc, Exton, PA) under a stereomicroscope (Model MZ6 with darkfield base, Leica Microsystems, Inc., Wetzlar, Germany). Once the experiments were completed the fluid was removed from the channel to settle the film down onto the substrate, and an image was taken to record the film length.

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

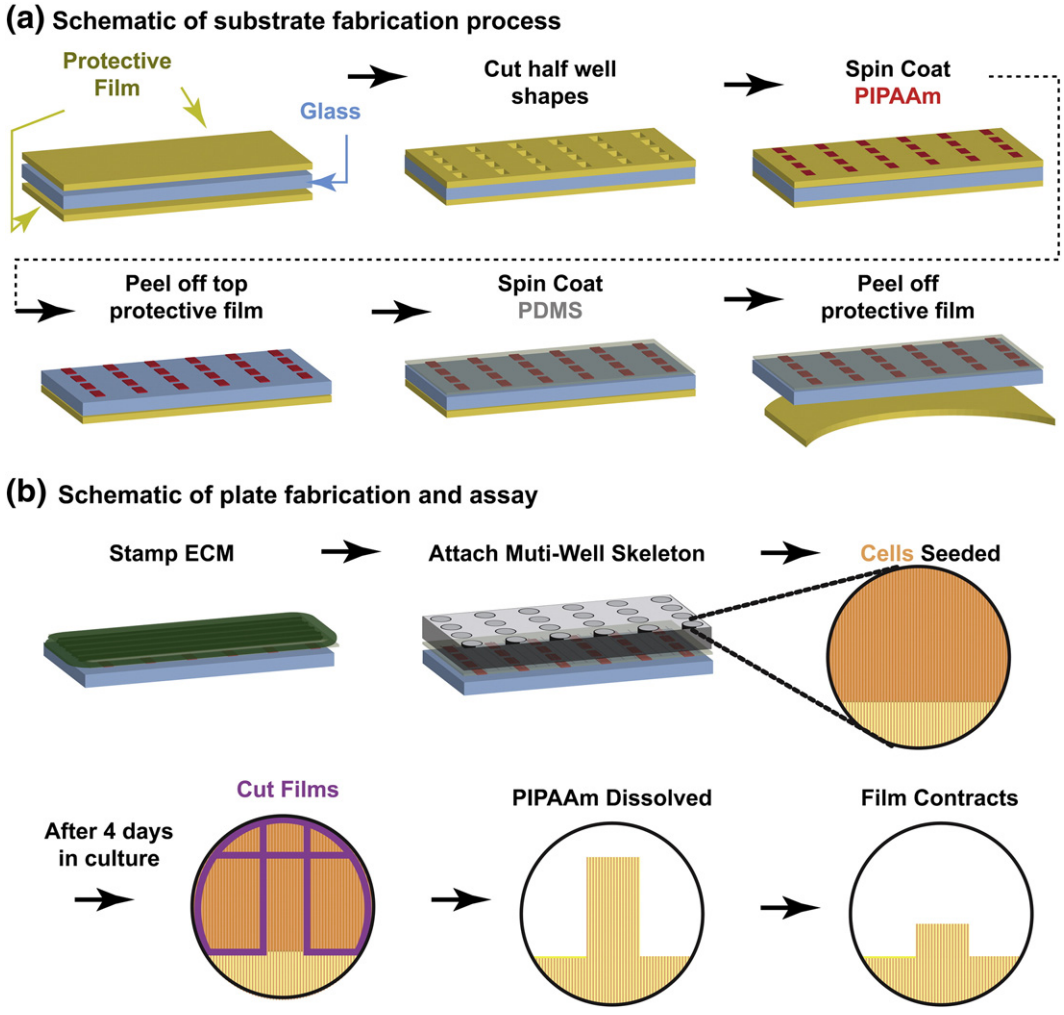

Fig. 2. Schematic of an MTF multi-well plate construction and assay (a) The plate was constructed by covering a glass section with protective film, cutting out islands and spin coating PIPAAm into them, then after the top protective film was peeled off, the PDMS was spin coated over the glass, and the bottom film was peeled off; (b) Fibronectin was stamped onto the substrate, and a 24-well plate skeleton was attached on top, the cells are seeded in each well, and after 4 days in culture the films are cut out and observed under a stereomicroscope.

2.10. Two-tissue MTF contractility experiments

To create a two-tissue MTF chip, a VSMC and cardiomyocyte chips were cultured separately, such that both were ready for the contractility experiments on the same day. Each chip was moved from the incubator to a normal Tyrode's solution, and cut the same way as described for a simple MTF chip experiment. The lengths of the films were recorded for both vMTFs and cMTFs through a stereomicroscope (Model MZ6 with darkfield base, Leica Microsystems, Inc., Wetzlar, Germany) with a Basler camera (A602f Basler Inc, Exton, PA), and both chips were affixed next to each other in a temperature controlled bath. The cardiomyocytes were stimulated at 2 Hz by field electrodes lowered into the bath. The field electrodes were mounted in a 35 mm Petri dish top, so the vertical separation between the tissue and the electrodes was ~5 mm. The two-tissue chip was imaged every 30 s for 3 min. Before ET-1 (50 nM) was added to stimulate VSMCs, a 100–120 fps movie was taken. The two-tissue chip was continuously imaged every 30 s before and after another high frame rate movie was taken and a ROCK Inhibitor (0.1 mM HA-1077) added. The third high frame rate movie was recorded 20 min after the ROCK Inhibitor was added to the solution.

2.11. Contractility experiments data analysis

All the images taken through the stereomicroscope with the Basler camera were analyzed in image processing software (ImageJ, NIH) and MatLab (Mathworks, Natick, MA). The images of MTFs flat on the glass were used to measure film length (L). The images or movies of bending MTFs were used to calculate the length of the projection of the films on the horizontal plane (x). The radius of curvature (r) of the films was numerically calculated at every time point from the following equation:

$$x = \begin{cases} r\sin\left(\frac{L}{r}\right) \in \frac{2L}{\pi} < x < L \\ r \in \frac{L}{2\pi} < x < \frac{2L}{\pi} \end{cases} \quad (1)$$

To calculate the stress the cells applied to the film, the MTF was modeled as a two layer plane strain beam as was previously described by Alford et al. (Alford et al., 2011, 2010). Briefly, the PDMS in the MTF was assumed to be the passive layer, while the myocytes were assumed to be the active layer that could undergo negative growth, i.e., contraction (Ramasubramanian & Taber, 2008; Rodriguez, Hoger, & McCulloch, 1994). To numerically calculate the stress the radius of curvature as a function of time, the thickness of the PDMS layer, and the average thickness of the cell layer was numerically processed in MatLab. For vMTF the stress was normalized to the initial stress prior to the addition of ET-1. For cMTF the peak systolic and diastolic stresses were defined as the average of the maxima and minima of the stress as a function of time, respectively. The active stress was defined as the difference between the diastolic and systolic stresses.

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

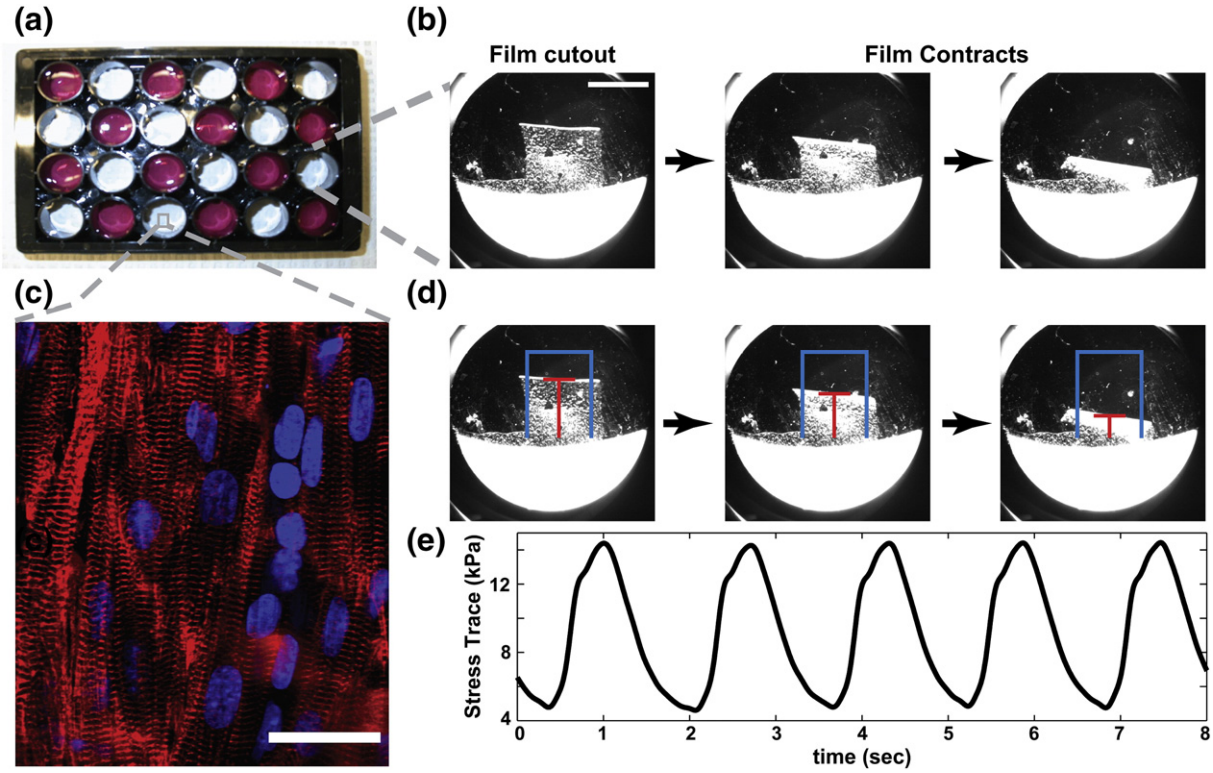

Fig. 3. (a) An assembled 24 well plate, with different colored liquid showed no leakage after 72 h; (b) Each well of the plate can have a film, which will spontaneously contract once it is released from the substrate, scale bar = 5 mm; (c) Other wells can be immunostained, in this example the nuclei (blue) and α-actinin (red) are shown, scale bar = 30 μm; (d) MTF in a plate can be tracked with the same set of software written for MTFs on a chip; (e) An example stress trace for a spontaneously contracting MTF in a 24-well plate.

2.12. Immunofluorescent staining and imaging

To ascertain the architecture of the VSMC, tissue non-MTF samples were fixed in 4% PFA with 0.01% Triton X-100 in PBS buffer for 10 min. The VSMCs were stained for actin (Alexa 488 Phalloidin, Molecular Probes, Carlsbad, CA) and nuclei (4′,6′-diamidino-2- phenylindole hydrochloride, DAPI, Molecular Probes, Carlsbad, CA). The tissues were imaged using a Leica DMI 6000B microscope (Leica Microsystems, Inc., Wetzlar, Germany) with a 40× plan-apochromat objective and a CoolSnap HQ CCD camera (Roper Scientific, Tucson, AZ).

For cMTFs in a multi-well plate, the cells were fixed (4% PFA and Triton X-100 for 10 min) and stained directly in the plate. The cardiomyocytes were stained for actin (Alexa 488 Phalloidin, Molecular Probes, Carlsbad, CA), nuclei (4′,6′-diamidino-2-phenylindole hydrochloride, DAPI, Molecular Probes, Carlsbad, CA), and sarcomeric α-actinin (clone EA-53, Sigma, St. Louis, MO). Secondary staining was done with tetramethylrhodamine-conjugated goat anti-mouse IgG (Alexa Fluor 594, Molecular Probes, Carlsbad, CA). Tissues were imaged with a LSM 5 LIVE laser scanning confocal microscope (Carl Zeiss, Dresden, Germany) with a 40× plan-apochromat objective.

3. Results

3.1. vMTF on a chip

We have previously demonstrated, with striated cardiac muscle, that muscular thin film technology is best adapted for higher-throughput methods if the films are mounted on a chip (Grosberg, Alford, et al., 2011). However, for a more general approach we wanted to demonstrate the chip's use with smooth muscle. To construct a simple MTF chip we cut out strips in a protective film using a razor blade, which covered a glass section. A sacrificial polymer, PIPAAm, was spin coated onto the exposed glass, and the top protective film strips were removed. PDMS was spin coated over the whole glass section, and cured for 8–12 h. The glass was then cut into separate chips with a diamond glass cutter (Fig. 1a). To recreate the anisotropic organization observed *in vivo*, the FN was patterned using a microcontact printing technique (Bray et al., 2008; Feinberg et al., 2007; Geisse et al., 2009). The pattern was chosen such that the smooth muscle cells would form a confluent anisotropic monolayer (Fig. 1b). Once a confluent cell layer was formed, multiple films were cut in each chip such that each film remained bound to the chip at one edge (Fig. 1b). As the cells contracted, the films bent up from the plane of the chip (Figs. 1b, 3D view), and their horizontal projection shortened (Fig. 1b, top view). By measuring the projection on the culture plane and the length of the films, it was possible to calculate the radius of curvature of the films and the stress produced by the cells. As an example of a smooth muscle contractility assay, we demonstrate VSMC MTF (vMTF) on a chip (Fig. 1c, insets). Endothelin-1 (ET-1) was added to the bath to induce a contraction. As the vascular tissue on the top of the film contracts, the film curves upward (Movie 1). Data was recorded for 12 min to ensure maximal contraction was reached, and then a ROCK inhibitor (HA-1077) was added to induce a complete relaxation (Alford et al., 2011). As the vascular tissue relaxes, the film the returns to lie flat in the plane of the cover slip. The resultant stress profile is in agreement with previous vMTF data (Fig. 1c) (Alford et al., 2011) in an assay designed to mimic the cut aortic ring assay (Uehata et al., 1997). The previous versions of the MTF experiments were easier to image (Alford et al., 2010; Feinberg et al., 2007), but the muscle on a chip contractility assay is more efficient because it requires less handling of the MTFs and is more repeatable. Additionally, it provides a platform for further extensions and applications of the MTF technology.

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

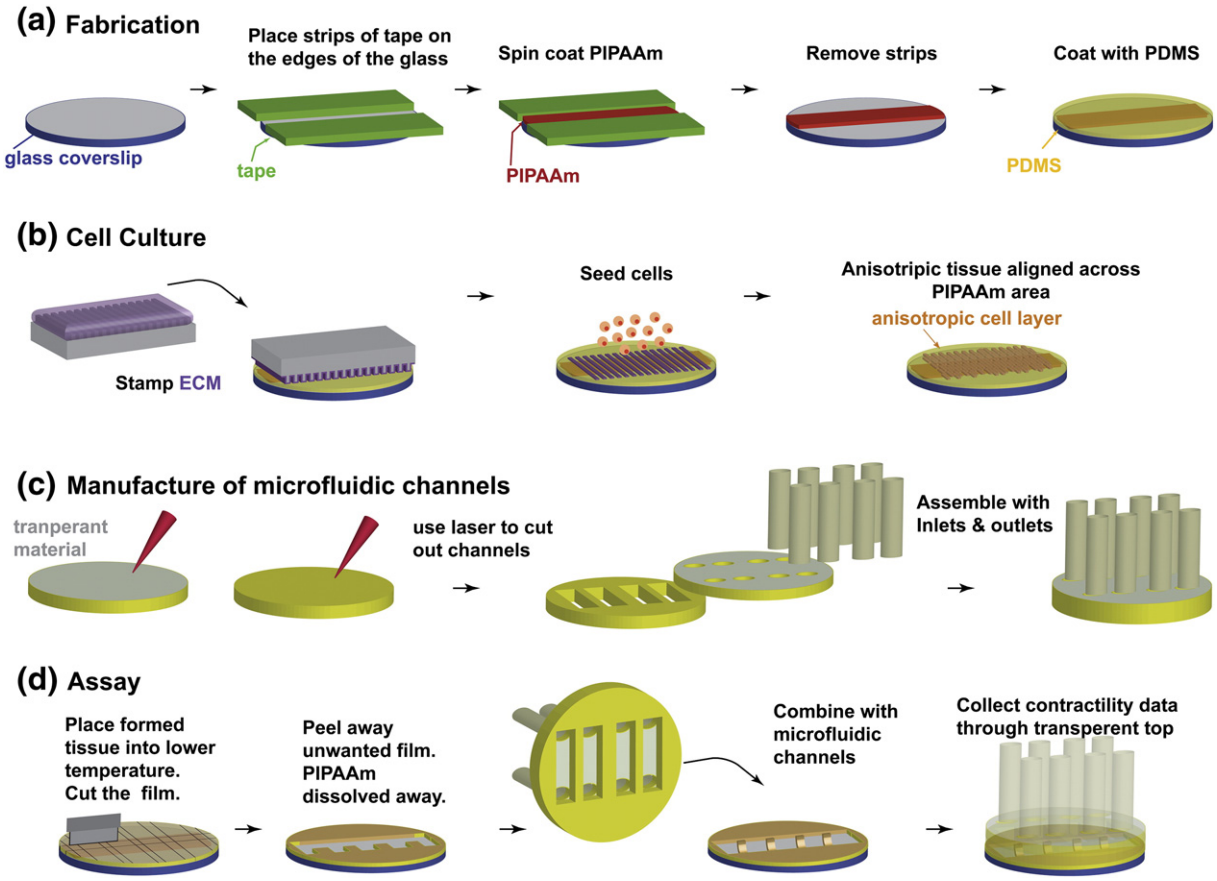

Fig. 4. Schematic of MTF on a chip and micro-fluidic top manufacturing and assembly. (a) Fabrication of MTF on a chip base starts with a glass substrate, tape is used to protect the edges as PIPAAm is spin coated over a portion of the glass, then the tape is removed and PDMS is spin coated over the whole area. (b) Cells are cultured on the base by patterning FN onto the PDMS, such that the cells grow into a confluent anisotropic layer. (c) The top portion of the device is manufactured by cutting channels and inlet/outlet openings with a laser; the different sections of the channels are assembled together to form the top portion of the chip. (d) After 4 days of cell culture the unwanted portions of the film are cut out and peeled away, leaving small muscular thin films attached to the substrate by one edge; the films are aligned with the microfluidic channels and the whole chip is assembled.

3.2. Multi-well plate MTF

MTFs in the planer configuration are amenable to a multi-well plate format. The plate was constructed by applying protective films to a section of glass (7.5×11 cm), cutting out half-well shapes, and spin coating PIPAAm onto the exposed glass. The top protective film was removed to spin coat PDMS over the whole glass section, and the bottom protective film was removed after 8 h of PDMS curing (Fig. 2a). After FN was patterned onto the substrate using microcontact printing, the top of the plate was attached using a thin coating of PDMS and secured with clear nail polish. To cure the adhesive PDMS, the assembled plate was placed into a sterile environment at 37 °C for at least 24 h prior to cell seeding. After the cells formed confluent anisotropic monolayers, a film was cut out in each well such that after the PIPAAm dissolved, the MTF remained attached to the plate by one edge. As the cells contracted the horizontal projection of a film was observed through the plate (Fig. 2b).

Plate integrity was demonstrated by filling alternate wells with clear and red colored media and observing no mixing over a 72 hour period in the incubator (Fig. 3a). In this example, cardiomyocytes were seeded in a 24-well plate to form laminar cardiac muscle after four days of culture. After a film was cut in each well of the plate, data of the spontaneously contracting tissues was collected (Fig. 3b, Movie 2). After the experiment the tissue was stained in the plate, and the anisotropic architecture was confirmed by fluorescent imaging of the sarcomere constructs (Fig. 3c). The movies of the film's kinematics were analyzed with the same customized software as for the vMTF on a chip, using ImageJ to track the projection (Fig. 3d) and MatLab to calculate the stress (Fig. 3e). These results illustrate the possibility of constructing MTF multi-well plates for providing higher throughput contractility data in a standardized format.

3.3. MTF in a fluidic channel

Beyond the standard multi-well plate format, it is possible to combine the MTF technology with fluidic chips. To construct the substrate for the MTF in a fluidic channel, we covered a 25 mm cover slip with two pieces of protective film 1 mm apart, spin coated PIPAAm, and removed the tape to spin coat PDMS on the whole cover slip (Fig. 4a). Fibronectin was patterned onto the substrate via microcontact printing such that the seeded myocytes organized to form an anisotropic monolayer (Fig. 4b). The fluidic-channel top was constructed with three sections matching the dimensions of the MTF substrate. The bottom section (1.5 mm thick) was made with one to four rectangular holes for the channels of approximately 1.5×15 mm dimensions. The transparent middle section (1.5 mm thick) had inlet–outlet holes co-localized with the ends of the channels in the bottom section. The top section was made out of hard tubing permanently adhered to the middle section over the inlet–outlet holes. All sections were held together with epoxy glue, and once fully assembled the fluidic channels could be imaged from the top through the middle section, while fluid could be exchanged through the inlet–outlet wells (Fig. 4c). The complete MTF in a fluidic channel chip was assembled after the cells had formed a confluent anisotropic monolayer. First,

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

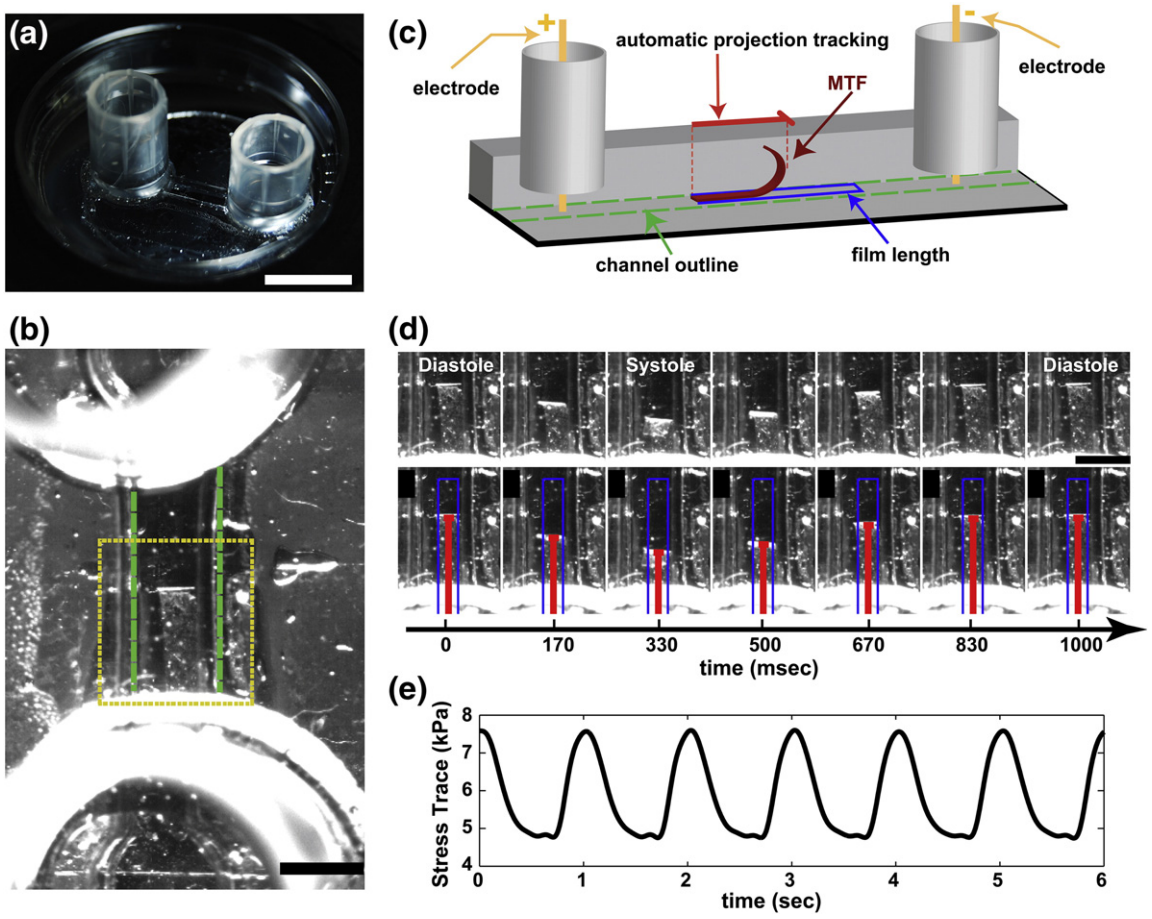

Fig. 5. (a) Photograph of a fluidic channel top, scale bar = 10 mm; (b) Photograph of a completed microfluidic device with the thin film inside, the channel is outlined in green, scale bar = 2 mm; (c) A schematic showing the microfluidic channel with the MTF inside; (d) top row—time lapse images of thin film as it bends during a full cycle (region outlined in yellow in (b)); bottom row—time lapse images of the bending film with the x-projection indicated in red, and the film length indicated in blue; (e) An example output of a contraction stress data for the film as a function of time (paced at 1 Hz).

muscular thin films were cut out in the substrate such that they were co-localized with the fluidic channel, and they remained attached to the substrate by one edge. Second, the fluidic-channel top was fixed to the MTF substrate with vacuum grease, and the film behavior was observed within the fluidic channel (Fig. 4d).

As proof of principle, we used cMTFs, and the experiments were performed four days after the cardiomyocytes were seeded. A single channel fluidic top was built (Fig. 5a), and the cMTF was positioned inside the middle of the channel and imaged through a stereo scope with a high speed camera (Fig. 5b). The film's projection was tracked with automated software, and the tissue could be paced by inserting electrodes in the inlet and outlet wells (Fig. 5c, Movie 3). By imaging through the top of the channel, it was possible to observe the systolic and diastolic behavior of the cMTF paced at 1 Hz (Fig. 5d). The same customized software used for simple MTF chips was used to produce stress traces for cMTF inside a fluidic channel (Fig. 5e). Thus, it is possible to integrate the MTF technology with fluidic chips, and as the technology develops further, multi-tissue microfluidic chips can be made for rapid testing.

3.4. Two-tissue MTF chip

The possibility of studying contractility of multiple tissue types simultaneously is one of the advantages of the MTF chip technology. A double chip with striated and smooth muscle was build to demonstrate this application of the technology. To build this chip, we used the same method as for building simple MTF chip substrates (Fig. 6a), but micropatterned FN to create anisotropic cardiomyocyte confluent tissue on one and VSM tissue on the other. Once both tissues were ready for contractility experiments, both chip halves were combined in the dish (Fig. 6a). The two-tissue chip was imaged every 30 s to capture the vascular tissue contraction, and fast (120 fps) movies were collected to record faster contractile rate of cardiac muscle. During the fast movies the cardiomyocytes were stimulated at 2 Hz by field electrodes lowered into the bath. The same custom software was used to track vMTFs and cMTFs. Prior to the addition of ET-1, the vMTFs (Fig. 6b(i)) were partially contracted with the projection (red) shorter than the length of the film (blue). The cardiomyocytes were beating strongly as can be seen from the frame taken at diastole (Fig. 6b(ii)), peak systole (Fig. 6b(iii)), and the resultant stress trace (Fig. 6b(iv)). Addition of 50 nM ET-1 significantly increased the curvature of the vMTFs (Fig. 6b(v)), but adversely affected the stress generated by the cardiomyocytes (Fig. 6b(vi)-(viii)). While some investigators have reported a positive cardiac inotropic effect of ET-1 (Takanashi & Endoh, 1991), there have also been reports of heterogeneity of response (Katoh et al., 1998; Kelso, Spiers, McDermott, Scholfield, & Silke, 1996). A negative inotropic response, similar to our results, has been demonstrated for the high ET-1 concentrations used in our study (Izumi, Miyamoto, Hori, Ozaki, & Karaki, 2000; Nishimaru, Miura, & Endoh, 2007). ROCK inhibitor relaxed the VSM such that the vMTFs lay almost flat, and their projections matched the films' length (Fig. 6b(ix)). As expected from previous studies

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

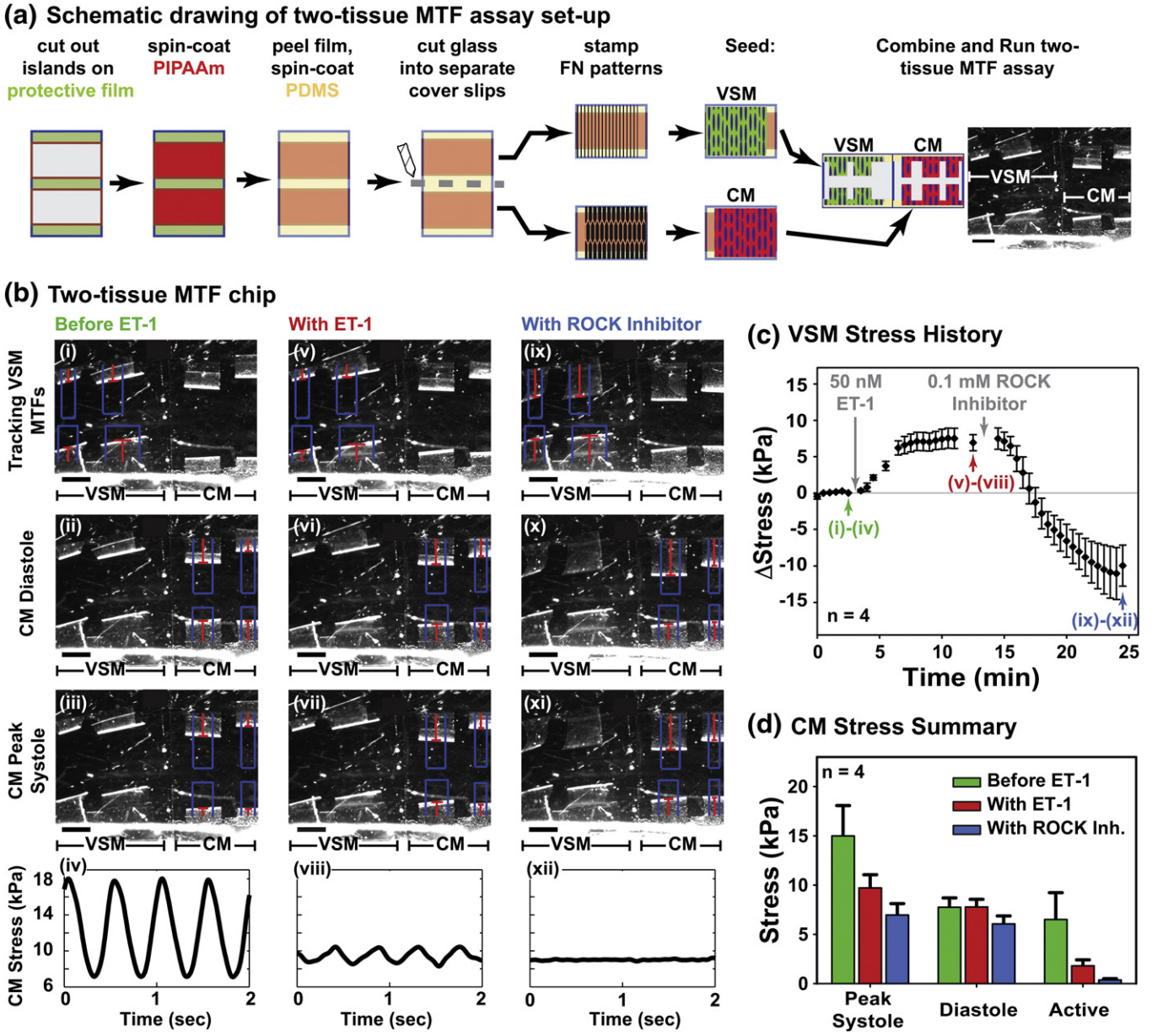

Fig. 6. (a) A schematic illustrating the assembly of a two-tissue MTF chip; (b) Results of a VSM (left) and cardiomyocytes (CM) (right) chip: (i–iv) prior to addition of ET-1; (v–viii) post ET-1 addition which induced a contraction of vMTFs; (ix–xii) post ROCK Inhibitor addition, which induced a relaxation in the vMTFs; (i, iv, ix) show the tracking of the vMTFs on the left side of the chip; (ii, vi, x) show the tracking of cMTFs at CM diastole; (iii, vii, xi) show the tracking of cMTFs and CM peak systole; (iv, viii, xii) show example CM stress traces; (c) VSM stress history ($n = 4$), addition of drugs indicated with grey arrows, and snapshots from (b) indicated with colored arrows; (d) Comparison of CM stresses at different time points of the experiment. All error bars standard error of the mean.

(Jacot et al., 2008), the inhibitor also negatively affected the contractility of the cardiomyocytes (Fig. 6b(x)–(xii)). The stress calculated for vMTFs had followed the expected trend (Alford et al., 2011), increasing with addition of ET-1 and then decreasing when the inhibitor was introduced in the bath solution (Fig. 6c). During the pacing of cardiomyocytes the contractility of VSMCs was not affected (points indicated with colored arrows in Fig. 6c). The average peak systolic stresses, diastolic stresses, and the difference (active stress) for the cMTFs was calculated for different VSMCs stimuli. The results showed a decrease in both systolic and active stress with the addition of the each drug (Fig. 6d). This illustrates the different behavior of cMTFs and vMTFs under exactly the same conditions, and shows the technological advantages of the MTF chip technology, which allows simultaneous experiments on multiple tissue types in the same environment.

4. Discussion

MTF technology provides a platform for measuring contractility of striated or smooth muscle tissue constructs with a controlled cellular architecture. The experiments with a vMTF chip showed how the technology can be extended to measurements of contractility in multiple MTFs simultaneously. The automated tracking and analysis codes provide a tool to rapidly calculate stress for all the films on a chip, and the technology of the MTF chip is adaptable to other platforms. Multiwell plates with MTFs may prove attractive to industrial use because they fit within automated analysis platforms. Furthermore, for even higher throughput, a 96-well plate (well diameter 6.4 mm) may be developed without changing the size of the MTFs (1×3 mm). The MTF in a fluidic channel chip demonstrates that this technology can be used for future "lab-in-a-chip" designs, which might prove more efficient than

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

the current industry standard of multi-well plates. Indeed, nanotoxicology measurements are becoming more important amid environmental concerns (Chen et al., 2008; Nemmar et al., 2002; Peters, Dockery, Muller, & Mittleman, 2001; Peters et al., 2004), and MTF microfluidic chips would be an ideal tool for *in vitro* measurements of the affects of pollutants on the cardiovascular system.

By varying the muscle type tested in these experiments, we demonstrated that the system was amenable to both striated and smooth muscle types. To further take advantage of this property, a two-tissue chip was tested. By combining vMTFs and cMTFs in the same experiment, it was possible to observe the effect of VSM stimuli on the cardiomyocytes and vice versa. This shows the potential for the MTF-on-a-chip technology to be a part of a multi-organ-on-a-chip microdevice. In the future the manufacture of these plates and chips can be automated providing a truly high-throughput platform for *in vitro* functional contractility measurements.

The microenvironment the cells experience impacts their function (Chen et al., 2005; Streeter et al., 1969; Vendelin, Bovendeerd, Engelbrecht, & Arts, 2002) and their gene expression (Chung et al., 2011). It is also known that *in vitro* geometrical guidance cues will control self-assembly of myocytes (Grosberg, Kuo, et al., 2011) and can reproduce some disease like gene expression (Chung et al., 2011). Unlike single cell systems (Cheng et al., 2006, 2010; Werdich et al., 2004; Zhao et al., 2007), complex tissue contractility assays (Kim et al., 2008; Linder et al., 2010; Park et al., 2005), and impedance plates (Guo et al., 2011), the range of MTF technology platforms presented here are well poised to fill the need for industrial experimental platforms that would explore different healthy and pathological cellular architecture.

Efficient *in vitro* cardiovascular functional measurements are a necessary tool for the development of drugs and toxicity studies. The MTF organ-on-chip platforms provide a flexibility to control cell architecture while integrating into both a multi-well plate and in-a-chip formats. Additionally, the ability to include multiple tissue types in one assay demonstrates the long range possibility of *in vitro* testing on multiple organ systems simultaneously.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.vascn.2012.04.001.

Author contributions

AG and KKP conceived and designed the experiments. APN and AG conducted and analyzed VSMC experiments. JAG designed and built the fluidic chip. MDB designed the multi-well plate construction. AG conducted and analyzed cardiac contractility experiments. AG and KKP wrote the paper.

Acknowledgment

This work has been supported by the Nanoscale Science and Engineering Center of the National Science Foundation under NSF award number PHY-0117795, the Harvard Materials Research Science and Engineering Center under NSF award number DMR-0213805, the Harvard Stem Cell Institute and GlaxoSmithKline, NIH/NINDS grant 1 U01 NS073474-01, and NIH-NHBLI grant 1 R01 HL079126. We are grateful to the Center of Nanoscale Systems at Harvard University for the use of their cleanroom facilities.

References

Adams, W. J., Pong, T., Geisse, N. A., Sheehy, S. P., Diop-Frimpong, B., & Parker, K. K. (2007). Engineering design of a cardiac myocyte. *Journal of Computer-Aided Materials Design*, *14*, 19–29.

Addae-Mensah, K. A., & Wikswo, J. P. (2008). Measurement techniques for cellular biomechanics *in vitro*. *Experimental Biology and Medicine*, *233*, 792–809.

Alford, P. W., Dabiri, B. E., Goss, J. A., Hemphill, M. A., Brigham, M. D., & Parker, K. K. (2011). Blast-induced phenotypic switching in cerebral vasospasm. *Proceedings of the National Academy of Sciences*, *108*, 12705–12710.

Alford, P. W., Feinberg, A. W., Sheehy, S. P., & Parker, K. K. (2010). Biohybrid thin films for measuring contractility in engineered cardiovascular muscle. *Biomaterials*, *31*, 3613–3621.

Bass, A., Kinter, L., & Williams, P. (2004). Origins, practices and future of safety pharmacology. *Journal of Pharmacological and Toxicological Methods*, *49*, 145–151.

Bray, M. A., Sheehy, S. P., & Parker, K. K. (2008). Sarcomere alignment is regulated by myocyte shape. *Cell Motility and the Cytoskeleton*, *65*, 641–651.

Chen, J. J., Liu, W., Zhang, H. Y., Lacy, L., Yang, X. X., Song, S. K., et al. (2005). Regional ventricular wall thickening reflects changes in cardiac fiber and sheet structure during contraction: Quantification with diffusion tensor MRI. *American Journal of Physiology. Heart and Circulatory Physiology*, *289*, H1898–H1907.

Chen, Z., Meng, H., Xing, G. M., Yuan, H., Zhao, F., Liu, R., et al. (2008). Age-related differences in pulmonary and cardiovascular responses to SiO2 nanoparticle inhalation: Nanotoxicity has susceptible population. *Environmental Science & Technology*, *42*, 8985–8992.

Cheng, W., Klauke, N., Sedgwick, H., Smith, G. L., & Cooper, J. M. (2006). Metabolic monitoring of the electrically stimulated single heart cell within a microfluidic platform. *Lab on a Chip*, *6*, 1424–1431.

Cheng, W., Klauke, N., Smith, G., & Cooper, J. M. (2010). Microfluidic cell arrays for metabolic monitoring of stimulated cardiomyocytes. *Electrophoresis*, *31*, 1405–1413.

Chung, C. -Y., Bien, H., Sobie, E. A., Dasari, V., McKinnon, D., Rosati, B., et al. (2011). Hypertrophic phenotype in cardiac cell assemblies solely by structural cues and ensuing self-organization. *The FASEB Journal*, *25*, 851–862.

Effron, M., Bhatnagar, G., Spurgeon, H., Ruano-Arroyo, G., & Lakatta, E. (1987). Changes in myosin isoenzymes, ATPase activity, and contraction duration in rat cardiac muscle with aging can be modulated by thyroxine. *Circulation Research*, *60*, 238.

Feinberg, A. W., Feigel, A., Shevkoplyas, S. S., Sheehy, S., Whitesides, G. M., & Parker, K. K. (2007). Muscular thin films for building actuators and powering devices. *Science*, *317*, 1366–1370.

Geisse, N. A., Sheehy, S. P., & Parker, K. K. (2009). Control of myocyte remodeling *in vitro* with engineered substrates. *In Vitro Cellular & Developmental Biology. Animal*, *45*, 343–350.

Grosberg, A., Alford, P. W., McCain, M. L., & Parker, K. K. (2011). Ensembles of engineered cardiac tissues for physiological and pharmacological study: Heart on a chip. *Lab on a Chip*, *11*, 4165–4173.

Grosberg, A., Kuo, P. L., Guo, C. L., Geisse, N. A., Bray, M. A., Adams, W. J., et al. (2011). Self-organization of muscle cell structure and function. *PLoS Computational Biology*, *7*.

Guo, L., Abrams, R., Babiarz, J. E., Cohen, J. D., Kameoka, S., Sanders, M. J., et al. (2011). Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell derived cardiomyocytes. *Toxicological Sciences*, *123*(1), 281–289.

Han, M., Wen, J. K., Zheng, B., Cheng, Y. H., & Zhang, C. X. (2006). Serum deprivation results in redifferentiation of human umbilical vascular smooth muscle cells. *American Journal of Physiology. Cell Physiology*, *291*, C50–C58.

Heeckt, P. F., Halfter, W. M., Schraut, W. H., Lee, K. K., & Bauer, A. J. (1993). Small bowel transplantation and chronic rejection alter rat intestinal smooth muscle structure and function. *Surgery*, *114*, 449–456 (discussion 456–457).

Izumi, M., Miyamoto, S., Hori, M., Ozaki, H., & Karaki, H. (2000). Negative inotropic effect of endothelin-1 in the mouse right ventricle. *European Journal of Pharmacology*, *396*, 109–117.

Jacot, J. G., McCulloch, A. D., & Omens, J. H. (2008). Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. *Biophysical Journal*, *95*, 3479–3487.

Kaneko, T., Kojima, K., & Yasuda, K. (2007). An on-chip cardiomyocyte cell network assay for stable drug screening regarding community effect of cell network size. *The Analyst*, *132*, 892–898.

Katoh, H., Terada, H., Iimuro, M., Sugiyama, S., Qing, K., Satoh, H., et al. (1998). Heterogeneity and underlying mechanism for inotropic action of endothelin-1 in rat ventricular myocytes. *British Journal of Pharmacology*, *123*, 1343–1350.

Kelso, E., Spiers, P., McDermott, B., Scholfield, N., & Silke, B. (1996). Dual effects of endothelin-1 on the L-type Ca2 + current in ventricular cardiomyocytes. *European Journal of Pharmacology*, *308*, 351–355.

Kim, J., Park, J., Na, K., Yang, S., Baek, J., Yoon, E., et al. (2008). Quantitative evaluation of cardiomyocyte contractility in a 3D microenvironment. *Journal of Biomechanics*, *41*, 2396–2401.

Lakatta, E., Gerstenblith, G., Angell, C., Shock, N., & Weisfeldt, M. (1975). Prolonged contraction duration in aged myocardium. *The Journal of Clinical Investigation*, *55*, 61.

Linder, P., Trzewik, J., Rueffer, M., Artmann, G. M., Digel, I., Kurz, R., et al. (2010). Contractile tension and beating rates of self-exciting monolayers and 3D-tissue constructs of neonatal rat cardiomyocytes. *Medical and Biological Engineering and Computing*, *48*, 59–65.

Nemmar, A., Hoet, P. H. M., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M. F., et al. (2002). Passage of inhaled particles into the blood circulation in humans. *Circulation*, *105*, 411–414.

Nishimaru, K., Miura, Y., & Endoh, M. (2007). Mechanisms of endothelin-1-induced decrease in contractility in adult mouse ventricular myocytes. *British Journal of Pharmacology*, *152*, 456–463.

Park, J., Ryu, J., Choi, S. K., Seo, E., Cha, J. M., Ryu, S., et al. (2005). Real-time measurement of the contractile forces of self-organized cardiomyocytes on hybrid biopolymer microcantilevers. *Analytical Chemistry*, *77*, 6571–6580.

Peters, A., Dockery, D. W., Muller, J. E., & Mittleman, M. A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. *Circulation*, *103*, 2810–2815.

Peters, A., von Klot, S., Heier, M., Trentinaglia, I., Hormann, A., Wichmann, H. E., et al. (2004). Exposure to traffic and the onset of myocardial infarction. *The New England Journal of Medicine*, *351*, 1721–1730.

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001

Ramasubramanian, A., & Taber, L. A. (2008). Computational modeling of morphogenesis regulated by mechanical feedback. *Biomechanics and Modeling in Mechanobiology*, *7*, 77–91.

Rodriguez, E. K., Hoger, A., & McCulloch, A. D. (1994). Stress-dependent finite growth in soft elastic tissues. *Journal of Biomechanics*, *27*, 455–467.

Streeter, D. D., Jr., Spotnitz, H. M., Patel, D. P., Ross, J., & Sonnenblick, E. H. (1969). Fiber orientation in the canine left ventricle during diastole and systole. *Circulation Research*, *24*, 339–347.

Takanashi, M., & Endoh, M. (1991). Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. *American Journal of Physiology. Heart and Circulatory Physiology*, *261*, H611–H619.

Tan, J. L., Tien, J., Pirone, D. M., Gray, D. S., Bhadriraju, K., & Chen, C. S. (2003). Cells lying on a bed of microneedles: An approach to isolate mechanical force. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 1484–1489.

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., et al. (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature*, *389*, 990–993.

Vendelin, M., Bovendeerd, P., Engelbrecht, J., & Arts, T. (2002). Optimizing ventricular fibers: Uniform strain or stress, but not ATP consumption, leads to high efficiency. *The American Journal of Physiology*, *283*, 1072–1081.

Werdich, A. A., Lima, E. A., Ivanov, B., Ges, I., Anderson, M. E., Wikswo, J. P., et al. (2004). A microfluidic device to confine a single cardiac myocyte in a sub-nanoliter volume on planar microelectrodes for extracellular potential recordings. *Lab on a Chip*, *4*, 357–362.

Zhao, Y., Lim, C. C., Sawyer, D. B., Liao, R. L., & Zhang, X. (2007). Simultaneous orientation and cellular force measurements in adult cardiac myocytes using three-dimensional polymeric microstructures. *Cell Motility and the Cytoskeleton*, *64*, 718–725.

Please cite this article as: Grosberg, A., et al., Muscle on a chip: *In vitro* contractility assays for smooth and striated muscle, *Journal of Pharmacological and Toxicological Methods* (2012), doi:10.1016/j.vascn.2012.04.001