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Title: Micropatterned Magneto-Rheological Elastomers to Drive Changes in Cardiomyocyte Alignment

Authors and Affiliations:
Ali H Lateef[1], Nesrine Bouhrira[2], Jia-Jye Lee[2], Alexia Vite[2], Kenneth B Margulies[2], Elise A Corbin[1,3,4]

[1]Department of Biomedical Engineering, University of Delaware
[2]Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania
[3]Department of Materials Science and Engineering, University of Delaware
[4]Nemours/Alfred I. duPont Hospital for Children

Corresponding Authors:
Elise A Corbin (ecorbin@udel.edu)

Email Addresses for All Authors:
Ali H Lateef (alateef@udel.edu)
Nesrine Bouhrira (nesrine.bouhrira@pennmedicine.upenn.edu)
Jia-Jye Lee (ajjlee@gmail.com)
Alexia Vite (alexiav@pennmedicine.upenn.edu)
Kenneth B Margulies (ken.margulies@pennmedicine.upenn.edu)
Elise A Corbin (ecorbin@udel.edu)

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 16
Number of Shots: 43

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Elise Corbin:** We're investigating how cardiomyocytes respond to dynamic, reversible changes in their mechanical environment, mimicking the fluctuations in stiffness and structure they experience in vivo.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

1.2. **Alexia Vite:** Since the development of the MRE, we've published several important studies in cardiology. Using these materials, we found that stiffness-driven hypertrophy in adult cardiomyocytes depends on the microtubule network. More recently, we uncovered more information about mechanical memory in both cardiac fibroblasts and iPSC-derived cardiomyocytes.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.3. **Elise Corbin:** The best part of using magnetorheological elastomers is their simplicity**.** Our protocol is as straightforward as it sounds—just a combination of off-the-shelf products working together to create something powerful. It's an easy-to-use approach that makes cutting-edge science accessible to everyone.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

How will your findings advance research in your field?

1.4. **Alexia Vite:** This paves the way for more realistic disease models, especially for conditions like heart failure, where the mechanics of the tissue are always shifting. It also helps researchers design smarter biomaterials that are highly tunable, dynamic, and reversible.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Elise Corbin:** Our team has mainly focused on heart disease, but recently we expanded the dynamic stiffness range of our MRE from as low as 5 kPa to as high as 400 kPa. That breakthrough opens the door for us to extend our research and collaborate on other organs beyond the heart.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Fabrication and Surface Coating of Patterned PDMS Stamps

Demonstrator: Ali Lateef

2.1. To begin, mix the base and curing agent in a 10 to 1 ratio to prepare 10 grams of 184 polydimethylsiloxane or PDMS *(P-D-M-S)* **[1]**. Pour 5 grams of the 184 PDMS into a 35-millimeter Petri dish **[2]**. Then place the dish in a desiccator **[3]** and degas for approximately 5 to 10 minutes until all bubbles have dissipated **[4]**.

2.1.1. WIDE: Talent mixing the base and curing agent in a flaktech container for speed mixer

2.1.2. Talent pouring PDMS into the 35 millimeter Petri dish.

2.1.3. Talent placing the dish into a desiccator.

2.1.4. Talent setting the vacuum pump.

2.2. Allow 184 PDMS to partially cure at 60 degrees Celsius in an oven, for 30 minutes **[1]**. When 5 minutes remain for curing, apply a thin layer of additional uncured 184 PDMS onto the surface of the diffraction grating **[2]**. Place it in a desiccator to degas for approximately 5 minutes **[3]**.

2.2.1. Talent placing the dish into the oven and closing the door.

2.2.2. Talent adding a thin PDMS layer to the diffraction grating using a pipette.

2.2.3. Talent placing the grating into the desiccator.

2.3. Now, remove the 35-millimeter Petri dish containing the partially cured 184 PDMS from the oven **[1]**. Flip the diffraction grating with PDMS onto the partially cured 184 PDMS in the dish **[2]**. Lightly press the diffraction grating until a small amount of PDMS surrounds it **[3]**.

2.3.1. Talent taking the Petri dish out of the oven.

2.3.2. Talent carefully inverting the grating and placing it onto the PDMS layer.

2.3.3. Talent pressing the diffraction grating until a small amount of PDMS surrounds it.

2.4. Use the remaining uncured 184 PDMS to backfill the dish surrounding the grating to ensure it stays in place while curing **[1]**. Then place the dish into an oven set at 60

degrees Celsius for 1.5 hours **[2]**.

2.4.1. Talent pipetting the remaining uncured 184 PDMS to backfill the dish surrounding the grating.

2.4.2. Talent placing the dish back into the oven.

2.5. After curing, remove the dish with the grating from the oven **[1]**. Use a scalpel to score around the diffraction grating **[2]**.

2.5.1. Talent opening the oven and retrieving the dish.

2.5.2. Closeup of talent making an incision around the grating with a scalpel.

2.6. Apply a small amount of isopropyl alcohol to penetrate underneath the diffraction grating **[1]**. Use forceps to pull the grating off the PDMS in the direction parallel to the patterns **[2]**.

2.6.1. Talent pipetting isopropyl alcohol onto the scored region.

2.6.2. Talent removing the grating using forceps, following the pattern lines.

2.7. Remove any excess PDMS, leaving only the patterned section intact **[1]**. Then cut the PDMS stamp to the desired size **[2]**. Mark the direction of the pattern on the MRE dish **[3].**

2.7.1. Talent trimming off surrounding PDMS with a scalpel or razor blade.

2.7.2. Talent slicing the patterned PDMS to the desired size.

2.7.3. Talent marking directionality of pattern on stamp.

2.8. To coat the stamp with silane, place the fabricated 184 PDMS stamp inside an oxygen plasma cleaner with the patterned surface facing up **[1]**. Treat the surface with 45 watts of oxygen plasma for 30 seconds **[2]**.

2.8.1. Talent placing the patterned PDMS stamp into the plasma cleaner with the patterned surface facing up.

2.8.2. Talent setting the plasma cleaner settings panel.
Videographer: Please capture the screen of the instrument for this shot

2.9. Place the treated 184 PDMS stamps inside a desiccator positioned in a fume hood **[1]**. Now, tear off the lid from a microcentrifuge tube **[2]**. Place the microcentrifuge tube lid next to the PDMS stamps inside the desiccator **[3]**.

2.9.1. Talent transferring the PDMS stamps into a desiccator and closing the lid.

2.9.2. Talent removing and separating the lid from a microcentrifuge tube.

2.9.3. Talent positioning the lid adjacent to the PDMS stamps inside the desiccator chamber.

2.10. Pipette 20 microliters of silane to the microcentrifuge tube lid **[1-TXT]**. Close the desiccator **[2]** and pull a vacuum to initiate vapor coating **[3-TXT]**.

2.10.1. Talent using a pipette to carefully dispense silane into the lid. **TXT: Trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane**

2.10.2. Talent closing the desiccator.

2.10.3. Talent activating the vacuum pump. **TXT: Allow the silane to coat the stamps from 1 h to overnight**

3. Preparation of Magnetorheological Elastomer Structures (MRE)

3.1. For the MRE *(M-R-E)* preparation, measure the desired amount of silicone thinner and Eco elastomer part B **[1-TXT].** Mix them at 2500 rpm in a speed mixer for 1 minute **[2]**.

3.1.1. Talent weighing silicone thinner and Eco elastomer part B. **TXT: MRE: Magnetorheological Elastomer**

3.1.2. Talent placing them in the speed mixer and starting the device.

3.2. Add Eco elastomer part A and carbonyl iron particles to the mixture **[1]** and mix again at 2500 rpm for 1 minute **[2]**.

3.2.1. Talent adding part A and carbonyl iron to the previous mix.

3.2.2. Talent placing the mixture in the speed mixer.

3.3. Using a transfer pipette with the tip cut off, dispense 5 grams of the MRE mixture into a new 35-millimeter Petri dish **[1]**. Degas in a desiccator for approximately 5 minutes **[2]**, then partially cure in an oven at 60 degrees Celsius for 10 minutes **[3]**.

3.3.1. Talent pipetting the MRE mixture into a dish using a modified pipette.

3.3.2. Talent placing the dish into a desiccator.

3.3.3. Talent transferring the dish to an oven.

3.4. When 5 minutes remain for curing, use a transfer pipette to add uncured MRE to just coat the patterned surface of the PDMS stamp **[1]**. Place the coated stamp in a desiccator to degas for 5 minutes **[2]**.

3.4.1. Talent coating the PDMS stamp surface with fresh MRE using a pipette.

3.4.2. Talent placing the coated stamp into a desiccator.

3.5. Remove the partially cured MRE dish from the oven **[1]**. Using forceps, flip the coated stamp face-down onto the MRE surface and press lightly **[2]**. Mark the direction of the pattern on the MRE dish **[3].** Place the assembly back into the oven at 60 degrees Celsius for an additional 25 minutes **[4]**.

3.5.1. Talent retrieving the Petri dish from the oven.

3.5.2. Talent inverting the coated stamp onto the dish and pressing lightly with fingers or tweezers.

3.5.3. Talent marking direction of pattern on dish.

3.5.4. Talent returning the full setup to the oven.

3.6. Once the material is fully cured, use a scalpel to score the MRE at the bottom edge of the stamp in a direction perpendicular to the pattern lines **[1]**. Apply a small amount of isopropyl alcohol onto the cut region around the stamp **[2]**. Then use forceps to pull the PDMS stamp off the MRE in the direction parallel to the patterns **[3]**.

3.6.1. Talent scoring the MRE at the bottom edge of the stamp using a scalpel.

3.6.2. Talent pipetting isopropyl alcohol around the scored area.

3.6.3. Talent lifting the stamp using forceps while keeping alignment with the pattern direction.

3.7. Sterilize the surface of the MRE by washing with 70% ethanol 3 times and allow the third wash to sit for 20 minutes **[1]**. Then, wash the MRE surface 3 times with sterile PBS **[2]**.

Added shot: Extra 1 Talent adding ethanol on the MRE.

Added shot: Extra 1 Talent adding PBS on the MRE.

3.8. Coat the surface of the MRE with 10 micrograms per milliliter fibronectin in PBS containing Magnesium and Calcium **[1]** for 1 hour at 37 degrees Celsius to promote cell adhesion to the device **[2]**.

Added shot: Extra 1 Talent adding fibronectin on the MRE for coating.

Added shot: Extra 2 Talent placing the sample in the incubator.

3.9. Finally, wash the MRE surface 3 times with PBS containing Magnesium and Calcium **[1]** and seed the neonatal rat cardiomyocytes at a density of 20,000 cells per square centimeter **[2].**

Added shot: Extra 4 Talent removing the fibronectin from MRE.

Added shot: Extra 5 Talent placing the sample in the incubator.

Results

4. Results

4.1. The fidelity of micro-pattern transfer from the master mold to the MRE substrate is demonstrated in this figure through normalized height profiles and quantitative measurements of feature pitch and height **[1]**.

4.1.1. LAB MEDIA: Figure 2.

4.2. The X-profile comparison showed that the normalized feature height profile of the MRE closely followed that of the master mold, though with slightly reduced peak heights and broader feature shapes **[1]**.

4.2.1. LAB MEDIA: Figure 2A. *Video Editor: Highlight the red dotted plot when the VO says "the normalized feature height profile of the MRE" and the black plot when the VO says "master mold".*

4.3. The average pitch of the MRE substrate was approximately 10 micrometers **[1]** and was not significantly different from the pitch of the master mold **[2].**

4.3.1. LAB MEDIA: Figure 2B. *Video Editor: Highlight the red bar.*

4.3.2. LAB MEDIA: Figure 2B. *Video Editor: Highlight both the bars.*

4.4. The average feature height on the MRE substrate was significantly lower than that of the master mold, with mean values of 3.81 micrometers **[1]** and 5.49 micrometers, respectively **[2].**

4.4.1. LAB MEDIA: Figure 2C. *Video Editor: Highlight the red bar.*

4.4.2. LAB MEDIA: Figure 2B. *Video Editor: Highlight the black bar.*

- Polydimethylsiloxane

Pronunciation link: https://www.howtopronounce.com/polydimethylsiloxane How To Pronounce+1
IPA (American): /ˌpɒliˌdaɪˌmɛθəlˈsɪlɒkˌseɪn/
Phonetic spelling: pol-ee-dye-meth-uhl-sil-ok-sane

- Magnetorheological (as in magnetorheological elastomer)

Pronunciation link: No confirmed link found (common dictionaries don't list "magnetorheological" separately)
IPA (American): /ˌmægnəʊroʊˌriːˈɒdʒɪkəl/
Phonetic spelling: mag-noh-roh-ree-o-jic-uhl

- Elastomer

Pronunciation link: https://www.merriam-webster.com/dictionary/elastomer (the “elastomer” entry)
IPA (American): /ɪˈlæstəˌmər/
Phonetic spelling: ih-las-toh-mer

- Desiccator

Pronunciation link: https://www.howtopronounce.com/desiccator How To Pronounce+1
IPA: /ˈdɛsɪˌkeɪtər/
Phonetic Spelling: des-ih-kay-ter

- Carbonyl (as in “carbonyl iron particles”)

Pronunciation link: https://www.howtopronounce.com/carbonyl How To Pronounce+1
IPA: /ˈkɑːrbənɪl/
Phonetic Spelling: kar-buh-nil

- Diffraction grating

Pronunciation link: https://www.howtopronounce.com/diffraction-grating How To Pronounce+1
IPA: /dɪˈfrækʃən ˈgreɪtɪŋ/
Phonetic Spelling: di-frak-shun gray-ting

- Silicone (as in “silicone thinner”)

Pronunciation link: https://dictionary.cambridge.org/pronunciation/english/silicone Cambridge Dictionary
IPA: /ˈsɪl.əˌkoʊn/
Phonetic Spelling: sil-uh-kohn

- Elastomer (as in “Eco elastomer part A/B”)

Pronunciation link: https://www.howtopronounce.com/elastomer How To Pronounce+1
IPA: /ɪˈlæstəmər/
Phonetic Spelling: ih-las-toh-mer