

Submission ID #: 69779

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21282643>

## **Title: A Novel Platform for In Vitro Cellular Stretching and Imaging**

### **Authors and Affiliations:**

**Benjamin M. Goykadosh<sup>\*</sup>, Suzanne E Stasiak<sup>\*</sup>, Vasuretha Chandar, Samuel R. Polio, Harikrishnan Parameswaran**

**Department of Bioengineering, Northeastern University**

**\*These authors contributed equally**

### **Corresponding Authors:**

Harikrishnan Parameswaran

[h.parameswaran@northeastern.edu](mailto:h.parameswaran@northeastern.edu)

### **Email Addresses for All Authors:**

Benjamin M. Goykadosh

[Goykadosh.b@northeastern.edu](mailto:Goykadosh.b@northeastern.edu)

Suzanne E Stasiak

[stasiak.s@northeastern.edu](mailto:stasiak.s@northeastern.edu)

Vasuretha Chandar

[chandar.v@northeastern.edu](mailto:chandar.v@northeastern.edu)

Samuel R. Polio

[s.polio@northeastern.edu](mailto:s.polio@northeastern.edu)

Harikrishnan Parameswaran

[h.parameswaran@northeastern.edu](mailto:h.parameswaran@northeastern.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? **Yes**

*Videographer: Please film the close-up shot of the computer screen for all shots labeled "SCREEN" as a back up*

I believe all microscope shots are listed as screen captures, that I am working on uploading. Please let me know if I have missed any that should be specific to the day the videographer is present.

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

**4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

### Current Protocol Length

Number of Steps: 20

Number of Shots: 40

# Introduction

---

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

**Authors:** Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

## INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Professor Harikrishnan Parameswaran**: Our goal was to develop a low-cost, Hoberman-inspired, microscope-compatible cell stretching device to investigate how mechanical stretch influences cellular function.
  - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.3.1*

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Vasuretha Chandar**: This device allows researchers to study live cell responses to mechanical stimuli mimicking physiological systems, such as a beating heart or breathing lungs.
  - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.6.1, 3.6.2*

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Benjamin Goykadosh**: Using our stretcher, researchers can conduct live-cell calcium imaging, traction force microscopy, and quantify cellular mechanical responses to isotropic or uniaxial stretch.
  - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.13.2*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Vasuretha Chandar**: A critical step is to ensure that gels cure on a level surface, as uneven thickness can shift the substrate out of focus and reduce optical clarity during live imaging.

- 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.10.1*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Benjamin Goykadosh:** Future studies can investigate stretch-induced cytoskeletal reorientation, pathological strain conditions relevant to asthma or lung injury, and mechanotransduction across diverse cell types.

- 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.***

**Ethics Title Card**

This research has been approved by the Biosafety Committee of Northeastern University

# Protocol

---

## 2. Fabrication of Tunable PDMS and NuSil Gel Substrates for Cell Culture

**Demonstrators:** Benjamin Goykadosh and Vasuretha Chandar

- 2.1. To begin, wear appropriate personal protective equipment [1]. Mix Sylgard 184 (*Sylgard-One-Eight-Four*) base and curing agent in a 10 to 1 ratio in a plastic dish [2]. With a tongue depressor, mix the components vigorously for 2 to 3 minutes [3].
  - 2.1.1. Talent wearing gloves, a lab coat, and eye protection.
  - 2.1.2. Talent adding Sylgard 184 base and curing agent into a plastic dish placed on the bench.
  - 2.1.3. Talent using a tongue depressor to vigorously mix the contents in the plastic dish.
- 2.2. Then place the dish in a degassing chamber for 1 hour [1].
  - 2.2.1. Talent placing the plastic dish containing the mixture into a degassing chamber.
- 2.3. Next, take the prepared 3D printed molds [1] and add 1.3 grams of the PDMS (*P-D-M-S*) mixture into the isotropic mold [2]. Then, add 0.5 grams of the PDMS mixture to the uniaxial mold [3]. Spread the gel evenly across the mold [4] and degas the filled mold for 1 hour [5].
  - 2.3.1. Shot of the molds.
  - 2.3.2. Talent measuring and adding 1.3 grams of mixture into the isotropic mold. **TEXT: PDMS: Polydimethylsiloxane:**
  - 2.3.3. Talent measuring and adding 0.5 grams of mixture into the uniaxial mold.
  - 2.3.4. Talent gently tilting or guiding the mold to spread the gel evenly across the surface. **NOTE: 2.3.4 is filmed together with 2.3.3**
  - 2.3.5. Talent placing the filled mold into the degassing chamber.
- 2.4. Allow the gel in the mold to settle on a flat surface at room temperature for 2 hours [1]. Then cure the mold in an oven at 60 degrees Celsius overnight [2].
  - 2.4.1. Talent placing the mold carefully on a flat laboratory surface.
  - 2.4.2. Talent placing the mold into an oven set to 60 degrees Celsius.
- 2.5. Next, mix parts A and B of NuSil Gel-8100 (*Gel-Eight-One-Hundred*) in equal proportions [1]. Mix the combined gel with Sylgard 184 crosslinker at 0.36 percent of the total volume to produce a substrate stiffness of 13 kilopascal [2]. Add no crosslinker to produce a substrate stiffness of 0.3 kilopascal [3]. Then, tumble mix the solution for 15 minutes [4]. **NOTE: The VO is edited for the additional shot**

- 2.5.1. Talent mixing parts A and B of NuSil Gel 8100 in a container.
- 2.5.2. Talent adding Sylgard 184 crosslinker to the mixture to achieve 0.36 percent of the total volume.
- 2.5.3. Shot of a separate container showing NuSil mixture prepared without crosslinker.

**ADDED SHOT:** Tumble mix the solution (from 2.5.2) for 15 minutes.

- 2.6. Place the NuSil mixture in a degassing chamber and degas for 30 minutes [1].
  - 2.6.1. Talent placing the NuSil mixture container into the degassing chamber.
- 2.7. Now carefully remove the PDMS gel from the custom mold [1] and place it onto a 40-millimeter coverslip without tearing it [2].
  - 2.7.1. Talent gently peeling the polydimethylsiloxane gel from the custom mold.
  - 2.7.2. Talent positioning the fragile gel onto a 40 millimeter coverslip.
- 2.8. Next, place the PDMS gel in the spin coater [1]. Pipette 100 microliters of the NuSil mixture onto the negative area of the isotropic PDMS gel [2]. Then pipette 50 microliters of the NuSil mixture onto the negative area of the uniaxial PDMS gel. After that, coat at 7 g for 50 seconds to attain a thickness of 100 micrometers [3]. **NOTE: The VO is edited for the moved shot**
  - 2.9.1. Talent placing the gel on the spin coater. **NOTE: This shot is moved here as per author's request**
  - 2.8.1. Talent pipetting 100 microliters of NuSil mixture onto the isotropic gel.
  - 2.8.2. Talent pipetting 50 microliters of NuSil mixture onto the uniaxial gel. **TXT:**
- ~~2.9. Using a spin coater, coat the respective PDMS gels with the NuSil mixture for 50 seconds at 7 g to attain a uniform substrate thickness of 100 micrometers [1].~~
  - ~~2.9.1. Talent placing the gel on the spin coater. **NOTE: This step is moved before 2.8.1**~~
- 2.10. Allow the coated gel to settle on a flat level surface at room temperature for 2 hours [1]. Place the gel in an oven and cure at 60 degrees Celsius overnight [2-TXT].
  - 2.10.1. Talent placing the coated gel carefully on a level laboratory surface.
  - 2.10.2. Talent placing the gel into an oven set to 60 degrees Celsius. **TXT: Store the completed cell culture dishes in covered petri dishes until use**
- 2.11. Now, seed human airway smooth muscle cells in elastic cell culture wells and incubate under standard conditions [1]. Afterwards, mix cells with the FLIPR (*Flipr*) Calcium 6 solution for imaging [2-TXT].
  - 2.11.1. Talent adding cells to the well of an elastic cell culture dish. . **TXT: Incubation: 37 °C, 5% CO<sub>2</sub>, 2 h**
  - 2.11.2. Talent adding calcium solution for imaging

~~2.12. Next, use an inverted microscope equipped with a camera, light engine, and a 10X dry objective [1].~~ **NOTE: Step 2.12 and 2.13 are moved before 3.5**

~~2.12.1. WIDE: Talent seated at the inverted microscope configured with the specified camera, light engine, and "10x 0.32 dry objective."~~

~~2.13. Set the excitation to 480/40 (Four Eighty by Forty) nanometers and the emission to 527/50 (Five Twenty Seven by Fifty) nanometers [1]. Acquire 16 bit images at a frame rate of 2 hertz during stretch experiments [2].~~

**Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: <https://review.jove.com/account/file-uploader?src=21282643>**

~~2.13.1. **SCREEN:** Display the microscope software interface showing the fluorescence settings panel while adjusting excitation to 480 slash 40 nanometers and emission to 527 slash 50 nanometers.~~

~~2.13.2. **SCREEN:** Show the acquisition settings window with image depth set to 16 bit and frame rate set to 2 hertz while live images are being captured.~~

~~*Videographer: Please film the close-up shot of the computer screen for all shots labeled "SCREEN" as a back up*~~

### 3. Traction Force Microscopy Analysis of Stretchable PDMS-NuSil Substrates

3.1. For traction force measurement, coat the PDMS-NuSil gel substrates with a layer of fluorescent beads to serve as fiducial markers [1].

3.1.1. Talent at the working bench with all the required reagents placed at the bench.

3.2. Prepare a 0.025 percent solution of 1-micrometer-diameter red, fluorescent carboxylate modified microspheres in HBSS [1-TXT]. Using a vortex mixer, vortex the bead solution for 10 seconds [2].

3.2.1. Talent diluting microspheres in Hank's Balanced Salt Solution to achieve the specified concentration. **TXT: Dilute in a 1:4000 ratio**

3.2.2. Talent placing the bead solution tube on a vortex mixer for 10 seconds.

3.3. Then pipette 880 microliters of the bead solution to the isotropic gel [1]. Add 420 microliters of the bead solution to the uniaxial gel [2].

3.3.1. Talent pipetting 880 microliters of bead solution onto the isotropic gel.

3.3.2. Talent pipetting 420 microliters of bead solution onto the uniaxial gel.

3.4. After incubating the gel at room temperature for 1 hour, carefully pour off the bead solution to preserve bead distribution [1]. Then use a cotton swab or laboratory wipe to gently blot any residual solution from the gel surface [2].

- 3.4.1. Talent gently pipetting off the bead solution from the soft gel.
- 3.4.2. Talent gently blotting the gel surface with a cotton swab.
- 2.12 Next, use an inverted microscope equipped with a camera, light engine, and a 10X dry objective [1].
  - 2.12.1 WIDE: Talent seated at the inverted microscope configured with the specified camera, light engine, and “10x 0.32 dry objective.
- 2.13. Set the excitation to 475/40 (*Four-Seventy-Five-by-Forty*) nanometers and the emission to 555/50 (*Five-Fifty-Five-by-Fifty*) nanometers [1]. Acquire 16-bit images at a frame rate of 2 hertz during stretch experiments [2].
  - 2.13.1 SCREEN: 69779\_screenshot\_1.mp4: 00:00-end
  - 2.13.2 SCREEN: 69779\_screenshot\_2.mp4: 00:00-end
- 3.5 Now, place isotropic gel on the stretcher [1] and then image the fluorescent beads using an inverted microscope equipped with a 10X dry objective [2]. NOTE: The VO is edited for the additional shot
  - Added Shot: Talent placing isotropic gel onto stretcher.
  - 3.5.1 SCREEN: 69779\_screenshot\_3.mp4: 00:05-00:15
- 3.6 Capture images in the pre-stretch condition [1]. Continuously capture images throughout the stretching protocol at the center of the substrate [2]. Refocus the microscope at the stretched position if necessary, before capturing images [3].
  - 3.6.1 SCREEN: 69779\_screenshot\_4.mp4: 00:00-00:08
  - 3.6.2 SCREEN: 69779\_screenshot\_4.mp4: 00:15-00:25
  - 3.6.3 Talent adjusting the focus knob to refocus at the stretched position.
- 3.7 Analyze the images using custom MATLAB code based on Fourier traction force microscopy to calculate displacement vectors and corresponding planar strain fields [1]. Represent the data as the average of N trials [2].
  - 3.7.1 SCREEN: 69779\_screenshot\_5.mp4: 00:00-00:08
  - 3.7.2 LAB MEDIA: 69779\_screenshot\_6.pdf

# Results

---

## 4 Results

4.1 Applying a 240-micrometre displacement caused fluorescent beads to shift radially outward from the center [1]. Traction force microscopy revealed an isotropic displacement field with vectors radiating outward from the center and increasing in magnitude with distance [2].

4.1.1 LAB MEDIA: Figure 4A.

4.1.2 LAB MEDIA: Figure 4A and B.

4.2 The isotropic displacement field generated a homogeneous planar strain field [1].

4.2.1 LAB MEDIA: Figure 4C.

4.3 Increasing the displacement to 1000 micrometers resulted in displacement vectors that increased in magnitude while maintaining radial orientation [1].

4.3.1 LAB MEDIA: Figure 4E. *Video editor: Highlight the longer arrows radiating outward from the center compared to the smaller displacement condition.*

4.4 The corresponding strain field at 1000 micrometers increased in magnitude while remaining radially symmetric [1].

4.4.1 LAB MEDIA: Figure 4D to F. *Video editor: Sequentially show images from D to F*

4.5 In uniaxial gels subjected to a 250 micrometer displacement, displacement vectors were predominantly aligned along a single axis consistent with uniaxial strain [1].

4.5.1 LAB MEDIA: Figure 5C and D *Video editor: Sequentially show images C and D*

4.6 The strain waveforms closely mirrored the programmed motor displacements and remained synchronized across 3 repeated cycles [1]. Strain values at maximum displacements remained consistent across 3 independent samples [2]. Applying a transient displacement up to 5555 micrometers produced strains ranging from greater than 0.1 percent to 12 percent [3].

4.6.1 LAB MEDIA: Figure 6A. *Video editor: Highlight the overlapping strain peaks across the 3 cycles*

4.6.2 LAB MEDIA: Figure 6B. *Video editor: Sequentially highlight trials 1 to 3*

4.6.3 LAB MEDIA: Figure 6C. *Video editor: Highlight the curve extending to the highest linear displacement*

4.7 A transient 0.5 percent strain induced an increase in intracellular calcium, evident as higher fluorescence intensity after stretch [1].

4.7.1 LAB MEDIA: Figure 7A. *Video editor: Show the left image and then the right.*

1. Sylgard  
Pronunciation link: No confirmed link found  
IPA: /'sɪl.gɑːrd/  
Phonetic Spelling: sil·gard
2. Degassing  
Pronunciation link: <https://www.merriam-webster.com/dictionary/degas>  
IPA: /diː'gæs/  
Phonetic Spelling: dee·gas
3. Isotropic  
Pronunciation link: <https://www.merriam-webster.com/dictionary/isotropic>  
IPA: /,aɪ.sə'trɑː.pɪk/  
Phonetic Spelling: eye·suh·traa·pik
4. Uniaxial  
Pronunciation link: <https://www.merriam-webster.com/dictionary/uniaxial>  
IPA: /,juː.ni'æk.si.əl/  
Phonetic Spelling: yoo·nee·ak·see·uhl
5. Polydimethylsiloxane  
Pronunciation link: No confirmed link found  
IPA: /,pɑː.li.daɪ.məθ.əl.sɪ'lə:k.seɪn/  
Phonetic Spelling: pah·lee·dye·meth·uhl·sih·lok·sayn
6. PDMS  
Pronunciation link: No confirmed link found  
IPA: /,piː.di.ɛm'ɛs/  
Phonetic Spelling: pee·dee·em·ess
7. NuSil  
Pronunciation link: No confirmed link found  
IPA: /'nuː.sɪl/  
Phonetic Spelling: noo·sil
8. Crosslinker  
Pronunciation link: <https://www.merriam-webster.com/dictionary/cross-link>  
IPA: /'krɒs.lɪŋ.kə/  
Phonetic Spelling: kraws·ling·ker
9. Kilopascal  
Pronunciation link: <https://www.merriam-webster.com/dictionary/kilopascal>  
IPA: /'kɪl.ou.pæs.kəl/  
Phonetic Spelling: kil·oh·pas·kuhl
10. Microliter  
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>  
IPA: /'maɪ.kroʊ.liː.tər/  
Phonetic Spelling: my·kroh·lee·ter
11. Micrometer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>  
IPA: /maɪ'krɑː.mə.tər/  
Phonetic Spelling: my·kraa·muh·ter

12. Centrifuge  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>  
 IPA: /'sen.trə.fjuːdʒ/  
 Phonetic Spelling: sen·truh·fyooj
13. Fluorescent  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>  
 IPA: /flʊ'res.ənt/  
 Phonetic Spelling: floor·es·uhnt
14. Carboxylate  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/carboxylate>  
 IPA: /kɑːr'baːk.səˌleɪt/  
 Phonetic Spelling: kar·bok·suh·layt
15. Microspheres  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microsphere>  
 IPA: /'maɪ.kroʊˌsfɪr/  
 Phonetic Spelling: my·kroh·sfeer
16. Vortex  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>  
 IPA: /'vɔːr.tɛks/  
 Phonetic Spelling: vor·teks
17. Fiducial  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fiducial>  
 IPA: /fɪ'duːʃi.əl/  
 Phonetic Spelling: fy·doo·shee·uhl
18. Traction  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/traction>  
 IPA: /'træk.ʃən/  
 Phonetic Spelling: trak·shuhn
19. Fourier  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Fourier>  
 IPA: /'fɔːr.i.ər/  
 Phonetic Spelling: foor·ee·ay
20. MATLAB  
 Pronunciation link: No confirmed link found  
 IPA: /'mæt.læb/  
 Phonetic Spelling: mat·lab
21. Displacement  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/displacement>  
 IPA: /dɪs'pleɪs.mənt/  
 Phonetic Spelling: dis·plays·muhnt
22. Isotropic  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/isotropic>  
 IPA: /,aɪ.sə'trɑː.pɪk/  
 Phonetic Spelling: eye·suh·traa·pik

23. Uniaxial

Pronunciation link: <https://www.merriam-webster.com/dictionary/uniaxial>

IPA: /ˌjuː.niˈæk.si.əl/

Phonetic Spelling: yoo·nee·ak·see·uhl

24. Intracellular

Pronunciation link: <https://www.merriam-webster.com/dictionary/intracellular>

IPA: /ˌɪn.trəˈsel.jə.lər/

Phonetic Spelling: in·truh·sel·yuh·ler