

2014 BioNanotechnology Summer Institute

Cancer Nanotechnology and Cell Mechanics

an NSF-IGERT and
NIH-CNTC collaboration

July 28–August 8, 2014

University of Illinois at Urbana-Champaign



MECHANOBIOLOGY LAB MODULE (MechB):

Response of Patterned Fibroblast Cytoskeleton to Substrate Stiffness 1: Hydrogel Preparation

Location: 3110 Digital Computing Laboratory (DCL)

Lead Instructor: Andrew Smith, Bioengineering

Lab Assistants: Anthony Fan, Mechanical Science and Engineering and Rishi Singh, Mechanical Science and Engineering

Purpose and Expected Outcome

The purpose of this module is to provide participants with hands-on experience in the synthesis of hydrogels and cellular micropatterning, as well as a basic understanding of how substrate elasticity modulates the cellular cytoskeleton. Participants will prepare hydrogels of varying stiffness, conjugate the hydrogels with extracellular matrix proteins in defined shapes using micro-contact printing, seed cells onto the patterned substrates, and image the products using optical microscopy. This module builds upon other experiences from summer institute modules in clean room fabrication, cell culture, and immunofluorescence staining, reflecting the interdisciplinary nature of mechanobiology. In the first session of this module, hydrogels will be prepared, and in the second session, the hydrogels will be functionalized with matrix proteins in patterns and cells will be seeded.

Background

The environment that a cell experiences in a tissue can widely vary in mechanical properties (stiffness/elastic properties) depending on tissue type and disease state. It has been shown that substrate stiffness influences cell behaviors, including locomotion, adhesion, and spreading [1-7]. We will prepare polyacrylamide (PA) hydrogel substrates that will provide a representative range of stiffness, with elasticity moduli of 1 or 40 kPa, to mimic the extracellular matrix in a cells' native tissue environment. Some things to note about PA hydrogels:

1. The modulus of elasticity of the substrate can be varied by changing the relative concentrations of acrylamide and bis-acrylamide.
2. The surface chemistry of PA and mechanical properties can be tuned independently.
3. PA is generally non-fouling – only adhesive molecules covalently attached to the surface of the gel will serve as ligands for cell attachment.
4. Immunofluorescence imaging at high magnifications through the gel is facile due to the thin, translucent quality of PA gels.

Module Workflow

Participants will learn about cellular biomechanics and modulation of matrix stiffness through hydrogel tuning. Participants will be split into 6 teams of two individuals and each team will make both a stiff and a compliant substrate. At the conclusion of this session, each participant group will be able to make hydrogels of different stiffness on glass coverslips. Due to time constraints, participants will perform tasks out of order. The correct order would be: (1) coverslip and slide preparation and (2) hydrogel preparation.

30 minutes – Briefing and introduction to section

60 minutes – Hydrogel preparation

20 minutes – Coverslip preparation

10 minutes – Slide preparation

Section 01 – Hydrogel preparation

Items needed:

1. Acrylamide stock solution, 40% in water
2. Bis-acrylamide stock solution, 2% in water
3. Deionized water
4. Ammonium persulfate (APS), 10% in water, prepared fresh
5. Tetramethylethylenediamine (TEMED)
6. Amino-silane treated glass coverslip
7. Chloro-silane treated glass slide
8. 35 mm petri dish

Protocol:

1. Mix acrylamide and bis-acrylamide solutions to their desired concentrations in deionized H₂O. *Two separate mixtures should be prepared: 3% acrylamide/0.1% bis-acrylamide for 1 kPa, and 8% acrylamide/0.48% bis-acrylamide for 40 kPa.*
2. Degas the mixture in a vacuum desiccator for 15 minutes to purge the solution of dissolved oxygen. *Oxygen in the solution will quench the subsequent free radical polymerization. Degassing the solution speeds up polymerization and ensures more uniform polymerization.*
3. The following steps must be performed rapidly:
 - a. To each mixture, add 1/100 total volume of APS and 1/1000 total volume of TEMED.
 - b. Vortex the polymerizing solutions.
 - c. Using a micropipettor, transfer 25 µl of the gel solution onto the treated side of the chloro-silanated glass slide and add the amino-silanated coverslip with the treated side down. See Figure 1 below. *The setup resembles a “sandwich” in which the polymerizing solution sits in between the chloro-silanated glass slide and the amino-silanated coverslip.*
4. Allow the gel to polymerize for 5-30 min. Monitor the unused solution to determine when the solution is fully polymerized. *Shorter times may result in insufficient polymerization of all available monomers and may cause the mechanical properties of the hydrogels to vary from the expected values. [At this time, begin Section 02]*

5. Remove the bottom glass slide, which should not bind to the hydrogel. Place the top coverslip-gel composite in a petri dish with deionized water. Make sure that the gel-coated side faces up and that the gels are completely immersed.
6. To remove un-polymerized acrylamide, rinse twice, each time for 5 minutes in deionized water. *These hydrogels can be stored for long periods of time without losing any of their mechanical properties by keeping them immersed in water at 4°C to maintain hydration.*

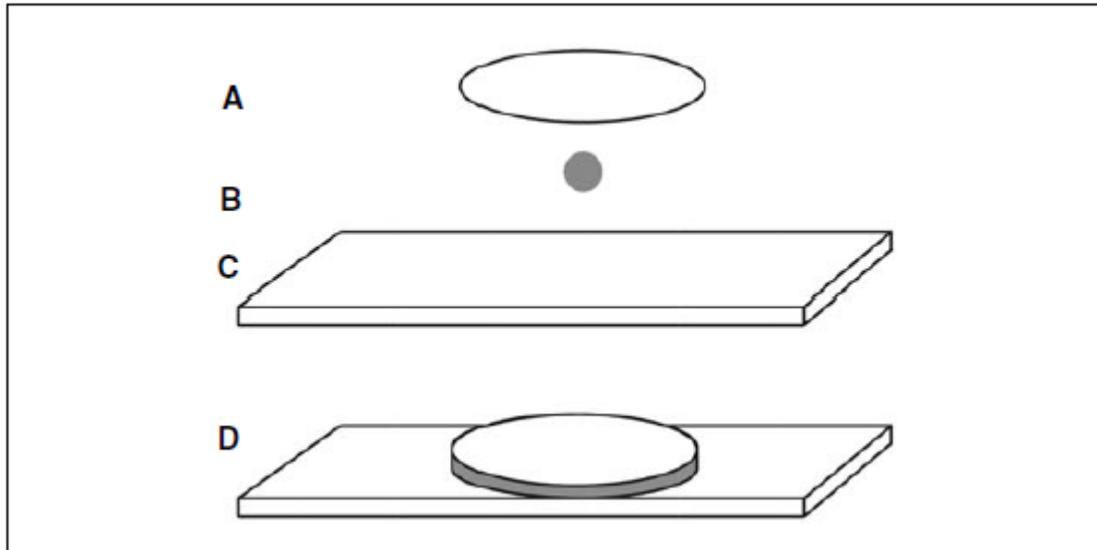


Figure 1: Schematic of hydrogel preparation. The gel-glass composite includes (A) the amino-silanated coverslip, (B) polymerizing solution, and (C) chloro-silanated glass slide. (D) The completed setup is shown.

Section 02 – Amino-silanated coverslip preparation

Items needed:

1. 25 mm coverslips
2. 3-Aminopropyltriethoxysilane (APES)
3. Glutaraldehyde, 0.5% in PBS
4. 0.1 M NaOH
5. Deionized water
6. 35 mm Petri dish

Protocol:

1. Place coverslip on a hot plate and add 500 μ l of 0.1 M NaOH to cover the entire surface.
2. Heat the coverslip at 80°C until the liquid entirely evaporates, leaving a semi-transparent film of sodium hydroxide. *If the film is not uniform, add a small amount of deionized water and evaporate again.*
3. Cool the coverslip and add 250 μ l APES to the surface and allow it to react for 5 minutes.
4. Rinse both sides of the coverslip with deionized water repeatedly to remove unreacted APES
5. Place the coverslip in 10 ml fresh distilled water in a petri dish for 5 minutes. Repeat once to complete cleaning of the surface.

6. Aspirate the water off of the coverslip and add 10 ml of glutaraldehyde solution and allow the solution to react for 30 minutes. [At this time, begin Section 03]
7. Aspirate the solution and dry the coverslip in air. *The coverslips are viable for 48 hours, but it is best to use the coverslips immediately after preparation.* [Return to Section 01, Step 5]

Section 03 – Chloro-silanated coverslip preparation

Items needed:

1. Dichlorodimethylsilane (DCDMS)
2. 25 x 75 mm glass slide
3. Deionized water

Protocol:

1. Spread about 100 μ l DCDMS on one side of the slide, ensuring that the solution coats the entire surface. Allow the solution to react for 5 minutes.
2. Remove excess DCDMS using a Kimwipe and rinse for 1 minute using deionized water. [Return to Section 02, Step 7]

Suggested References

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- [3] Hazeltine, L. B., Simmons, C. S., Salick, M. R., Lian, X., Badur, M. G., Han, W., Delgado, S. M., et al. (2012). Effects of substrate mechanics on contractility of cardiomyocytes generated from human pluripotent stem cells. *International journal of cell biology*, 2012, 508294. doi:10.1155/2012/508294
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- [6] Yuan Ye, K., Sullivan, K. E., & Black, L. D. (2011). Encapsulation of cardiomyocytes in a fibrin hydrogel for cardiac tissue engineering. *Journal of visualized experiments : JoVE*, (55), e3251. Retrieved from <http://www.jove.com/video/3251/encapsulation-cardiomyocytes-fibrin-hydrogel-for-cardiac-tissue>
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MECHANOBIOLOGY LAB MODULE (MechB):

Response of Patterned Fibroblast Cytoskeleton to Substrate Stiffness 2: Micro-Contact Printing and Cell Seeding

Location: 3110 Digital Computing Laboratory (DCL)

Lead Instructor: Andrew Smith, Bioengineering

Lab Assistants: Anthony Fan, Mechanical Science and Engineering and Rishi Singh, Mechanical Science and Engineering

Background

Using knowledge on making PA gels of different stiffness from Mechanobiology Session 1, students can explore some common cell seeding techniques to achieve geometric confinement. One particular well known method originally invented by Prof. George Whitesides from Harvard University is known as micro-contact printing. This method utilizes expertise from cleanroom fabrication to generate high resolution micro-patterned wafer which acts as a mold for Polydimethylsiloxane (PDMS), a polymer resin that can conform to almost anything. Upon polymerization, PDMS can act as a stamp for the transfer of protein patterns onto a substrate. This process makes cells adhere selectively at only the printed region, defined by the micro-pattern (or the inverse of the micro-pattern). This is achieved by simply bringing the PDMS stamp and the substrate to contact to transfer chemicals from the stamp to the substrate, hence the name micro-contact printing.

Module Workflow

Due to time constraints, participants will perform tasks out of order so that final products can be inspected at the end of the session. The correct order would be: (1) PA gel functionalization, (2) micro-contact printing, and (3) cell seeding.

15 minutes – Briefing

30 minutes – Cell seeding

30 minutes – Substrate functionalization

30 minutes – Micro-contact printing

20 minutes – Inspection

Section 01—Cell Seeding

Items needed:

1. Cell pellet
2. Petri dishes containing hydrogel/coverslips with pattern functionalization
3. Cell media

Protocol:

1. Remove the liquid from the hydrogels.
2. Add 1ml of cell medium to centrifuge tube with cell pellet and resuspend cells, pipette up and down until the pellet disappears. Try to avoid bubbles by not utilizing the full range of pipettor until final discharge.
3. Pipette 1ml of cell-media mixture onto the PA gels with patterned protein functionalization.
4. Return petri dish to the incubator. [Begin Section 02]
5. After approx. 30 minutes see whether cells have attached using the microscope.
6. If cells are attached, remove media, rinse dish with 1 ml 1xPBS, add 1 ml fresh media.
7. Return petri dish back into the incubator and inspect at the end of the session.

Section 02—Substrate Functionalization

Items needed:

1. PA hydrogels on glass coverslips
2. Sulfo-SANPAH stock solution, 0.1 M in DMSO (frozen)
3. HEPES buffer, 200 mM
4. Ultraviolet lamp

Protocol:

1. Using a Pasteur pipette or aspirator, remove the excess water from the PA hydrogel. *Avoid drying the gel.*
2. Perform the following three steps rapidly due to the short half-life of sulfo-SANPAH in water (~5 min).
 - a. Thaw the sulfo-SANPAH solution.
 - b. Using a micropipettor, dilute sulfo-SANPAH to 1 mM with HEPES buffer.
 - c. Using a micropipettor, transfer the solution to cover the entire gel (~200 μ l).
3. Expose the gel to the ultraviolet lamp for 5 minutes. Sulfo-SANPAH changes from orange to brown. *Be careful to not expose your skin to the ultraviolet light.*
4. Dip the UV-treated coverslips in a beaker with fresh deionized water and remove excess water by drying with a jet of air for 5 minutes. [Return to Section 01, Step 4]

Section 03—Micro Contact Printing

Items needed:

1. Diluted fibronectin (FN), 50 µg/mL in PBS
2. Fluorescent dye in PBS
3. PDMS stamps
4. Functionalized gel substrates
5. Pre-prepared PDMS wetted with FN (optional)

Protocol:

1. Mix dye and FN.
2. Pipette appropriate amount of FN solution to cover the entire stamp(s), and allow the protein to diffuse into the stamps for 30 mins or less at 37°C in the incubator. *Because PDMS is hydrophobic, it might be difficult for the FN solution to wet the surface initially. Students can use pipette tips to draw on the stamps for capillary forces to hold FN solution over the entire surface.*
 - a. *NOTE: if a plasma generator is available, a brief 3 minute plasma treatment of PDMS stamps will make the PDMS surface hydrophilic.*
3. Blow dry both the gel(s) and stamp(s). The quality of the patterns depends on the dryness of both stamp(s) and gel(s). Ensure that they are as dry as possible while maintaining the integrity of gel(s). *Any wetness will make stamp lose specificity since the remaining solution, which is not constricted by the geometric features of the stamps, will also transfer to the substrate.*
4. Place patterned side of stamp(s) onto desired location on gel(s). *Try to do this in one shot, again because of pattern specificity.*
5. Gently tap on stamp(s) for 90 seconds each.
6. Use tweezers to gently peel off stamp.
7. Inspect the pattern under a fluorescence microscope.
8. Submerge gel(s) in PBS.

Section 04—Imaging

Items needed:

1. Microscope slides of stained patterned cells (DAPI, cytoskeleton)
2. Previously seeded cells (From section “cell seeding”)

Protocol:

1. Take turn to look at the products: stained cells prepared by TAs, and previously seeded cells roughly 1.5 hrs ago. A portion of these could be done during waiting time in previous sections.

Suggested References

- [1] Bajaj, P. *et al.* Patterning the differentiation of C2C12 skeletal myoblasts. *Integrative biology : quantitative biosciences from nano to macro* **3**, 897-909 (2011).
- [2] Engler, A. J. *et al.* Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *The Journal of cell biology* **166**, 877-87 (2004).
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