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Title: Accessible Silicone Chip-to-Membrane Sealing Procedure for Flexible, Reliable Bonding

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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? **Yes, 10 minute walk**
- 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. **Yes**

Current Protocol Length

Number of Steps: 12

Number of Shots: 27

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**.

Videographer's NOTE: Quite a bit of extra shots, especially CU's, to make the actions stand out a bit clearer. All shots are numbered including the extra additions.

4 headshots of the authors

5 interview pieces in 4K

2 testimonial in 4K

29 shots for the protocol in HD (extra CU's not counted as separate shots; included in the numbered shots with slate)

One shot does not have a slate number

INTRODUCTION:

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

- 1.1. **Brady Rae:** We aim to fill a gap in Organ-on-Chip design, providing a new sealing procedure for PDMS devices with porous membranes.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Brady Rae:** Currently, chip sealing is challenging. Our process forces silicone through the membrane, which means you only deal with silicone-silicone bonding.

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

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

- 1.3. **Brady Rae:** If the membrane is porous, such as for a lung- or gut-on-chip, then PDMS, toluene, and a vacuum can be applied.

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

CONCLUSION:

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

- 1.4. **Brady Rae**: This procedure facilitates the production of Organ-on-Chips in the majority of research environments, accessibility for new developers, and lower-income labs.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Brady Rae**: Epithelial cells can be grown at the air-liquid interface, co-cultured with other cell types, and their barrier integrity readily investigated.
 - 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your article address?

- 1.6. **Brady Rae, Post-Doc, University of Groningen**: (authors will present their testimonial statements live)

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

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Brady Rae, Post-Doc, University of Groningen**: (authors will present their testimonial statements live)

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

Protocol

2. Fabrication and Assembly of a PDMS Microfluidic Chip with PET or PC Membrane Bonding

Demonstrator: Brady Rae

- 2.1. To begin, thoroughly mix liquid PDMS base with curing agent in a 10 to 1 ratio [1]. Using a vacuum chamber, degas the mixture [2] until all air bubbles are removed [3]. **NOTE:** The VO is edited for the additional shot

2.1.1. WIDE: Talent pouring polydimethylsiloxane base and curing agent into a container and mixing thoroughly. **TXT: PDMS: Polydimethylsiloxane** **NOTE:** Additional close-ups showing the label of the PDMS and curing agent as well as the mixing process.

2.1.2. Talent placing the container into a vacuum chamber for degassing. **NOTE:** Additional close up of the bubbles rising in the container due to the vacuum.

Added shot 2.1.2.1. Shot of degassed PDMS mixture

- 2.2. Using a wide nozzle syringe, fill the molds with the degassed PDMS mixture [1]. Place the filled molds in a vacuum chamber and degas for approximately 10 minutes [2] until all bubbles are removed [3]. Using a dry oven, cure the molds for 2 hours at 65 degrees Celsius [4]. **NOTE:** The VO is edited for the additional shot

2.2.1. Talent dispensing polydimethylsiloxane into the molds with a wide nozzle syringe. **NOTE:** Wide shot of PDMS syringing, followed by close-up of injection into molds. 2.2.1.1: close-up, two loads of the syringe, 1x2mL 1x2.4mL

2.2.2. Shot of the molds being placed into a vacuum chamber for degassing. **NOTE:** Close-up of bubbles rising in the molds due to vacuum.

Added shot: 2.2.2.1 close-up of degassed molds

2.2.3. Talent placing the molds into a dry oven, pre-heated to 65 degrees Celsius.

- 2.3. With a scalpel, remove the solid chip halves from the molds [1]. Trim the flashing from the edges of each chip half [2]. Then use a biopsy punch to add four inlets to the top half at each end of the chamber and at the two adjacent rings, indicating the bottom chamber inlet locations [3]. **NOTE:** The VO is edited for the deleted shot

2.3.1. Talent lifting the cured polydimethylsiloxane chip halves from the molds with a scalpel.

2.3.2. Talent cutting away excess flashing from the chip edges.

2.3.3. Talent using a biopsy punch to create inlet holes in the designated positions on the chip half.

- 2.3.4. ~~Shot digitally highlighting the bottom chamber inlets.~~ **NOTE: Not filmed as the mentioned information is already there in 2.3.3**
- 2.4. Next, place a 3D printed stencil over a PET or PC membrane [1]. Using a scalpel, cut the membrane into shape while firmly holding the stencil in place [2].
- 2.4.1. Talent positioning the stencil over the membrane sheet on the bench. **TEXT: PET: Polyethylene Terephthalate; PC: Polycarbonate** **NOTE: Additional optional close-up to clarify positioning**
- 2.4.2. Talent cutting the membrane along the stencil outline with a scalpel. **NOTE: Two extra angles to show how the cutting works around hands, extra top down shot for detailed view**
- 2.5. Alternatively, prepare an extracted ECM membrane and dehydrate it [1]. Place the stencil over the membrane [2] and cut it into shape while firmly holding the stencil in place [3].
- 2.5.1. Talent showing the wet membrane and its dehydrated equivalent. **NOTE: Close-ups for details of membranes**
- 2.5.2. Talent positioning the stencil over the membrane sheet on the bench .
- 2.5.3. Talent cutting the membrane along the stencil outline with a scalpel.
- 2.6. Now, wear protective goggles and nitrile gloves [1]. Mix liquid PDMS with toluene in a 7 to 5 ratio to prepare the mortar [2]. Using a wide nozzle syringe, mix thoroughly by syringing up and down until consistent [3]. **NOTE: The VO is rearranged for the moved shot**
- 2.6.3. Talent wearing protective goggles and nitrile gloves while handling the mixture.
- 2.6.1. Talent combining polydimethylsiloxane and toluene in a container. **NOTE: 2.6.1 is filmed in 2 takes, one adding PDMS in a syringe showing the volume, one collecting the toluene in a 1mL pipette**
- 2.6.2. Talent drawing the mixture up and down in a wide nozzle syringe to ensure thorough mixing. **NOTE: This shot is filmed in 2 takes, one over the shoulder of the mixing, one of the mixing up and down up close (this one is important to show thorough and complete mixing)**
- 2.6.3. ~~Talent wearing protective goggles and nitrile gloves while handling the mixture.~~ **NOTE: This shot is moved before 2.6.1 as per authors request**
- 2.7. Place all laboratory materials used in handling toluene in a fume hood for 24 hours to permit evaporation [1]. Dispose of the materials in approved and labeled containers through specialist waste services [2].
- 2.7.1. Shot of used pipette tips and containers placed inside a fume hood.
- 2.7.2. Talent placing dried waste materials into labeled disposal containers.

- 2.8. Now, use a pipette tip to swiftly apply the prepared PDMS mortar to the surface of the chip half where the membrane will be placed [1].
- 2.8.1. Talent applying a thin layer of mortar onto the designated chip surface with a pipette tip. NOTE: Additional closeup for clarity of positioning
- 2.9. Using clean forceps, hold the prepared membrane at one end [1]. Position the membrane tip onto the wet mortar at the correct location on the chip and allow it to fall into place [2].
- 2.9.1. Talent gripping the membrane edge with clean forceps. NOTE: Closeup to visualise the membrane
- 2.9.2. Shot of the membrane being aligned and gently lowered onto the wet mortar surface.
- 2.10. Gently hold the membrane in place [1]. Reapply PDMS mortar over the top of the membrane, moving away from the chamber with each stroke to remove bubbles and secure the membrane without shifting its position [2].
- 2.10.1. Talent stabilizing the membrane with forceps.
- 2.10.2. Talent spreading mortar over the membrane surface in controlled strokes away from the chamber. NOTE: Additional closeup for clarity of positioning
- 2.11. Immediately place the membrane-bound chip halves into a vacuum chamber after mortar application [1-TXT].
- 2.11.1. Talent placing the assembled chip halves into a vacuum chamber. **TXT: Cure at RT, 25 mBar, 72 h**
- 2.12. Turn on the vacuum pump, pressure modulator, and plasma oven. Set the chamber pressure to 320 millibars, then place both the membrane-bound chip half and the second chip half inside for 30 seconds of oxygen plasma treatment [1]. After removing the chips from the vacuum chamber, swiftly press the two halves together under light hand pressure to complete chip assembly [2]. NOTE: The authors added additional details in the shot, hence the VO is edited as per the shots
- 2.12.1. Talent placing the chips in a oxygen plasma chamber. NOTE: The authors divided 2.12.1 into 5 shot, showing the vacuum pump turned on, the pressure modulator turned on, the plasma oven turned on, the pressure of 320 mbar, placing them inside. Optional shot of the blue plasma light in the oven.
- 2.12.2. Talent aligning and pressing the two chip halves together by hand under light pressure. NOTE: 3 shots, removing the chips, pressing them together, over shoulder of the alignment and testing of the connection.

Results

3. Results

- 3.1. Membranes with dyed mortar demonstrated intrusion of liquid PDMS into the 0.4 micrometer pores after room temperature vacuum sealing [1]
 - 3.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the small oval-marked regions*
- 3.2. Basement membrane-containing extracellular matrix scaffolds were successfully bound within the chip device [1].
 - 3.2.1. LAB MEDIA: Figure 6. *Video editor: Sequentially show A to C*
- 3.3. The extracellular matrix membrane withstood 1 millimeter of horizontal stretch induced by vacuum overnight [1].
 - 3.3.1. LAB MEDIA: Figure 7. *Video editor: Show image A (top and bottom) then image B (top and bottom)*
- 3.4. After 10 days of apical air exposure, Calu-3 (*Cal-u-three*) epithelial cells differentiated into mucus-producing cells as demonstrated by Alcian blue staining [1].
 - 3.4.1. LAB MEDIA: Figure 8. *Video editor: Show A then B*
- 3.5. In co-culture on the extracellular matrix membrane, epithelial cells formed a confluent monolayer on the inner side [1] while fibroblasts were distributed less densely on the outer surface and retained a spindle-like morphology [2].
 - 3.5.1. LAB MEDIA: Figure 10C. *Video editor: Highlight ii and iii*
 - 3.5.2. LAB MEDIA: Figure 10C. *Video editor: Highlight i and iv*