FINAL SCRIPT: APPROVED FOR FILMING

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Title: Fabrication of a Master Mold for Microneedles with a Micron-Sized Air-Vent Hole

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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? **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: 19
Number of Shots: 32

Introduction

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

INTRODUCTION:

1.1. **Hyerin Ahn:** Currently, advanced microfabrication, silicon micromachining, vacuum-assisted molding, and high-resolution microscopy are used to fabricate and characterize microneedles.

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

1.2. **Hyerin Ahn:** The major challenges are to prevent air entrapment during viscous polymer casting and achieve consistent microneedle tip sharpness.

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

CONCLUSION:

1.3. **Sangjun Pyo:** We fabricated air-vent–assisted master molds that are defect-free. These sharp-tipped solid and hollow microneedles are efficient and offer 100% yield.

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

1.4. **Sangjun Pyo:** Our protocol overcomes the hurdle of uncontrolled air entrapment during viscous polymer casting that limits microneedle tip fidelity and yield.

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

1.5. **Jaehyeong Kim:** Oue method also enables reliable tip formation without complex equipment, using air venting and simple vacuum-assisted casting.

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. Polymer Solution Preparation for Microneedle Fabrication

Demonstrator: Hyerin Ahn

2.1. To begin, aspirate 9 milliliters of distilled water into a 10-milliliter disposable syringe **[1]** and add it into a 50-milliliter conical centrifuge tube **[2]**.

2.1.1. WIDE: Talent holding a 10 milliliter disposable syringe and drawing 9 milliliters of distilled water into it.

2.1.2. Talent adding the water to a 50 milliliter conical centrifuge tube.

2.2. Using an S/T *(S-T)* spoon and greaseproof paper, weigh 1 gram of gelatin powder on a precision balance **[1]** and transfer it into the conical tube containing water **[2]**.

2.2.1. Talent weighing gelatin powder on a greaseproof paper on a precision balance and using an S/T spoon.

2.2.2. Talent lifting the greaseproof paper and pouring the gelatin powder into the conical tube containing water.

2.3. With the S/T spoon, break apart any clumps of gelatin by stirring the mixture in a clockwise direction for 5 minutes **[1]**, ensuring that the rotating solution does not spill **[2]**.

2.3.1. Talent stirring the gelatin mixture clockwise inside the conical tube using the S/T spoon.

2.3.2. Close-up shot of the solution after gelatin is mixed completely.

2.4. Now, fill a water bath with 4 liters of distilled water **[1]**.

2.4.1. Talent pouring distilled water into the water bath until it reaches 4 liters.

2.5. Set the water bath temperature to 60 degrees Celsius **[1]** and wait for approximately 30 minutes until it reaches the target temperature **[2]**.

2.5.1. Talent setting the temperature.

2.5.2. Shot of the water bath displaying 60 degrees Celsius.

2.6. Now, place the tube containing the gelatin solution in the water bath **[1]** and incubate for 30 minutes to dissolve the gelatin **[2]**.

2.6.1. Talent lowering the conical tube containing the gelatin solution into the water bath.

2.6.2. Shot of the tube after gelatin is dissolved.

3. Fabrication of Solid and Hollow Microneedles

Demonstrator: Sangjun Pyo

3.1. Stick double-sided non-woven tape on two microscope slides **[1]** and lay them flat inside a Petri dish, spacing them 7 millimeters apart **[2-TXT]**.

3.1.1. Close-up of the talent applying double-sided non-woven tape on the slide.

3.1.2. Talent placing two microscope slides flat inside a Petri dish with a visible 7 millimeter gap. **TXT: Slide dimensions: 76 mm × 26 mm × 1 mm**

3.2. Then, place the fabricated silicon master mold on top of the two microscope slides **[1]**, with its backside containing the air-vent hole facing downward **[2]**.

3.2.1. Talent positioning the silicon master mold on top of the two microscope slides.

3.2.2. Close-up of the mold being oriented so that the air-vent hole-containing backside faces downward.

3.3. Adjust the position of the mold so that the air-vent hole is centered over the gap between the two slides **[1]**.

3.3.1. Close-up of the talent gently shifting the mold to align the air-vent hole directly over the central gap.

3.4. Now, place the Petri dish containing the silicon master mold at the center of a vacuum desiccator measuring approximately 260 by 260 by 100 millimeters **[1]**.

3.4.1. Talent placing the Petri dish with the mold into the center of the vacuum desiccator.

3.5. Then, using a 1 milliliter disposable syringe, apply 0.3 milliliters of methanol to the center of the silicon master mold **[1]** and gently move the syringe tip along the surface of the mold to distribute the methanol evenly toward the edges **[2]**.

3.5.1. Talent dispensing 0.3 milliliters of methanol from a 1 milliliter disposable syringe onto the center of the silicon master mold.

3.5.2. Close-up of the talent guiding the syringe tip across the mold surface to spread the methanol evenly.

3.6. Seal the vacuum desiccator **[1]** and use a vacuum pump to apply a vacuum of minus 80 kilopascal for 2 minutes to let the methanol fully infiltrate the silicon master mold **[2]**.

3.6.1. Talent placing the lid onto the vacuum desiccator to seal it.

3.6.2. Talent operating the vacuum pump to apply minus 80 kilopascal to the desiccator.

3.7. Once done, release the vacuum to remove remaining bubbles in the methanol **[1]**.

3.7.1. Talent releasing the vacuum from the desiccator.

3.8. Next, remove the prepared gelatin solution from the water bath **[1]** and wipe water from the outside of the tube using a cleanroom wipe **[2]**.

3.8.1. Talent lifting the conical tube containing the gelatin solution out of the water bath.

3.8.2. Talent wiping condensation from the outside of the tube with a cleanroom wipe.

3.9. For solid microneedles, use a 20 to 200 microliter micropipette to dispense 100 microliters of the gelatin solution onto the center of the silicon master mold **[1-TXT]**.

3.9.1. Talent pipetting 100 microliters of gelatin solution onto the center of the mold for solid microneedles. **TXT: Hollow microneedles: 40 µL gelatin solution**

3.10. Then, seal the vacuum desiccator **[1]** and apply a vacuum of minus 80 kilopascal for 2 minutes **[2]**.

3.10.1. Talent sealing the vacuum desiccator lid.

3.10.2. Talent setting minus 80 kilopascal on the vacuum pump.

3.11. After releasing the vacuum, incubate the Petri dish flat in a 60 degrees Celsius water bath for 2 minutes **[1]**.

3.11.1. Talent carefully lowering the leveled Petri dish into the water bath.

3.12. Then, carefully remove the Petri dish from the water bath without tilting it **[1]**.

3.12.1. Talent lifting the Petri dish straight up out of the water bath without tilting. **Videographer's NOTE**: FX6_0473.MXF is a failed shot of 3.12.1

FX6_0472.MXF is a failed shot of 3.12.2

3.13. Finally, using a pair of tweezers, touch the surface of the gelatin solution **[1]** and spread the solution evenly to the edges of the mold **[2]**, ensuring the entire mold surface is coated with a thin layer **[3]**.

3.13.1. Close-up of tweezers lightly contacting the gelatin surface.

3.13.2. Talent guiding the gelatin solution outward toward the edges of the mold.

3.13.3. Top-down shot showing the mold surface evenly coated with a thin gelatin layer.

Results

4. Results

4.1. The silicon master mold with an air-vent hole was successfully fabricated using anisotropic wet etching, forming a 5 by 5 microneedle array layout with distinct V-grooves meeting at the cavity tip **[1]**, and the anisotropic etch profile was confirmed via a cross-sectional scanning electron microscopy image, showing upper and lower etch depths **[2]**.

4.1.1. LAB MEDIA: Figure 3. *Video editor: Focus on A*.

4.1.2. LAB MEDIA: Figure 3. *Video editor: Focus on C*.

4.2. In the airvent hole-equipped master mold, the hole was successfully etched at the center of the cavity tip, as shown in the top-view scanning electron microscopy image **[1]**. These molds produced a 100% cavity yield with clearly defined pyramidal cavities and a distinct air-vent hole at the tip across all 25 cavities **[2]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the small dark hole at the center of the pyramid in B*.

4.2.2. LAB MEDIA: Table 1. *Video editor: Highlight the row for "AVH"showing 25 / 25 (100.0%) cavity yield*.

4.3. Microneedles fabricated using a mold without an air-vent hole showed blunt tips due to trapped air, despite vacuum application for over 10 minutes **[1]**.

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the dull tip of the microneedle and the absence of a sharp point*.

4.4. When using a mold with an air-vent hole, sharp microneedle tips formed after only 2 minutes under vacuum due to successful air venting during casting **[1]**.

4.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the sharp tip of the microneedle in the center of the image*.

4.5. A 5 by 5 microneedle array fabricated using the AVH-equipped mold showed a 100% fabrication yield with bubble-free, sharply defined tips **[1]**.

4.5.1. LAB MEDIA: Figure 4C.

4.6. When casting 40 microliters of gelatin into molds with an air-vent hole, microneedles with hollow interiors were formed due to gelatin flowing toward the venting point during curing **[1]**.

4.6.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the transparent arch-shaped microneedle structure*.

1. Aspirate
   Pronunciation link: https://www.merriam-webster.com/dictionary/aspirate
   IPA: /ˈæs.pə.reɪt/
   Phonetic Spelling: as·puh·rayt
2. Milliliters
   Pronunciation link: https://www.merriam-webster.com/dictionary/milliliter
   IPA: /ˈmɪl.əˌliː.tɚ/
   Phonetic Spelling: mill·uh·lee·ter
3. Disposable
   Pronunciation link: https://www.merriam-webster.com/dictionary/disposable
   IPA: /dɪˈspoʊ.zə.bəl/
   Phonetic Spelling: dih·spoh·zuh·buhl
4. Conical
   Pronunciation link: https://www.merriam-webster.com/dictionary/conical
   IPA: /ˈkɑː.nɪ.kəl/
   Phonetic Spelling: kah·nih·kuhl
5. Centrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   IPA: /ˈsɛn.trəˌfjuːdʒ/
   Phonetic Spelling: sen·truh·fyooj
6. Gelatin
   Pronunciation link: https://www.merriam-webster.com/dictionary/gelatin
   IPA: /ˈdʒɛl.ə.tɪn/
   Phonetic Spelling: jel·uh·tin
7. Precision
   Pronunciation link: https://www.merriam-webster.com/dictionary/precision
   IPA: /prɪˈsɪʒ.ən/
   Phonetic Spelling: pruh·sizh·uhn
8. Incubate
   Pronunciation link: https://www.merriam-webster.com/dictionary/incubate
   IPA: /ˈɪn.kjəˌbeɪt/
   Phonetic Spelling: in·kyuh·bayt
9. Fabrication
   Pronunciation link: https://www.merriam-webster.com/dictionary/fabrication

IPA: /ˌfæb.rɪˈkeɪ.ʃən/
Phonetic Spelling: fab·rih·kay·shuhn
10. Microneedles
Pronunciation link: https://www.merriam-webster.com/dictionary/microneedle
IPA: /ˈmaɪ.kroʊˌniː.dəl/
Phonetic Spelling: my·kroh·nee·duhl
11. Nonwoven
Pronunciation link: https://www.merriam-webster.com/dictionary/nonwoven
IPA: /ˌnɑːnˈwoʊ.vən/
Phonetic Spelling: non·woh·vuhn
12. Petri
Pronunciation link: https://www.merriam-webster.com/dictionary/Petri
IPA: /ˈpiː.tri/
Phonetic Spelling: pee·tree
13. Silicon
Pronunciation link: https://www.merriam-webster.com/dictionary/silicon
IPA: /ˈsɪl.ɪ.kən/
Phonetic Spelling: sil·ih·kuhn
14. Desiccator
Pronunciation link: https://www.merriam-webster.com/dictionary/desiccator
IPA: /ˈdɛs.ɪˌkeɪ.tɚ/
Phonetic Spelling: dess·ih·kay·ter
15. Methanol
Pronunciation link: https://www.merriam-webster.com/dictionary/methanol
IPA: /ˈmɛθ.əˌnɑːl/
Phonetic Spelling: meth·uh·nahl
16. Kilopascal
Pronunciation link: https://www.merriam-webster.com/dictionary/kilopascal
IPA: /ˈkɪl.oʊˌpæs.kəl/
Phonetic Spelling: kil·oh·pas·kuhl
17. Microliter
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪ.kroʊˌliː.tɚ/
Phonetic Spelling: my·kroh·lee·ter
18. Micropipette
Pronunciation link: https://www.merriam-webster.com/dictionary/micropipette
IPA: /ˌmaɪ.kroʊ.pɪˈpɛt/
Phonetic Spelling: my·kroh·pih·pet
19. Anisotropic
Pronunciation link: https://www.merriam-webster.com/dictionary/anisotropic
IPA: /ˌæn.aɪ.səˈtrɑː.pɪk/
Phonetic Spelling: an·eye·suh·trah·pik
20. Etching
Pronunciation link: https://www.merriam-webster.com/dictionary/etching

IPA: /ˈɛtʃ.ɪŋ/
Phonetic Spelling: etch·ing

21. Microscopy
    Pronunciation link: https://www.merriam-webster.com/dictionary/microscopy
    IPA: /maɪˈkrɑː.skə.pi/
    Phonetic Spelling: my·krah·skuh·pee
22. Pyramidal
    Pronunciation link: https://www.merriam-webster.com/dictionary/pyramidal
    IPA: /pɪˈræm.ɪ.dəl/
    Phonetic Spelling: puh·ram·ih·duhl
23. Cavity
    Pronunciation link: https://www.merriam-webster.com/dictionary/cavity
    IPA: /ˈkæv.ə.ti/
    Phonetic Spelling: kav·uh·tee
24. Yield
    Pronunciation link: https://www.merriam-webster.com/dictionary/yield
    IPA: /jiːld/
    Phonetic Spelling: yeeld
25. Venting
    Pronunciation link: https://www.merriam-webster.com/dictionary/venting
    IPA: /ˈvɛn.tɪŋ/
    Phonetic Spelling: ven·ting
26. Curing
    Pronunciation link: https://www.merriam-webster.com/dictionary/curing
    IPA: /ˈkjʊr.ɪŋ/
    Phonetic Spelling: kyur·ing