STAR Protocols

Protocol

Fabricating self-powered microfluidic devices via 3D printing for manipulating fluid flow

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Highlights

Detailed protocol for fabricating self-powered microfluidic devices via 3D print

The protocol includes fabrication approaches for both PDMS and non-PDMS materials

Diffusion model provides design parameters for controlling the flow kinetics

Potential to advance droplet-based, portable, point-of-care device applications

Advances in 3D printing technologies allow fabrication of complex structures at micron resolution. Here, we describe two approaches of fabricating self-powered microfluidic devices utilizing 3D printing: PDMS (polydimethylsiloxane)-based microfluidic devices with a built-in vacuum pocket fabricated by soft lithography using a 3D-printed mold, and non-PDMS microfluidic devices operating by a removable vacuum battery fabricated by 3D-printed materials. These microfluidic devices can be used for controlling blood flow and separating blood plasma.

Protocol

Fabricating self-powered microfluidic devices via 3D printing for manipulating fluid flow

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SUMMARY

Advances in 3D printing technologies allow fabrication of complex structures at micron resolution. Here, we describe two approaches of fabricating self-powered microfluidic devices utilizing 3D printing: PDMS (polydimethylsiloxane)-based microfluidic devices with a built-in vacuum pocket fabricated by soft lithography using a 3D-printed mold, and non-PDMS microfluidic devices operating by a removable vacuum battery fabricated by 3D-printed materials. These microfluidic devices can be used for controlling blood flow and separating blood plasma.

For complete details on the use and execution of this protocol, please refer to Woo et al. (2021).

BEFORE YOU BEGIN

The protocol below describes steps for 3D printing and post-treatments of the 3D printed materials.

Design, 3D printing, and curing a mold for PDMS soft lithography

⊙ Timing: 2–3 days

1. Design the microfluidic device including a microchannel and built-in vacuum pocket.
2. Draw a mold of the device using standard CAD software (e.g., AutoCAD, FreeCAD, Blender, SketchUp, etc.).
3. Export the 3D mold design to a lithography file (STL or OBJ file format).
4. 3D printing of the mold.
   a. Upload the lithography file to the 3D printer via an attached computer or USB drive. In this work, we have used a Form 3(+) SLA 3D printer (Formlabs).
   b. Adjust the printing parameters such as the use of auxiliary supporting structure and the printing resolution. The auxiliary support structure helps print the mold while printing, and thus is recommended to use. We have used the XY resolution of 25 microns.
   c. Install the resins in the 3D printer and mount the build platform. We have used a clear resin (RS-F2-GPCL-04, Formlabs).
   d. Start printing the mold.
   e. When 3D printing is completed, remove the printed mold from the platform using a scraper.
5. Washing the printed mold (Figure 1).
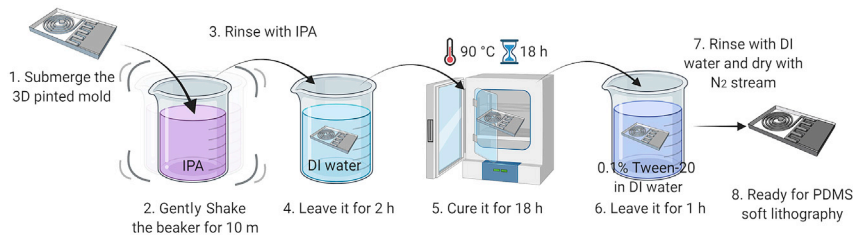
Figure 1. Washing and post-curing steps of the 3D printed mold for PDMS soft lithography

    a. Prepare a beaker bigger than the mold size for an isopropyl alcohol (IPA) bath.

    b. Fill IPA in the beaker and submerge the printed mold into the beaker.

    c. Gently shake the beaker to remove the remaining liquid resin from the printed mold for 10 min.

    d. Take out the mold from the IPA beaker.

    e. Rinse the mold with fresh IPA for further cleaning.

    f. Inspect the mold carefully. In particular, the liquid resin residue may remain at the corners and small gaps of the mold.

    g. Submerge the mold in deionized water for 2 h.

6. Post-curing the 3D printed mold (Figure 1).

    a. Set a vacuum oven temperature to 90°C.

    b. Put the mold in the oven and cure it for 18 h.

    c. Submerge the mold in the 0.1% (v/v) Tween-20 in deionized water for 1 h to remove oily materials exuded on the mold surface during the heating process.

    d. Rinse the mold with deionized water.

    e. Dry the mold with a nitrogen gas stream.

⚠ CRITICAL: The post-printing process is a key for PDMS soft lithography. The remaining liquid resin at any surface on the mold must be removed completely. Otherwise, the resin residue prohibits PDMS curing, which leads to poor surface finish and distorted structure of the PDMS device.

Design and process acrylic sheets for a device holder

⏱ Timing: 8 h

7. Design a PDMS device holder consisting of top and bottom pieces using the standard CAD software.

    a. The top piece requires an inlet port that fits the PDMS microfluidic device.

    b. Make 6–10 screw holes around the edge of both top and bottom pieces.

8. Export the 3D holder model to a design file (DXF or DWG file format).

9. Upload the design file to a laser cutter (Universal Laser Systems).

10. Mount the acrylic sheet or plate.

11. Run the cutter software to cut the acrylic sheet according to the design.

Optional: The holder pieces could be fabricated with various materials including 3D printing materials and aluminum. Also, making threads on the bottom piece via a tap drilling method allows direct screw attachments, instead of using nuts.

⚠ CRITICAL: The top piece of holder material should be transparent for the recording of fluid dynamics and stiff to prevent deformation when assembling the device with screws and nuts. We have used thick acrylic sheets for the top piece and the laser cutter to cut them

clean and smooth cut surface. Machining could be an alternative way to process acrylic materials. However, it should be careful to avoid generating scratches, debris, or melting acrylic materials while machining.

KEY RESOURCES TABLE

| REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sylgard™ 184 Silicon Elastomer Kit (PDMS)	Dow Corning	Cat#2065622
Clear Resin for 3D printer	Formlabs	Cat#RS-F2-GPCL-04
Isopropyl Alcohol (IPA)	Thermo Fisher Scientific	Cat#A416-4
Tween-20	Thermo Fisher Scientific	Cat#J20605.AP
Vacuum Grease	Dow Corning	Cat#1597418
Software and algorithms		
ImageJ Software	National Institutes of Health	https://imagej.nih.gov/ij/index.html
Tracker Software	Open Source Physics	https://physlets.org/tracker/
AutoCAD	Autodesk	https://www.autodesk.com/products/autocad
Others		
Thermo Napco Vacuum Oven	Precision Scientific	Model#5831
Hand Vacuum Pump	Actron	Cat#CP7830
Form 3 SLA 3D printer	Formlabs	Cat#PKG-F3-WSVC
Laser Engraver/Cutter	Universal Laser Systems	Cat#PLS6150
Spin Coater	Laurell	Cat#WS-400B-6NPP
Nitrogen Spray Gun	Entegris	Cat#421-42-11
Polystyrene Petri Dish (1,500 mm × 15 mm)	Fisherbrand	Cat#FB0875714
Steel Hex Nut (4–40 Thread size)	McMaster-Carr	Cat#91841A005
Steel Socket Head Screw (4–40 Thread size)	McMaster-Carr	Cat#92196A113
Clear Scratch-/UV-Resistant Cast Acrylic Sheets, 6″ × 12″ × 1/8″	McMaster-Carr	Cat#8560K275
Clear Scratch-/UV-Resistant Cast Acrylic Sheets, 6″ × 12″ × 1/4″	McMaster-Carr	Cat#8560K359
Pipe and Conduit Thread Tap (1/4 NPT)	McMaster-Carr	Cat#2538A42
USB camera with 8MP SONLYCOMS sensor	Inswan	Model#INS-1
1/4 Tube Connector with Valve	Grainger	Cat#4DGP9

STEP-BY-STEP METHOD DETAILS
Soft lithography of PDMS with the mold

⊙ **Timing: 2–3 h**

These steps describe how to prepare PDMS and fabricate PDMS devices with the mold.

1. Prepare the mixture of PDMS and the curing agent at a 10:1 volume ratio (Cong and Pan, 2008; Mata et al., 2005).
   a. Put a weight boat on a scale and set it to zero.
   b. Pour PDMS into the weight boat using a stainless steel lab spatula.
   c. Pour the curing agent into the PDMS using a dropper.
   d. Vigorously stir the PDMS mixture using the spatula for 3 min.
2. Put the boat containing the PDMS mixture into the vacuum oven.
3. Set the vacuum oven pressure to 20 kPa at room temperature (25°C).
4. Degas the PDMS mixture for 30 min to ensure there is no air bubble formed at the surface of the PDMS mixture. Our vacuum oven is equipped with a front window, allowing us to observe the degassing process.
5. Prepare the resin-free, completely cured mold on the flat surface of a table.
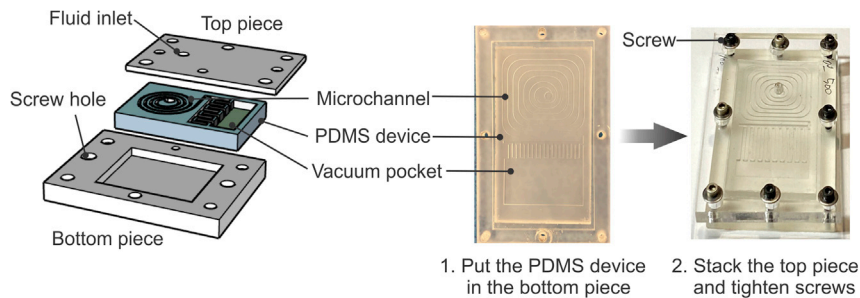
Figure 2. Assembling of the PDMS device with the acrylic holder

6. Pour the degassed PDMS mixture into the mold until the PDMS fills up.
7. Put the mold filled up with the degassed PDMS into the vacuum oven.
8. Set the vacuum oven pressure to 20 kPa at room temperature (25°C).
9. Degas the PDMS mixture in the mold for 30 min.
10. Change the vacuum oven pressure to 101.325 kPa (1 atm) and temperature to 85°C for curing.
11. Cure the PDMS mixture in the mold in the vacuum oven for 1 h.
12. Take out the mold from the oven and let it cool down to room temperature.
13. Take off the cured PDMS from the mold.

> △ CRITICAL: During the degassing process, the pressure in the vacuum oven should be adjusted properly. If the pressure is too low, the liquid in the weight boat (or any container) comes out together with the air bubbles. In contrast, the degassing process takes a long time to remove air molecules from the PDMS mixture at high pressure.

Assembling the PDMS device and the acrylic holder

⏱ Timing: 1 h

These steps describe the screw mechanism, caution, and alternative methods.

14. Set the bottom piece of the holder on a flat surface of the table.
15. Align the PDMS device in the center of the bottom piece (Figure 2).
16. Cover the PDMS with the top piece of the holder.
17. Align screw holes around the edge and the fluid inlet hole.
18. Insert screws into screw holes around the edge.
19. Loosely tighten all screws and then further tighten each screw with nuts in the zig-zag direction.

> △ CRITICAL: Because the 3D printed PDMS device is soft, special care should be taken during the assembly of the device using a screw mechanism. The overtightening of screws can squeeze and deform the PDMS device, leading to the failure of proper device operation. In contrast, loosely tightened screws allow air leaks, causing unsuccessful degassing of the vacuum pocket and uncontrollable fluid flow in the microchannel. Although the screw mechanism is a cheap and easy method to fabricate devices in the laboratory, this method needs hands-on experience. Instead, a hinge-like 4 side locking mechanism used in most food containers with airtight lids or clamps is an alternative way to improve the reliability of the device fabrication.

Video recording and device operation

⏱ Timing: 2–3 h

These steps describe the particular sequence of preparing the devices and performing the fluid trajectory measurements.

20. Set up a digital camera and light source for recording the fluid trajectory.
    a. Use a white background below the device for clear recording.
    b. Adjust the focus of the camera on the microchannel in the device.
21. Set the vacuum oven pressure to 50 kPa and temperature to room temperature (25°C).
22. Place the assembled device into the vacuum oven.
23. Degas the device for 1.5 h.
24. Place the degassed device under the camera.
25. Start recording in the camera.
26. Begin the experiment by dropping a fluid into the inlet of the device.
27. After completing the experiment, stop recording the camera.
28. Check the video recordings for the data analysis.

> ⚠ CRITICAL: Prepare the experimental setup for video recording before the final degassing process of the PDMS device, in order to avoid diffusion of air molecules into the PDMS in ambient conditions, which can cause deterioration of the vacuum power.

### Alternative method: Fabricating non-PDMS, self-powered microfluidic device using a removable vacuum battery

The following steps describe an alternative method to fabricate a self-powered microfluidic device using non-PDMS materials. Here, we have used the 3D printed materials to fabricate all parts of the device, which includes a microchannel embedded device body and a removable vacuum battery.

Design and 3D printing of the microfluidic device and vacuum battery

⊙ Timing: 2–3 days

These steps describe the fabrication, post-treatment, and assembling of parts.

29. Design the device body consisting of the microchannel and several holes.
    a. The top piece of the body includes holes for fluid inlet, screws, and vacuum battery attachment.
    b. The bottom piece of the body includes the microchannel for fluid flow and holes for screw assembly.
30. Design the vacuum battery to drive the fluid flow in the microchannel of the 3D printed, non-PDMS device. The vacuum battery includes the following components (Figure 3).
    a. Empty volume (space) to control the vacuum level.
    b. A vacuum pumping port on the top (or side) of the vacuum battery for attaching a hand pump.
    c. Open area on the bottom of the vacuum battery for the diffusion of air molecules in the microchannel through a thin PDMS membrane.
    d. Screw holes for attaching the vacuum battery to the device body.
31. Print the device body and vacuum battery design via the 3D printer.
32. Wash the 3D printed body and vacuum battery by following the washing step described above (Figure 1).
33. Dry them with a nitrogen gas stream.
34. Make threads on the bottom piece of the device body and vacuum battery.
    a. Hold the bottom piece or vacuum battery securely using a bench vise.
    b. Insert a tip drill and manually turn it gently to make threads.
35. Prepare a PDMS membrane (thickness of 500 μm).
    a. Prepare the mixture of PDMS and the curing agent at a 10:1 volume ratio as described above.
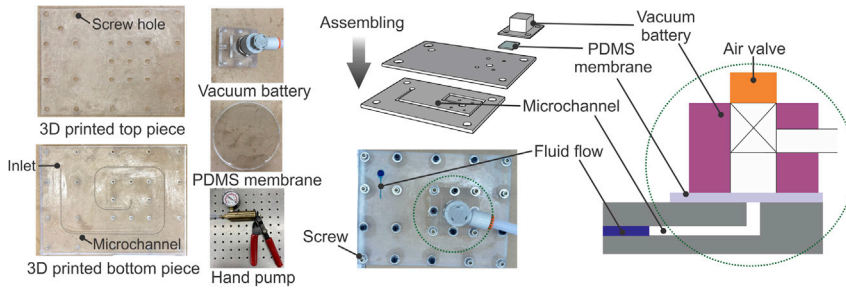
Figure 3. 3D printed, non-PDMS device parts and assembling, along with the removable vacuum battery

  b. Place down a polystyrene Petri dish upside down on the spinner chuck.
  c. Pour 30 g of the PDMS onto the Petri dish.
  d. Set the spin speed of the spin coater to 200 rpm and time to 30 s.
  e. Run the spin coater.
  f. When spinning is completed, take the Petri dish out from the spin coater.
  g. Set the vacuum oven pressure to 101.325 kPa (1 atm) and temperature to 85°C for curing.
  h. Cure the PDMS on the Petri dish in the vacuum oven for 1 h.
  i. Carefully peel off the cured PDMS membrane from the Petri dish.
  j. Keep the membrane clean before use.
36. Assemble the device body and vacuum battery (Figure 3).
  a. Place the bottom piece of the device body on a flat surface of the table.
  b. Evenly spread the vacuum grease on the surface.
  c. Stack the top piece of the device body on the top of the bottom piece.
  d. Make sure the alignment of all screw holes of the top and bottom pieces.
  e. Insert screws into the holes and tighten them up. Tighten screws properly as described above.
  f. Place the PDMS membrane on the hole for the vacuum battery.
  g. Stack the vacuum battery on the PDMS membrane.
 h. Insert screws and tighten them up. Tighten screws properly as described above.

  ⚠ CRITICAL: Ensure that all screws are properly tightened in the 3D printed materials. The overtightening of screws can crack or break the device during assembling, which allows an air leak like the case of loosely tightened screws. The diffusion of air molecules out of the microchannel reduces the power of the vacuum battery, responsible for driving the fluid flow in the microchannel.

Device operation and data acquisition

  ⏱ Timing: 1 h

These steps describe the particular sequence of preparing the devices and performing the fluid trajectory measurements.

37. Set up the CCD camera and light for recording the fluid trajectory as described above.
38. Attach a hand pump to the vacuum battery.
39. Squeeze the lever repeatedly to remove the air in the vacuum battery (<1 min).
40. When the vacuum gauge reaches 50 kPa, stop the squeezing.
41. Detach the hand pump from the vacuum battery.
42. Close the air valve to close the hole.
43. Put the degassed device under the camera.
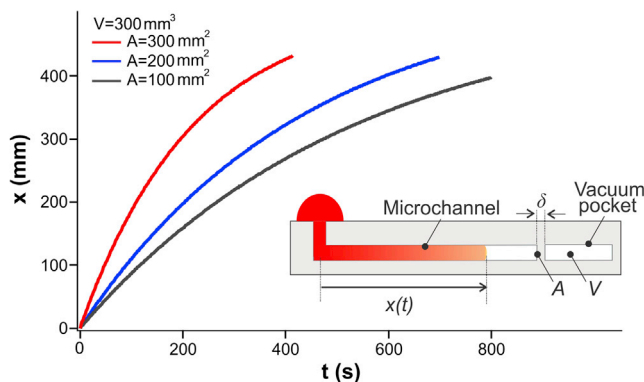
Figure 4. Typical time-dependent fluid dynamics driven by the vacuum pocket/battery

44. Start recording in the camera.
45. Begin the experiment by dropping a fluid into the inlet of the device.
46. After completing the experiment, stop recording the camera.
47. Check the video recordings for the data analysis.

> *Optional:* The removable vacuum battery could be attached to conventional microfluidic devices fabricated with silicon or glass materials. Furthermore, the vacuum battery could be used to drive the fluid flow for the PDMS-based microfluidic devices described above, instead of using the built-in vacuum pocket.

EXPECTED OUTCOMES

By following this protocol, two types of self-powered, microfluidic devices made of either PDMS or non-PDMS materials using the 3D printing method, rather than the optical lithography method, will be fabricated to carry out microfluidic experiments (Figures 2 and 3). Both devices operate without the need for an external power source such as a syringe pump or peristaltic pump. During the experiments, the fluid flow as a function of time will be recorded for further analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

The time-dependent fluid distance curves obtained from the experiments fit our molecular diffusion model given by (Woo et al., 2021).

$$x(t) = x_0\left(1 - e^{-tAD_0/\delta V}\right),$$

where t is time, x_0 is a terminal distance, D_0 is the diffusion coefficient of PDMS (Mäki et al., 2015), δ is the thickness of thin PDMS partition, A is the interfacial area between the microchannel and vacuum pocket/battery, and V is the volume of the vacuum pocket/battery. By fitting the curve to the model, kinetic parameters governing the flow dynamics can be extracted, which will be utilized to tune the fluid flow by adjusting the vacuum pocket/battery geometry, δ, A, and V. Figure 4 displays exemplary time-dependent flow distance curves fitted to the model with various A at given V. It should be noted that the flow of this protocol is not capillary-driven (Saha et al., 2009), but vacuum-driven. The PDMS and non-PDMS materials used here to fabricate devices are unmodified and untreated. Thus, the capillary action has no or finite effect on the fluid kinetics of the vacuum-driven, unmodified, hydrophobic PDMS and non-PDMS microfluidic devices (Ramalingam et al., 2016).

LIMITATIONS

Although the 3D printing method provides a great advantage for fabricating PDMS mold and non-PDMS microfluidic devices over the optical lithography method, the printing resolution and laser spot size of the 3D printer limit the overall resolution of the device structure including the microchannel. For example, we were able to reliably print a complex mold structure with a lateral resolution of

500 μm using the Form 3(+) printer. However, the 3D printed structure below 500 μm was inconsistent and unreproducible. Higher resolution printing depends on the complexity of the device design.

TROUBLESHOOTING

Problem 1
A little resin remains at the corners or narrow structure of 3D printed materials because the resin was incompletely polymerized by UV exposure (step 5 in the before you begin section).

Potential solution
After printing and cleaning, the 3D printed materials should be inspected thoroughly with a magnifying glass. Once liquid or gel-like residue on the surface is found, spray IPA at the residue to eliminate it. If the residue remains after IPA spraying, a long and sharp tip like a needle can be used to clean and remove the residue manually.

Problem 2
Breaking PDMS devices while taking them off from the mold (step 13).

Potential solution
To obtain intact PDMS devices without breaking, take the cured PDMS off from the mold carefully utilizing a flat and blunt end of a laboratory stainless steel spatula. Insert the spatula between the PDMS and the mold to make a small gap, then pull off the device completely from one corner.

Problem 3
No fluid flow in the microchannel of the PDMS-based microfluidic device (step 19).

Potential solution
The uneven pressure between the PDMS device and acrylic holder potentially creates a small gap, through which air molecules diffuse into the microchannel. Disassemble all parts and reassemble them with proper tightening of screws to keep the even pressure between the PDMS and holder.

Problem 4
Air leak due to broken thread in the vacuum battery (step 34).

Potential solution
Special care is needed during the thread fabrication for the pumping port in the vacuum battery body. Because the 3D printed materials and structures are not as hard as metals, chips generated during the tapping process could damage the thread. When tapping holes, turn the tap slowly back and forth direction frequently to remove chips and debris.

Problem 5
No fluid flow in the microchannel of the non-PDMS device with the vacuum battery (step 36).

Potential solution
Similar to the PDMS-based microfluidic device, the major cause of the problem could be the air leak of non-PDMS devices. Depending on the thickness of the 3D printed materials, they could be easily deformed. Examine the device thoroughly for deformation of the device. Alternatively, a thicker design of the 3D printing materials (> 5 mm) could prevent the deformation.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Yongki Choi (yongki.choi@ndsu.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any new code. The published article includes all details required for this study.

AUTHOR CONTRIBUTIONS

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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