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Highlights

Fabrication of
lithographically
defined multipore
structures in dielectric
membranes

Polydimethylsiloxane
flow cells for
facilitating liquid
processes

Characterization of
salinity gradient-
mediated ion
transport through
multipore
membranes

Optimizations of
multipore structures
for osmotic power
generators

Nanopore is an emerging energy-harvesting device that can create electricity directly from salt solutions. Here, we present a protocol for the preparation and structure optimization of solid-state multipore osmotic power generators. We describe steps for sculpting multiple pores at well-defined positions in a thin SiNx membrane using electron-beam lithography. We also detail an imprinting technique to form polydimethylsiloxane blocks with fluidic channels bonded to the multipore membrane. This approach facilitates repeated liquid-exchange processes involved in ionic current measurements.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Protocol

Protocol for preparation of solid-state multipore osmotic power generators

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SUMMARY

Nanopore is an emerging energy-harvesting device that can create electricity directly from salt solutions. Here, we present a protocol for the preparation and structure optimization of solid-state multipore osmotic power generators. We describe steps for sculpting multiple pores at well-defined positions in a thin SiNx membrane using electron-beam lithography. We also detail an imprinting technique to form polydimethylsiloxane blocks with fluidic channels bonded to the multipore membrane. This approach facilitates repeated liquid-exchange processes involved in ionic current measurements.
For complete details on the use and execution of this protocol, please refer to Tsutsui et al.[1]

BEFORE YOU BEGIN

The protocol below describes the steps for fabrication and performance optimization of SiN_x multipore osmotic power generators with NaCl solutions. This recipe may also be applicable for preparing the nanopore energy harvesters in other membrane materials and electrolytes.

Preparation for chrome photomask

⏱ Timing: 1–2 days

Preparation steps before starting the nanopore fabrication. You can also choose to skip these steps and use other procedures such as mask-less lithography to directly delineate the patterns on the wafer so as to create the photo masks (A), (B), and (C).

1. Chromium coating on a quartz substrate.
   a. Clean a commercial quartz substrate with *N,N*-dimethylformamide (DMF) in an ultrasonic bath for 15 min.
   b. Rinse the quartz in Isopropyl alcohol (IPA) and dry it with nitrogen.
   c. Set the cleaned quartz on a stage of the radio frequency (RF) magnetron sputtering system (Sanyu Electron SVC-700LRF) and wait until the vacuum level reaches 5×10^{-4} Pa.
   d. Deposit a sub-100 nm thick chromium layer at 50 W under an argon flow rate of 8 sccm.
   e. Air-leak the chamber and take out the sample.
2. Photolithography and chromium etching
   a. Apply 10 mL of photoresist AZ5214E on the chromium layer.

b. Spin-coat the photoresist at 3000 rpm for 60 s.

c. Pre-bake the photoresist on a hot plate at 100 degrees Celsius for 60 s.

d. Set the chrome mask to the maskless aligner with exposure dose of 100 $\mu C/m^2$ (MLA 150, Heidelberg Instruments).

e. Dip the substrate in an NMD-3 for 10–15 s for the development, rinse with water, and dry it with nitrogen.

f. Etch the exposed chromium with a mixture solution of $(NH_4)_2[Ce(NO_3)_6]$ and $HClO_4$ ($(NH_4)_2[Ce(NO_3)_6]$ 100 g, $HClO_4$ 20 mL, H_2O 600 mL) for 45 s.

Chromium etchant

Reagent	Amount
$(NH_4)_2[Ce(NO_3)_6]$	100 g
$HClO_4$	20 mL
H_2O	600 mL
Total	620 mL

Storage temperature: 10–20 degrees Celsius; storage maximum time: 2 days.

g. Rinse in water and dry it with nitrogen.

h. Clean the chrome photomask with UV cleaner (Samco).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
10× Phosphate buffered saline in liquid form	Nippon Gene	Cat# 314-90185
Potassium hydroxide	Aldrich	Cat#: 306568
SU-8 3000 photoresist	Microchem	N/A
SU-8 developer	Microchem	Cat# Y020100
PDMS (SYLGARD™ 184 Silicone Elastomer)	Dow	N/A
Ethanol	FUJIFILM Wako Pure Chemical Corporation	Cat#: 057-00451
Isopropyl alcohol (IPA)	FUJIFILM Wako Pure Chemical Corporation	Cat#: 169-28121
N,N Dimethylformamide (DMF)	FUJIFILM Wako Pure Chemical Corporation	Cat#: 045-02911
Acetone	FUJIFILM Wako Pure Chemical Corporation	Cat#: 019-00353
Quartz substrate	Shin-Etsu Chemical	N/A
Chromium etchant A: $(NH_4)_2[Ce(NO_3)_6]$	FUJIFILM Wako Pure Chemical Corporation	Cat#: 036-01742
Chromium etchant B: $HClO_4$	FUJIFILM Wako Pure Chemical Corporation	Cat: # 162-00695
Ag/AgCl Ink	BAS Inc.	Cat#: 011464
Pt electrodes	Nilaco	Cat#: PT-351481
AZ-5214E photoresist	Merck	N/A
Silica tube	AS ONE Corporation	CAT#: 5-4032-01
TSMR photoresist	Tokyo Ohka Kogyo Co., Ltd.	N/A
NMD-3 developer	Tokyo Ohka Kogyo Co., Ltd.	N/A
Electron beam resist	Zeon	ZEP-520A
Developer for ZEP-520A	Zeon	ZED-N50
Deionized (DI) water	Merck Millipore	Milli-Q ® IQ 7000 Ultrapure Lab Water System
Tetraammonium Cerium(IV) Sulfate Dihydrate	FUJIFILM Wako Pure Chemical Corporation	Cat#: 014-12292
Perchloric Acid	FUJIFILM Wako Pure Chemical Corporation	Cat#: 162-00695
Gold, Chrome, Silicon oxide, zinc oxide targets (3N)	FKD Factory	N/A
SiN_x-coated 4 inch silicon wafer	Electronics & Materials Co.	N/A

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
LabVIEW	National Instruments	N/A
Visual Basic 6.0	Microsoft	N/A
CAD software (AutoCAD)	Autodesk	version 2019
OriginPro	OriginLab Corporation	N/A
Other		
Spin coater	Misaka	MS-A150
UV cleaner	SAMCO	UV-1
Maskless aligner	Heidelberg Instruments	MLA 150
RF magnetron sputtering deposition system	Sanyu Electron	SVC-700LRF
Reactive ion etching system	Samco	RIE-10NR
Electron beam lithography system	Elionix	N/A
Automatic dicing saw	Disco	DAD322
Double-sided mask aligner	Union	PEM-800
LF plasma cleaner	Femto Science	CUTE
Picoammeter/Source	Keithley	6487
1 mm biopsy puncher	Kai Medical	BPP-10F
Dry Nitrogen	N/A	N/A
Oven	N/A	N/A
Hot plate	N/A	N/A
Ultrasonic bath	N/A	N/A
Optical microscope	Olympus	N/A
Scanning electron microscope	JEOL	7900

△ CRITICAL: Potassium hydroxide solution is highly alkaline. Wear protective gloves and glasses to avoid contact with skin and eyes.

DMF, ethanol, IPA, and acetone are flammable chemicals.

An aqueous solution of KOH is toxic.

One should wear protective equipment products (eye shields, face shields, and gloves) when using these chemicals.

Chromium etchant is a very strong oxidant and causes severe skin burns and eye damage.

Alternatives: General chemicals such as ethanol, IPA, and acetone can be purchased from other sources.

The instruments are also not restricted to those listed here but can be replaced by others of similar performances.
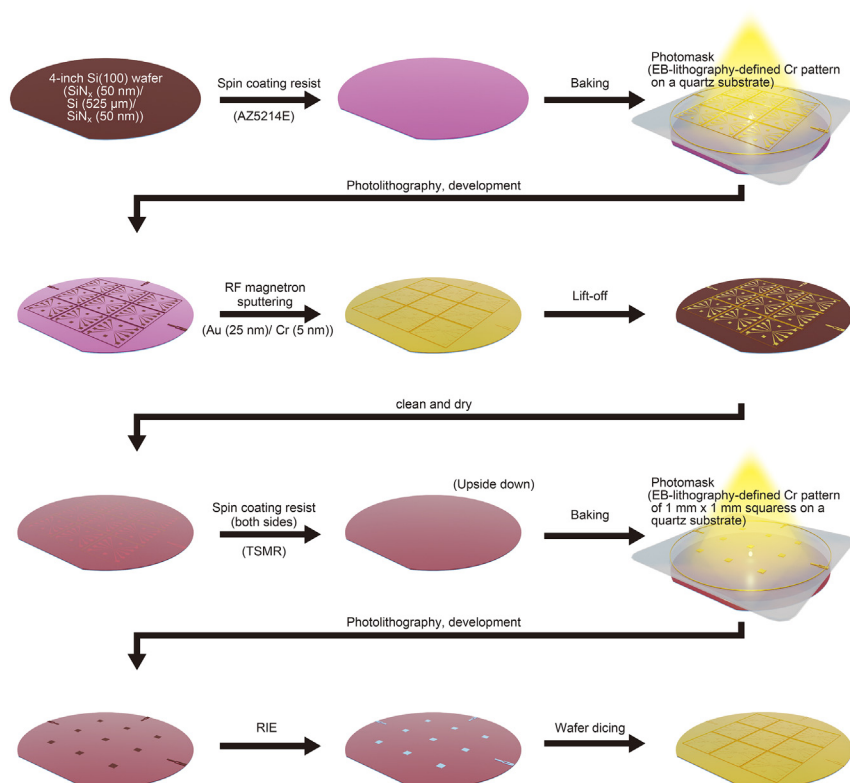
STEP-BY-STEP METHOD DETAILS
Part 1: Fabrication of multipore membranes

⊙ Timing: 12 days

The fabrication of multipore membranes follows the steps described in detail below that includes substrate cleaning processes in addition to the electron beam drawing and dry etching.

1. Form a metal pattern by photolithography (Figure 1).

Figure 1. Steps for forming metal patterns using photolithography

a. Apply 5 mL of photoresist AZ5214E to the 4-inch silicon wafer both sides coated with SiN_x layers by low-pressure chemical vapor deposition.

b. Spin-coat the photoresist at 5000 rpm for 60 s.

c. Pre-bake the photoresist on a hot plate at 100 degrees Celsius for 60 s.

d. Set the chrome photomask (A) to the double-sided mask aligner (Union PEM-800).

e. Place the silicon wafer on a stage of the mask aligner.

f. Lift the stage to make a contact between the photomask and the silicon wafer surface.

g. Irradiate with UV light for 10 s.

h. Post-bake the AZ5214E photoresist on a hot plate at 120 degrees Celsius for 60 s.

i. Dip the silicon wafer in an NMD-3 developer for 60 s, rinse it with water, and dry with nitrogen.

j. Set the silicon wafer on a stage of the RF magnetron sputtering system (Sanyu Electron SVC-700LRF) and wait until the vacuum level reaches 5×10^{-4} Pa.

k. Sputter a 5 nm thick Cr layer at 50 W under an argon flow rate of 8 sccm.

l. Sputter a 25 nm thick Au layer at 50 W under an argon flow rate of 8 sccm.

m. Air-leak the chamber and take out the sample.

n. Immerse the wafer in DMF overnight (10–12 h).

o. Lift off the photoresist by ultrasonication for 15 min.

p. Rinse the silicon wafer with IPA, ethanol, and acetone, and dry it with nitrogen.

q. Apply 5 mL of photoresist TSMR to the top side of the silicon wafer to protect the metal pattern.

r. Spin-coat the photoresist TSMR at 2000 rpm for 60 s.

s. Pre-bake the photoresist TSMR on a hot plate at 90 degrees Celsius for 90 s.

t. Repeat step 1(q) – 1(s) for the other side of the silicon wafer.

u. Put the metal-patterned silicon wafer face down on a stage of the mask aligner.

v. Irradiate the chrome photomask (B) with UV light for 10 s.
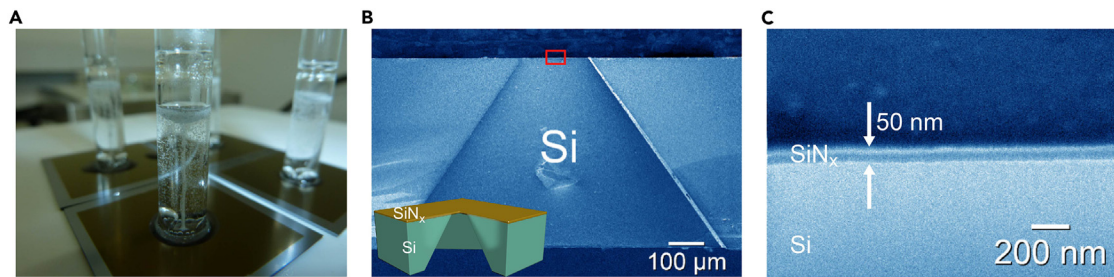
Figure 2. Steps for forming SiN$_x$ membranes by wet etching
(A) Si etching using high-temperature KOH solution.
(B) Cross-sectional view of the Si chip after the wet etching.
(C) A magnified view of the red rectangular region in (B) showing a 50 nm thick SiN$_x$ membrane.

  w. Post-bake the TSMR photoresist on a hot plate at 110 degrees Celsius for 90 s.

  x. Dip the silicon wafer in an NMD-3 for 10–15 s, rinse with water, and dry it with nitrogen.

  y. Remove the exposed 1 mm × 1 mm square region of the SiN$_x$ layer by RIE with CHF$_3$ etchant gas.

  z. Cut the silicon wafer with the dicer.

2. Forming a free-standing SiN$_x$ membrane by wet etching of the Si layer (Figure 2).

  a. Bond a silica tube on the SiN$_x$-removed surface of the silicon chip by pasting SYLGARD™ 184 and heating at 150 degrees Celsius for 10 min on a hot plate.

  b. Pour KOH 25 wt. % into the silica tube after cooling to below 50 degrees Celsius.

  c. Heat the hot plate to 80 degrees Celsius for about 3 h. Bubbles may come out due to the dissolution of Si in the KOH solution.

  d. Check whether the etching is complete by flashing light from the bottom.

  Note: If the Si is completely removed, you can clearly see the light coming through the square SiN$_x$ membrane. Do not attempt to look from the top of the silica tube directly while heating as the KOH vapor is hazardous.

  e. Take out the Si chip and remove the silica tube.

  f. Rinse the Si chip with DI water.

  g. Remove residual polydimethylsiloxane (PDMS) on the Si chip if there is any.

  h. Clean the Si chip in a water bath for 15 min.

  i. Rinse the Si chip with ethanol and acetone.

  j. Dry with nitrogen.

  k. Expose the photoresist on the other side to UV light for 10 s.

  l. Dip the Si chip in a developer.

  m. Rinse the Si chip with ethanol and acetone.

  n. Dry with nitrogen.

  o. Remove residual resist with a UV cleaner.

3. Patterning multipores by electron beam lithography.

  a. Apply 1 mL of electron beam resist ZEP520A to the Si chip surface with the metal pattern.

  b. Spin-coat the electron beam resist at 5000 rpm for 60 s.

  c. Pre-bake the photoresist on a hot plate at 180 degrees Celsius for 180 s.

  d. Load the Si chip on a stage of the electron beam lithography system (Elionix).

  e. Adjust beam alignment and focus.

  f. Define the position to delineate a multipore pattern by finding and setting the specific positions of the metal patterns of cross-like motifs at 8 μm away from the center.

  g. Start electron beam drawing (Dose: 120 μC/cm^2).

  h. Take out the Si chip and dip it into a developer.

    i. Dry with nitrogen.
4. Opening multipores by reactive ion etching.
   a. Load the Si chip in RIE-10NR, Samco.
   b. Purge the chamber with etchant gas RIE.
   c. Etch-out the SiN_x under 100 W via the ZEP520A mask.
   d. Take out the Si chip and keep it in DMF overnight (10–12 h) to remove the residual resist.
   e. Rinse with IPA, ethanol, and acetone.
   f. Dry with air.
   g. Check the structure by SEM observation to confirm that the nanopores are opened (optional).
   h. Store the chip in a vacuum dry desiccator.

⚠ CRITICAL: All the steps for the photolithography should be performed in a clean room with yellow illumination. When checking the multipore structures with SEM, one should not observe the pores at high magnifications nor capture the high-resolution micrographic images as it may contaminate the membrane surface. One should wear protective equipment products such as eye shields, face shields, and gloves when using DMF, KOH solution, and other organic solvents.

Part 2: Preparation for ionic current measurements

⏱ Timing: 2 days

The steps below are for preparing conduits to facilitate the liquid flow into the multipores, which include mold fabrication and PDMS curing (Figure 3).

5. Manufacturing an SU-8 mold for PDMS flow cell.
   a. Spin-coat SU-8 3000 on a 4-inch Si wafer at 2000 rpm for 60 s.
   b. Pre-bake the Si wafer at 90 degrees Celsius for 1 h.
   c. Set a metal mask (C) to the double-Sided Mask Aligner (PEM-800, Union).
   d. Put the Si wafer on the stage.
   e. Lift the stage to make a contact between the metal mask and the membrane surface.
   f. Irradiate with UV light for 10 s.
   g. Soft-bake the SU-8 3000 on a hot plate at 90 degrees Celsius for 30 min.
   h. Dip the Si wafer in SU-8 developer and rinsing with IPA.
   i. Dry with nitrogen.
   j. Hard-bake the SU-8 3000 on a hot plate at 150 degrees Celsius for 15 min.
6. Preparation of Ag/AgCl electrodes
   a. Cut a Pt wire of 0.8 mm diameter into about 20 mm long rods.
   b. Take out the Ag/AgCl paste from a refrigerator and wait until it returns to room temperature (10–25 degrees Celsius).
   c. Paste the Ag/AgCl on the tips of the Pt rods.
   d. Heat the Ag/AgCl-pasted Pt rods in an oven at 180 degrees Celsius for 6 h.
7. PDMS curing
   a. Put the SU-8 mold on a petri dish.
   b. Mix the base and curing agent of SYLGARD™ 184 at 10 to 1 weight ratio in a plastic container.
   c. Place the container in a desiccator and evacuate for 1 h to remove bubbles.
   d. Pour the mixture solution on the SU-8 mold.
   e. Put the petri dish in an oven and heat to 90 degrees Celsius for 8 h for solidification.
8. PDMS bonding
   a. Cut out a 20 mm × 20 mm block from the PDMS on the SU-8 mold with a laser knife.
   b. Punch holes at three locations of the I-trench with a 1 mm biopsy puncher.
   c. Prepare another PDMS block by repeating steps 8(a) – 8(b).
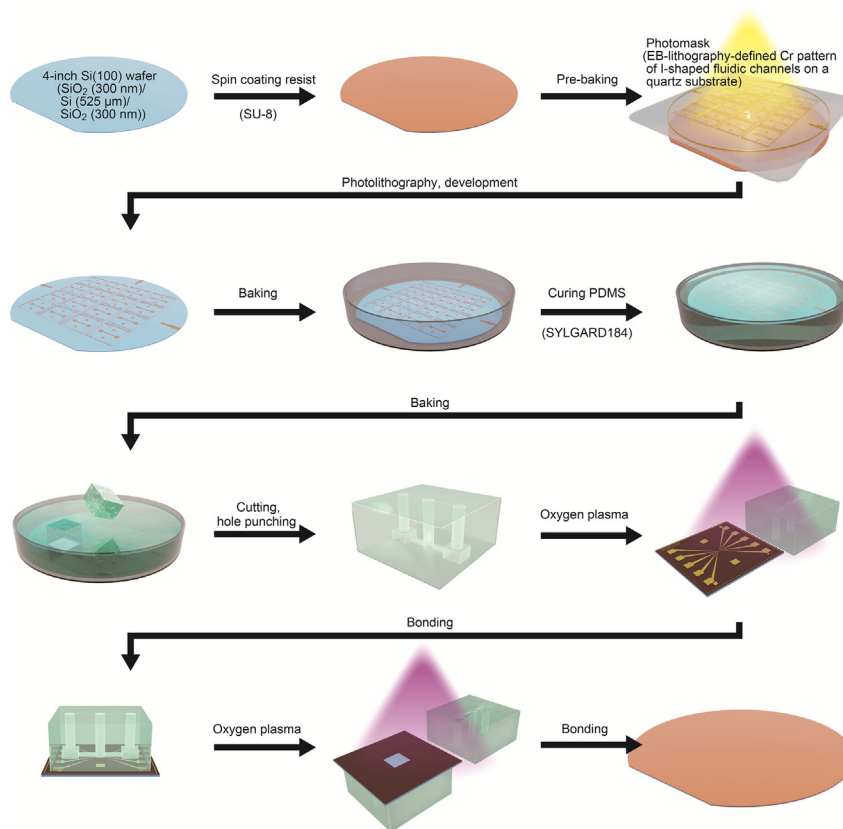
Figure 3. Steps for forming PDMS blocks

d. Put one of the PDMS blocks and the multipore chip in the oxygen plasma system FEMTO (Power: 50 W; Exposure time: 30 s).
e. Expose them to oxygen plasma at 100 W under a gas flow rate of 10 sccm for 30 s.
f. Take out the samples and bond them together under an optical microscope.
g. Repeat steps 8(d) – 8(f) to bond the other PDMS block at the other side of the multipore chip.

Part 3: Evaluation of ion selectivity in single nanopore

© Timing: 2 days

This section describes the steps for the evaluation of ion selectivity in nanopores. For this, single pores of various diameters from 10 μm to 20 nm are formed by the aforementioned electron beam lithography-based fabrication procedure. After bonding the PDMS blocks, the ionic current through these pores is measured followed by data analyses (Figure 4).

9. Nanopore structure analysis by open pore conductance measurements.
   a. Insert two Ag/AgCl rods in one of the holes in the PDMS blocks.
   b. Add 10 x phosphate-buffered saline (PBS) at both sides of the membrane through the remaining holes in the PDMS blocks (PBS is in liquid form and used as purchased).
   c. Out the membrane chip on a stage in the Faraday cage.
   d. Connect the Ag/AgCl electrodes to the picoammeter/source Keithley 6487.
   e. Measure the ionic current under several cycles of voltage scans from -1 V to 1 V at 26 V/s with 2 mV steps.
   f. Estimate the open pore conductance by a linear fit to the ionic current versus voltage curves.
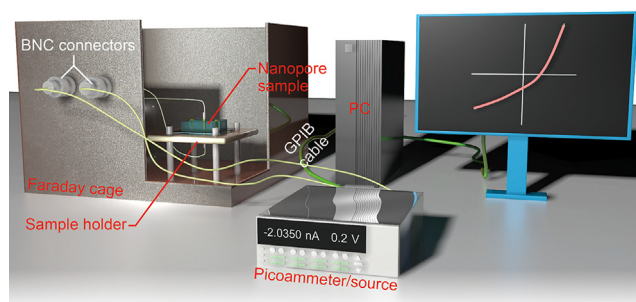
Figure 4. Ionic current measurement setup

g. Compare the open pore conductance to that predicted by the Maxwell's model to confirm whether the nanopore is of the right size.

Note: If the conductance is higher by more than a factor of 2, it is regarded as a rupture of the thin membrane. On the other hand, it may also happen that the conductance is nearly zero in the case when the pore is not opened or not wettable enough to admit water flow, which is more often the case for nanopores smaller than 100 nm. In such cases, one should replace the nanopore chip to a new one at this point.

10. Measure the salt concentration gradient-mediated ionic current characteristics (Figure 5).
    a. Take out one of the Ag/AgCl electrodes from the PDMS block.
    b. Prepare 5 x PBS by diluting 10 x PBS with DI water.
    c. Pour 1 mL of 5 x PBS several times through the holes so as to ensure that the salt concentration is changed from that of 10 x PBS.
    d. Flow 100 mL of 10 x PBS at the other side of the membrane.
    e. Measure the ionic current under several cycles of voltage scans from -1 V to 1 V at 26 V/s with 2 mV steps.
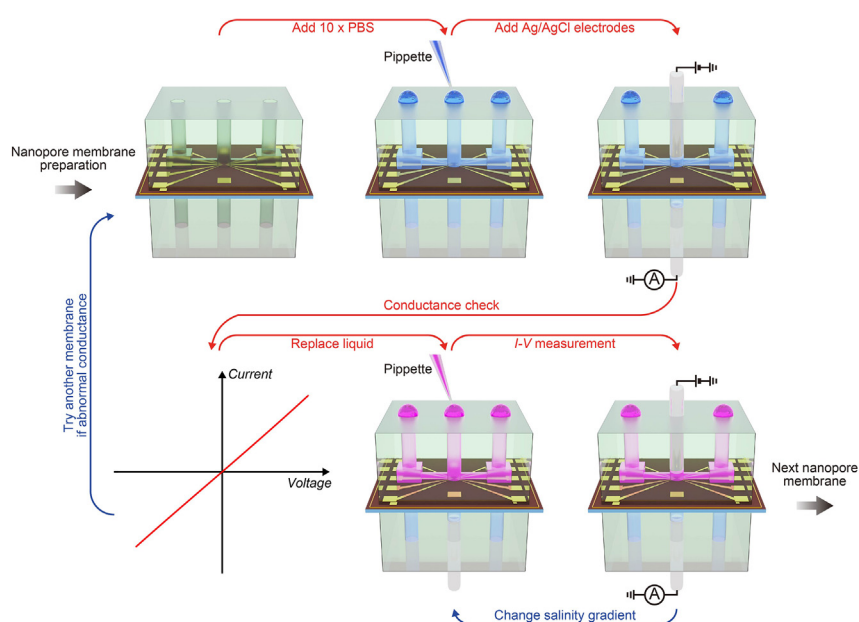
Figure 5. Steps for characterizing ion transport properties of multipore membranes

    f. Repeat step 10(a) – 10(d) for 2 x PBS, 1 x PBS, 0.5 x PBS, 0.2 x PBS, 0.1 x PBS, 0.05 x PBS, 0.02 x PBS, and 0.01 x PBS.

11. Estimate ion selectivity.

    a. Repeat 9 for pores of diameters 10 μm, 3 μm, 1 μm, 300 nm, 100 nm, 50 nm, 30 nm, 20 nm, and 10 nm.

    b. Obtain the open circuit voltage from the intersects at zero current in each ionic current versus voltage curves measured.

    c. Create semi-log plots of the open circuit voltage as a function of the salt concentration ratio at both sides of the membranes.

    d. Confirm that the plots are nearly the same for the 10 μm and 3 μm.

    e. Use the open circuit voltage of the 10 μm micropore as the electrode potential.

    f. Subtract the electrode potential from the open circuit voltage to obtain the osmotic potential.

    g. Create semi-log plots of osmotic potential as a function of the salt concentration ratio at both sides of the membranes.

    h. Evaluate the ion selectivity from the slopes of the semi-log plots.

Part 4: Multipore structure optimizations

⏱ Timing: 2 days

This section describes the steps for the optimization of multipore structures for osmotic power generation. For this, various numbers of nanopores of researcher-defined size and shape are sculpted at a specified area of the dielectric membrane via the aforementioned electron beam lithography-based fabrication procedure. For instance, the multipore layout can be 2 × 2, 4 × 4, 6 × 6, 8 × 8, 10 × 10, 20 × 20, and 40 × 40 in a 10 μm square region in a SiN_x membrane with constant distances between the neighboring pores. After bonding the PDMS blocks, the ionic current through these pores is measured followed by data analyses.

12. Measure the ionic current characteristics of the multipore membrane.

    a. Insert two Ag/AgCl rods in one of the holes in the PDMS blocks.

    b. Add 10 x PBS at both sides of the membrane through the remaining holes in the PDMS blocks.

    c. The membrane chip is put on a stage in the Faraday cage.

    d. Connect the Ag/AgCl electrodes to the picoammeter/source Keithley6487.

    e. Measure the ionic current under several cycles of voltage scans from -1 V to 1 V at 26 mV/s with 2 mV steps.

    f. Estimate the open pore conductance by a linear fit to the ionic current versus voltage curves.

    g. Compare the conductance to that predicted by the Maxwell's model to confirm whether the multiple pores are formed as designed.

Note: If the conductance is higher by more than a factor of 2, it is regarded as a rupture of the thin membrane. On the other hand, it may also happen that the conductance is anomalously low in the case when a part of the whole number of the pores are not opened or not wettable enough to admit water flow, which is more often the case for nanopores smaller than 100 nm. In such cases, one should replace the nanopore chip with a new one at this point.

    h. Take out one of the Ag/AgCl electrodes from the PDMS block.

    i. Pour 1 mL of 0.01 x PBS several times through the holes so as to ensure that the salt concentration is changed from that of 10 x PBS.

    j. Flow 100 mL of 10 x PBS at the other side of the membrane.

    k. Measure the ionic current under several cycles of voltage scans from -1 V to 1 V at 26 V/s with 2 mV steps.
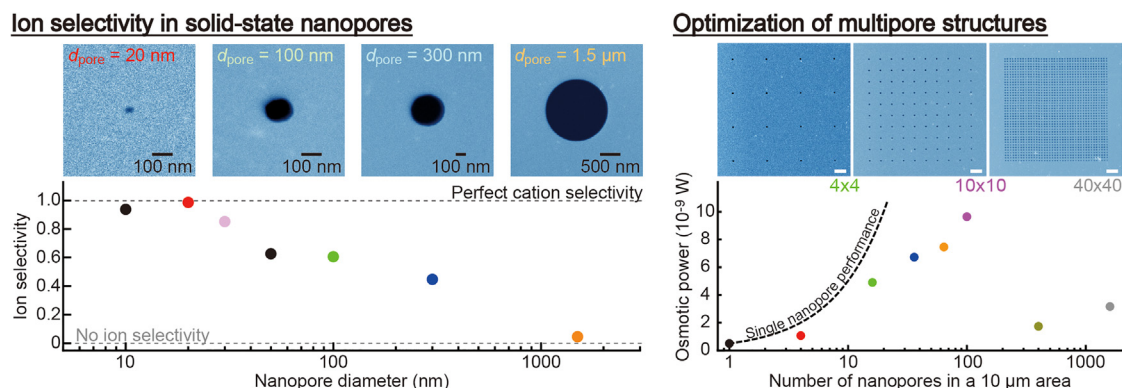
Figure 6. Expected results
Scale bars in the scanning electron micrographs of multipore structures denote 1 μm. Adapted with permission from Ref.,[2] Elsevier.

13. Finding an optimal multipore design for osmotic power generation.
    a. Repeat 13 for multipores of different number densities (e.g., 2 × 2, 4 × 4, 6 × 6, 8 × 8, 10 × 10, 20 × 20, and 40 × 40 in a 10 μm square region) as well as single 10 μm micropore for the calibration of the electrode potential.
    b. Subtract the electrode potential from the voltage data for the multipore membranes.
    c. Obtain the osmotic voltage and current from the intersects at zero current and voltage, respectively, in each ionic current versus voltage curve measured.
    d. Calculate the osmotic power as products of the osmotic voltage and current.
    e. Plot the osmotic power as a function of the number of pores in the membrane.
    f. Find an optimal number density of multipores with respect to the osmotic power output.

EXPECTED OUTCOMES

Based on this protocol, multiple nanopores of well-defined diameters and spacing are formed in a dielectric membrane on a silicon wafer, and its ion selectivity as well as the osmotic power density are evaluated (Figure 6). It provides the maximal osmotic power density of 25 W/m^2 with 100 nm-sized multipores at 1% porosity in a 40 nm thick SiN$_x$ membrane.[1]

LIMITATIONS

Fabrication of nanopores smaller than 20 nm is difficult with this protocol. Also, it may not be applicable for membranes thicker than 100 nm due to that the electron beam resist cannot endure the RIE for opening pores.

TROUBLESHOOTING
Problem 1
Membrane size variation (Part 1).

Potential solution
The membrane becomes larger over time during the KOH etching of Si. To suppress the over-etching, one can check whether the membrane is formed by shining light from the membrane side. If the membrane is formed, a square-like pattern can be found on the other side as the thin SiN$_x$ transmits the light. On contrary, when Si is not removed completely, it blocks the light and so cannot see such a pattern under light irradiation. Caution: never look from above when the chip is still at high temperature since the vapor of the highly-alkaline solution is hazardous.

Problem 2
Membrane rupture (Part 1).

Potential solution

SiN$_x$ membranes may rupture during the fabrication and measurement processes depending on the low-pressure chemical vapor deposition conditions. If the residual pressure in the membrane is appropriate, they are tough enough to endure the nitrogen blowing for cleaning and the liquid injections during the ionic current measurements as long as the thickness is larger than 20 nm. When they are thinner than 10 nm, the membranes become corrugated and easy to break. In either case, never put them for ultrasonication.

Problem 3

Contamination of nanopores during SEM observations (Part 1).

Potential solution

One can use SEM to check the size and shape of nanopores. However, the surface tends to become contaminated when irradiated by the electron beam that usually shrinks the pore[2] and also changes the surface properties. Therefore, it should be avoided to observe them at high magnification for a long time, or check the pore structure only by the open pore conductance in a well-defined electrolyte solution.

Problem 4

Sticking PDMS blocks on an SU-8 mold (Part 2).

Potential solution

Cleaning of an SU-8 mold is an important step. However, never try to clean the surface with a plasma treatment. Although it helps for the cleaning, it also activates the surface so that the cured PDMS becomes strongly bonded on the mold and never be cut out.

Problem 5

Mis-aligned PDMS channel on the nanopore membrane (Part 2).

Potential solution

Always use a microscope of appropriate magnification when bonding the PDMS block on the nanopore chip. Significant misalignment results in complete sealing of the pore by the polymer. It is also not recommended to try the bonding more than one time as it raises the risk of liquid leakage from the PDMS channels.

Problem 6

Buffer leakage due to weak bonding of PDMS blocks to the nanopore chip (Part 2).

Potential solution

Optimize the conditions of the oxygen plasma treatments. Generally, the surfaces of the nanopore chip and the PDMS block may not be activated sufficiently when the power to generate the plasma is too weak and/or the exposure time is too short. At the same time, too strong power may also damage the PDMS surface that also leads to weak bonding to the SiN$_x$ surface. We add to note that the optimal power and the exposure time may vary depending on the facility used.

Problem 7

Air bubbles in PDMS channels and/or the Si trench (Part 3).

Potential solution

Flow buffer with pipette several times until the air bubbles disappear. It is also important to put the buffer as swiftly as possible after the PDMS bonding as the polymer and the SiN$_x$ surfaces become more hydrophobic over time, which makes it more likely to have the bubbles in the channels.

Problem 8

No ionic current (Part 3).

Potential solution

Make sure the cables are connected to the right places. Try re-inserting the Ag/AgCl electrodes into the PDMS blocks. Try flowing buffer with a pipette so as to remove air bubbles as well as contaminants such as dust particles.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Makusu Tsutsui (tsutsui@sanken.osaka-u.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

No data or code were generated in this study.

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

Conceptualization, M.T., T.K.; methodology, M.T., K.Y.; investigation, M.T., K.Y., Y.H.; writing – original Draft, M.T., T.K.; writing – review & editing, M.T., T.K.; funding acquisition, M.T., K.Y.; resources, M.T., K.Y.; supervision, T.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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