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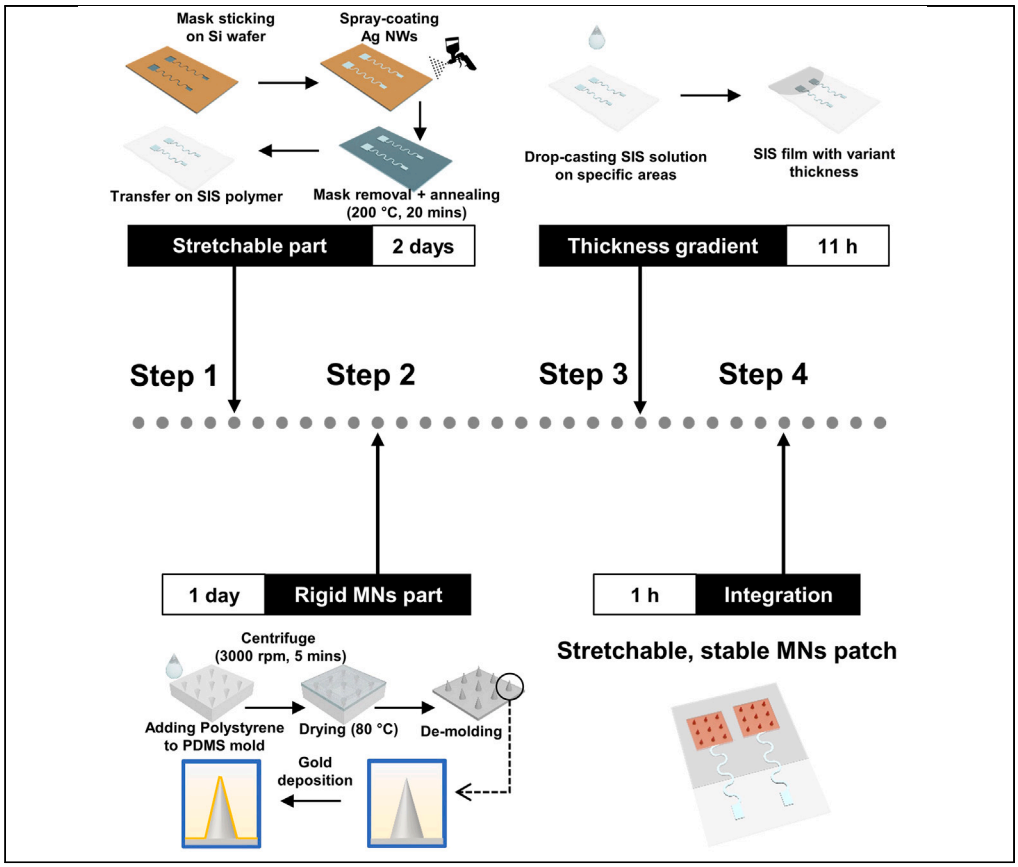
Protocol to fabricate wearable stretchable microneedle-based sensors



Creating highly stretchable and robust electrodes while retaining conductivity and stability is challenging. Furthermore, combining these elastic parts with rigid ones brings its own problems due to the discrepancy in firmness between the flexible patches and rigid constructions. Here, we present a protocol to create a stable, conductive, and flexible microneedle sensor patch. We describe steps for using polystyrene-block-polyisoprene-block-polystyrene with silver nanowires, besides fabricating rigid microneedles and combining them together using a thickness-gradient strategy.

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

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Highlights

Step-by-step protocol to fabricate stretchable microneedles (MNs)-based sensors patch

Procedures for fabricating the stretchable soft polymer substrate

Procedures for fabricating stretchable Ag NWs electrodes and rigid MNs

Integration of rigid MNs with soft substrate by thickness-gradient strategy

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Protocol to fabricate wearable stretchable microneedle-based sensors

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SUMMARY

Creating highly stretchable and robust electrodes while retaining conductivity and stability is challenging. Furthermore, combining these elastic parts with rigid ones brings its own problems due to the discrepancy in firmness between the flexible patches and rigid constructions. Here, we present a protocol to create a stable, conductive, and flexible microneedle sensor patch. We describe steps for using polystyrene-block-polyisoprene-block-polystyrene with silver nanowires, besides fabricating rigid microneedles and combining them together using a thickness-gradient strategy.

For complete details on the use and execution of this protocol, please refer to Zheng et al. (2022).[1]

BEFORE YOU BEGIN

This protocol describes the specific steps of fabricating a stretchable, conductive substrate by utilizing a soft polystyrene-block-polyisoprene-block-polystyrene (SIS) polymer with silver nanowires (Ag NWs) on the one hand, and forming rigid metal nanostructures (microneedles [MNs]) coated with gold, on the other hand. Subsequently, the two components are incorporated using a thickness-gradient technique to form a wearable, conformable, and stretchable MNs sensor patch. Our previous study has created an extended-gate field-effect transistor biosensor with stretchable and skin-conformal MNs that enables real-time and minimally invasive monitoring of sodium levels in the interstitial fluid (ISF). This biosensor presents outstanding sensitivity, fast response, and mechanical stability while worn on the body.[1] Developments in wearable and stretchable electronics have garnered significant interest in advanced healthcare technologies. Presently, the devices utilized for health monitoring tend to be cumbersome, heavy, pricey, and inflexible, thus rendering them ill-suited for long-term monitoring, skin compatibility, and patient comfort.[2] To tackle this issue, researchers have produced flexible and stretchable electronics using soft materials such as thermoplastic elastomers, silicones, and conductive hydrogels with greater conformability to the skin and higher adaptability.[3–6] These electronics provide a better interface between the skin and the device, which makes them a perfect fit for wearable applications, such as biomechanical sensors, e-skin for diagnostics and therapy, and allow for the real-time tracking of bodily signals such as chemical, biological, and physical indicators.[1,7–22] The method we propose in this protocol is simple, easy, cost-effective, and provides a practical solution to a significant challenge that can be applied in real-life applications. Moreover, it doesn't require complex procedures or heavy instruments, making it affordable and feasible to implement without the need for special expertise. This approach can also be used to create secure patches by uniting rigid and soft parts, leading to the development of sweat sensors made with flat electrodes instead of needles, forming dependable sweat sensor

patches. Additionally, attaching other inflexible electrical components with wearable devices, such as batteries, transistors, resistors, and more, can be accomplished with the procedure described in this protocol, generating more durable and firm structures for long-term utilization. The methods in this protocol can be applied to different diagnostic applications by modifying the MNs with diverse recognition elements, for example, enzymes, antibodies, and selective membranes.[23,24] In addition, the protocol can be employed to make MNs on pliable patches for drug administration and treatment uses, transcending diagnostic functions, thus enlarging the variety of potential applications.[9,25–27]

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Polystyrene-block-polyisoprene-block-polystyrene (SIS), styrene 22 wt %	Sigma-Aldrich	CAS: 25038-32-8
Toluene	Bio-Lab, Ltd.	CAS: 108-88-3
N,N-dimethylformamide (DMF)	Bio-Lab, Ltd.	CAS: 68-12-2
Polydimethylsiloxane (PDMS) - Sylgard 184 Silicone Elastomer Kit	Dow, Inc.	CAS: 9006-65-9
Trichloro(hexyl)silane, 97%	Sigma-Aldrich	CAS: 928-65-4
Acetone	Bio-Lab, Ltd.	CAS: 67-64-1
Ethanol	Bio-Lab, Ltd.	CAS: 64-17-5
Isopropyl alcohol (IPA)	Bio-Lab, Ltd.	CAS: 67-63-0
Poly(vinylpyrrolidone) (PVP)	Sigma-Aldrich	CAS: 9003-39-8
Ethyl glycol (EG)	Bio-Lab, Ltd.	CAS: 107-21-1
Silver nitrate ($AgNO_3$)	CARLO ERBA	CAS: 7761-88-8
Chloroform	Bio-Lab, Ltd.	CAS: 67-66-3
1H,1H,2H,2H-perfluorodecanethiol	Sigma-Aldrich	CAS: 34143-74-3
Polystyrene (MW: 280,000)	Sigma-Aldrich	CAS: 9003-53-6
Other		
Laser cutter (VLS 4.60)	Universal Laser Systems (ULS)	https://www.ulsinc.com/product-matrix
Plasma system FEMTO PCCE	Electronic diener	https://www.plasma.com/en/low-pressureplasma-femto/
Centrifuge	Thermo Fisher Scientific	https://www.fishersci.be/shop/products/sl-16-centrifuge-series/p-4527753
Ultrasound bath	Elma, Elmasonic S30H	NA
Digit Multimeter	Agilent, 34411A	NA
Spin coater	Laurell	https://www.laurell.com/spin-coater/?model=WS-400-6NPP
Milli-Q water (18.2 MΩ cm [25°C])	Millipore, Bedford, MA, USA	https://www.merckmillipore.com/INTL/en/product/Direct-Q-3-UV-Water-Purification-System,MM_NF-ZRQSVP3WW,MM_NF-ZRQSVP3WW
Hot plate stirrer	VELP SA20510061 AREC.X	NA
Vacuum drying oven	Electrotherm group, model DZF	NA
Light microscope	Olympus, model BX51M	NA
E-beam evaporation system	Evatec, cat. no. BAK-501	NA

MATERIALS AND EQUIPMENT

Reagent	Final concentration	Amount
SIS in toluene	100 mg/mL	100 mL
Ag NWs in IPA	10 mg/mL	10 mL
Polystyrene in DMF	200 mg/mL	80 mL
Note: The solutions can be stored at 25°C for 12 months		

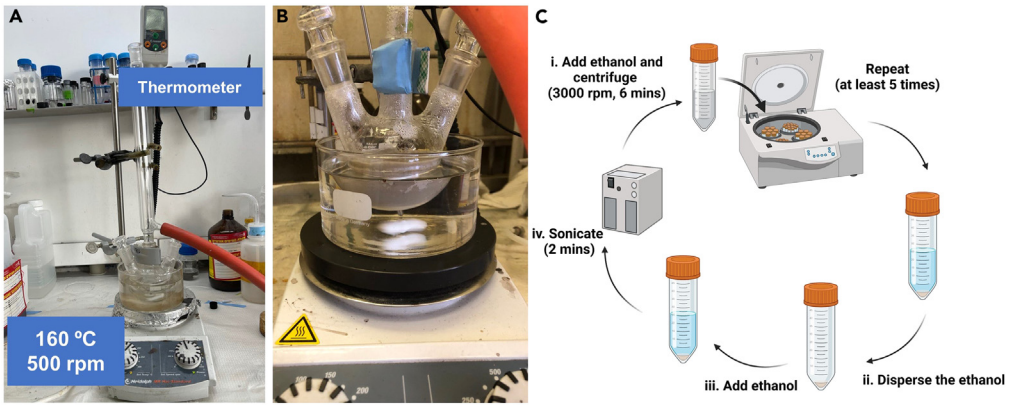

Figure 1. Synthesis and purification procedure of the Ag NWs

(A) The setting of the synthesis system, including all required parts.

(B) The final step in the synthesis to obtain the gray-silver product.

(C) Schematic of the purification procedures of the Ag NWs (The illustration was created with BioRender.com).

STEP-BY-STEP METHOD DETAILS

Note: The fabrication procedure of the wearable stretchable MN-based patch comprises four main steps. First, the fabrication of the soft stretchable part involves preparing the electrodes by fabricating Ag NWs using a mask through spray coating techniques and annealing at 200°C to achieve conductive electrodes. These electrodes are then transformed into the stretchable SIS substrate. Second, the rigid MNs part is fabricated. This involves designing and preparing PDMS molds using a laser cutter, applying a polymeric solution (polystyrene), drying at 80°C, and demolding to obtain the rigid polymeric MNs templates. A gold deposition is then applied to the electrodes to prepare different biosensors through various modifications. Third, the SIS film is updated using a thickness-gradient strategy. This ensures that the side electrodes are included in the thin part, while the rigid MNs component is loaded onto the thick part. Fourth, the stretchable film created using the thickness-gradient strategy is integrated with the rigid MNs component to obtain a stable, wearable stretchable MN-based patch. In each step, the material types can be optimized and changed based on the requirements. For example, different types of soft, stretchable, and flexible polymers (e.g., styrene ethylene butylene styrene) can be used for fabricating stretchable patches. Various materials can be used for fabricating the electrodes, such as carbon nanotubes and gold nanowires. The thickness-gradient strategy can be employed to combine different types of rigid structures for diverse applications. Additionally, the rigid component can include other types of structures, such as flat components or structures made of different materials apart from polystyrene polymers, including polycaprolactone, polylactic acid, and others. Furthermore, the MNs can be deposited with metals other than gold, such as silver, platinum, and nickel, expanding their range of applications.

Fabrication of Ag NWs

Timing: 2 days

The synthesis of Ag NWs included purification and modification with thiol. The following steps demonstrate the procedure of synthesizing Ag NWs, purification steps, and thiol modification, as shown in Figure 1.[28]

1. Wash all the glassware with IPA, acetone, and ethanol, then dry in the oven at 80°C for 10 min.

CAUTION: Wear gloves and goggles, and handle under a chemical hood.

2. Measure 2.49 gr of PVP, put it in a beaker, and mix it with 40 mL of EG. Add a stir bar, place on a hot plate at 60°C, and cover with aluminum foil.

   Note: Wait for solids to dissolve. It can take 10–30 min.

3. Measure 2.54 gr of $AgNO_3$, put it in a beaker, and mix it with 10 mL of EG. Add a stir bar and place on a hot plate at 60°C.

   Note: Wait for solids to dissolve. It can take 10–30 min.

4. Prepare $FeCl_3$ and NaCl solution:
   a. 43.83 mg $FeCl_3$ + 5 mL purified water
   b. 43.83 mg NaCl + 5 mL purified water
5. Prepare a three-neck round bottom flask stand.
   a. Half submerge the three-neck round bottom flask in silicon oil.
   b. Heat to 160°C, and stir constantly using a stir bar at 500 rpm.

   Note: Be sure to set the thermometer to 160°C to monitor the temperature continuously.

   ⚠ CRITICAL: When heating the round flask at 160°C, the temperature should be controlled and monitored using a thermometer. Be careful not to touch or get too close to the hot plate and the silicon oil to avoid injuries and burns.

CAUTION: Handle under a chemical hood and be careful of the hot plate.

6. Add a condensation tube on the top of the three-neck round bottom flask and flush dry air inside.
7. Add the EG + PVP solution, including the stir bar, to the three-neck round bottom flask and stir until it reaches 160°C.
8. When the temperature reaches 160°C, add 100 μL $FeCl_3$ to the flask and wait 5 min.
9. After 5 min, add 100 μL NaCl.
10. Add the $AgNO_3$ + EG solution in 15 μL/s increments.
11. After adding all the $AgNO_3$ + EG solution, wait 1.75 h for the reaction.
12. Stop heating and let cool for 15 min (keep stirring).
13. Move the reacted solution to a clean beaker and add 200 mL methanol to stop the reaction.

    Note: Keep the solution in the hood until it cools down to room temperature (20°C–25°C).

    ⏸ **Pause point:** The solution should be kept in a chemical hood until it reaches room temperature (20°C–25°C).

14. Divide the solution into plastic tubes and add ethanol.
    a. Shake the tubes and sonicate for 2 min.
    b. Centrifuge the tubes at 1006 × *g* for 6 min, 25°C.
    c. Disperse the solvent and add new ethanol.
    d. Wash 5 times at least to obtain pure Ag NWs.

CAUTION: Discharge the solvent waste in a proper chemical waste in a chemical hood.

15. Spray some Ag NWs onto A4 paper and check the conductivity. Ohm (Ω) or kilo ohm (KΩ) is good.

CAUTION: Perform the spray coating of Ag NWs under a chemical hood.

16. Weight 28 mL glass jars (without cups) and write down the weight of each jar for later calculating the Ag NWs weight.
    a. Drain the waste after the last centrifuge run.
    b. Add around 3 mL of ethanol to clean the plastic tubes of silver and transfer it to the glass jars.

Note: Make sure not to fill the glass jars to the top. Fill each jar to 1/4 of its max.

17. Dry ethanol in the glass jars using a vacuum oven at 50°C for 2–4 h.
18. Calculate the Ag NWs' weight.

Ag NWs thiol modification

19. Suspend 50 mg of dry Ag NWs in 5 mL ethanol/chloroform 1:1 V/V (10 mg/mL) and sonication for 5 min.
20. Add 5 μL of the thiol group 1H,1H,2H,2H-perfluorodecanethiol (10 mg Ag NWs: 1 micron thiol) to obtain Ag NWs modified with 1H,1H,2H,2H-perfluorodecanethiol (Ag NWs-F)
21. Sonicate the Ag NWs-F solution for 10 min.
22. Heat the solution to 80°C–90°C and stir overnight for 12–14 h.

CAUTION: Handle under a chemical hood and be careful of the hot plate.

23. Centrifuge and wash three times with ethanol/chloroform solution: Sonicate (2 min), centrifuge (1006 × *g* for 6 min, 25°C).

CAUTION: Wear gloves and goggles and handle under a chemical hood while using chloroform.

24. Weight 28 mL glass jars (without cups) and write down the weight of each jar for later calculating the modified Ag NWs' weight.
    a. Drain the waste after the last centrifuge run.
    b. Add around 3 mL of ethanol to clean the plastic tubes of silver.
    c. Transfer the solutions to the glass jars.
25. Dry the ethanol in the glass jars using a vacuum oven at 50°C for 2–4 h.
26. Calculate the weight of the final product Ag NWs-F.

Fabrication of the stretchable polymer-The soft part

Timing: 2 days

The fabrication of the soft stretchable part included forming a thin film of SIS and preparing the electrode by spray coating techniques. The following steps describe the fabrication process of the stretchable polymer part, as shown in Figure 2.

Spraying of Ag NWs electrodes

27. Clean the surface of a silicon (Si) wafer using an oxygen plasma system.

Note: Use these parameters: O_2 50% + Argon (Ar) 50%: Pumping down for 3 min, gas supply 2 min, plasma 3 min, flushing 1 min, and venting for 30 s.

28. In a glass plate, add 80 mL of toluene and then add 8 drops of trichloro(hexyl)silane, 97%.

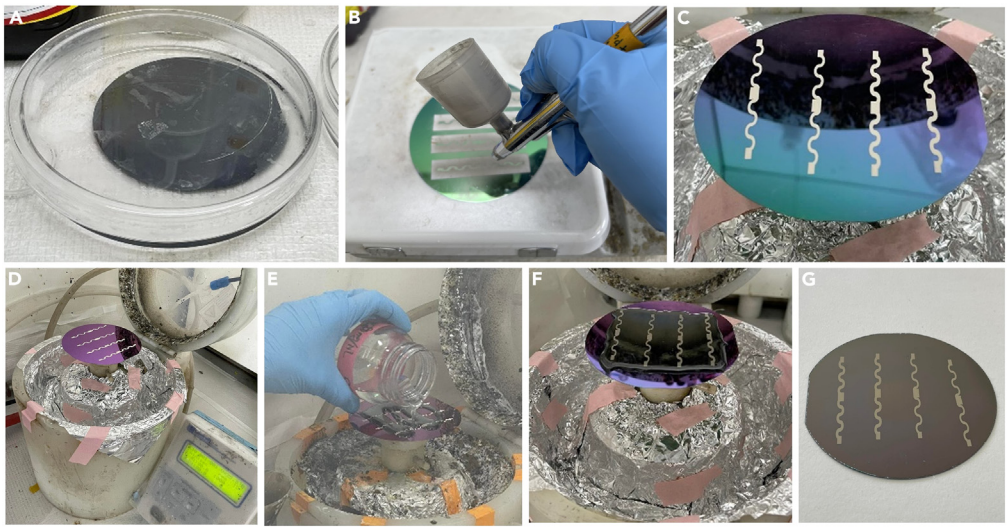

Figure 2. Preparing the Ag NWs electrodes

(A) Hydrophobic modification of the Si wafer by immersing it in toluene, including trichloro(hexyl)silane.
(B) Spray coating of the Ag NWs-F electrodes using a mask.
(C) The sprayed Ag NWs electrodes on the Si wafer after annealing at 200°C for 20 min.
(D) Setting the parameters of the spin-coater to coat with a thin layer of SIS.
(E) Adding 100 mg/mL of SIS on the Si wafer.
(F) Spreading the SIS on the Si wafer to cover the whole area of electrodes.
(G) The obtained electrodes covered with a very thin layer of SIS.

CAUTION: Wear gloves and goggles, and handle under a chemical hood.

29. Add the Si wafer inside the solution and cover the plate for 30 min.
30. Wash the Si wafer with toluene and acetone and dry it with dry air to obtain a hydrophobic Si wafer.

CAUTION: Wear gloves and goggles, dry, and handle under a chemical hood.

31. Use the laser cutter to create a mask using matte self-adhesive paper with the desired shape of the electrodes.

CAUTION: Avoid looking directly at the laser beam during use. Exposure to the laser beam may cause severe eye damage.

32. Stick the mask on the hydrophobic Si wafer and spray-coat Ag NWs-F using a spray gun.
33. Remove the mask and anneal the electrodes using a hot plate at 200°C for 20 min to obtain a conductive Ag NWs electrode.

CAUTION: Be careful of the hot plate and perform in a chemical hood.

34. Spin-coat 100 mg/mL SIS in toluene on the Ag NWs-F on the Si wafer with 1000 rpm for 60 s.

CAUTION: Wear gloves and goggles and perform under a chemical hood.

Preparing the soft stretchable polymer with the Ag NWs electrode

35. Prepare a thin SIS film by pouring 100 mg/mL SIS in toluene into a Teflon template and let dry (for 12–14 h).

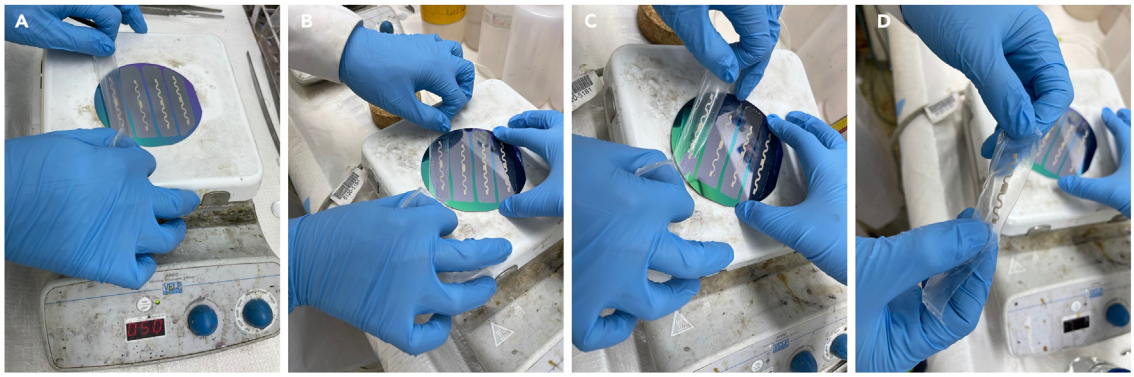

Figure 3. Transforming the Ag NWs electrodes to a soft SIS film

(A) Adding the SIS/Ag NWs-F/Si wafer on a heat plate at 50°C and stretching a pre-prepared soft SIS film.

(B) Pressing the stretched SIS film on the SIS/Ag NWs electrodes.

(C) Peeling off the SIS film carefully and slowly from the Si wafer.

(D) The SIS film with the transformed Ag NWs electrode.

CAUTION: Wear gloves and goggles and perform under a chemical hood.

Pause point: The Teflon containing the SIS should be kept on a stable surface and dried in a chemical hood for 12–14 h.

36. Pre-stretched the obtained thin SIS film to 100% of the original length and press it gently using a round tip spatula onto the SIS/Ag NWs-F/Si wafer (prepared in step 34) at 50°C for 30 s (Figure 3).
37. Peel off the SIS film carefully and slowly to ensure the Ag NWs electrode is entirely transformed into the film. Troubleshooting.
38. Check the conductivity of the transformed Ag NWs electrode by stretching the film to different lengths using a Digit Multimeter (Figure 4). Troubleshooting.

Fabrication of MNs—The rigid part

Timing: 1 day

The fabrication of the rigid MNs part included casting polystyrene solution into a PDMS mold, centrifuge to fill the polymer inside the mold, then heating the molds and de-molding to receive the rigid MNs similar to previously described scalable manufacturing procedures.[29,30] The MNs are then deposited with gold to obtain the final gold MNs, as shown in Figure 5.

Preparing the polystyrene rigid MNs

39. Prepare a mold of PDMS for the MNs using a laser cutter to carve holes.

Note: Use these parameters: 10% power, 3% speed, 1000 points per inch (PPI), and a Z-axis of 1.8 mm to make even holes inside the PDMS polymer. The diameter of each hole is around 458 μm × 358 μm, and the length is equal to 1153 μm. Troubleshooting.

40. Add the PDMS molds inside a polystyrene solution in DMF (200 mg/mL) and then centrifuge at 1006 × *g* for 3 min.
41. Move the PDMS molds filled with polystyrene solution onto a heat plate, drop-cast more polystyrene solution on the top of the molds, and dry at 80°C for 12–14 h.

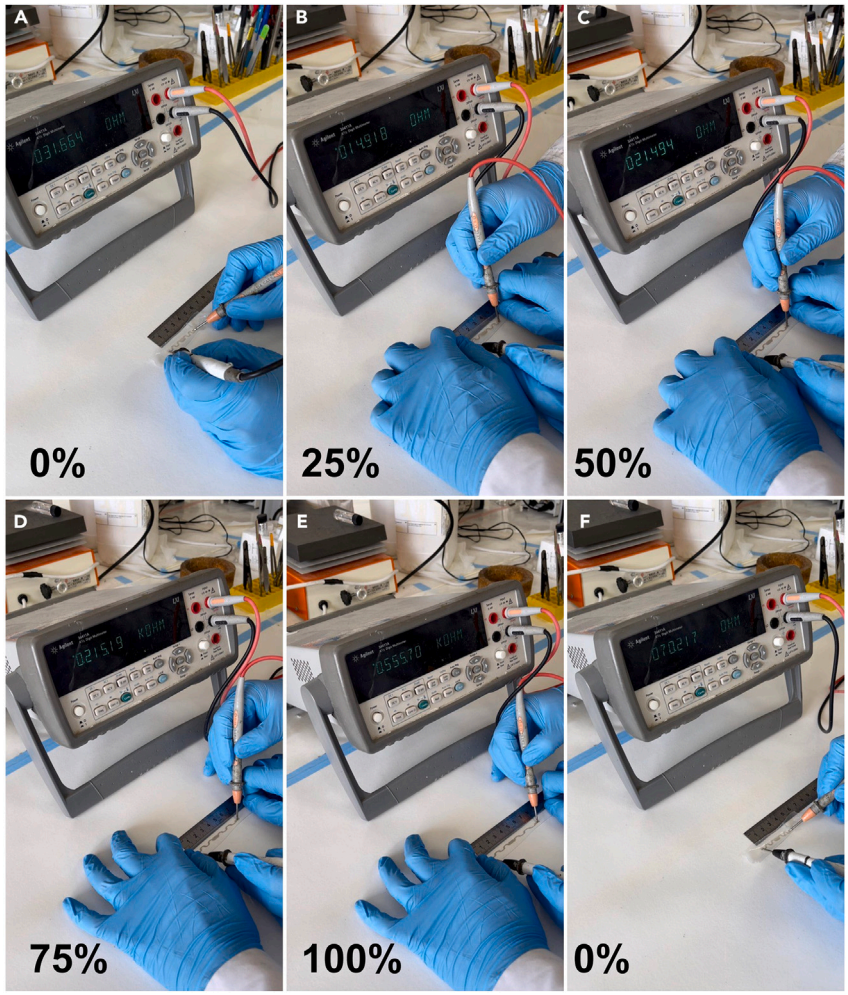

Figure 4. Checking the conductivity of the transformed Ag NWs electrode on the SIS film in different stretching lengths

(A) Initial length: 0% stretching.
(B) 25% stretching.
(C) 50% stretching.
(D) 75% stretching.
(E) 100% stretching.
(F) Back to the initial length: 0% stretching.

⚠ CRITICAL: When adding the PDMS molds filled with polystyrene on the hot plate, be careful not to touch the hot plate to avoid injuries and burns. Also, avoid creating bubbles when drop-casting more polystyrene to get a uniform MN without holes. Troubleshooting.

42. De-mold the template to get rigid polystyrene MNs.

Preparing the gold-coated MNs

43. Cover the rigid polystyrene MNs substrate with an electrode mask and stick them well on a Si wafer using a tape.
44. Evaporate a thin layer of titanium (Ti) (10 nm) as an adhesion layer using a thermal evaporation technique with a pressure of $5*10^{-7}$ Torr and a rate of 3A/s.

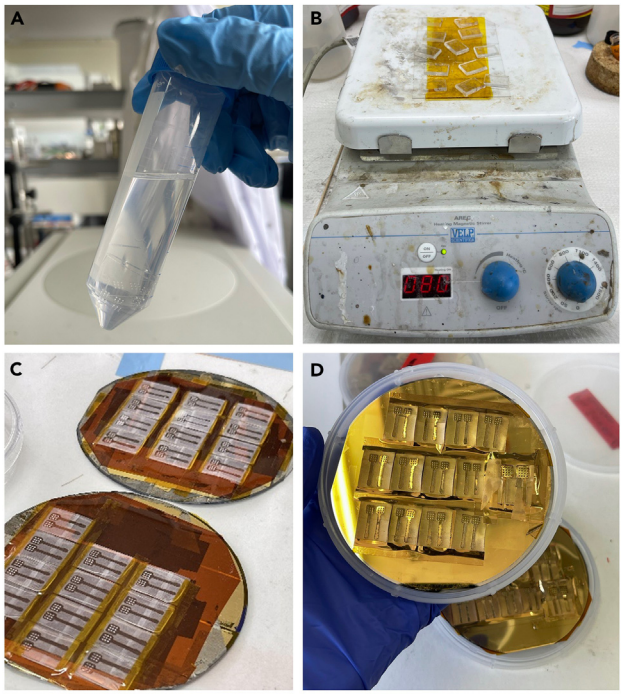

Figure 5. Fabrication procedure of MNs—The rigid part
(A) Adding the PDMS MNs molds into the polystyrene solution to centrifuge.
(B) Adding the PDMS molds filled with polystyrene solution on a heat plate at 80°C.
(C) Sticking the de-molded polystyrene MNs on the Si wafer using a mask and tape.
(D) The MNs after evaporation of gold.

45. Deposit a gold layer with a thickness of 300 nm to receive the gold MNs by a thermal evaporation technique using a pressure of $5*10^{-7}$ Torr with a rate of 7A/s.[31]

Note: For fabricating biosensors, one of the gold-coated MN arrays should be used as a sensing electrode and the other as a reference electrode. Based on the desired target analyte, different biomolecules can be modified in the sensing area, such as enzymes, antibodies, and ion-selective membranes (ISMs) (Figure 6A). For preparing a reference electrode, the other gold-coated MN electrode is deposited with Ag NWs followed by drop-casting 10 μL of 0.05 M $FeCl_3$ for 1 min, then washing using pure water. A solution of NaCl and poly(vinyl butyral) (PVB) in methanol is then drop-cast and dried for 30 min to obtain the Ag/AgCl reference electrode (Figure 6B).

Combining the soft polymer with the rigid MNs

Timing: 12 h

The soft SIS polymer with variant thickness was combined with the rigid MNs part by sticking the MNs on the thick parts of the polymer. To connect between the Ag NWs electrodes on the thin, soft part and the MNs component on the rigid part, a silver paste was used, and the connection was performed on the rigid part of the patch to keep the connection stable after stretching, as shown in Figures 7 and 8.

Combining the soft SIS film with the rigid MNs using a thickness-gradient strategy

46. After transforming the Ag NWs electrode to the soft SIS film, drop-cast 100–200 μL SIS solution on specific locations onto the backside of the prepared stretchable Ag NWs electrode and let it dry to create a variant thickness substance (See steps 35–38).

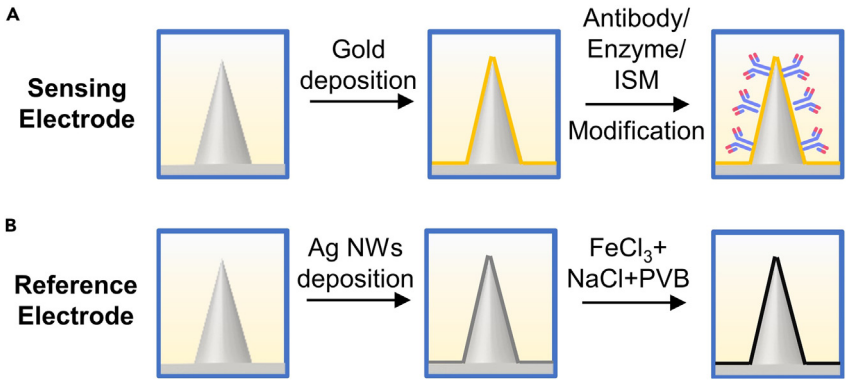

Figure 6. Schematic of the fabrication of MN-based biosensors
(A) Fabrication of the sensing electrode.
(B) Fabrication of the reference electrode.

47. Fix the rigid MNs on the thick part of the SIS film with a small amount of SIS solution.
48. Connect the gold-coated MNs with the Ag NWs electrode using silver paste. Troubleshooting.

EXPECTED OUTCOMES

This protocol provides a procedure for fabricating a wearable stretchable MNs sensor patch. We present the step-by-step procedures for manufacturing the final patch by dividing the protocol into different parts to demonstrate each step individually. First, we fabricate the soft polymeric part by synthesizing Ag NWs and incorporating them into the soft substrate. Using a mask, we spray-coat Ag NWs onto a hydrophobic Si wafer and then transfer them to a pre-stretched SIS film. We provide instructions on how to perform this transfer while maintaining the conductivity of the electrodes after stretching. Second, we demonstrate the method for fabricating the rigid MNs component. This includes fabricating the rigid MNs substrate using PDMS molds and polystyrene solutions, followed by the deposition of gold to form the gold rigid MNs electrodes. Finally, we integrate the soft polymer substrate, which includes the stretchable Ag NWs electrode, with the rigid MNs component using the thickness gradient strategy. In this strategy, we drop-cast additional SIS solution in specific places on the SIS substrate to form a film with varying thicknesses that adheres to the rigid component. In terms of outcomes, we present various results obtained from the different steps of the protocol, illustrating the applicability and functionality of the MN-based sensor patch fabricated using this protocol. The primary outcome of the 'fabrication of Ag NWs' step is creating a highly conductive Ag NWs network, homogeneously spray-coated for creating the conductive electrodes (Figure 9A). For the 'fabrication of MNs - The rigid part' step, the SEM image demonstrates the rigid MN array deposited with gold (Figure 9B), which can then be modified with various

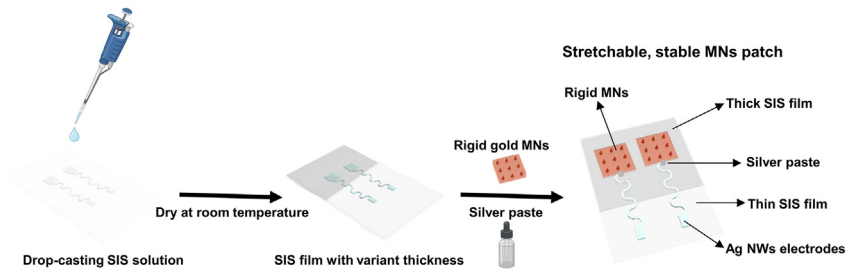

Figure 7. Schematic of the procedure for integrating the rigid MNs with the soft stretchable substrate using the thickness-gradient strategy
The illustration was created with BioRender.com.

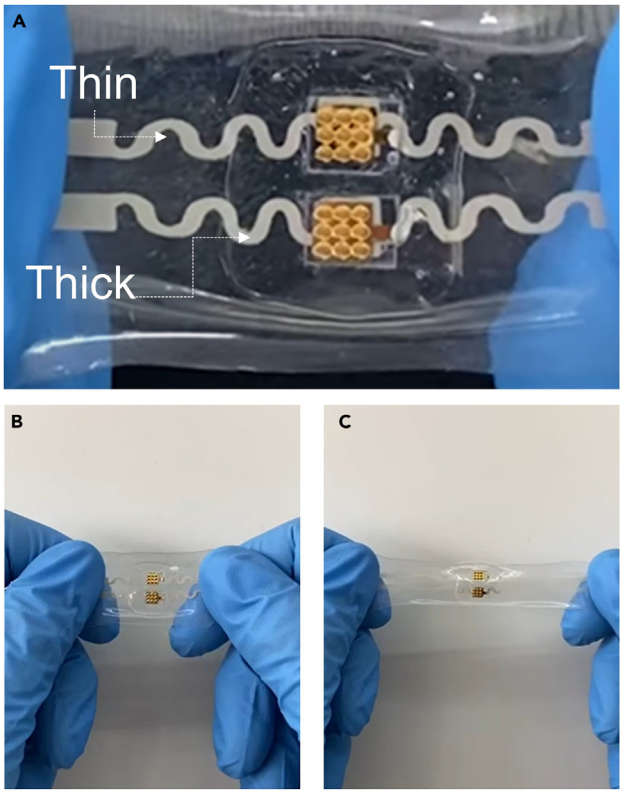

Figure 8. The stretchable stable MNs patch prepared by the thickness-gradient strategy

(A) SIS film with variant thicknesses prepared with the thickness-gradient strategy. Reproduced with permission.[1] Copyright © 2022 Wiley-VCH GmbH.

(B) Combined rigid-MNs part with the soft SIS thin part using the thickness-gradient strategy.

(C) Stretching the combined MNs rigid and soft SIS thin parts while maintaining the patch's stability.

biomolecules and materials based on the desired sensing application (as described previously in Figure 6).

The primary outcomes of the steps 'fabrication of the stretchable polymer - the soft part' and 'combining the soft polymer with the rigid MNs' are demonstrated in Figure 10. Figure 10A demonstrates a photo showcasing the combined patch, and Figure 10B depicts the patch's skin conformability when worn on the arm. Additionally, we present results illustrating the applicability of the thickness-gradient strategy, which aids in integrating the soft and rigid parts to create a stable MN-based sensor patch. The rigid MN part is loaded on the thick part of the patch, whereas the Ag NWs are sprayed on the thin parts of the patch. Simulation and stretching results depict that the thick part loaded with the rigid MN remains stable upon stretching, while the thin parts, including the stretchable Ag NWs electrodes, undergo stretching (Figure 10 C1-3 and D1-3)

Additional primary outcome of the step 'combining the soft polymer with the rigid MNs' including the results that validate the overall applicability of our proposed protocol are presented in Figure 11. We tested the MN sensor patch, modified with a sodium-selective membrane, to detect sodium ions. Building upon our previous application, the sensor exhibited excellent electrical performance and responsiveness to elevated concentrations of sodium, demonstrated by increased current values (Figure 11A). Moreover, it demonstrated exceptional repeatability and reversibility upon using low and high concentrations of sodium, affirming the successful modification and functionality of the MNs and the stability of the stretchable patch across three testing cycles (Figure 11B). We further conducted on-body tests by inserting and peeling off the fabricated patch and testing the response

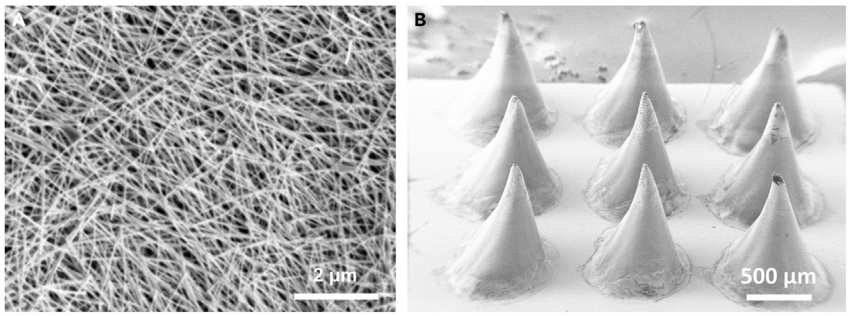

Figure 9. The fabrication of Ag NWs and rigid MNs

(A) SEM image of the conductive network of spray-coated Ag NWs for creating the conductive electrodes.

(B) SEM image of the fabricated rigid MNs, deposited with gold.

over three cycles. The results displayed remarkable responsiveness, mechanical stability, sensing reproducibility, reversibility, and durability (Figure 11C). Finally, to showcase the applicability with metals beyond gold, we deposited the MNs with platinum (Pt) and nickel (Ni) and modified the MNs with a sodium-selective membrane. Testing against elevated concentrations of sodium revealed excellent electrical performance and stability (Figure 11D). This underlines the versatility of our protocol for integrating different metals, broadening the range of potential applications.

LIMITATIONS

Despite the applicability and generalizability of this protocol and its provision of a simple, cost-effective, and easy method to overcome the challenge of combining rigid and soft components, several limitations still need to be considered. The main limitation of this protocol is maintaining the stability and conductivity of Ag NWs on the stretched soft SIS film after multiple cycles of stretching. Repeated stretching may decrease the mechanical stability of the fabricated structure over time and limit the device's applicability. Therefore, we recommend not stretching the soft part beyond 100% of its initial length to overcome this limitation. Another limitation is maintaining the conductivity of the entire patch, especially in the connection between the stretched Ag NWs electrodes and the MNs, which may break under strong mechanical forces caused by stretching or bending. We addressed these limitations and provided suggested solutions in the "Troubleshooting" section.

TROUBLESHOOTING

Problem 1

The fabricated Ag NWs are not conductive or have low conductivity (A resistance equal to or higher than MΩ). This may stem from insufficient cleaning and purification of the Ag NWs and/or the fabrication procedure of the Ag NWs wasn't performed correctly (related to step 15).

Potential solution

Make sure to follow the synthesis steps accurately. Clean and purify the Ag NWs using ethanol for a greater number of cycles (more than five cycles). Chloroform and methanol can also be used in the cleaning process by using pure chloroform, pure methanol, and 1:1 chloroform: methanol, and then clean with ethanol until the PVP polymer is dissolved and the conductivity reaches Ω to KΩ.

Problem 2

The transformation of the Ag NWs from the Si wafer to the pre-stretched SIS polymer is incomplete. Some parts of the sprayed Ag NWs remain on the Si wafer and\or the transferred Ag NWs electrode on the SIS film is not conducive. This may stem from different reasons: The transfer was performed without heating, the pressing was not done well or thoroughly thus missing some parts or edges due to air bubbles or insufficient contact between the pre-stretched SIS polymer and the sprayed electrodes on the Si wafer, the Si wafer wasn't cleaned well as described in step 27; there was

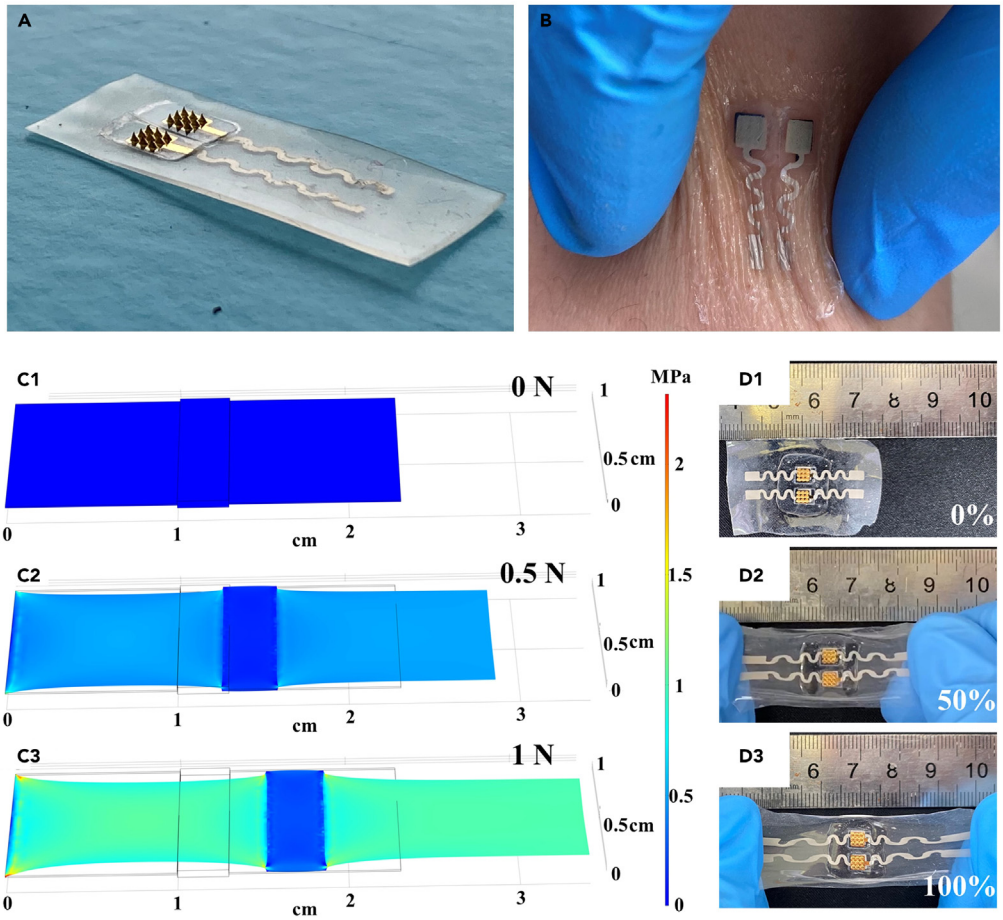

Figure 10. The performance of the integrated stretchable stable MNs patch prepared by the thickness-gradient strategy

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(A) Photo of the combined MN-based sensor patch.

(B) Photo of the skin conformal MN-based sensor patch.

(C1-C3) Simulation of stretching the stretchable patch.

(D1-D3) Photos for stretching the combined soft, stretchable SIS film with the rigid MNs.

contamination or defects on the surface of the Si wafer, and the hydrophobic modification of the Si wafer wasn't performed properly, as described in step 28 (related to step 37).

Potential solution

Control the temperature and press the pre-stretched SIS polymer well over the sprayed Ag NWs on the Si wafer. Performing the process at room temperature (20°C–25°C) will not enable a full transfer of the Ag NWs from the Si wafer to the stretched SIS film. 50°C was found to be the ideal temperature for a complete transformation and good conductivity.

Problem 3

A deformation occurs to the pre-stretched SIS film in the transformation step, and the stretched polymer doesn't go back to the original length and structure before stretching. This may occur due to using a high temperature for the transformation process, such as 80°C which causes a deformation of the stretched SIS polymer, and the film doesn't return to its original length due to heating. It's already known that high temperatures decrease the elasticity of SIS polymers[32] (related to step 37).

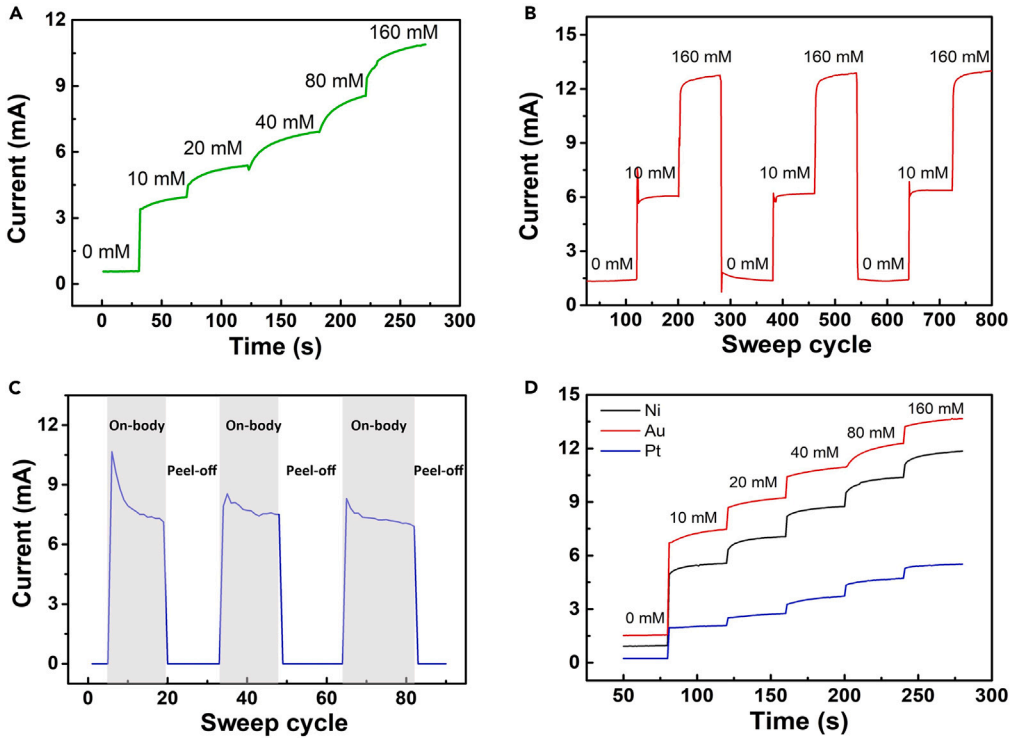

Figure 11. The electrical performance of the fabricated MN-based sensor patch

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(A) The electrical performance of the MN-based sensor patch modified with sodium-selective membrane.

(B) The repeatability and reversibility of the MN-based sensor patch for detecting sodium.

(C) The mechanical stability and responsiveness of the fabricated MN-based sensor patch.

(D) Fabrication of the MN-based sensor patch using different metals.

Potential solution

Control and use lower temperatures in this process. We found that 50°C is the ideal temperature. The deformation of the heated stretched SIS at 80°C is presented in Figure 12.

Problem 4

The Ag NWs electrode on the soft SIS polymer is not conductive even though the transformation was performed as described in step 37. This may stem from improper spray-coating of the Ag NWs on the Si wafer in step 32 (related to step 38).

Potential solution

Before annealing, check the spraying quality and uniformity using a light microscope to ensure the Ag NWs are sprayed well and entirely on the Si wafer. Incomplete spraying and coverage may lead to unseen holes on the electrodes and lead to non-conductive or low-conductivity electrodes.

Problem 5

The carved holes in the PDMS mold aren't even and\or don't stand with the correct diameters and length dimensions. This may occur since the power of different laser cutters can vary, causing different results for the same parameters (related to step 39).

Potential solution

Optimizations have to be done based on the laser cutter type. When the same laser cutter is used with the same parameters, it can also produce different results. Therefore, calibrations should be performed frequently to ensure the same results.

Figure 12. A deformed SIS substrate with the Ag NWs electrode after heating at 80°C

Problem 6

The polystyrene MNs may be filled with bubbles besides being too thin, weak, and easy to break. This may occur since one round of centrifugation wasn't enough and/or the filled PDMS molds were applied upside down on the heat plate (The holes were face-down to the plate) (related to step 41).

Potential solution

Perform a second round of centrifugation in case the molds were filled with bubbles. When moving the PDMS molds filled with polystyrene solution onto a heat plate, ensure the holes are on the top to prevent the solvent from leaking out of the mold. Then, add more polystyrene solution on the top of the PDMS mold (make sure you don't add a solvent with bubbles) and heat. 80°C is the preferred temperature because a lower temperature will take a longer time to dry, and a higher temperature will cause bubbles inside the MNs.

Problem 7

The fabricated combined patch, which integrates the MNs on the thick part and the Ag NWs electrodes on the thin part, is not conducive after stretching the patch. This can occur because applying the silver paste on the thin part of the patch may cause the breakage of the semi-rigid dry paste after multiple stretching cycles (related to step 48).

Potential solution

Apply the silver paste on the thick part, in the connection point between the soft-rigid parts, to ensure the dry paste remains stable and doesn't break over stretching.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prof. Hossam Haick (hhossam@technion.ac.il).

Materials availability

This study did not generate new unique reagents.

Data and code availability

No data or code was generated in this study.

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AUTHOR CONTRIBUTIONS

R.O. developed the protocol, designed and performed the experiments, designed the figures, and wrote the manuscript. Y.Z. developed the protocol, designed and performed the experiments, reviewed and edited the manuscript, and supervised the work. H.H. supervised the work, reviewed and edited the manuscript, and secured funding for the work.

DECLARATION OF INTERESTS

The Technion - Israel Institute of Technology holds a patent for this invention (TECH/092 USP). H.H., Y.Z., and R.O. are the inventors of the patent.

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