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Preparing a 3D Printed Implant for Acute In Vivo Electrophysiology V.1

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https://doi.org/10.1101/2023.11.12.566771

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We use this protocol and it's

working

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Abstract

When performing acute electrophysiology experiments in mice, replacing the skull with a 3D printed implant several weeks in advance can substantially improve recording quality. Prior to surgery, these implants must be coated with a layer of silicone to maintain brain health. This protocol describes the steps necessary to apply this silicone layer in advance of a cranial windowing procedure.

Image Attribution

Images modified with permission from Bennett et al. (2024) bioRxiv (DOI: 10.1101/2023.11.12.566771)



Materials

Custom parts

- Custom 3D printed implant
- Custom 3D printed stamping tool (e.g. Allen Institute Part #0274-910-21)

Standard equipment

- Glass beaker
- Microwave
- Degasser
- Autoclave

Standard materials

- Luer-Lock Syringe, 1ml (VWR BD-309628 or equivalent)
- 25G blunt-tip needle (Fisher NC0481462 or equivalent)
- 70% ethanol

Protocol materials

Making Putty Amazon Catalog #B01DO4S1JA Step 1 SORTA-Clear 18 Smooth-On, Inc. Step 6

Before start

The implants need to be designed and printed in advance, for more details see Bennett et al. (2024) bioRxiv (DOI: 10.1101/2023.11.12.566771):



Coat the implant

1d 1h 51m

1 Prepare the

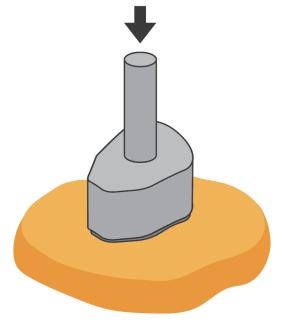
Impressive Re-Usable Mold Making Putty **Smooth-On, Inc. Catalog #**B01DO4S1JA . Put the solid putty on a flat surface (like the bottom of a glass beaker) and heat the putty in the micro-wave oven. Check the softness of the putty every 30 s to prevent overheating. Stop heating when the putty is hot to the touch and easily malleable.

2 Manipulate the putty to create a flat surface with no creases.

5m

3 Disinfect the implant by soaking in 70% ethanol for a few seconds.

4 2m

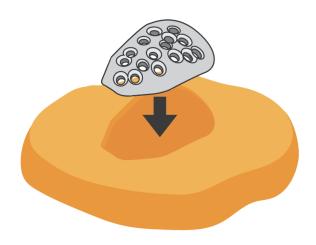


Use the 3D-printed stamping tool to create indentations in the putty. One indentation is needed for each implant being prepared.

5

10m





Insert the implants into the indentations. Gently press the implants into place, so the bottom of the implant fully makes contact with the putty. Do not press too hard, as you do not want the putty to fill the holes in the implant.

6 Prepare the SORTA-Clear 18 Smooth-On, Inc. (including Part A and Part B).

15m

6.1 In a beaker, weigh out $\Delta 20 g$ of Part A, and $\Delta 2 g$ of Part B.

5m

6.2 Mix the two parts together thoroughly to combine them, until the mixture is homogeneous.

1m

6.3 Use a degasser to remove bubbles from the mixture. Keep the mixture in the degassing chamber until you see the mixture rise, then collapse as the bubbles burst. Wait another minute after the collapse before removing the mixture from the degasser.

9m

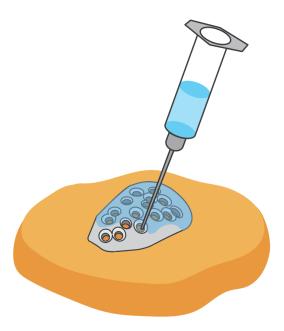
7 Draw the degassed SORTA-Clear into a 1 mL Luer-lock syringe. Attach a 25G blunt needle.

1m

8

1h





Cover the top of the implants with SORTA-Clear.

- 8.1 Place the putty with the implants under a dissection scope to better see what you're doing.
- 8.2 Fill each hole in the implant with SORTA-Clear using the 25G needle. Take care not to create bubbles by dispensing SORTA-Clear near the hole and allowing it to flow into the hole on its own. If bubbles do form, try to dislodge them with the needle.
- 8.3 When each hole is filled, cover the entire top surface of the implant (excluding the outer lip) with a layer of SORTA-Clear.

Note

The SORTA-Clear should not extend onto the outer lip of the implant, as this part is cemented into place during surgery. Having SORTA-Clear here will interfere with the implantation and lead to weak attachment of the implant to the skull.

- 8.4 Repeat for each implant. You have about an hour to work with the SORTA-Clear before it cures too much to work with. If it is getting very difficult to push it out of the syringe, make a fresh batch.
- Allow the SORTA-Clear to fully cure for at least 24:00:00 before removing the implants from the putty and implanting them into animals.

1d



Sterilizing the implant

5m

Coated implants are then cleaned by rinsing in 70% ethanol, placed into individual autoclave bags, and autoclaved for 00:05:00 (131°C, 14.7 psi). Note that autoclaving at higher temperatures can lead to defects in the implant.

5m

Citations

Corbett Bennett, Ben Ouellette, Tamina K Ramirez, Alex Cahoon, Hannah Cabasco, Hannah Belski, Ryan Gillis, Conor Grasso, Robert Howard, Tye Johnson, Henry Loeffler, Heston Smith, David Sullivan, Allison Williford, Shiella Caldejon, Severine Durand, Samuel Gale, Alan Guthrie, Vivian Ha, Warren Han, Ben Hardcastle, Ethan McBride, Chris Mochizuki, Arjun Sridhar, Lucas Suarez, Jackie Swapp, Joshua Wilkes, Colin Farrell, Peter A. Groblewski, Shawn R Olsen. SHIELD: Skull-shaped hemispheric implants enabling large-scale electrophysiology datasets in the mouse brain https://doi.org/10.1101/2023.11.12.566771