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Scalable Fabrication Framework of Implantable Ultrathin and Flexible Probes with Biodegradable Sacrificial Layers

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Abstract

For long-term biocompatibility and performance, implanted probes need to further reduce their size and mechanical stiffness to match that of the surrounding cells, which, however, makes accurate and minimally invasive insertion operations difficult due to lack of rigidity and brings additional complications in assembling and surgery. Here, we report a scalable fabrication framework of implantable probes utilizing biodegradable sacrificial layers to address this challenge. Briefly, the integrated biodegradable sacrificial layer can dissolve in physiological fluids shortly after implantation, which allows the in situ formation of functional ultrathin film structures off of the initial small and rigid supporting backbone. We show that the dissolution of this layer does not affect the viability and excitability of neuron cells in vitro. We have demonstrated two types of probes that can be used out of the box, including (1) a compact probe that spontaneously forms three-dimensional bend-up devices only after implantation and (2) an ultraflexible probe as thin as 2 μm attached to a small silicon shaft that can be accurately delivered into the tissue and then get fully released in situ without altering its shape and position because the support is fully retracted. We have obtained a >93% yield of the bend-up structure, and its geometry and stiffness can be systematically tuned. The robustness of the ultraflexible probe has been tested in tissue-mimicking agarose gels with <1% fluctuation in the test resistance. Our work provides a general strategy to prepare ultrasmall and flexible implantable probes that allow high insertion accuracy and minimal surgical damages with the best biocompatibility.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nano-lett.7b02851. Additional details on the experimental materials and methods. Figures showing fabrication procedures, action potentials, the Mg dissolution effect, and in vitro astrocyte response tests. (PDF)

Keywords

Implantable bioprobes; flexible ultrathin electrodes; biodegradable; biocompatibility

Implantable electrodes have been the central unit of implanted sensors and electronics for biomedical research, diagnostics, and therapy. Overall, there are two categories of commercialized electrodes that have been used in implantation. First, rigid microscale electrodes, including twisted microwires, tungsten microelectrodes, Michigan probes, and Utah probes,^{1–4} are most widely used when accurate positioning into the tissue are required. The major challenge for the chronic use of these probes is the tissue reaction and scar formation around the probe due to the huge mechanical mismatch with the tissue and micromotions relative to the tissue after the implantation, such that the active cells tend to migrate away from the implanted electrodes, which eventually leads to significant degradation of performance.^{5–7} Second, flexible thin-film probes are used in situations in which they can conform to the shape of the tissue or organ so that it could bring less irritation to the live cells and better biocompatibility.^{8–12} However, the devices typically can only get in touch with the outmost surface of the tissue, and it is generally difficult to deliver the flexible structure deep below the surface to get closer to the active cells, which reduces the resolution and quality of signals.

Recent advances in nanobioelectronics and bioresorbable electronics have triggered a new wave of innovations for the interfacing of artificial devices to live cells and tissues.^{13–18} For example, silicon nanowire based active nanoscale field-effect transistor (nanoFET) sensors,^{8,19–21} vertical nanowire electrode arrays,^{22–24} and silicon nanobelt devices²⁵ brings higher sensitivity, smaller size, and stronger interaction with cells.^{14,15} Furthermore, several new strategies have been explored to package nanomaterial-enabled devices in flexible forms and to deliver them inside tissue rather than just making a surface contact.^{26–35} Specifically, Xie et al. developed procedures to implant three-dimensional ultraflexible macroporous nano-electronic probes into the brain shortly after freezing the probes in liquid nitrogen so that the rigidity of the probes is temporarily strong enough to penetrate the tissue before thawing in position.²⁸ In addition, Liu et al. and Hong et al. have recently demonstrated injectable flexible electronics into tissues, for which packed microporous mesh electronics is directed through a syringe by a flux of liquid and multiplexed connection can be made using conductive ink printing technique.^{29,30} This technique enables the

delivery of a large-area network of devices with high yield into hidden or opaque regions with the aid of field of view control. The device mesh typically forms a cylinder with a diameter of a couple of hundred micrometers up to 1 mm because the soft probes need to be first separated from the fabrication substrate and then delivered as suspended in medium. Significantly, Zhou et al. showed recently that such a highly porous structure could lead to much less inflammation and damage to surrounding neurons and no chronic immune response or a negative effect on the natural distribution of neurons.³¹ However, Hwang et al. proposed transient electronics for implanted application that utilizes biodegradable materials, also known as bioabsorbable materials that are soluble in aqueous solutions or biofluids, specifically for functional components, connection wires, and packaging, and the whole circuit will be absorbed by the tissue and disappear after an extended period.³² In addition, for the implantation of ultrathin injectable optoelectronics and sensors, Kim et al. and Koh et al. designed a process for picking up and transferring thin-film devices onto an epoxy-based needle and gluing them together with silk-based solution so that the needle is strong enough to bring the thin-film structure into the tissue and the film can detach soon after the silk glue or cellulose adhesive layer is dissolved.^{33,34} The precision of insertion is well maintained, whereas the surgical lesion and damage of the tissue is typically wider than 100 μm , up to 1 mm in size, possibly because the choice of materials of the supporting structure requires a larger geometry for enough rigidity, and the dissolvable layer is incompatible with lithographic process that also limits the scaling down of the device size. Most recently, Luan et al. demonstrated an ultraflexible nanoelectronic probe that can be implanted with surgical damage as small as 7 μm with the aid of an insertion shuttle made of tungsten wire or carbon fiber.³⁵ Nevertheless, such process requires an elaborated focus ion beam sharpening of each shuttle and an assembling procedure for each insertion.

All of these pioneering and trending studies demonstrate the importance of matching the size and mechanical properties of the implanted functional devices with live cells and tissues and the desire to minimize the impact of lesion while maintaining the feasibility of accurate surgery with rigidity. However, it remains a rather difficult task to reconcile the request for scaling down the size of probe, maintaining the mechanical strength required for accurate implantation surgery while having the core functional structure remain small and flexible enough for better interfacing with cells. In addition, all existing implantable flexible devices require additional preparation of a carrier and support facility, a manual assembly procedure, or both, which increases the difficulty in scaled up production, and complicates practical uses. To address these existing challenges, we report here a general fabrication framework utilizing an inorganic biodegradable sacrificial layer that can be integrated in a unified top-down lithography procedure for preparing ultrasmall probes, which can be used out-of-box, to accurately deliver ultraflexible devices in deep tissue with minimal lesion by the in situ formation of the flexible functional structures only after the surgery process.

The schematics of the fabrication procedures and the probe structure are shown in Figure 1. Specifically, there are three main fabrication stages. In stage I, a double-sided polished (100) silicon substrate with 150 nm Si_3N_4 coating is first patterned with a biodegradable metal sacrificial layer (discussed in more details later in the paper) before a thin-film device structure is fabricated on the top using standard top-down lithographic procedures. The device layer features bonding areas that will be used for external connections and a long arm

of device(s) that fits into the final shape and dimensions of the probe. In stage II, a reactive ion etching (RIE) process from the top side is used to carve out the probe body with a patterned photoresist layer as the mask, leaving a thin neck connection between the bonding area and the outer frame area. The depth of the RIE in this step defines the final thickness of the probe, typically in the range of 10–30 μm . In stage III, the whole probe is shaped by a backside RIE process, which uniformly reduces the thickness of the whole substrate from the back until the area that has been thinned in the previous stage is completely removed and the probe body is isolated in full suspension. The resulting probe will have a thin neck connection to the supporting frame and can be easily detached by a notch. In summary, three layers are defined all by lithography in this process, including (1) the top device layer, which typically has conductive metal connections passivated by insulating polymer shells, (2) the middle biodegradable sacrificial layer, and (3) the 10–30 μm thick rigid silicon beam that provides the mechanical strength with the smallest cross-section profile for the precise insertion surgery. Here for simplicity without losing generality, we use metal electrode pairs passivated by SU-8 polymer to demonstrate the feasibility of our proposed strategy and surgical procedures (for details in fabrication, see the Supporting Information).

There are several key considerations of this design. First, we choose silicon to construct the initial supporting structure, which can be easily shaped for optimal surgical insertion by standard lithographic processes and has a Young's modulus of ~ 165 GPa, so that the critical dimension of the probe can be shrunk to the 10 μm level and still provide enough rigidity for implantation. Second, the biodegradable material for the sacrificial layer is chosen on the basis of several criteria. (1) It must be compatible with top-down lithography procedures so that the functional devices can be miniaturized and accurately aligned in a unified fabrication protocol for scalable production. (2) The dissolution of the material must only involve the physiological biofluids without the assistance from additional chemicals or enzymes. (3) The outcome of the dissolution of the material must not affect the physiological status and functionality of the neighboring cells. Third, the gradual dissolution of the sacrificial layer in physiological environment triggers the formation, separation, and release of the flexible and functional devices from the rigid supporting beam, which all happen in situ within 15–30 min after the implantation, such that the precise adjustment of the probe position can be performed after insertion without prolonged waiting or surgical complications. Lastly, these fabrication procedures can be generally used for preparing thin-film probes of arbitrary sizes and shapes for accurate implantation, and the surgical procedures we demonstrate below can be adopted for either a single probe or for a probe of arrays of device arms.

There have been many reports on integrating biodegradable materials into implantable or surface-mounting electronic devices.^{32,36} Most widely studied materials used for packaging or gluing components are organically based, such as polyglycolic acid, poly L-lactic acid, and silk fibroin.^{37–39} To date, these materials either are not compatible with top-down lithography or, in the case of photo-cross-linkable silk protein, require additional enzyme protease XIV to initiate a very slow degradation that is not practical for use with live cells in vivo.⁴⁰ In addition, carboxymethyl cellulose (CMC)- or polyethylene glycol (PEG)-based dissolvable needles of hundreds of micrometers in diameters have been proposed to deliver thin film devices into tissue, but the size of the lesion is overall very large due to the weak

mechanical strength and the expansion of probe volume after insertion during the dissolution process could potentially cause secondary damages.^{41–43} However, inorganic biodegradable materials, including metals, such as Mg, AZ31 Mg alloy, Zn, Fe, W, Mo, and semiconductor and dielectric materials, such as Si, SiO₂, MgO, and ZnO, have been investigated for use as electrodes, connections, or surface coatings, but no study using them as sacrificial layer has been reported.^{44,45} Here, for the first time, we choose Mg to construct a biodegradable sacrificial layer because it has a decent dissolution rates of $4.8 \pm 2.2 \mu\text{m/h}$ in simulated body fluids and the least concern of toxicity to live cells when fully digested within a short time.⁴⁵ The dissolution time, shelf life, and biocompatibility of the Mg-based sacrificial layer are investigated as shown in Figure 2.

Specifically, we have tested the dissolution time in $1\times$ phosphate buffer solution (PBS) of a 100 nm Mg layer deposited on a blank substrate when it was freshly prepared and after 10 weeks of storage in an ambient environment (relative humidity controlled below 10%). In both cases, the Mg layer dissolved cleanly within 2 min (Figure 2a) and did not show any degradation of its quality. When a top layer of thin-film devices is fabricated, the dissolution is limited to progress only from the sidewalls of the Mg layer. Therefore, the full undercut etching of the sacrificial layer and release of device structures of tens of millimeters in width and length is typically slowed down such that it would take 15–30 min, and all probes have demonstrated >6 months of shelf life in our tests. In addition, the impact on live cells from the dissolution of the Mg sacrificial layer is evaluated in vitro by a cell viability test. As shown in Figure 2b, a 100 nm Mg layer was deposited on a $15 \text{ mm} \times 15 \text{ mm}$ control silicon substrate, where the edges of the substrate were covered by a $10 \mu\text{m}$ thick SU-8 frame spacer by photolithography. The SU-8 spacer simulates the typical thickness of an implanted probe and the spacing between the sacrificial layer and neighboring live cells and helps avoid direct mechanical damage to the cells in this test. The chip was then flipped over to make contact with E18 rat cortical neurons (Genlantis) 7 days in vitro (DIV) cultured on a coverslip in a 12-well plate. After culturing for 20 h, the cells were then stained by LIVE/DEAD cell viability kit no. 1 (Thermo Fisher Scientific, L7013; see the Supporting Information), fixed, and evaluated by epi-fluorescence. Figure 2c shows a typical image of the fluorescence signals from the samples for which the red signals showed the dead cells and green signals showed the live cells. For cells cultured with the Mg-coated substrates, the viability was $97.7 \pm 0.3\%$, which is statistically the same as the $97.3 \pm 0.6\%$ of the control group (Figure 2d). The local Mg^{2+} concentration increase is estimated as follows. For simplicity, we use a 1D model in which Mg is dissolved from an infinite plane and start diffusion in one direction. Given the diffusion coefficient of Mg^{2+} in solution as $7.05 \times 10^{-6} \text{ cm}^2/\text{sec}$, within 10 min, the diffusion distance of Mg^{2+} in free medium will be $\sim 950 \mu\text{m}$. Because inside the tissue, the diffusion would be constrained by the surrounding cells, we can assume a diffusion distance an order of magnitude smaller, $\sim 100 \mu\text{m}$, which gives us a higher limit of the impact. For a Mg film of 100 nm, the average local concentration increase is about 0.04 mM. Because the extracellular Mg^{2+} concentration ranges from 2 mM for the brain to 1.2 mM for the heart,^{46,47} such a concentration increase is less than 3.5% down to 2%. Because in real scenarios, the diffusion would be a 3D model, the above numbers define the higher limit of concentration change. In addition, the increase of extracellular Mg^{2+} concentration generally will introduce suppression of neuron activities or cardioprotective

effects.^{48,49} The in vitro study of survival effects on hippocampal neurons of Mg^{2+} showed that a >1 mM increase of concentration would start to show trend of neuronal loss ($p = 0.07$),⁵⁰ which is well above our estimated value. Therefore, we believe that the dissolution of Mg sacrificial layer would have a minimal impact on neuron viability.

In addition, we investigated the excitability of neurons that have been cocultured with Mg substrate with the same procedure in Figure 2b. Specifically, the Mg substrate was introduced to E18 rat hippocampal neurons 8 DIV, and whole-cell patch current-clamping was performed on 14 DIV together with a control group. The action potentials were analyzed and compared following the method used by Dribben et al. in their investigation on Mg^{2+} effect on neurons,⁵⁰ as shown in Figure 2e. The threshold (Figure 2f), full width at half-maximum (fwhm) (Figure 2g), and peak amplitude (Figure 2h) of the action potentials did not show differences between the two groups. Therefore, the local Mg^{2+} concentration increase caused by the dissolution of the Mg sacrificial layer was not significant enough to change the neuron excitability. Lastly, we have also performed preliminary in vitro evaluation of the response from E18 rat astrocytes to the probes. Specifically, the viability for astrocytes co-cultured with Mg-evaporated substrate was $97.4 \pm 0.8\%$ versus $97.5 \pm 0.7\%$ in the control group ($p = 0.75$) (Figure S4), and the proliferation rates of astrocytes within $200 \mu\text{m}$ from the edge of the probe was $60 \pm 10\%$ versus $53 \pm 30\%$ in the control group ($p = 0.76$) (Figure S5). Because immune response leads to significantly higher astrocyte proliferation,^{51,52} the astrocytes proliferation rate can be used as an indicator of immune response to probe. Compared to reported astrocyte enrichment of up to $100\text{--}300 \mu\text{m}$ from the surface of implanted electrode 2 weeks post-injection,⁵¹ our probe design does not seem to induce significant astrocytes immune response. In summary, our preliminary in vitro data suggest that our biodegradable sacrificial layer would not negatively impact the physiological status of neurons cells. Nevertheless, in vivo immune histochemical study of the tissue response to the dissolution of the Mg sacrificial layer will be necessary to fully evaluate the biocompatibility of our design and will be performed in a follow-up study.

Based on the proposed fabrication procedures, we demonstrate here two types of designs of probes for different applications. In type I probes, a 3D bend-up structure is spontaneously formed on the thin silicon supporting shaft shortly after the probe is exposed to physiological fluids due to the integrated stress within the thin film structure. In type II probes, the whole ultrathin device layer is accurately released with all rigid structures completely removed soon after surgery.

Figure 3a shows the schematics of the basic structure of a type I probe. The Mg sacrificial layer was defined locally (red area in Figure 3a) below the end terminals of the thin-film device, and the rest of the top layer was directly anchored on the silicon supporting shaft. The part of the device arms on top of the Mg layer was composed of Cr/Pd/Cr (1.5/75/50 nm). When the Mg layer is dissolved in physiological solution, this free ending of the device will turn from the planar structure into a 3D bend-up structure due to the built-in stress, similar to the bend-up structure utilizing Ni as sacrificial layers, as reported previously.¹⁹ The deflection of the dual-metal film is proportional to the product of the average intrinsic stress and the film thickness. To obtain the required geometry, tensile stress in metal film is required. In addition, the two layers of metals should have comparable stress-thickness

product to generate similar deflection to avoid deformation or separation of the two films. We used 75 nm Pd and 50 nm Cr as optimal combination for our bend-up structure, which have tensile intrinsic stress and provide 4.4×10^{-4} and 4.9×10^{-4} dyn/cm, respectively.⁵³ This design would allow us to insert the probe into the tissue, and the functional thin-film device will be able to spontaneously form a 3D structure that deviates away from the lesion caused by the surgery so that only the flexible part of the device will get in contact with the active cells in the surrounding space (Figure 3a right). Arrays of locally bend-up structures on a planar silicon substrate have been fabricated to test the dissolution time and the yield of the formation of the bend-up structure in PBS solution. All samples have shown consistent dissolution time within 20 min and yield of >93% (Figure 3b) independent of the shelf life of the samples (ranging from 2 weeks to 6 months).

To have the functional devices of the type I probe positioned in tissue properly, the bend-up height and the bend-up moment need to be tuned such that the devices can be positioned closer to the active cells with optimal stiffness. This can be achieved by adjusting the dimensions of the electrodes. Specifically, we kept the distance between the electrode pair fixed as $S = 8 \mu\text{m}$ for a small cross-section profile and observed that the bend-up height increased with the length L of the electrodes for different electrode width W (Figure 3c). This can be understood by the same model used in previous reports. The total bend-up stiffness per unit width D of the structure can be calculated as $D = \alpha_s \cdot D_s + \alpha_m \cdot D_m$, where α_s and α_m are the area fraction of the SU-8 polymer and metal part, D_s and D_m are the bending stiffness per unit width of polymer and metal part, respectively.⁵⁴ In our design, $\alpha_m = W/(W + 2)$, where W is the width of metal electrodes. Because D can also be expressed as $D = M/(c \cdot d)$, where M is the bending moment, d is the total width, and c is the curvature of the bending, we have the following. (1) When bending against the same load, such as in our tests in free medium, the curvature would remain constant, and bend-up height h increased with the length of the electrodes. (2) As the width of the metal electrodes increased, α_m and D_m increased accordingly, which led to increased curvature and, thus, higher bend-up height under the same load and electrode length. (3) For the same electrode length and bend-up height, increasing the width of the metal electrodes would lead to a higher bending moment, which means that the structure would be able to penetrate into tougher tissue structures. In addition, from Figure 3c, we can see that for the same length of the electrodes, the bend-up height increased significantly only when W was above $2 \mu\text{m}$. For $W = 2 \mu\text{m}$, the bend-up height followed fairly close to the case when $W = 1 \mu\text{m}$. This can be attributed to the larger contribution of $\alpha_s \cdot D_s$ when α_m is small.

A photo of an as-fabricated probe is shown in Figure 3d in which multiple local bend-up structures have been defined along the length of the probe body. Such probes have been tested in vitro to interface with individual neurons, as shown in Figure 3e. The probe was mounted on a micromanipulator with an angle of $25\text{--}30^\circ$ and the thin-film electrode pairs facing down. After the insertion of the probe into the neuron recording medium, the Mg sacrificial layer was completely dissolved within 15 min and the thin-film electrodes bent downward by $\sim 20 \mu\text{m}$. The ends of the electrodes were originally aligned with the tip edge of the probe before the bend up and in the image it was clearly retracted as a result of the bend-up and the projection of the imaging angle. The device can be used to target and contact individual neurons for localized stimulation (see the Supporting Information). The

limit for such an in vitro use of the probe is that the access angle of the probe must be kept shallow as the rigid tip of the silicon shaft will interfere with the contact when the angle is too steep.

In addition, type II probes are designed to accurately implant fully flexible devices without any rigid structure remaining in the tissue at all. As shown in Figure 4a, type II probes have all of the functional connections and devices integrated within the top thin-film structure, which is fabricated on the Mg sacrificial layer covering the entire surface of the rigid silicon shaft. The as-prepared whole probe is rigid and robust enough that it easily bonds reliably with external wire connections directly and can be mounted on a micromanipulator. After the insertion of the probe into the tissue, the whole top film structure is released from the silicon shaft, typically within 30 min, and the rigid shaft can be retracted following the exact same path of insertion from the tissue, leaving only the thin film staying with its shape and position unaltered. Here, for the first time, we demonstrate high level of fidelity with keeping the shape and position of the thin-film probe when the thickness was as low as 2 μm , with the rigid shaft structure itself as thin as 10 μm , both of which are much smaller than previously reported results. Figure 4b–e shows the whole process of implanting the thin-film probe into a tissue-mimicking agarose gel (1 wt %, Young's modulus 40 kPa), demonstrating (1) the as-prepared whole probe mounted on a micromanipulator holder with wire connections before insertion, (2) the whole probe inserted inside the gel, (3) the release of the top thin-film structure and retraction of the rigid silicon shaft after 30 min, and (4) the full retraction of the silicon shaft with the thin-film probe remaining in the gel.

In addition, to track the integrity of the film structure and the functional device, we have used electron-beam lithography (EBL) to connect the metal electrode pairs at the very end with a thin gold film of dimensions 2 μm wide $\times$ 2 μm long $\times$ 50 nm thick so that the resistance through the whole length of the electrodes can be tracked during the whole operation. The calculated resistance based on geometry of the electrodes was ~ 1430 ohm, and the measured result before the operation was 1390 ohm (Figure 4b,f(i)), consistent with the estimation. As the probe was inserted into the gel, we observed no distortion of the shape or the direction (Figure 4c), which showed that the rigidity of the 10 μm thick silicon shaft was enough to deliver the full structure into tissues with similar Young's modulus to the gel used here. The resistance between the electrode pair was constant during this process (Figure 4f(ii)). After 30 min, the silicon shaft was gently retracted fully from the gel following the same path, while the thin-film probe was left inside of the gel (Figure 4d,f(iii)). As the silicon shaft was fully retracted, the position and shape of the 2 μm thin film probe showed no distortion in shape nor size, with a consistently constant resistance reading (Figure 4e,f(iv)). It is important to note that the gold bridge we defined by EBL was only supported by a 500 nm SU-8 layer on the bottom, and therefore, in our test, the thinnest part of the probe was only 550 nm. The $<1\%$ change in the resistance of the test probe throughout the whole process proves the robustness of our design and protocol for implantation surgeries.

Our biodegradable sacrificial layer and choice of Si for the supporting structure also put some limitations in some applications. For example, our sacrificial layer dissolves quickly in high-ionic-strength solution to minimize the waiting time during surgery. This also makes

post-fabrication treatment in aqueous solution difficult such as conductive polymer coating, electrochemical preparation of Pt black coating, etc. For applications requiring these additional treatments of the core devices, the extra steps of masking and of protection and deprotection would be necessary. In addition, similar to existing commercialized products (for example, neuroprobes from Neuronexus Inc.) that uses silicon as the supporting structure, the low fracture strength of silicon makes the backbone subject to potential breaking during insertion when the probe becomes too long. Therefore, our current design is suitable for implantations within 10 mm depth from the surface of the tissue. For deeper insertion applications, additional supporting material and structure may be needed. Other fabrication-friendly materials such as SiC and Si₃N₄ could be explored with proper etching protocols. In addition, this study focuses on the material and structure designs and surgical strategy for implantable devices. Therefore, for simplicity and clarity, we only used single-channel probes with the smallest features (~1 μm) for our demonstrations of robustness. However, multiplexing of devices is an oft-desired feature, and additional follow-up study will be needed to find an optimal solution for our designs. For example, for our type I probe, because the rigid structure of the Si backbone will remain after surgery, conventional wire-bonding and sealing methods can be directly applied like in commercial implantable probes, whereas for our type II probe, a more-delicate high-resolution process that works better with thin-film structures may be necessary. The conductive ink printing methods explored by Hong et al.³⁰ could be a potential solution.

In summary, we have reported a fabrication framework and surgery procedures to prepare flexible ultrathin-film devices that can be delivered accurately into tissue-mimicking gel samples with a biodegradable sacrificial layer that allows (1) the in situ formation of 3D bend-up structures after the surgery and (2) the full release of the whole thin-film probe within the sample and the whole rigid structure completely removed afterward. We have systematically studied the behavior of the bend-up structure with Mg sacrificial layers in biological fluids and demonstrated that film probes as thin as 2 μm can be delivered accurately with intact geometry inside tissue, and the lesion caused by the retractable rigid delivery structure can be as thin as 10 μm using our techniques. The in vivo biocompatibility of our probes remains to be verified in a follow-up study, but our in vitro test results and the estimated <0.04 mM increase in local Mg²⁺ concentration suggest that our design could provide a general outside-the-box solution for accurately implanting ultrathin and flexible device arrays that is compatible with scalable top-down production.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Jog M, Connolly C, Kubota Y, Iyengar D, Garrido L, Harlan R, Graybiel A. *J Neurosci Methods*. 2002; 117:141–152. [PubMed: 12100979]
2. Hubel DH. *Science*. 1957; 125:549–550. [PubMed: 17793797]
3. Kipke DR, Vetter R, Williams J, Hetke J. *IEEE Trans Neural Syst Rehabil Eng*. 2003; 11:151–155. [PubMed: 12899260]
4. Normann R, Maynard E, Rousche P, Warren D. *Vision Res*. 1999; 39:2577–2587. [PubMed: 10396626]
5. Polikov V, Tresco P, Reichert W. *J Neurosci Methods*. 2005; 148:1–18. [PubMed: 16198003]
6. Gilletti A, Muthuswamy J. *J Neural Eng*. 2006; 3:189–195. [PubMed: 16921202]
7. Seymour JP, Kipke DR. *Biomaterials*. 2007; 28:3594–3607. [PubMed: 17517431]
8. Timko BP, Cohen-Karni T, Yu G, Qing Q, Tian B, Lieber CM. *Nano Lett*. 2009; 9:914–918. [PubMed: 19170614]
9. Kim D, Vimenti J, Amsden JJ, Xiao J, Vigeland L, Kim Y, Blanco JA, Panilaitis B, Frechette ES, Contreras D, Kaplan DL, Omenetto FG, Huang Y, Hwang K, Zakin MR, Litt B, Rogers JA. *Nat Mater*. 2010; 9:511–517. [PubMed: 20400953]
10. Kozai TDY, Kipke DR. *J Neurosci Methods*. 2009; 184:199–205. [PubMed: 19666051]
11. Rousche P, Pellinen D, Pivin D, Williams J, Vetter R, Kipke DR. *IEEE Trans Biomed Eng*. 2001; 48:361–371. [PubMed: 11327505]
12. Khodagholy D, Gelinis JN, Thesen T, Doyle W, Devinsky O, Malliaras GG, Buzsaki G. *Nat Neurosci*. 2014; 18:310–315. [PubMed: 25531570]
13. Rivnay J, Wang H, Fenno L, Deisseroth K, Malliaras GG. *Sci Adv*. 2017; 3:e1601649. [PubMed: 28630894]
14. Duan X, Fu TM, Liu J, Lieber CM. *Nano Today*. 2013; 8:351–373. [PubMed: 24073014]
15. Tian B, Lieber CM. *Annu Rev Anal Chem*. 2013; 6:31–51.
16. Duan X, Lieber CM. *Nano Res*. 2015; 8:1–22.
17. Zhang A, Lieber CM. *Chem Rev*. 2016; 116:215–257. [PubMed: 26691648]
18. Zhou W, Dai X, Lieber CM. *Rep Prog Phys*. 2017; 80:016701. [PubMed: 27823988]
19. Tian B, Cohen-Karni T, Qing Q, Duan X, Xie P, Lieber CM. *Science*. 2010; 329:830–834. [PubMed: 20705858]
20. Qing Q, Jiang Z, Xu L, Gao R, Mai L, Lieber CM. *Nat Nanotechnol*. 2013; 9:142–147. [PubMed: 24336402]
21. Duan X, Gao R, Xie P, Cohen-Karni T, Qing Q, Choe HS, Tian B, Jiang X, Lieber CM. *Nat Nanotechnol*. 2011; 7:174–179. [PubMed: 22179566]
22. Xie C, Hanson L, Cui Y, Cui B. *Proc Natl Acad Sci U S A*. 2011; 108:3894–3899. [PubMed: 21368157]
23. Hanson L, Zhao W, Lou H, Lin ZC, Lee SW, Chowdary P, Cui Y, Cui B. *Nat Nanotechnol*. 2015; 10:554–562. [PubMed: 25984833]
24. Xie X, Xu AM, Angle MR, Tayebi N, Verma P, Melosh NA. *Nano Lett*. 2013; 13:6002–6008. [PubMed: 24237230]
25. Kim DH, Lu N, Ma R, Kim Y, Kim R, Wang S, Wu J, Won SM, Tao H, Islam A, Yu KJ, Kim T, Chowdhury R, Ying M, Xu L, Li M, Chung H, Keum H, McCormick M, Liu P, Zhang Y, Omenetto FG, Huang Y, Coleman T, Rogers JA. *Science*. 2011; 333:838–843. [PubMed: 21836009]
26. Kim D, Ghaffari R, Lu N, Rogers JA. *Annu Rev Biomed Eng*. 2012; 14:113–128. [PubMed: 22524391]
27. Choi C, Choi MK, Hyeon T, Kim D. *Chem Nano Mater*. 2016; 2:1006–1017.
28. Xie C, Liu J, Fu T, Dai X, Zhou W, Lieber CM. *Nat Mater*. 2015; 14:1286–1292. [PubMed: 26436341]
29. Liu J, Fu T, Cheng Z, Hong G, Zhou T, Jin L, Duvvuri M, Jiang Z, Kruskal P, Xie C, Suo Z, Fang Y, Lieber CM. *Nat Nanotechnol*. 2015; 10:629–637. [PubMed: 26053995]

30. Hong G, Fu T, Zhou T, Schuhmann TG, Huang J, Lieber CM. *Nano Lett.* 2015; 15:6979–6984. [PubMed: 26317328]
31. Zhou T, Hong G, Fu T, Yang X, Schuhmann TG, Viveros RD, Lieber CM. *Proc Natl Acad Sci U S A.* 2017; 114:5894–5899. [PubMed: 28533392]
32. Hwang S, Tao H, Kim D, Cheng H, Song J, Rill E, Brenckle MA, Panilaitis B, Won SM, Kim Y, Song YM, Yu KJ, Ameen A, Li R, Su Y, Yang M, Kaplan DL, Zakin MR, Slepian MJ, Huang Y, Omenetto FG, Rogers JA. *Science.* 2012; 337:1640–1644. [PubMed: 23019646]
33. Kim T, McCall JG, Jung YH, Huang X, Siuda ER, Li Y, Song J, Song YM, Pao HA, Kim R, Lu C, Lee SD, Song I, Shin G, Al-Hasani R, Kim S, Tan MP, Huang Y, Omenetto FG, Rogers JA, Bruchas MR. *Science.* 2013; 340:211–216. [PubMed: 23580530]
34. Koh A, Gutbrod SR, Meyers JD, Lu C, Webb RC, Shin G, Li Y, Kang S, Huang Y, Efimov IR, Rogers JA. *Adv Healthcare Mater.* 2016; 5:373–381.
35. Luan L, Wei X, Zhao Z, Siegel JJ, Potnis O, Tuppen CA, Lin S, Kazmi S, Fowler RA, Holloway S, Dunn AK, Chitwood RA, Xie C. *Sci Adv.* 2017; 3:e1601966. [PubMed: 28246640]
36. Hwang S, Lee CH, Cheng H, Jeong J, Kang S, Kim J, Shin J, Yang J, Liu Z, Ameer GA, Huang Y, Rogers JA. *Nano Lett.* 2015; 15:2801–2808. [PubMed: 25706246]
37. Gunatillake P, Mayadunne R, Adhikari R. *Biotechnol Annu Rev.* 2006; 12:301–347. [PubMed: 17045198]
38. Kim UJ, Park J, Kim HJ, Wada M, Kaplan D. *Biomaterials.* 2005; 26:2775–2785. [PubMed: 15585282]
39. Nair LS, Laurencin CT. *Prog Polym Sci.* 2007; 32:762–798.
40. Kurland NE, Dey T, Wang C, Kundu SC, Yadavalli VK. *Adv Mater.* 2014; 26:4431–4437. [PubMed: 24737390]
41. Khilwani R, Gilgunn PJ, Kozai TDY, Ong XC, Korkmaz E, Gunalan PK, Cui XT, Fedder GK, Ozdoganlar OB. *Biomed Microdevices.* 2016; 18:97. [PubMed: 27778225]
42. Barz, F., Ruther, P., Takeuchi, S., Paul, O. Flexible silicon-polymer neural probe rigidified by dissolvable insertion vehicle for high-resolution neural recording with improved duration. *Proceedings of The 28th International Conference on Micro Electro Mechanical Systems*; Estoril, Portugal. Jan 18–22, 2015; New York: IEEE; 2015. p. 636-639.
43. Kozai TDY, Gugel Z, Li X, Gilgunn PJ, Khilwani R, Ozdoganlar OB, Fedder GK, Weber DJ, Cui XT. *Biomaterials.* 2014; 35:9255–9268. [PubMed: 25128375]
44. Yin L, Cheng H, Mao S, Haasch R, Liu Y, Xie X, Hwang S, Jain H, Kang S, Su Y, Li R, Huang Y, Rogers JA. *Adv Funct Mater.* 2014; 24:645–658.
45. Hwang S, Park G, Cheng H, Song J, Kang S, Yin L, Kim J, Omenetto FG, Huang Y, Lee K, Rogers JA. *Adv Mater.* 2014; 26:1992–2000. [PubMed: 24677058]
46. Veloso D, Guynn RW, Oskarsson M, Veech RL. *J Biol Chem.* 1973; 248:4811–4819. [PubMed: 4718747]
47. Taylor J, Vigneron D, Murphy-Boesch J, Nelson S, Kessler H, Coia L, Curran W, Brown T. *Proc Natl Acad Sci U S A.* 1991; 88:6810–6814. [PubMed: 1650484]
48. Sato Y, Fukuda J. *Clin Calcium.* 2004; 14:50–57. [PubMed: 15577097]
49. Watanabe M, Wu JR, Li CZ, Okada T. *Exp Clin Cardiol.* 2004; 9:181–185. [PubMed: 19641723]
50. Dribben WH, Eisenman LN, Mennerick S. *Cell Death Dis.* 2010; 1:e63. [PubMed: 21364668]
51. Fu TM, Hong G, Zhou T, Schuhmann TG, Viveros RD, Lieber CM. *Nat Methods.* 2016; 13:875–882. [PubMed: 27571550]
52. Chesik D, Glazenburg L, Keyser JD, Wilczak N. *J Neurochem.* 2007; 100:1555–1564. [PubMed: 17348863]
53. Klokholm E, Berry B. *J Electrochem Soc.* 1968; 115:823–826.
54. Tian B, Liu J, Dvir T, Jin L, Tsui JH, Qing Q, Suo Z, Langer R, Kohane DS, Lieber CM. *Nat Mater.* 2012; 11:986–994. [PubMed: 22922448]

Figure 1.

Schematics of fabrication procedures and probe structure. (I) On a 300 μm thick double-side polished (100) silicon substrate with 150 nm Si_3N_4 coating (1), the biodegradable sacrificial layer and thin-film device layer with bonding areas (2) are fabricated successively using standard top-down lithographic procedures. (II) The probe body is carved out by a top-side RIE process. During the RIE, a photoresist pattern is used as the etching mask to define and protect the probe profile as well as the outer frame area (4), while the opened area (3) is thinned for 10–30 μm . (III) The silicon substrate is uniformly etched by a back-side RIE process until the thinned open area in stage (II) is completely removed, resulting in a fully isolated probe structure that has a narrow neck connection (5) to the outer supporting frame. Inset: cross-section of the probe structure. The thin-film device layer (6) and biodegradable metal sacrificial layer (7) are on top of a 10–30 μm thick silicon shaft (8). For clarity, the dimensions are not drawn to scale.

Figure 2.

Characterization of Mg biodegradable sacrificial layer. (a) Shelf life and dissolution time test. (1) A 100 nm Mg layer was thermally evaporated on a blank silicon substrate. (2) The freshly prepared sample was then stored in ambient environment (humidity <10%) for 10 weeks, and the metal film did not show quality degradation. (3) After 10 weeks of storage, the Mg layer can be dissolved in 1× PBS within 2 min. (b) Cell viability test setup. A 100 nm Mg layer (2) was deposited on a 15 × 15 mm silicon substrate in which the edges were covered by 10 μm thick SU8-frame spacer (1) mimicking the typical distance from a cell to the sacrificial layer on probe. This silicon substrate was flipped over and placed on a coverslip on which the seven DIV rat cortical neurons were cultured. Scale bar: 5 mm. (c) Typical fluorescence microscope image of cells cultured with Mg-evaporated substrates for 20 h. Green color represents live cells and red signals represent dead cells. (d) Cell viability of control group and cells cultured with Mg-evaporated substrates. The viability of cells cultured Mg-evaporated substrate was $97.7 \pm 0.3\%$. Control group viability is $97.3 \pm 0.6\%$; $n = 9$; total number of cells analyzed = 1280; *, $p = 0.22$. (e) Representative traces (red) of action potentials of the control group and Mg substrate co-cultured group. A total of five cells from each group were patched in current-clamp mode (current traces are in blue). (f) The action potential threshold of control group (-27 ± 3 mV) and cells cultured with Mg substrate (-28 ± 4 mV); $p = 0.61$. (g) Full width at half-maximum (fwhm) of the control group (4.7 ± 0.9 ms) and cells cultured with Mg substrate (4.7 ± 0.8 ms); $p = 0.96$. (h) Spike potential amplitude of control group (71 ± 6 mV) and cells cultured with Mg substrate (75 ± 11 mV); $p = 0.47$.

Figure 3.

Probe with 3D flexible thin-film bend-up structure. (a) Schematics of probe implantation and in situ formation of bend-up structure. Left: The backside of silicon shaft is glued to a probe holder of micromanipulator (brown). The bonding area is connected to external instrumentation by silver paste (green) and conductive wires (blue). Zoom (dashed black circle) of basic structures at probe tip showing the thin-film flexible structure (blue) is held by Mg biodegradable sacrificial layer (red) before implantation. Right: in situ formation of 3D flexible thin-film bend-up structure. The black arrow indicates the insertion direction. Zoom (dashed black circle) of thin-film device shows the functional thin-film device spontaneously forms a 3D bend-up structure after implantation. (b) Bright-field optical microscope images of bend-up structures. (i) Array of bend-up electrodes in $1\times$ PBS. (ii, iii) Close-up view of a thin-film bend-up electrode structure. Microscope was focused on the (ii) electrode tip and (iii) anchor part of a same electrode structure, respectively. Images were taken using a water-immersion lens. (c) Bend-up height of the thin-film structure as a function of electrode dimensions. The bend-up height increased with electrode length and width. The measurement was done in PBS solution for metal layer composed of Cr/Pd/Cr (1.5/ 75/50 nm). Inset, top-down schematics of electrodes (red) geometry in thin-film structures. L, W, and S represent the electrode length, width, and distance between electrode pair. (d) Left: Camera image of probes connected to a supporting frame with a thin neck structure. Right: Zoomed-in view of electrode site at probe tip. Image was taken by bright-

field optical microscope. Scale bar: 30 μm . (e) Microscope image of in situ formation of 3D thin-film bend-up structure in neuron cell culture medium. Electrodes tip deviated from its original position (indicated by blue arrow) to form a 3D structure, the apex of which is indicated by the red arrow. Scale bar: 30 μm .

Figure 4.

Ultraflexible probe implantation. (a) Schematics of ultraflexible probe assembly and implantation procedures. Left: Silicon shaft (gray) of the probe is glued to probe holder of a micro-manipulator (brown). Bonding area on the thin-film device side is connected to external instrumentation by silver paste (green) and conductive wires (blue). Right: The thin-film probe (magenta) fully delaminates from the silicon shaft, typically in 30 min after insertion into tissue. The shaft can be fully retracted from the tissue, leaving the position and the shape of device intact. (b–e) Stereomicroscope images show the whole process of implanting probe into 1% agarose gel mimicking brain tissue environment and detachment of the thin-film probe. (b) The bonding areas on thin-film probe (1) were connected to recording instruments and the silicon shaft was glued to probe holder of micromanipulator (2). Scale bar: 2 mm. (c) The probe (3) was inserted into tissue-mimicking gel. Scale bar: 2 mm. (d) 30 min after insertion, the sacrificial layer dissolved. The thin-film probe (4) was fully released from the silicon shaft (5), which is then fully retracted from the gel, leaving only the thin-film probe (4) and the connected wires (6) in the gel. Scale bar: 2 mm. (e) The thin-film probe (7) in the gel. Scale bar: 2 mm. (f) Resistance recorded from an electrode pair that is shorted at the tip in the thin-film layer during the insertion and thin-film detachment procedures. (i) The time when the probe enters the gel. (ii) The time when the

probe is completely inserted in the gel. (iii, iv) The beginning of pulling silicon shaft and the fully extraction of silicon shaft, respectively.

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