

Gold Coated SU-8-Based Microelectrodes for *In Vivo* Electrophysiological Studies

Rapid Prototyping and Protocol-Specific Microelectrode Designs

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Abstract—Microelectrodes are widely used to record biological signals from living tissue, namely in neuroscience where researchers need to record brain signals *in vivo*. Several types of microelectrodes are available in the market. Unfortunately, most of the microelectrode developing processes are time consuming and expensive. This work presents a rapid low-cost prototyping technology for protocol-specific microelectrode designs. The suggested fabrication process combines SU-8 photoresist patterning in multiple layers with the deposition of intermediate conductive layers (Au layer in the presented case). The fabricated microelectrodes were specifically designed for an electrophysiological experiment that required simultaneous recording in the pre-frontal cortex (PFC) and stimulation in the hippocampus of the rat brain. After electrode implantation surgery in an anesthetized rat, local-field potentials (LFP) from the PFC were acquired. The power spectra of the LFPs using the SU-8-based microelectrode and using a commercially available Pt-Ir electrode are comparable in a great extent. Staining of perfusion fixed brain slices showed that the electrodes targeted the correct brain regions. SU-8-based microelectrodes appear to be valuable as a rapid prototyping technology to design low-cost protocol-specific microelectrodes.

Keywords—microelectrodes; SU-8; rapid prototyping electrodes; electrophysiology; LFP

I. INTRODUCTION

The high demand for microelectrodes specifically designed for each experimental protocol that requires recording and stimulation of neural tissue is driving new electrode technologies [1]. However, the development process of new electrode designs are often time consuming and expensive (e.g. neural probes, electrode arrays, wire-coated electrodes), since it either requires the optimization of a great number of fabrication parameters to achieve structures with high-aspect-ratio (e.g. silicon etching processes) or the alternative fabrication processes are usually expensive (e.g. reactive ion etching) [2].

This work presents an alternative electrode technology for animal studies *in vivo*. The new invasive electrodes are

intended to be a low-cost solution for electrophysiological studies and may be completely redesigned on an experiment-basis in a short-time. Thus, specific electrode designs are provided for each experimental protocol, depending on the brain regions to be recorded and/or stimulated. The new electrodes presented in Figure 1 are needle-shaped and fabricated in a single structure of SU-8 photoresist in order to combine high-aspect-ratio and tough structures with small acquisition points able to acquire from neural correlates. Although the SU-8 allows the fabrication of tough needles, this material is flexible enough to penetrate the neural tissue smoothly and with low tissue damage [3], [4]. Besides, SU-8 also allows high resolution structural patterning with high aspect-ratio through photolithography techniques [3].

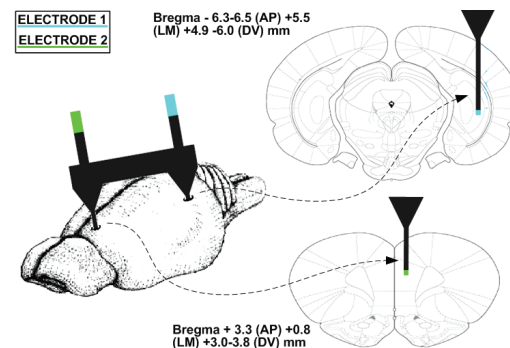


Figure 1. Needle-shaped electrodes specifically designed for an electrophysiological study which requires recording and stimulation of PFC (electrode 1) and hippocampus (electrode 2) respectively (adapted from [5]).

The next sections present the characteristics of the SU-8 photoresist, provide an overview of the electrodes' fabrication process, and present the results from the electrophysiological tests. The results section demonstrates the effective acquisition of local-field potentials in anesthetized animals with an example of the SU-8-based electrodes specifically designed for a protocol that requires simultaneous recording and stimulation of the pre-frontal

cortex (PFC) and the hippocampus, respectively, as depicted in Figure 1.

II. SU-8 PHOTORESIST

The SU-8 100 from *MicroChem Corporation* is a photoresist that consists of chemically amplified epoxy with high functionality, high optical transparency and sensitivity to ultra-violet (UV) radiation. Cured films or developed structures are highly resistant to solvents, acids and bases and have excellent thermal stability, making it well suited for permanent use applications. SU-8 can be patterned with aspect-ratios as high as 20, and with thicknesses between nanometers to 1 mm [6]. This material has been used to fabricate a wide variety of devices, including cantilever-based biosensors [7], low-cost point-of-care diagnostic equipments [8][9], biopotential electrodes [10], and micro-valves [11]. In comparison with other hard materials, the SU-8 photoresist presents the best compromise between mechanical characteristics and easiness of shape definition to be used as physical support for biopotential electrodes. There is a high number of candidate materials to be used as the conductive film of biopotential electrodes. Some examples include aluminum (Al) [12], platinum (Pt) and its alloys [13], gold (Au) [1] and stainless steel [14]. These materials also present a high biocompatibility and a stable electrochemical behavior for a wide range of biopotentials. Gold was the selected material to coat the SU-8 electrode due to its excellent surface inertness and because it provides no native oxide [10,15]. Moreover, and as it will be described in greater detail, the film thickness employed facilitates the co-patterning of SU-8 and gold (Au).

III. FABRICATION PROCESS

The Figure 2 shows a schematic drawing with the structure of the proposed SU-8-based microelectrode, where it is possible to observe the required layers (SU-8/Au/SU-8). A gold conductive layer must be deposited in the first SU-8 layer in order to provide the electrical contact with the neural tissue. The deposition process of the conductive layer cannot damage the SU-8 or even change its mechanical properties. Thus, the Au conductive layer was selected in order to keep the SU-8 layer intact.

A. Patterning of the SU-8 Photoresist

The structure is composed by three layers and was fabricated combining photolithography techniques for the SU-8 structural layers and a thermal evaporation technique for the deposition of the gold electrode layer. The steps of the fabrication process are summarized in Figure 3. After a proper cleaning of the glass substrate, a thin layer of Omnicoat (from *MicroChem Corporation*) (a) is deposited by spin-coating to allow an easy stirring of the structure from the glass substrate at the final stage of the process (h). After that, a 200 μm film of SU-8 100 is spin coated (b), soft-baked and exposed to UV radiation (c). In this step, the SU-8 layer acquires the desired structural pattern using a specific mask. Finally, a development process is necessary and a SU-8 developer (from *MicroChem Corporation*) is usually used in order to obtain the final structure. However, the need to deposit a gold film and another layer of SU-8 means that this step can only be performed at the end of the photolithography process of the second SU-8 layer. At this

point, a thin gold layer (d) is deposited on top of the first SU-8 layer through a thermal evaporation technique. Three successive depositions are performed in order to prevent heating the SU-8 layer and subsequent deterioration of its properties and the structural pattern held in (c). In addition, a mask is used to protect specific areas and separate the two needles of the structure that acquire distinct signals from different brain regions. Then, a new layer of SU-8 is deposited in step (e) and processed in the same way as the first SU-8 layer. However, in this case, the mask is designed to protect specific parts of the underneath gold layer, which will act as electrode contacts to measure and acquire the brain signals. In step (g), all the non-exposed SU 8 is developed, the gold deposited over the non-exposed SU-8 layer is removed and the electrode contacts are exposed. Thus, the final structure is obtained.

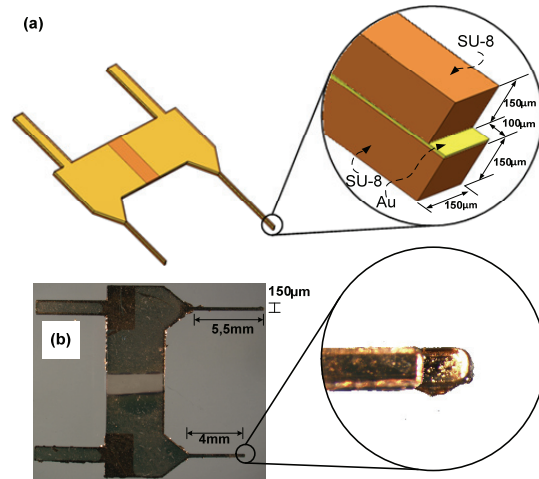


Figure 2. SU-8 electrode; (a) Schematic drawing of the proposed SU-8-based electrode and needle tip in detail; (b) SU-8 electrode fabricated with the needle tip in detail.

B. Au Depositions by Thermal Evaporation

The deposition process of the selected conductive material must be compatible with the requirements to maintain the structural integrity of the SU-8. Thus, the temperature during the depositions must be low enough to avoid the degradation of SU-8 properties. The adhesion between the SU-8 and the conductive layer must be good in order to avoid the electrode disaggregation with the consequent layers splitting. Because of its soft mechanical characteristics that can trade with success the former constraints, gold can be deposited by evaporation with rates in the range $0.6\text{-}0.8 \text{ nm}\cdot\text{s}^{-1}$. These deposition rates are compatible with the desired thickness of the Au layer: 40-50 nm. The resistivity of the Au film for the whole electrode was estimated to be $3.09 \times 10^{-10} \Omega \times \text{mm}$. If the evaporation process is fast enough (less than one minute) then it can be done without significantly heating the substrate (SU-8). Repeating this process (after decreasing the temperature of the substrate) as desired to deposit successive layers of gold with the desired thickness is an effective strategy. Moreover, since the gold is a soft metal and the SU-8 presents a high Young's Modulus (approx. 3 GPa [16]) in comparison to brain tissue's (3-10 KPa [17]), a good mechanical match is achieved and the danger of the electrode bending is low.

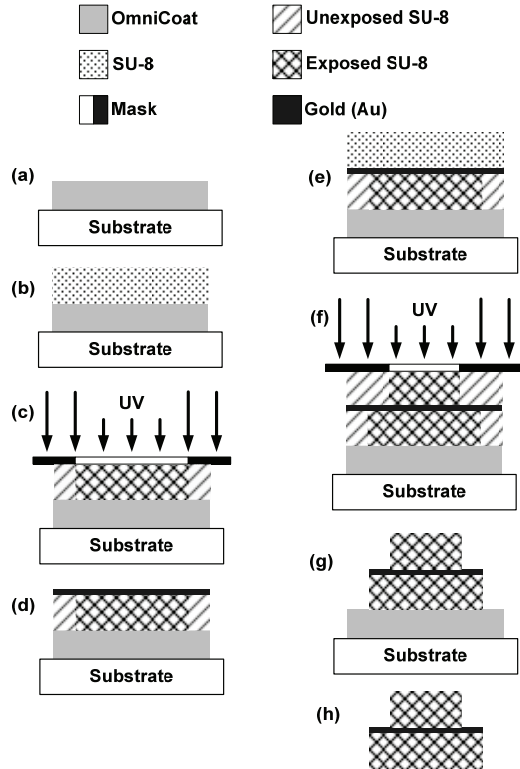


Figure 3. Fabrication steps of SU-8 electrodes.

IV. ELECTROPHYSIOLOGICAL TESTS

In order to test an example of the newly developed gold coated SU-8-based microelectrodes, *in vivo* recordings from rat brain were performed as described below.

A. Animals

Male Wistar rats (Charles-River Laboratories, Barcelona, Spain), weighing 300–400 g and aged 3 months were housed (two per cage) under standard laboratory conditions (12 h light: 12 h dark cycle, at 22°C, relative humidity of 55%; free access to food and water). All procedures were carried out in accordance with European Union Directive 86/609/EEC on animal welfare and experimentation.

B. Electrophysiology

The experimental protocol to test the produced electrodes follows the procedure of electrode implantation and recording extracellular field potentials that has been described previously [18]. Briefly, one rat (weighing 350–400 g) was anesthetized with sodium pentobarbital (60 mg/kg, *i.p.*, supplemented each 40 min throughout the experiment) and placed in a stereotaxic frame. Rectal temperature was monitored to 37°C by means of a homeothermic warming blanket. The regions addressed were the prelimbic portion (PL) of the PFC and the CA1 region of the hippocampus, thus requiring 4 and 5.5 mm long needles respectively (Figure 2). These two regions are intimately related to the computation of complex cognitive functions such as information integration, learning, memory processing and behaviour flexibility [19]. The SU-8 recording electrode tips were inserted simultaneously in the PL (coordinates, 3.3 mm anterior to bregma, 0.8 mm lateral

to the midline, 3.0–3.8 mm below cortical surface) and in the CA1 (coordinates, 6.3–6.5 mm posterior to bregma, 5.5 mm lateral to the midline, 4.9–6.0 mm below cortical surface) sub-regions, according to Paxinos and Watson [5]. While the new SU-8-based microelectrode was implanted in the mentioned regions of the right hemisphere, a commercially available bipolar platinum-iridium (Pt-Ir) microelectrode (0.5 MΩ at 1 kHz), Science Products GmbH, Germany, was implanted in the PL region of left hemisphere (same coordinates as the SU-8-based microelectrode). A stainless steel screw electrode inserted through a burr hole (coordinates, 3.0 mm anterior to lambda; 3.0 mm lateral to the midline) served as reference for both Pt-Ir and Au SU-8-based electrodes. The LFP signals were acquired through both the Au SU-8-based and Pt-Ir microelectrodes from the PL by means of a LP511 AC Amplifier (gain=5000; band-pass filtered at 0.3–3,000 Hz), Grass, UK, connected to a Micro 1401 mkII digital-to-analogue converter, CED, Cambridge, UK, stored and analyzed in a lab computer using Signal software, CED, Cambridge, UK. Recordings at 1000 samples/s were performed during 100 seconds. In order to avoid 50 Hz interference, the whole set-up was placed inside of a Faraday cage.

V. RESULTS

Although the electrical activity of each PL area is different from its hemispherical counterpart, the LFPs acquired by the SU-8 based microelectrode were compared with the LFPs acquired by the commercially available Pt-Ir microelectrode in terms of frequency content. In order to assess the performance of the Au SU-8-based microelectrode, the single-ended signal collected from a Pt-Ir microelectrode referenced to the stainless steel screw establishes a LFP standard in this study. The Figures 4(a) and 4(c) illustrate the LFP single-ended signals recorded from the PL of a rat using one Pt-Ir microelectrode and the new SU-8-based gold-coated microelectrode, respectively, both referred to the stainless steel screw. Figures 4(b) and 4(d) present the power spectrum of the LFP signal acquired by both electrodes during 100 seconds after being digitally high-pass filtered at 3 Hz (4th order Butterworth filter). As shown on Figures 4(b) and 4(d), the power of the LFP signal is highly concentrated in the 4–20 Hz frequency range for both electrode types. This range comprises frequency bands commonly observed in anaesthetized rats like theta (4–8 Hz), alpha (8–12 Hz) and lower beta bands (12–20 Hz). The recordings of the Au SU-8 microelectrode compared well to the recordings obtained using the commercially available Pt-Ir electrode, in terms of the frequency bands with highest power. The signal recorded with the Au SU-8 electrode is apparently noisier than the signal obtained with the Pt-Ir electrode but the high-frequency contents may be related with multi-unit activity due to the fact that the Au SU-8-based electrode has a larger recording surface. Impedance measures of the Au SU-8-based electrode in a saline solution are not presented as the procedures are ongoing at present. These measures will be more informative regarding the technical properties of the new electrode excluding the biological activity effects. The brain slices shown in Figure 5 indicate the electrode trajectories during implantation surgery and demonstrate that the Au SU-8-based needles were correctly placed on PL and CA1 sub-regions.

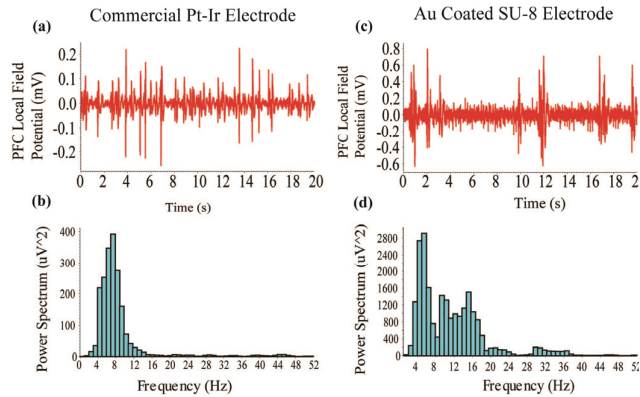


Figure 4. Local field potential signal in pre-frontal cortex acquired from (a) Pt-Ir electrode and (c) SU-8 electrode; Power spectra of the LFP signal in the 0-54 Hz frequency range for (b) Pt-Ir electrode and (d) SU-8 electrode.

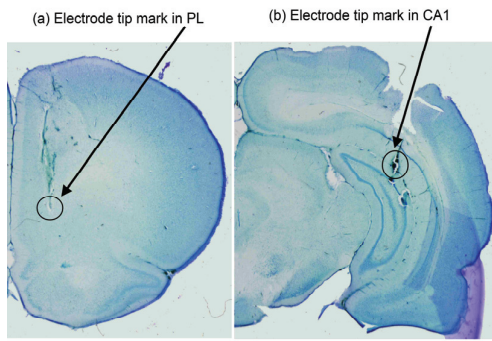


Figure 5. Brain slices showing the electrodes trajectory during implantation surgery in (a) PL and (b) in CA1.

VI. DISCUSSION AND CONCLUSIONS

This paper presented the fabrication and testing of a protocol-specific microelectrode design based on SU-8 photoresist. This methodology is suitable for rapid prototyping of needle-shaped structures for acquisition and stimulation of brain tissue. The SU-8 photoresist seems to be appropriate to achieve electrodes with simultaneous high degree of flexibility and high-aspect-ratio structures for invasive brain recordings. The SU-8 is also tough and our lithographic process allows the patterning of “exotic” shapes. Once the fabrication process of a specific electrode design is well established, its adaptation to other protocols (shapes and design) is a fast and low-cost procedure. The design of the electrode presented considered two needles with different lengths to reach the different brain regions of interest (PL and CA1 in this example) to a particular experimental protocol. Therefore, this work suggests the SU-8-based microelectrodes as a rapid prototyping technology to design low-cost protocol-specific microelectrodes.

ACKNOWLEDGMENT

L. R. Jacinto and J. F. Oliveira are supported by the Portuguese Foundation for Science and Technology under the Grant references SFRH/BD/40459/2007 and SFRH/BPD/66151/2009, respectively, and Centre Algoritmi.

This work was supported by FCT with the reference FCOMP-01-0124-FEDER-010909 (FCT/PTDC/SAU-BEB/100392/2008)

REFERENCES

- [1] S.F. Cogan, “Neural stimulation and recording electrodes,” *Annual Review of Biomedical Engineering*, vol. 10, 2008, pp. 275-309.
- [2] P. Griss and P. Enoksson, “Micromachined electrodes for biopotential measurements,” *Journal of Microelectronic Systems*, vol. 10, 2001, pp. 10-16.
- [3] A. Altuna, G. Gabriel, M.J. Rodriguez, R. Vilares, R. Villa, M. Tijero, M. Battle, F.J. Blanco, J. Berganzo, and L.J. Fernandez, “Study of functional viability of SU-8-based microneedles for neural applications,” *Journal of Micromechanics and Microengineering*, vol. 19, 2009, p. 025007.
- [4] R. Safavieh, M.P. la Roca, M.A. Qasaimeh, M. Mirzaei, and D. Juncker, “Straight SU-8 pins,” *Journal of Micromechanics and Microengineering*, vol. 20, May. 2010, p. 055001.
- [5] V.G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, 2005.
- [6] G. Liu, Y. Tian, and Y. Kan, “Fabrication of high-aspect-ratio microstructures using SU8 photoresist,” *Microsystem Technologies*, vol. 11, Apr. 2005, pp. 343-346.
- [7] M. Nordström, S. Keller, M. Lillemose, A. Johansson, S. Dohn, D. Haefliger, G. Blagoi, M. Havsteen-Jakobsen, and A. Boisen, “SU-8 Cantilevers for Bio/chemical Sensing; Fabrication, Characterisation and Development of Novel Read-out Methods,” *Sensors*, vol. 8, Mar. 2008, pp. 1595-1612.
- [8] P. Abgrall, A.-M. Gue, V. Conedera, N.-T. Nguyen, and H. Camon, “SU-8 as a structural material for labs-on-chips and microelectromechanical systems,” *Electrophoresis*, vol. 28, Dec. 2007, pp. 4539-4551.
- [9] J.M. Ruano-López, M. Agirregabiria, G. Olabarria, D. Verdoy, D.D. Bang, M. Bu, A. Wolff, A. Voigt, J.A. Dziuban, R. Walczak, and J. Berganzo, “The SmartBioPhone, a point of care vision under development through two European projects: OPTOLABCARD and LABONFOIL,” *Lab on a chip*, vol. 9, Jun. 2009, pp. 1495-9.
- [10] S.-H. Cho, H.M. Lu, L. Caulier, M.I. Romero-Ortega, J.-B. Lee, and G.A. Hughes, “Biocompatible SU-8-Based Microprobes for Recording Neural Spike Signals From Regenerated Peripheral Nerve Fibers,” *IEEE Sensors Journal*, vol. 8, Nov. 2008, pp. 1830-1836.
- [11] A. Ezkerra, L.J. Fernández, K. Mayora, and J.M. Ruano-López, “Fabrication of SU-8 free-standing structures embedded in microchannels for microfluidic control,” *Journal of Micromechanics and Microengineering*, vol. 17, Nov. 2007, pp. 2264-2271.
- [12] A. Lopez and P. Richardson, “Capacitive Electrocardiographic and Bioelectric Electrodes,” *IEEE Transactions on Biomedical Engineering*, vol. 16, 1969, pp. 99-99.
- [13] Ivorra, “Minimally invasive silicon probe for electrical impedance measurements in small animals,” *Biosensors & Bioelectronics*, vol. 19, 2003, pp. 391-399.
- [14] A Searle and L Kirkup, “A direct comparison of wet, dry and insulating bioelectric recording electrodes,” *Physiological Measurement*, vol. 21, 2000, p. 271.
- [15] A. Altuna, G. Gabriel, L. Menéndez de la Prida, M. Tijero, A. Guimerá, J. Berganzo, R. Salido, R. Villa, and L.J. Fernández, “SU-8-based microneedles for in vitro neural applications,” *Journal of Micromechanics and Microengineering*, vol. 20, Jun. 2010, p. 064014.
- [16] J. Hammacher, a Fuele, J. Flaemig, J. Saupé, B. Loechel, and J. Grimm, “Stress engineering and mechanical properties of SU-8-layers for mechanical applications,” *Microsystem Technologies*, vol. 14, Jan. 2008, pp. 1515-1523.
- [17] Z. Taylor and K. Miller, “Reassessment of brain elasticity for analysis of biomechanisms of hydrocephalus,” *Journal of Biomechanics*, vol. 37, Aug. 2004, pp. 1263-9.
- [18] J.J. Cerqueira, F. Mailliet, O.F.X. Almeida, T.M. Jay, and N. Sousa, “The prefrontal cortex as a key target of the maladaptive response to stress,” *The Journal of Neuroscience*, vol. 27, 2007, pp. 2781-7.
- [19] P.S. Goldman-Rakic, “Architecture of the prefrontal cortex and the central executive,” *Annals of the New York Academy of Sciences*, vol. 769, Dec. 1995, pp. 71-83.