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Published in:
Small

DOI:
10.1002/smll.201900582

Published: 17/05/2019

Please cite the original version:
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Keywords: flexible electronics, subdural electrodes, neural recording, electrocorticography, epilepsy

Flexible electronics that can form tight interfaces with neural tissues hold great promise for improving the diagnosis and treatment of neurological disorders and advancing brain/machine interfaces. Here, the facile fabrication of a novel flexible micropillar electrode array (µPEA) is described based on a biotemplate method. The flexible and compliant µPEA can readily integrate with the soft surface of rat cerebral cortex. Moreover, the recording sites of the µPEA consist of protruding micropillars with nanoscale surface roughness that ensure tight interfacing and efficient electrical coupling with the nervous system. As a result, the flexible µPEA allows for in vivo multi-channel recordings of epileptiform activity with high signal-to-noise ratio (SNR) of 252 ± 35. The ease of preparation, high flexibility, and biocompatibility make the µPEA an attractive tool for in vivo spatiotemporal mapping of neural activity.
1. Introduction

Neural electrode techniques have dramatically improved our understanding of brain functions and have become a clinical tool for the diagnosis and treatment of neurological disorders.\[1-8\] In particular, epilepsy is a common neurological disorder that affects about 1% of the general population and 4% of children.\[9\] Clinically, about 30% of patients with epilepsy respond poorly to antiepileptic drugs and have to undergo surgical resection of the epileptic foci. Subdual intraoperative electrocorticography (ECoG) has been routinely applied in patients with intractable epilepsy for both preoperative localization of the epileptogenic focus and assessment of postsurgical outcome.\[10-12\] ECoG recordings are typically performed with subdural electrode arrays that are placed directly on the surface of the cerebral cortex.\[13\] Subdural electrodes are much less invasive than penetrating depth electrodes that cause tissue damage and elicit pronounced foreign body response.\[14,15\] At the same time, subdural electrodes can record neural activity with higher temporal and spatial resolution than scalp electrodes because of their close proximity to neural tissues.\[16\] For these reasons, subdural ECoG recordings have also been considered as an attractive candidate to build next-generation brain-machine interface (BMI).\[17,18\]

The interfaces between electrodes and neural tissues play an essential role in neural activity recordings because extracellular signals decay rapidly with distance.\[16,19\] A key challenge for creating tight interfaces between conventional subdural electrodes and neural tissues has been their large mechanical mismatch.\[20\] The poor contacts between conventional rigid electrodes and soft tissues lead to poor electrical coupling at their interfaces. As a result, neural activity signals can be notably attenuated by the cerebrospinal fluid (CSF) in the electrode-tissue gaps.\[21\] In addition, micromotion between rigid electrodes and soft tissues has been shown to elicit shear-induced inflammatory response at the implantation sites.\[22\]

Recent advances in material science and engineering have resulted in significant progress in the development of biocompatible electrodes.\[23\] In particular, to improve the contacts between electrodes and soft, curvilinear neural tissues, extensive efforts have been made to develop flexible
subdural electrodes based on polymer substrates, including parylene C,[24, 25] polyimide (PI),[26, 27] polydimethylsiloxane (PDMS),[28] and nanocellulose paper.[29] Flexible subdural electrodes can form greatly improved contacts with neural tissues compared to their rigid counterparts. In addition, they were shown to reduce the foreign body response of the brain tissue in chronic applications.[30] Nevertheless, the moduli of the polymer substrates are in MPa to GPa range,[31] which is still orders of magnitude higher than that of the brain. This can cause shear force and micromotion at the electrode-tissue interfaces, which limits the accuracy and stability of subdural ECoG recordings.

As electrical intervention becomes more widely recognized in clinics, there is a growing need to develop advanced subdural electrode arrays that can form tight and stable neural interfaces.[24, 32, 33] Here, we introduce a new flexible subdural micropillar electrode array that enables the formation of tight neural interfaces and thus stable neural activity recordings. The recording sites of the µPEA consist of protruding microscale pillars with nanoscale roughness obtained by replicating the surface of a lotus leaf. Due to the introduction of the hierarchical surface structure, the impedance of the micropillar electrodes was effectively reduced compared to that of planar electrodes. In addition, the protruding micropillars ensure tight interfacing and effective electrical coupling between the electrodes and the cerebral cortex of rat brain. As a result, the µPEAs have been successfully demonstrated for stable multi-channel recordings of epileptiform activity with high SNR of 252 ± 35.

2. Results and discussion

2.1. Bio-template fabrication of µPEAs

The fabrication process of a µPEA is illustrated in Figure 1. We chose lotus leaf as the bio-template material due to its multiscale hierarchical surface structure. As shown in the scanning electron microscopy (SEM) image in Figure 1-(i), the surface of a lotus leaf consists of distributed microscale pillars with nanoscale roughness.[34, 35] PI was used as the target substrate material because of its high flexibility and good biocompatibility.[36] We designed a two-step molding process to selectively replicate the micropillars of the lotus leaf to the recording sites of the
electrode array. First, a mixture of PDMS base and curing agent was poured onto a flat lotus leaf and cured to form a negative template with microscale voids (Figure 1-(iii)). The PDMS was cured at a relatively low temperature of 50 °C and further treated with oxygen plasma for high adhesiveness. The PDMS negative template was then covered by a PI mask with a 3x3 hole array. The PI mask/PDMS was heated and pressed to further increase their adhesion. A PI precursor solution was then spin-coated onto the surface of the PI mask/PDMS to form a second PI layer. The strong adhesion between the PDMS template and PI mask was essential to avoid any diffusion of the PI solution into the area covered by the mask. As a result, only the micropillars in the open hole areas were selectively replicated to the second PI layer (Figure S1, Supporting Information). After curing and peeling off the PDMS negative template, a PI substrate with spatially patterned hierarchical micropillars was obtained (Figure 1-(vi)). Finally, a Cr/Au electrode layer was deposited onto the PI substrate, and then a SU-8 insulating layer was photolithographically defined to expose the active recording sites with patterned micropillars (Figure 1-(viii)) and encapsulate the interconnect lines.

**Figure 2a** shows an as-prepared µPEA with a final thickness of 18 µm. The µPEA is highly flexible and can be bent repeatedly without loss of structural integrity. The µPEA consists of nine recording sites arranged in a 3x3 matrix with 1 mm spacing (Figure 2b). Each recording site has a diameter of 120 µm (Figure 2c). Figure 2d illustrates the SEM images of a typical electrode before and after SU-8 passivation. The micropillars of the lotus leaf has been successfully transferred to the recording site of the electrode. The enlarged SEM images in Figure 2e and Figure S2 (Supporting Information) show that the micropillars are covered by numerous nanoscale wrinkles. The height of the nanowrinkles is in the range of 100-200 nm. 3D confocal images further confirm that only the recording sites of the µPEA consist of protruding micropillars (Figure 2f). The height of the micropillars range from 6 to 17 µm and the average spacing between adjacent micropillars is about 20 µm. Moreover, no change in the micropillar structure was observed after SU-8 passivation of the interconnect lines, indicating the robustness of the replicated micropillar structures. Figure 2g
shows a cross-sectional SEM image of a planar interconnect line which consists of a three-layer sandwich structure of SU-8/Au/PI. No debonding was observed at the SU-8/Au/PI interfaces, which thus confirms the effective passivation of the interconnect lines.

2.2. Electrochemical characterizations of μPEAs

The performance of neural electrodes is mainly limited by the thermal noise that arises from the impedance at the electrode-electrolyte interface. The impedance at the electrode-electrolyte interface is inversely proportional to the effective electrode area. For this reason, considerable efforts have been made to increase the effective electrode areas for noise reduction. Next, we characterized the impedance of our micropillar electrodes. Both micropillar and planar electrodes were fabricated on the same PI substrate for direct comparison. Figure 3a shows representative electrochemical impedance spectra (EIS) of a micropillar electrode and a planar electrode measured in phosphate buffered saline (PBS, 1X, HyClone Laboratories). The micropillar electrode exhibits an apparent impedance reduction compared with the planar one. The reduced impedance of the micropillar electrode can be attributed to the increased electrode area from the hierarchical surface structures of the recording site. Figure 3b summarized the averaged impedance of 6 micropillar electrodes and 27 planar electrodes measured at 1 kHz. The average impedance of the planar electrodes at 1 kHz was 192.26 ± 131.51 kΩ. The large variation in impedance could be due to surface contamination (e.g., resist residues), leading to poor wettability. The average impedance of the micropillar electrodes at 1 kHz was 45 ± 3 kΩ and about 4.3 times lower than that of the planar electrodes. The reduced impedance of the micropillar electrodes is thus expected to lead to reduced thermal noise in neural activity recordings.

2.3. In vivo subdural surface recordings in rat brain

The high flexibility and protruding micropillar structures make the μPEAs an attractive candidate for in vivo neural interfacing and recordings. Next, we applied the μPEAs for in vivo subdural recordings of penicillin-induced epileptiform activity in rat cortex. As an antagonist of the
gamma-aminobutyric acid (GABA) receptor, penicillin induces epileptiform activity by preventing GABA-mediated inhibitory control of pyramidal neurons.\textsuperscript{[40]}

As shown in Figure 4a, the \( \mu \)PEA conformally covered a large cortical area of the rat brain, including parietal association cortex (PC), primary visual cortex (V1), and secondary visual cortex (V2) (Figure S3, Supporting Information). Notably, the protruding micropillars at the recording sites were engulfed by the neural tissue (Figure S4, Supporting Information), which resulted in greatly improved electrical coupling between the electrodes and neural tissues. Figure 4b shows a representative real-time signal recorded by a micropillar electrode, \( Ch-9 \), and Figure 4c is the normalized time-frequency spectral analysis of the time-series data. As shown in Figure 4d, three periods, including basal period, latent period, and epileptiform activity period, could be clearly identified, consistent with former study.\textsuperscript{[41]} The epileptiform activity period began with increasing discharges arising from the hyperexcitability and hypersynchrony of neuronal activity. As shown in Figure 4c, there was an associated increase in the spectral power between 5-25 Hz. The amplitude of the discharges reached a maximum of 3.5 millivolts at 0.5 h after penicillin injection (Figure S5a, Supporting Information), and the \( \mu \)PEA allowed stable recording of the epileptiform signals over 3h (Figure S5b, Supporting Information). As shown in Figure S5c,d (Supporting Information), the discharging frequency decreased from 0.8 spikes per second in the initial period to 0.2 spikes per second in the late period of the epilepsy. The high signal amplitude could be attributed to the tight interfaces and efficient electrical coupling between the protruding micropillars and neural tissue. This, combined with the reduced impedance and noise level of the micropillar electrodes, allowed epileptiform activity recordings with SNR ranges from 150 to 300 (Figure S6, Supporting Information).

Figure 4e,f shows simultaneous multichannel recordings by the \( \mu \)PEA. We applied a thresholding method to detect epileptiform discharges recorded by the nine channels, from which spike-times were extracted (Figure 4g). Spike-time delays at different channel locations were then calculated relative to the spike-time of \( Ch-9 \) (Figure S7, Supporting Information). Figure 4h
summarized the spike-time delays of 50 epileptiform discharges recorded in a 255 seconds window. The spike-time delays ranged from -9 to 13 ms and showed a clear dependence on the channel locations, consistent with former studies.\[42, 43\] This allowed us to construct a pattern map by plotting the average spike-time delays as a function of each channel’s location. As shown in Figure 4i, the propagation characteristics of the epileptiform discharges across the recorded cortical regions can be clearly identified. These results confirm that flexible µPEAs can serve as a facile tool to study the spatiotemporal dynamics of neural activity. Moreover, to investigate the biocompatibility of the flexible µPEAs, we chronically implanted micropillar electrodes in mouse brain. Immunohistochemical analysis shows that the implanted micropillar electrodes elicited little inflammation response after four weeks’ implantation (Figure S8, Supporting Information), indicating the long-term biocompatibility of the µPEAs. We simultaneously recorded neural signals from rat cortex with a µPEA and a planar electrode array (PEA) and compared their noise quantitatively. As shown in Figure S9 (Supporting Information), the averaged noise of the planar electrodes is 55 μV. On the other hand, the averaged noise of the micropillar electrodes is only 22 μV, which is 2.5 times lower than that of the planar electrodes. We further compared the SNR of µPEA and PEA during epileptiform activity recordings. As shown in Figure S10 (Supporting Information), the averaged SNR of the micropillar electrodes is 234, while that of the planar electrodes is only 149. These results confirm that the micropillar electrode arrays can provide reduced noise and improved SNR in neural recordings compared to conventional planar electrode arrays.

3. Conclusion

In summary, we developed a facile bio-template method for the fabrication of flexible µPEAs. The introduction of the hierarchical surface structure effectively reduced the impedance of the micropillar electrodes. Moreover, a tight electrode-neural interface was obtained due to the engulfment of the micropillars by neural tissues. As a result, the µPEAs allowed stable subdural recordings of neural activity with high SNR. Multichannel recordings further revealed the
propagation characteristics of epileptiform activity across the cerebral cortex. These results show that flexible µPEAs can offer new opportunities to study tempo-spatial dynamics of neural activities. Moreover, owing to their good biocompatibility, flexible µPEAs hold promise for clinical diagnosis of epilepsy.

4. Experimental Section

**Preparation of PDMS negative templates:** PDMS base (SYLGARD 184, Dow Corning) was mixed with curing agent in the ratio of 10:1 (w/w) to form a mixture and degassed overnight in vacuum. The PDMS mixture was poured onto a lotus leaf and cured at 50 °C for 1 h. After peeling off the lotus leaf, a PDMS negative template was obtained.

**Fabrication of PI masks:** The detailed fabrication process of the PI masks is illustrated in Figure S11 in the Supporting Information. A 100 nm-thick aluminum (Al) sacrificial layer was first deposited onto a silicon wafer by RF sputtering (Lab-18 Sputtering System, Kurt J. Lesker). Then, a PI precursor solution (20 wt%, U-Varnish, UBE) was spin-coated onto the Al layer at 2000 rpm for 1 min. After removal of solvent at 120 °C for 10 min and curing at 200 °C for 30 min in vacuum, a 5 µm thick PI film was obtained. The PI film was patterned by photolithography (MJB4, SUSS Microtec) using SUN-1150P resist (SUNTIFIC) and etched by reactive ion etching (RIE) (Etchlab 200, SENTECH Instruments) to obtain a 3 × 3 hole array. After the removal of the Al sacrificial layer in HCl (5 wt%), a free-standing PI mask with a 3 × 3 hole array was obtained.

**Fabrication of PI substrates with spatially patterned micropillars:** A PDMS negative template was treated with oxygen plasma (PJ Plasma Surface Treatment System, AST Products, Inc.) at 100 w for 5 min. Then, a PI mask with a 3x3 hole array was transferred onto the PDMS template. The PI mask/PDMS was heated at 140 °C for 30 min under a pressure of ~20 kPa to increase their adhesion. A PI precursor solution was then spin-coated onto the PI mask/PDMS. The system was heated at 120 °C for 20 min for the removal of the solvent and then 200 °C for 30 min in vacuum for curing.
After peeling off the PDMS template, a PI substrate with spatially patterned micropillars was obtained.

*Fabrication of flexible μPEAs and PEAs:* Neural electrode arrays were defined on a patterned PI substrate by thermal deposition of Cr/Au (10 nm/200 nm) (SBC-2, KYKY Technology Co., China) through a shadow mask. The recording sites were aligned with the patterned micropillar regions of the PI substrate. Then a SU-8 passivation layer was defined by photolithography to encapsulate the planar interconnect lines. The μPEA was bonded to a flexible flat cable (FFC) through anisotropic conductive film (ACF) (MF-331, Hitachi Chemical co., ltd.). PEAs were prepared using the same fabrication parameters, except that planar PI was used as substrates.

*Structural and electrochemical characterizations of μPEAs:* 3D confocal images of the micropillar electrodes were acquired on a laser confocal microscope (LEXT OLS4000, Olympus). SEM images were collected using a FEI Nova NanoSEM 430 system and Hitachi-SU8220, with the samples 45° tilted. Cross-sections of the electrodes were obtained by focused ion beam (FIB) etching using a FIB/SEM dual-beam system (Nova Nanolab 200, FEI), and SEM image was collected with the same system. Before FIB etching, a thin platinum layer was in-situ deposited on the sample using a CH$_3$C$_5$H$_4$Pt(CH$_3$)$_3$ gas source in the dual-beam system.

EIS of the electrodes was measured using an electrochemical workstation (Reference 3000, Gamry Instruments). The frequency was varied from 1 Hz to 100 kHz using an AC excitation potential of 10 mV. The EIS were measured in PBS. A Platinum (Pt) rod and an Ag/AgCl electrode (CHI111, CH Instruments) were used as counter and reference electrode, respectively.

*In vivo neural activity recordings of Sprague-Dawley rats:* A Sprague-Dawley rat was anaesthetized with intraperitoneally administered urethane (1.4 g/kg weight) and fixed in a stereotaxic apparatus. Craniotomy was performed and the left hemisphere was exposed, and the dura matter was carefully removed. A flexible μPEA was placed on the exposed subdural surface of the rat cortex. The FFC
of the µPEA was connected to a data acquisition system (Cerebus, Blackrock microsystems) through a custom printed circuit board (PCB). The rat was intraperitoneally administrated with penicillin G sodium (2,000,000 IU/kg weight) to induce epileptiform activity. The neural signals were recorded at a sampling rate of 1 kHz. All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Center for Nanoscience and Technology, China.

The recorded data was processed with NeuroExplorer software and MATLAB. First, normalized time-frequency spectral analysis was conducted with NeuroExplorer software. Then the data was filtered through a bandpass Butterworth filter (1 Hz–100 Hz), and then 50 Hz and 100 Hz Notch filter in NeuroExplorer. The filtered data series were sent to MATLAB, followed by spike detection and spike time analysis with custom MATLAB scripts. A thresholding method was applied for spike sorting. The spike-time was defined as the time point with the maximum amplitude. The spike-time delay at each recording channel was calculated with respect to channel 9 (Ch-9). For the SNR calculation during epileptiform activity, the non-spike periods of a filtered signal were divided into windows of 50 ms, and standard deviation (STD) of the windows were calculated and then an average STD was obtained. SNR was defined as the ratio between the peak-to-peak amplitude of an epilepsy discharge and the average STD.

Statistical Methods: For the impedance measurement in Figure 3b, 6 micropillar electrodes and 27 planar electrodes were measured. The error bars represent the standard deviation from different series of measurements. Student's t-test was performed to evaluate the statistical significance of the difference between planar and micropillar electrodes, and a p-value less than 0.01 was considered statistically significant.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (21790393, 21673057), the Frontier Research Program of the Chinese Academy of Sciences (QYZDB-SSW-SLH044). M. D. Du and S. L. Guan contributed equally to this work.
References


Figure 1. Fabrication of flexible μPEA through a two-step molding process. i) A flat lotus leaf. Inset, SEM image of a lotus leaf; (ii) PDMS was poured on the surface of lotus leaf and cured; (iii) PDMS negative template with random distributed microcavities. Inset, SEM image of a PDMS negative template; (iv) PDMS negative template covered with PI mask; (v) PI was spin-coated on the template and cured; (vi) PI substrate with spatially patterned hierarchical micropillars; (vii, viii) μPEA before and after SU-8 passivation. Scale bars: 10 µm.
Figure 2. Structure of flexible μPEA. a) A flexible μPEA. Scale bar: 5 mm. b) Optical image of the 3 × 3 micropillar electrode array. Scale bar: 500 μm. c) Optical image of the recording site marked with dashed blue box in inset (b). Scale bar: 50 μm. d) SEM image of an electrode (i) before and (ii) after passivation. Scale bars: 50 μm. e) Magnified SEM image of the area marked with dashed red box in insets (d-(i)). Scale bar: 10 μm. f) 3D confocal images of the electrode in inset (d-(i)) before and (d-(ii)) after passivation. Scale bars: 50 μm. g) Cross-sectional SEM image of the interconnect line marked with dashed orange line in inset (d-(ii)). Scale bar: 10 μm.
Figure 3. Impedance of micropillar and planar electrodes. a) EIS of micropillar and planar electrodes measured in PBS. b) Averaged impedance of micropillar and planar electrodes.
Figure 4. In vivo neural activity recordings with μPEA. a-(i) Optical image of a μPEA conformally attached onto the cortical surface of rat brain and a-(ii) schematic illustration of the tight interface between micropillars and brain tissue. Scale bar in (i): 1 mm. b) Representative real-time recording of neural activity by a micropillar electrode (Ch-9). c) Normalized time-frequency spectrum of the time-series data in inset (b). d-(i) Neural signals recorded during the basal period, d-(ii) latent period, and d-(iii) epileptiform activity period, respectively. e) Multichannel recordings of epileptiform activity with the μPEA. f) Array layout of the voltage traces during an epileptiform discharge, as marked with the dashed black box in inset (e). g) Overlaid voltage traces. The colored dots marked the spike-times. h) Spike-time delays of 50 discharges recorded by the nine channels in a 255-s window. i) Array layout of the average spike-time delays determined from inset (h).