Easy-to-Fabricate Conducting Polymer Microelectrode Arrays

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The field of bioelectronics explores the interface of electronic devices with biomolecules and living tissues, with the aim of developing new tools for both diagnosis and therapy. In this context, neuroscience has played a pivotal role in driving the development of such devices, since most of the information processed and transmitted in any animal nervous system is of electrochemical origin.[1] Pacemakers and cochlear implants are becoming common reliable tools in clinics, replacing less effective pharmacotherapies,[2,3] while stimulation of nerves in specific regions of the brain has been successfully applied in the treatment of various pathologies.[4,5] For a further development of such in vivo devices and practices, however, a deeper understanding of the basis of signaling mechanisms is needed. Most of the available technologies and research platforms rely on the use of microelectrode arrays (MEAs), able to stimulate and record electrical signals down to the single-cell level. MEAs have become an indispensable tool in the study of different properties of neural networks such as network formation, network dynamics and signal processing. A big advantage of MEAs is that they can be arranged in planar structures using thin film technology, allowing for in vitro investigations of neuronal cultures and brain slices.[6–8] Advancements in these MEA applications require a decrease in the size and spacing between electrodes in order to match the dimensions of neuronal networks, while maintaining a very high quality signal. Commercially available MEAs are based on micron-sized metallic electrodes produced through photolithography using traditional silicon patterning processes, which makes them expensive and restricted to rigid substrates. Importantly, as the size of the electrodes decreases, both signal quality and stimulation ability drop due to an increase in the electrical impedance at the electrode/electrolyte (i.e., physiological media) interface. This effect can be simplistically attributed to a decrease in the interfacial capacitance which is, in turn, directly correlated with the area of the electrode. For this reason, meso- and nanoporous electrodes with extremely high surface area have been the subject of intense research in the last years, leading to high-quality neural recordings.[9] In these cases, the electrodes were based on metals such as Au, Pt and Ir, or intrinsically nanostructured materials such as carbon nanotubes or graphene.[10–16] The incorporation of these materials in planar MEAs, however, adds great complexity to the device fabrication making them an impractical alternative to flat electrodes.

Recently, conducting polymers have emerged as one of the most promising candidates for the next generation MEAs for both in vitro and in vivo applications.[17–19] Several studies have demonstrated their low impedance in physiological environment, which derives from the combination of high electrical conductivity and ion permeability. These properties are believed to increase the effective electrochemical surface area of the electrodes, consequently reducing their impedance.[20] Furthermore, conducting polymers diminish the foreign-body response in vivo, enhancing the stability of electrophysiological activity recordings.[17,21] Conducting polymers have several advantages over their inorganic counterparts, most importantly i) ease of processability by simple wet deposition methods (such as spin casting and screen printing) at low temperature directly on flexible substrates, and ii) the ability to tune materials properties through chemical synthesis or by blending with active molecules. The latter has been demonstrated in the preparation of novel functional materials capable of delivering neurotransmitters and stimulating cell growth and neuron differentiation.[22,23] These unique properties will lead to a new generation of prosthetic devices with unique functionalities and form-factors.[24] In addition to the outstanding properties of conducting polymers, a customized fabrication protocol compatible with these materials is still needed. Conjugated polymers are sensitive to harsh solvents and to high-energy processes which are necessary for conventional patterning methods that rely almost exclusively on lift-off or plasma etching techniques. In situ electropolymerization has been successfully used in order to prepare polymer films on pre-patterned MEAs,[25] while other methods use complicated patterning approaches in order to define active polymer electrodes.[26] For these reasons, a reliable and simple process to prepare polymer MEAs without compromising their properties is still needed.

Here, we present a novel approach to define polymer MEAs by photolithography, patterning at the same time the recording electrodes as well as the insulating layer. The process is extremely versatile and can be used to pattern various organic materials onto a variety of substrates (rigid and flexible). In fact, only a simple mechanical peel-off is involved in the definition

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of the microelectrodes, thus preserving the properties of the organic materials in use. As a case study we present the fabrication of MEAs based on poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS), which is established as the state-of-the art conducting polymer because of its high conductivity. The insulating material employed is parylene-C [poly(para-chloroxylylene)], which is characterized by excellent moisture, chemical and dielectric barrier properties. Both PEDOT:PSS and parylene are, moreover, stable in physiological conditions and biocompatible, making them a convenient choice for applications in neuroprosthetics. We validated the MEAs by in vitro recording of electrophysiological signals from rat brain slices, showing that our platform is capable of monitoring spontaneous single-unit activities modulated by drug perfusion onto the brain tissue.

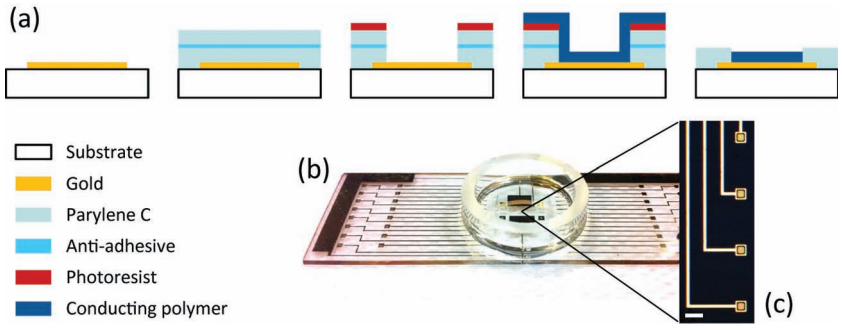

Figure 1. (a) Scheme of the fabrication process illustrating the main processing steps. (b) Photograph of MEA fabricated on a 3 × 1 inch microscope slide with a culture chamber ring attached. (c) Optical microscopy image showing the electrodes (scale bar 50 µm).

A schematic of the device fabrication is depicted in **Figure 1**. The process starts with the patterning of the gold pads and interconnects via photolithography and lift-off. Samples are then coated with a 2 µm thick parylene layer, which will be the insulating coating of the MEA. 3-(trimethoxysilyl)propyl methacrylate (A-174 Silane) is used as an adhesion promoter for parylene on the substrate. A dilute commercial cleaning solution is then spin-coated onto the parylene, forming a thin layer that acts as an anti-adhesive. Next, a second sacrificial 2 µm Parylene layer is deposited and windows corresponding to the microelectrodes and the contact pads are opened by photolithography and plasma etching. PEDOT:PSS is then deposited by spin- or blade-coating, forming 380 nm thick films (details in the Supplementary Information). After a short annealing step at 110 °C, the second Parylene layer is peeled-off, defining the final polymer MEA structure. After fabrication, devices are soaked and rinsed in deionized water to remove the remaining anti-adhesive and other low molecular weight residues.

This novel fabrication method is extremely versatile; it can be modified depending on the particular type of targeted application. Beyond the size and geometry of the MEAs, which can be easily modified through photolithography, it allows the use of different insulating and conducting layers. The arrays presented here consist in 16 square electrodes with a side of 20 µm (geometric surface area of 400 µm^2), and a center-to-center spacing of 160 µm (Figure 1b), a spatial resolution which allows the monitoring of signal propagation among adjacent neurons. Electrodes are connected through 10 µm wide metal lines to the pads which are arranged in DIP16 geometry, making it compatible with most common electronics. A glass ring is fixed on the MEA, forming the chamber which houses the cell culture or the tissue slice.

The polymer MEA is initially investigated using impedance spectroscopy, comparing the electrochemical properties of the PEDOT:PSS-coated electrodes with control electrodes (bare gold). Measurements were made in 0.1 M phosphate buffer saline (PBS) solution by applying a 10 mV RMS sine wave with frequencies varied logarithmically from 10 Hz to 10 kHz, the range of interest for most of electrophysiological signals. The Bode plots of the impedance magnitude for PEDOT:PSS and gold electrodes are depicted in **Figure 2**a. The impedance of the gold electrodes increases monotonically as the frequency of the signal decreases, with a value of approximately 20 MΩ at 10 Hz and of about 400 kΩ at 1 kHz. The latter frequency is of particular importance since it corresponds to the range for recording action potentials. The PEDOT:PSS coating drastically decreases the microelectrode impedance, remaining more than one order of magnitude lower compared to the control metal electrodes through the 10 Hz–1 kHz range. At 1 kHz, the impedance of PEDOT:PSS electrodes is as low as 23 kΩ, consistent

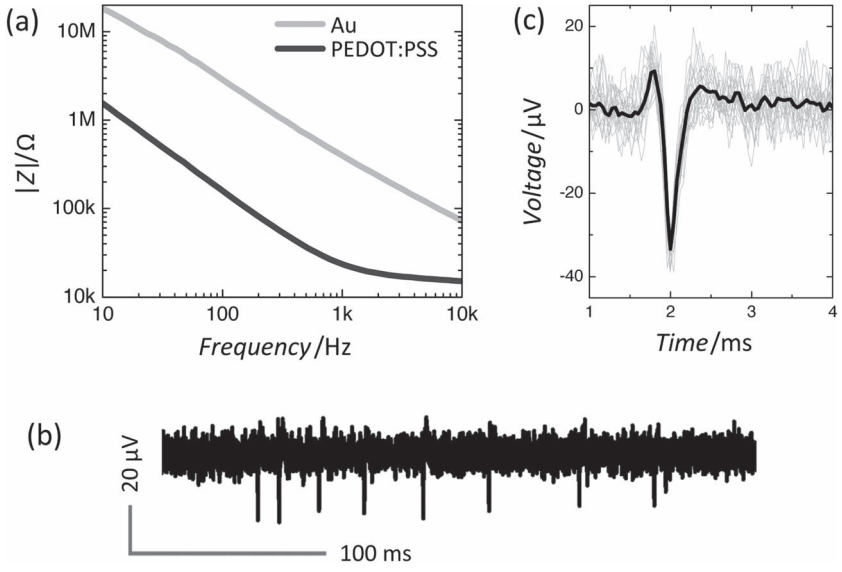

Figure 2. (a) Bode plot of impedance magnitude |Z| versus frequency for a PEDOT:PSS coated electrode and a gold electrode. (b) Extracellular recordings of a rat hippocampus slice from a single electrode, showing unit activities stimulated by perfusion of 1 µM carbachol enriched media. (c) Average signal obtained from 20 recorded action potentials.

with previous reports,[21] which validates the potential of simple solution-processed conducting polymer electrodes as candidates for electrophysiology recording.

In vitro signal recording from freshly prepared rat hippocampus slices was performed with a multi-channel amplifier chip (Intan Technologies, US) placed directly on the device and connected to the patterned pads through gold springs. The neuronal networks of hippocampus slices in oxygenated media can survive for several hours maintaining its activity. This characteristic makes brain slices a convenient model of in vivo electrophysiology. Various types of signals can be measured, the most significant being action potentials and local field potentials (LFP). LFPs are slow bands (1–200 Hz) reflecting the synaptic activities of a relatively large population of cells and their amplitude can vary considerably from hundreds of μV to a few mV. As previously mentioned, action potentials occur at higher frequency, but their amplitude is considerably smaller (<100 μV) since they originate from single neurons. For this reason the recording of action potentials represents a great challenge for MEAs, where often the amplitude of the noise can overwhelm the desired signal. Therefore we focused on the recording of such signals, which is common procedure for validating novel MEA designs. Since the frequency of neural action potentials is known to be about 1 kHz, a high-pass filter of 200 Hz was used during recording. The system was mounted on an optical microscope in order to control the position of the brain slice with respect to the electrodes. To enhance the coupling between the brain slice and the MEA, a small platinum grid is placed on top of the slice itself. A perfusion system allows for a continuous feeding of the slices from an external oxygenated media bottle connected to a peristaltic pump. Figure 2b shows single channel recordings from a polymer electrode in contact with the slice. The background noise is ±10 μV peak-to-peak (3.4 μV RMS). These values are similar or even lower compared to previously reported data for both nanostructured metal[9] and polymer electrodes,[20] in agreement with the low measured impedance of the PEDOT:PSS. In order to stimulate firing from the brain tissue, the chamber solution was replaced with media enriched in carbachol (CCh, 1 μM), a cholinomimetic drug known to stimulate firing from CA1 pyramidal neurons in vitro.[27] As reported in the Figure 2b, extracellular single-unit activity could be clearly recorded and discriminated from the background signal fluctuations. The amplitude of the signals ranged from 40 to 60 μV, as expected from measurements of brain slices. Extracellular measurements of neurons sitting on top of microelectrodes are known to give signals up to 100 μV, since the amplitude is directly correlated with the distance between the firing cell and the electrode. One of the disadvantages of tissue slices is that a layer of dead or damaged cells is always present, acting as an insulating buffer layer, and augmenting the distance between the recording electrode and viable firing neurons. This phenomenon explains the magnitude of the signals recorded in our experiments. Nevertheless, action potentials were easily recorded and, by averaging 20 consecutive spikes (Figure 2c), the depolarization peak together with the consecutive signal hyperpolarization could be clearly resolved. One of the most important characteristic of MEAs is the ability of simultaneous recording of signals in different regions of the nervous tissue and to observe differential effects of compounds according to the region of interest. As a further validation of our MEA design, we conducted a more systematic experiment monitoring the electrophysiological activity localized in the Stratum Pyramidale of the CA1 region of the hippocampus, which is characterized by a high density of pyramidal neurons. The slice was positioned in such a way that 4 PEDOT:PSS microelectrodes were overlapped with different parts of the CA1 region (**Figure 3**a,b). Recordings were performed with these electrodes continuously to monitor hippocampal activity while CCh was introduced into the culture chamber.

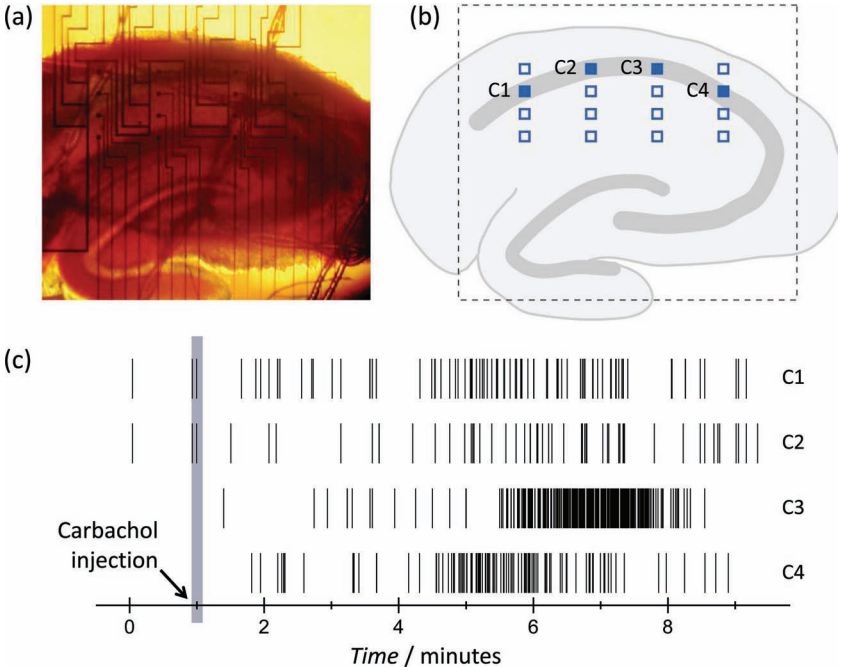

Figure 3. (a) Microscopy image of a hippocampus slice sitting on top of the PEDOT:PSS MEA. The thin net used to immobilize the tissue is also visible. (b) Schematic representation of the position of the MEA with respect to the brain slice. The highlighted and labeled electrodes were used to monitor the firing in the Stratum Pyramidale of the CA1 region of the hippocampus. (c) Action potentials recorded with the PEDOT:PSS MEA electrodes during perfusion of CCh.

The signals were analyzed by setting a threshold voltage of −35 μV and thus monitoring the number of events (action potentials) exceeding that value (Figure 3c). The initial spontaneous firing rate was very low, with few signals appearing from the recording of the 4 microelectrodes. The addition of 1 μM CCh, marked in Figure 3c, leads to a progressive increase in the firing rate, as observed in the 3 minutes following drug injection. An enhancement in activity was measured after approximately 4 minutes, which corresponds to the time needed for CCh to diffuse into the brain tissue, with a peak of the firing rate at about 5 to 7 minutes after injection. This experiment

further demonstrates the ability of conducting polymer MEA to efficiently monitor electrophysiological signals and highlights their potential in the field of basic neuroscience and pharmacology.

In conclusion, we developed a novel fabrication process which allows the preparation of planar microstructures on virtually any kind of substrate. The critical step is a mechanical peel-off and does not involve the use of chemical or ion etching of the active layer, making the process compatible with any sensitive conducting/semiconducting materials. In particular, with this technology it is possible to easily create MEAs incorporating conducting polymers such as PEDOT:PSS, electrically isolated from the environment through a thin parylene layer. The resulting electrodes have been electrochemically characterized, showing the low impedance necessary for high quality recording of electrophysiological signals. These claims were validated by recording the spontaneous electrical activity from rat hippocampus brain slices, thereby demonstrating the low noise recording of single neuron activity. The effect of drug diffusion into the hippocampus slice has been clearly observed by recording enhanced neuronal firing rates, highlighting the potential of this platform in drug screening and general pharmacology. This work shows that our general fabrication process can provide convenient and low-cost platforms that are promising alternatives to commercial MEAs.

Experimental Section

Microelectrode Array Fabrication: The fabrication process, outlined in Figure 1a, includes the deposition and patterning of metal, parylene and PEDOT:PSS. Glass slides are extensively cleaned using chemical and plasma methods. Shipley 1813 photoresist is spin coated and exposed to UV light using a SUSS MBJ4 contact aligner, and then developed using MF-26 developer. This is followed by the deposition of 5 nm of titanium and 100 nm of gold using a metal evaporator. Lift-off is performed by immersion of the samples in acetone:isopropanol (3:1) mixture for 2 hours. Parylene C layers are deposited using a SCS Labcoater 2 to a thickness of 2 µm. 3-(trimethoxysilyl)propyl methacrylate (A-174 Silane) is used as an adhesion promoter for the first parylene coating on the metal patterned substrate. A dilute solution of industrial cleaner (Micro-90) is spin coated in between the two parylene layers, acting as an anti-adhesive for the second parylene film. Substrates are then subsequently patterned with a 5 µm thick layer of AZ9260 photoresist and AZ developer (AZ Electronic Materials), while openings are made by reactive ion etching with an O_2 plasma using an Oxford 80 Plasmalab plus. For the preparation of the PEDOT:PSS films, 20 mL of aqueous dispersion (Clevios PH-1000 from Heraeus Holding GmbH, details at www.clevios.com) are mixed with ethylene glycol (5 mL), dodecyl benzene sulfonic acid (DBSA, 50 µL), and 1 wt% of (3-Glycidyloxypropyl) trimethoxysilane (GOPS), and the resulting dispersion is spin-coated resulting in a 380 nm thick film. Ethylene glycol is added in order to enhance the conductivity of PEDOT:PSS, while DBSA helps to adjust the surface tension and thus the coating properties of the suspension. GOPS is a common surface adhesion promoter as well as a polymer cross-linking agent and enhances the stability of PEDOT:PSS films in aqueous environments. Finally, the sacrificial parylene layer is peeled-off leaving behind the intact MEA structure. Devices are subsequently baked at 140 °C for 1 h and then immersed in phosphate buffered saline (PBS) to remove any excess of low molecular weight compounds.

Preparation of acute adult rat hippocampal slices and solutions: Experiments are carried out with 17- to 21-day-old Sprague-Dawley rats (Elevage Janvier, Le Genest St Isle, France). Animals are housed and used in accordance with French and European legislation for animal care. The rats are sacrificed by fast decapitation, without previous anesthesia. The brain is quickly removed and soaked in ice-cold oxygenated buffer (composition in mM: KCl 2.6, NaH_2PO_4 1.25, $MgCl_2$ 10, $CaCl_2$ 0.5, $NaHCO_3$ 26, glucose 10, saccharose 212.7). Hippocampus slices (400 µm) are cut with a MacIlwain tissue-chopper and incubated at room temperature for at least 1 h in Artificial Cerebro-Spinal Fluid (ACSF, composition in mM: NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 25, D-glucose 11) for recovery. During experiments, slices are continuously perfused with oxygenated ACSF (composition in mM: NaCl 124, KCl 5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, D-glucose 10). Carbachol (carbamylcholine chloride, Sigma Aldrich) is prepared as a 30 mM stock solution in deionized water and stored at −20 °C in polypropylene tubes. One aliquot is thawed on each day of experiment to prepare the 1000X concentrated intermediate solution in DMSO (at 1 mM). The final DMSO concentration is adjusted to 0.1% in all the perfused ACSF solutions.

Electrical and electrophysiological recordings: Impedance measurements are performed with a potentiostat (Autolab PGSTAT128N) in a three electrode configuration using platinum and Ag/AgCl as counter and reference electrode, respectively. The brain slice is placed in the center of the polymer MEA and a U-shaped platinum wire with a nylon net is carefully placed on the slice to immobilize it. A continuous flux of oxygenated ACSF solution is provided with a peristaltic pump. The whole system is mounted on an inverted microscope for alignment of electrodes with selected regions of the brain tissue. All data were recorded with an amplifier board (RHA2116, Intan technologies) connected to the MEA and to a PC through an interface board (RHA2000, Intan technologies). Sampling rate was 25 kHz and recordings were high-pass filtered at 200 Hz. All analysis was performed using custom-written tools in Matlab (Mathworks).

Supporting Information

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

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