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Two-photon polymerization lithography enabling the fabrication of PEDOT:PSS 3D structures for bioelectronic applications†

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Conductive 3D platforms have gained increasing attention in bioelectronics thanks to the improvement in the cell-chip coupling. PEDOT:PSS is nowadays widely employed in bioelectronic applications thanks to its electrical and mechanical properties. In this work, an innovative fabrication method for the realization of PEDOT:PSS-based conductive micropillars and 3D cage-like structures is presented, combining two-photon lithography and electrodeposition techniques.

Bioelectronic devices are electronic platforms that directly interact with biological systems, such as cells, tissues, or living systems, in order to monitor cellular electrical activity¹ or modulate cellular behavior² through the application of an external electrical field.³ Here, a crucial role is played by the interface between cells and electrodes that determines the effectiveness of the electrical coupling. In particular, the presence of a cleft between the membrane and the electrode represents a major limitation in the transduction of electrical signals.⁴ Several strategies have been developed to reduce the cleft at the interface, including the fabrication of protruding bioinspired microstructured electrodes featuring holes,⁵ grooves⁶ and pillars.⁷ The topography-mediated rearrangement of the cell membrane and cytoskeletal machinery, promoting the engulfment of the electrode, have been shown to improve the cell-chip coupling.⁸

Interestingly, organic conductive materials have been widely employed in bioelectronic devices thanks to their physical-chemical properties compared to inorganic materials, ranging from a higher versatility in synthesis and functionalization

to fabrication and processability.⁹ Nowadays, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) is one of the most widely used conjugated polymers in several bioelectronic applications.¹⁰ It displays mechanical properties close to biological systems¹¹ and shows a mixed ionic/electronic conduction mechanism,¹⁰ with hole conductivity above 1000 S cm⁻¹,¹² which is fundamental for an efficient cell-chip communication.¹³

Combining the beneficial effects of 3D microelectrodes in reducing the cleft and improving cellular adhesion with the properties of PEDOT:PSS can provide a step forward in the development of next generation bioelectronic and biomedical devices. Indeed, recently 3D PEDOT:PSS-based microstructures have been successfully developed by means of inkjet printing,¹⁴ 3D printing,¹⁵ direct writing,¹⁶ replica molding¹⁷ and nanosphere lithography,¹⁸ and with the support of photocurable hydrogels.¹⁹ An interesting approach for the fabrication of 3D microelectrodes involves the fabrication of gold mushroom-like microstructures (with 1 μm in diameter) coated *via* electrodeposition of PEDOT:PSS precursors.²⁰ However, as the 3D processing of PEDOT:PSS remains challenging, there is still a need to develop versatile patterning procedures that allow the integration of the material with microscale devices adapting to different geometries.

In recent years, two-photon polymerization (2PP) lithography has emerged as an effective method for the fabrication of 3D microstructures for numerous cell-material interfacing applications²¹ thanks to its high resolution (down to 100 nm)²¹ and design flexibility that enables the fabrication of complex 3D architectures without any geometrical constraint.²²

In this work, we propose an innovative approach for the fabrication of conductive micropillars and 3D cage-like scaffolds exploiting 2PP and electrodeposition of PEDOT:PSS *via* cyclic voltammetry (CV). The proposed technique allows for the fabrication of PEDOT:PSS-coated micropillars as well as complex 3D structures that can fulfill any geometrical requirement, paving the way towards the development of 3D organic-based

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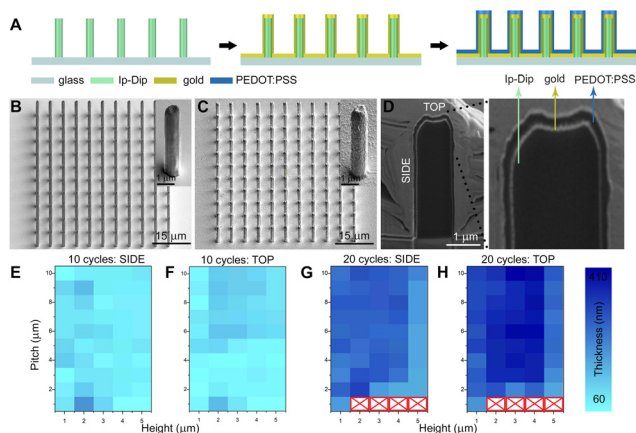


Fig. 1 PEDOT:PSS micropillar array fabrication and thickness analysis. (A) Schematics of fabrication of micropillars *via* 2PP, coating with gold, and electrodeposition of PEDOT:PSS. (B) Scanning electron micrographs of a representative array (height 5 μm , pitch 4 μm) before and (C) after PEDOT:PSS electrodeposition. (D) Scanning electron micrograph of a PEDOT:PSS-coated micropillar cross-section. The thickness of the PEDOT:PSS film electrodeposited on different micropillar arrays is reported as a function of height and pitch: (E) 10 CV cycles measured on the side surface, (F) 10 CV cycles measured on the top surface, (G) 20 CV cycles measured on the side surface, and (H) 20 CV cycles measured on the top surface.

electrodes that can be tightly interfaced with biological systems and therefore can find a variety of applications in biosensing and cellular recording/stimulation.

The proposed fabrication process is schematically shown in Fig. 1A (see also Experimental section, ESI[†]). In this work, non-conductive micropillar arrays were fabricated by means of 2PP, employing the commercially available Ip-Dip negative photoresist: in particular, the micropillar diameter was 1 μm , while micropillars of different heights (from 1 μm to 5 μm) and pitches (from 1 μm to 10 μm) were fabricated. A layer of gold was then deposited *via* sputtering onto the micropillars and PEDOT:PSS was subsequently electrodeposited *via* a 3-electrode CV procedure (Fig. S1, ESI[†]).²³ During the deposition process, the position of the working electrode (WE) can influence the film growth and assembly; here we first characterized PEDOT:PSS planar films obtained with a vertical and horizontal setting of the WE to determine the optimal conditions for the following functionalization of the micropillars (Fig. S2, ESI[†]).

By locating the WE above the electrolytic solution (Fig. S2E, ESI[†]), the electrodeposited PEDOT:PSS films displayed a uniform morphology (Fig. S2F, ESI[†]) and an average thickness of 72 ± 3 nm and 105 ± 5 nm when 10 and 20 CV iterations were performed, respectively (Fig. S3, ESI[†]). The latter configuration was then exploited to achieve a conformal PEDOT:PSS coating on micropillars arrays, preserving the initial geometry for both 10 and 20 applied CV deposition cycles (Fig. 1B and C, details in Fig. S4 and S5, ESI[†]). However, arrays with 1 μm pitch and micropillar heights from 2 to 5 μm showed a significant delamination from the substrate when 20 CV cycles were applied, probably due to an overgrowth of PEDOT:PSS that incorporates the arrays (Fig. S5, ESI[†]).

As the electrochemical properties of conjugated polymer films and electrodes strongly depend on their thickness and volume,²⁴ the obtained 3D PEDOT:PSS micropillar geometry was further characterized by focused ion beam (FIB) cross sectioning and SEM imaging (Fig. 1D, Experimental section, ESI[†]). In particular, the film thickness was evaluated at the side and top surfaces of the micropillars and the resulting measurements are reported in Fig. 1E–H as a function of height and pitch. The micropillars obtained with 10 applied CV cycles exhibit an average thickness of ~ 60 nm to ~ 200 nm and the highest PEDOT:PSS thickness was found on the top of the micropillars in arrays with increasing pitch from 5 to 10 μm . The thickness was homogeneous on the micropillar side across arrays with a height from 4 to 5 μm and was higher in arrays with a height from 1 to 2 μm . In addition, micropillars obtained with 20 applied CV cycles displayed an average PEDOT:PSS film thickness ranging from ~ 180 nm to ~ 410 nm. In particular, the highest thickness (up to 410 nm) was found on the top surface of micropillars of height from 2 to 4 μm and pitch from 3 to 10 μm . Conversely, the film thickness on the side of the micropillars reached a maximum height of 330 nm. Interestingly, the overall PEDOT:PSS film thickness of the arrays with micropillars of 5 μm in height is lower, reaching a maximum of 290 nm. The overall higher PEDOT:PSS thickness observed on micropillar arrays (compared to the planar surface) can be ascribed to the larger electroactive surface area.²⁵ In turn, the thickness difference found at the top and side of the micropillars may derive from the higher electric field induced by the tip effect.²⁶

Then, the electrochemical impedance of the PEDOT:PSS-coated micropillars was investigated by means of EIS (Experimental section, ESI[†]). Bode plots measured for planar gold films (black trace) and PEDOT:PSS films electrodeposited with 10 and 20 applied CV cycles (red and blue trace, respectively) are reported in Fig. 2A and Fig. S6A (ESI[†]): here, at low frequencies a capacitive-dominated behavior emerges, as the modulus of the impedance decreases linearly with the frequency. In particular, the Bode modulus decreases faster when the capacitance of the substrate is higher. At higher frequencies, the modulus of the spectra is constant, suggesting a transition towards a more resistive-dominated behavior.²⁷ It is worth noting that the PEDOT:PSS coating induces a shift of the capacitive-dominated behavior towards lower frequencies. In addition, larger shifts are induced by thicker polymeric layers (Fig. 2A, red and blue traces), due to the increase of the capacitance with the film thickness.²⁷

Furthermore, EIS measurements were performed on gold-coated and PEDOT:PSS-coated micropillar arrays of different height (1 and 4 μm) and pitch (1 and 10 μm) referred to as h_1p_1 , h_1p_{10} , and h_4p_{10} . Representative impedance spectra are shown in Fig. 2B (more details in Fig. S6B, ESI[†]). Here, in contrast to the planar case, where the film thickness has also an effect on the impedance (Fig. 2A, red and blue traces), the Bode moduli corresponding to the micropillar arrays are similar regardless of the thickness of the polymeric film (Fig. 2B, red and blue traces), probably due to comparable values of capacitance as also shown in Fig. 2C.



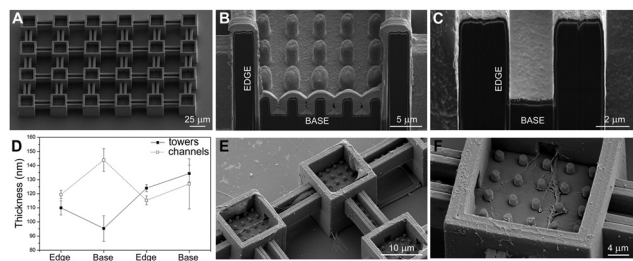


Fig. 4 PEDOT:PSS-coated cage-like scaffold fabrication outcome, thickness analysis and cell-scaffold interaction. (A) Scanning electron micrograph of the 3D cage-like structures coated with 20 applied CV cycles. Scanning electron micrographs of cross-sections performed on different scaffold regions: (B) tower with pillars and (C) flat channel. (D) PEDOT:PSS thickness analysis on 3D structures: each graph shows the mean values and standard deviations depending on the cross-sectioned scaffold region. (E) Scanning electron micrograph of primary neurons cultured on 3D coated scaffolds and (F) close-up of a neurite wrapping around a pillar.

respectively. Conversely, the PEDOT:PSS film thickness on the flat channels was higher than the thickness on channels with pillars, with an average thickness of 144 ± 8 nm and 127 ± 17 nm, respectively. In addition, the high standard deviation indicates the lower reproducibility of the PEDOT:PSS deposition on such narrow elements, compared to the other substrate regions (Fig. 4D). Furthermore, the biocompatibility of the PEDOT:PSS-coated 3D cage-like structures was assessed (details in the Experimental section, ESI[†]). As expected, cell survival was high both for HT-22 cells and primary cortical neurons (Fig. S11A and B, respectively, ESI[†]), which suggests that the spatial arrangement and morphology of the substrate have no effect on cell viability. Finally, cellular adhesion and interaction with the 3D structures was investigated *via* SEM imaging (Fig. 4E and F). The substrates support cellular adhesion, confining and guiding neurite outgrowth throughout the entire scaffold. Moreover, the presence of micropillars provides anchorage points for the elongation and growth of neuronal processes, allowing for a close cell-chip coupling, fundamental for an effective bioelectronic device.

In conclusion, in this work, an innovative and versatile fabrication method for 3D PEDOT:PSS-coated microstructures combining 2PP and electrodeposition techniques is reported. This method was employed for the fabrication of conductive micropillar arrays with various aspect ratios and pitches as well as complex 3D cage-like structures to demonstrate the versatility of the proposed technique. First, the resulting thickness of PEDOT:PSS according to the applied CV cycles on the micropillars was characterized by means of FIB cross-sectioning and SEM imaging. Subsequently, the electrochemical properties of the coated micropillars were investigated by means of EIS revealing that the presence of the 3D structures mediates the capacitance of the PEDOT:PSS film and is not dependent on the thickness. These findings demonstrate that the PEDOT:PSS-coated micropillars are suitable as electrodes to be implemented in bioelectronic devices for both sensing and stimulation of neuronal tissues. Additionally, we assessed the cell viability of HT22 cells confirming the biocompatible nature of PEDOT:PSS. Furthermore, it was observed that micropillar arrays can act as topographical guidance and that cage-like scaffolds were

able to confine and guide neurite outgrowth. The results show that this fabrication method is suitable for the realization of conductive 3D structures of any kind of geometry, overcoming the limits of 2D cell cultures.

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Conflicts of interest

There are no conflicts to declare.

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