Composite microelectrodes from PEDOT and carbon nanotubes enable advanced neuronal recording, stimulation and sensing

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ABSTRACT

In this work, we present composite microelectrodes to enable advanced neuronal recording stimulation and sensing. The composite from conducting polymer PEDOT (poly(3,4-ethylenedioxythiohene)) and CNT (carbon nanotubes) shows significantly lower impedance (< 20 kΩ for d = 30 µm) and higher capacitance (4-10 mF/cm^2) when compared to state-of-the-art neuronal electrodes. *In vitro* testing with different cell cultures reveals excellent biocompatibility an improved signal quality with lower noise and higher signal amplitudes. The high capacitance enables improved charge transfer. Significantly higher amounts of charge can be transferred over a long period of time without impairing electrical properties. Most interestingly, we could demonstrate that PEDOT-CNT electrodes can also be used as neurotransmitter sensors. In summary, results demonstrate exceptional performance of PEDOT-CNT mircoelectrodes enabling their application in neurotechnology.

Keywords: neurotechnology, PEDOT, CNT, microelectrode array

1 INTRODUCTION

Electrical recording and stimulation of brain tissue *in vitro* and *in vivo* is used to gain a better understanding of diseases like Parkinson's or epilepsy in order to develop new therapy options. Typically, microelectrode arrays (MEA) comprising electrodes fabricated from metal based materials are used for this purpose [1, 2]. In order to improve signal-to-noise ratio, stimulation and sensing properties as well as cell viability, microelectrodes were modified with poly(3,4-ethylenedioxythiophene) (PEDOT) and carbon nanotubes (CNTs). Previous reports had demonstrated the suitability of PEDOT as a material for micro-neural interfaces [3]. Furthermore, exceptional viability of cells and their efficient integration with layers composed of CNTs previously have been observed [4-6]. Moreover, CNT modified electrodes provide for enhanced sensing properties towards neurotransmitters such as dopamine [7]. PEDOT-CNT composite materials are therefore considered attractive candidates for electrodes in neurotechnology.

2 MATERIALS AND METHODS

PEDOT-CNT microelectrode arrays were fabricated as described before [8]. Briefly, electropolymerization was carried out to deposit PEDOT-CNT composite on gold microelectrodes (MEA from NMI-TT GmbH, electrode diameter of 30 µm) using suspensions of ethylene-diethoxythiophene (EDOT, 0.02 M), poly(sodium-p-styrenesulfonate) (PSS, $M_w \approx 70{,}000$ g/mol; 1 %) and single walled carbon nanotubes (0.03 %). Pure PEDOT coatings were electropolymerized using the same concentrations in the absence of CNTs. PSS fulfills 3 different functions: it serves (i) as a conducting salt during electrodeposition, (ii) as dispersant for CNTs by polymer wrapping [9] and (iii) as a counterion for the positively charged PEDOT chains. The electrochemical deposition was performed under ambient conditions in a three electrode system with galvanodynamic control applying a final current density of 2 mA/cm^2 and potentials of ca. 0.7 V vs. Ag/AgCl (chlorinated silver wire). Charge densities of 30-110 mC/cm^2 were passed during deposition in order to control the amount of deposited material on the electrodes. Employing an 8-channel potentiostat/ galvanostat it is also possible to coat all 59 electrodes of the MEA in a fast and reproducible way. This method is highly promising allowing integration into commercial production processes.

To characterize the material morphology, coatings were analyzed with scanning electron microscopy (SEM) for surface analysis and imaging of the cross sections prepared by focused ion beam etching (FIB).

Adhesion of the coatings was tested by the tape adhesion test (Cross-cut test ISO 2409:2007(E)) and optical as well as electrochemical characterization before and afterwards. Also microelectrodes underwent extensive rinsing with different solvents (water, ethanol, isopropanol, acetone), and typical sterilization procedures such as autoclaving (121°C, 20 min) as well as standard UV-irradiation over night to examine stability.

Electrochemical characteristics were determined by impedance spectroscopy, cyclic voltammetry and voltage pulsing.

To proof the applicability in neurotechnology, two different primary neuronal cell systems were employed. Firstly, primary sensory neurons isolated from neonatal rat dorsal root ganglia (DRG) were seeded on MEAs and cultivated for 48 h. Spontaneous activity as well as action potentials after chemical stimulation with 1 µM capsaicin were recorded (MEA1060-BC, 1100x amplifier, Multi Channel Systems). Secondly, cortical neuronal networks were cultured on MEA to proof biocompatibility over a longer period of time (up to two months) and to confirm applicability. Cortical tissue was obtained from newborn rats (Sprague-Dawley) within 24h after birth, mechanically and enzymatically dissociated following standard procedures [10]. Recordings were conducted at the age of 19 days *in vitro* (MEA1060-Up-BC, Multi Channel Systems) (see [11] for details).

For neurotransmitter sensing, different concentrations (0.5-100 µM) of dopamine in phosphate buffered saline (PBS) were measured using square wave voltammetry. The parameters were optimized to 25 mV amplitude, 4 mV step size and 100 ms pulse width. After preconditioning for 1 min, the potential was scanned from -200 to 500 mV.

3 RESULTS AND DISCUSSION

3.1 Material characterization

Gold microelectrodes are homogeneously coated by electropolymerization in a reproducibly manner. In figure 1 a) a typical gold MEA is shown, comprised of PEDOT-CNT coated microelectrodes displayed in figure 1 b). Figure 1 c) shows a cross section of the coating on top of the gold microelectrode prepared by FIB/SEM revealing the rough and porous morphology of PEDOT-CNT. In comparison, pure PEDOT microelectrodes show a smoother and thus smaller surface area [8].

Coated electrodes show extraordinarily low impedance (< 20 kΩ for d = 30 µm) and significantly higher capacitance (4-10 mF/cm^2) when compared to state-of-the-art MEA electrodes. PEDOT-CNT MEAs also exhibit significantly higher charge transfer capacitance when compared to state-of-the art MEAs which provides for the possibility of stimulating cells by extremely low voltages thus avoiding tissue damage and side reactions. Constantly high charge per pulse can be transferred over 3.6 million pulses without impairing the electrochemical characteristics and without delamination of the coatings. Details on the material characterization can be found in Gerwig et al., 2012 [8].

3.2 Stability and reproducibility

As described in the methods section, electrodes were examined concerning stability and adhesion. Typical procedures employed in cell culture were chosen as test methods. Extensive rinsing with solvents, autoclaving, UV iradiation over night as well as repeated cell cultures did not cause delamination or impair the electrochemical properties significantly [8].

This robustness and reproducibility render PEDOT-CNT microelectrodes applicable to real world applications.

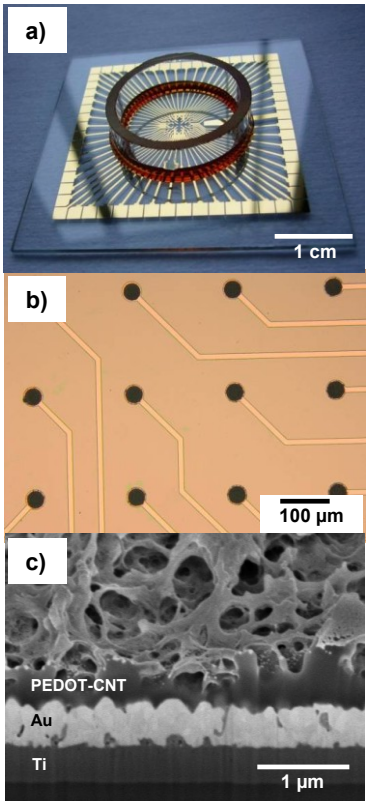

Figure 1: a) Fotograph of a microelectrode array with 59 gold microelectrodes with diameter of 30 µm. b) Micrograph of homogeneously coated PEDOT-CNT microelectrodes. c) Micrograph of the cross section of PEDOT-CNT microelectrode prepared by FIB/SEM.

3.3 Neuronal recordings

Primary sensory neurons from dorsal root ganglia (DRG) serve as an *in vitro* model for physiological, pharmacological and molecular studies in the nociceptive (pain receptive) and proprioceptive (detection of the motion and position of the organism) system [12, 13]. In this work, cultured primary sensory neurons served as analytical tool to examine biocompatibility and funtionality of PEDOT-CNT MEAs. Firstly, noise analysis was carried out by recording during a period of time with no or little activity. In Figure 2, the root mean square (rms) of the noise is depicted as mean from 2 (TiN) and 3 (PEDOT-CNT) different samples for two different experimant days using

the same set of substrates. In Experiment 1, noise was already by 32 % lower for PEDOT-CNT when compared to TiN. When reusing the same substrates, this difference in noise was even higher for the second experiment. This representative data shows, that noise is significantly lower when recording using PEDOT-CNT MEAs even when substrates are used repeatedly. Secondly, spontaneous activity as well as chemically evoked activity was recorded revealing higher signal amplitudes when using PEDOT-CNT electrodes as compared to TiN [14].

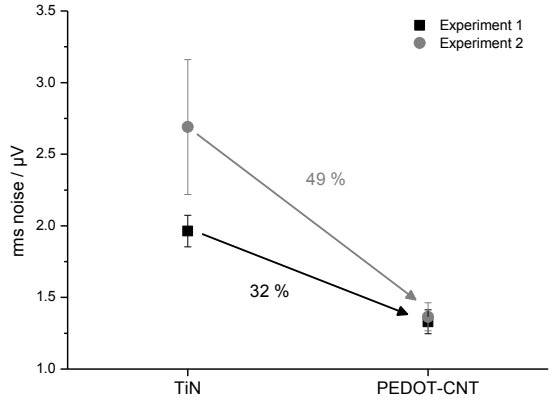

Figure 2: Root mean square (rms) of the noise of recordings from dorsal root ganglia. The grey and black markers show data from different experiment days on the same set of substrates (TiN: n = 2, PEDOT-CNT n = 3).

Primary dissociated neurons are cultivated *in vitro* to study important brain functions and dysfunctions. As these cells are cultivated over an extended period of time until they develop highly interconnected networks, they served as model system to examine biocompatibility as well as functionality.

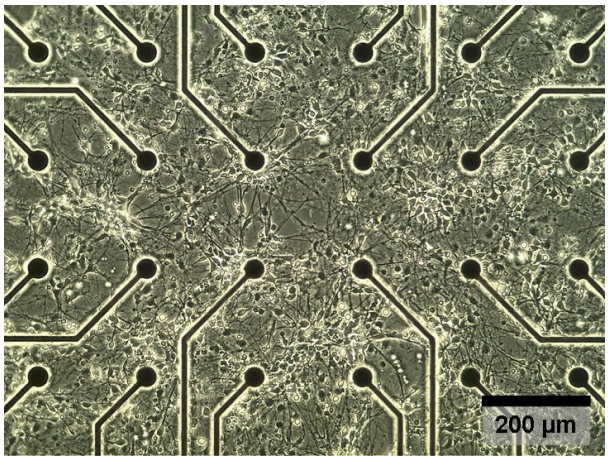

Figure 3: Cortical neuronal networks grown on MEA.

In Figure 3, such a well developed highly branched cortical network grown on a MEA is displayed.

The cells were viable and active for at least two months and developed a network on both types of MEA (TiN and PEDOT-CNT). Recordings were taken on day 19 of culture. From the overall activity pattern (e.g. the inter-network burst interval distribution) it was seen, that the network showed no unusual behavior on PEDOT-CNT electrodes when compared to standard TiN.

Spike amplitudes of about 50-60 µV are generally observed with state-of-the-art MEAs whereas spikes with amplitudes exceeding 100 µV are relatively rare. On PEDOT-CNT, however, spikes with amplitudes of well over 100 µV were regularly detected indicating improved coupling of the cells to the electrodes. A selection of recordings from the most active electrodes from TiN and PEDOT-CNT MEAs are assembled in Figure 4.

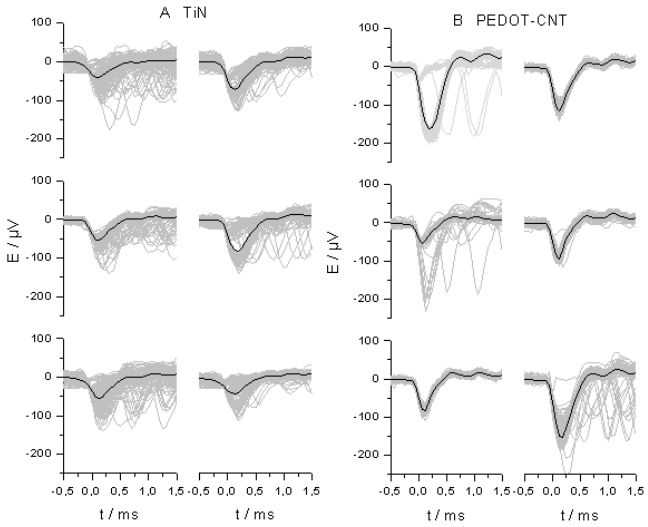

Figure 4: Section of recordings from primary cortical networks on A) TiN and B) PEDOT-CNT MEAs. The traces of the six most active electrodes of each type of MEA were selected. The black trace shows the average of all traces, respectively.

3.4 Neurotransmitter sensing

Most interestingly, we could also demonstrate that the PEDOT-CNT electrodes can be used as sensors to detect neurotransmitters. Sub-micromolar concentrations of dopamine and other neurotransmitters could be detected using square wave voltammetry at PEDOT-CNT electrodes. In Figure 5, representative square wave voltammograms are depicted for pure PEDOT and PEDOT-CNT composite microelectrodes. Incorporation of CNT increases the recorded current and thus improves the sensitivity towards dopamine. Calibration over a concentration range of 0.2 to 5 µM yields sensitivities of 0.09 nA μM^{-1} for PEDOT and 0.18 nA μM^{-1} for PEDOT-CNT.

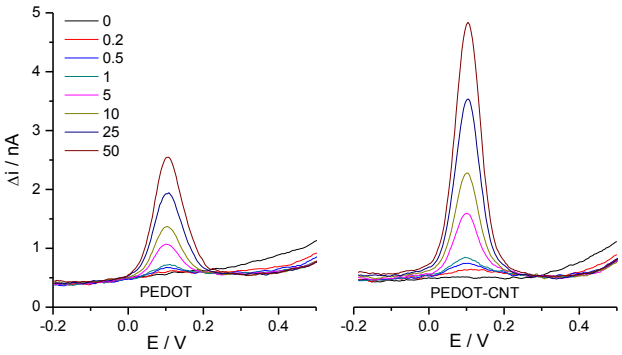

Figure 5: Representative square wave voltammograms (SWV) recorded in PBS solutions of dopamine of different concentrations (0.2 to 50 µM) at a PEDOT (left) and a PEDOT-CNT (right) microelectrode.

4 CONCLUSIONS

In summary, results demonstrate exceptional performance of nanostructured PEDOT-CNT micro-electrodes. Based on these results we anticipate significant improvements in applications in in vitro neurotechnology as well as in neuroprostheses to become possible in the future.

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