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Nanostructured platinum grass enables superior impedance reduction for neural microelectrodes

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Abstract

Micro-sized electrodes are essential for highly sensitive communication at the neural interface with superior spatial resolution. However, such small electrodes inevitably suffer from high electrical impedance and thus high levels of thermal noise deteriorating the signal to noise ratio. In order to overcome this problem, a nanostructured Pt-coating was introduced as add-on functionalization for impedance reduction of small electrodes. In comparison to platinum black deposition, all used chemicals in the deposition process are free from cytotoxic components. The grass-like nanostructure was found to reduce the impedance by almost two orders of magnitude compared to untreated samples which was lower than what could be achieved with conventional electrode coatings like IrOx or PEDOT. The realization of the Pt-grass coating is performed via a simple electrochemical process which can be applied to virtually any possible electrode type and accordingly shows potential as a universal impedance reduction strategy. Elution tests revealed non-toxicity of the Pt-grass and the coating was found to exhibit strong adhesion to the metallized substrate.

Keywords:

- Nanostructure
- Electrode
- Platinum
- Neural Prosthesis
- Electrical stimulation
- Interface

1. Introduction

Selective recording of neuronal signals, as well as the systematic stimulation of neurons, enable researchers to better understand basic neuronal functions. Furthermore such technology provides a versatile tool box [1] for applications in diagnostics, therapy and rehabilitation. Current research addresses pain therapy by means of spinal cord stimulation [2], deep brain stimulation to treat symptoms of Parkinson’s disease and severe psychiatric disorders [3, 4], epilepsy diagnostics, artificial limb control [5], lost body function replacement [6] and modulation [7] or the development of brain computer interfaces [8, 9], for example. All of these applications are enabled by electrodes that mediate the connection between the biological and the technical side of the neural interface. The amount and diversity of such electrodes seem almost unlimited owing to the fact that different applications demand different electrode types with respect to their size, invasiveness, selectivity, materials and performance. Aiming for the best signal to noise ratio (SNR) with the highest selectivity, it comes close at hand to use implantable micro-sized electrode arrays with multiple recording and stimulation sites close to the neuronal tissue. The fabrication of such electrodes, being preferably small enough for communication with single neurons, is technologically feasible regarding the overall dimensions of the implant. However, a size reduction of the actual conducting site is inevitably accompanied by an increase in the impedance of the electrode, so that small electrodes suffer from low signal to noise ratios. Furthermore, with higher impedance, higher voltages are needed to reach the necessary current-density for triggering a neuronal response. The voltages are however restricted to the range where no detrimental electrochemical reactions occur to either the biological or the technical side. Since tissue reactions are very application specific, in practice these voltage limits are commonly determined by the so called “water-window”. This parameter is individually defined for each material and represents the potential-range where oxidation or reduction currents will not lead to the formation of hydrogen or oxygen at the electrode interface (e.g. -0.6 V to 0.9 V vs. Ag/AgCl for Pt in aqueous solution). Taking these restrictions into account, the size of an electrode for clinical use is in fact determined by a trade-off between high selectivity (obtained by small size) and optimized electrical characteristics, the latter demanding a large contact surface to the electrolyte. Hughes et al. [10] described an electrode of 20x20 μm^2 to be the smallest suitable size to meet this trade-off.

In order to improve the performance of small electrodes, especially with respect to decreasing the impedance, various strategies have been investigated. Thereby a distinction between surface and bulk processes can be made. In the latter, the metallization of an electrode (bulk material) is selectively removed by laser roughening or metal etching, yielding an enlarged active surface area and respectively lower impedance compared to untreated surfaces of equal dimensions [11-13]. The surface modification in contrast relies on the additional deposition of either a material that provides a highly roughened surface (topographical approach) or a material that features additional electrochemical means to improve the impedance and charge delivery capacity (chemical approach). Relevant work is e.g. reported by Park et al. [14] using 3D structures and Marrese et al. [15] introducing platinum black for modification of the topography. The latter method provides an impedance reduction of more than one order of magnitude, however, practical applicability of Pt-black is limited as consequence of the low mechanical stability [16-18]. In addition, Pt-black has been reported to cause cytotoxic reactions if traces of lead, used in the electrolyte during fabrication, elute from the final coating *in vivo* [19]. While acute animal experiments were considered feasible, use of Pt-black for chronic human implants was not recommended [19]. A similar approach using non-toxic components during film formation has been introduced by Zhou 2005, known as Pt gray, however the roughness was found substantially lower compared to Pt-black [20]. Cui et al. [21] and Cogan et al. [22] demonstrated successfully the reduction of the electrode impedance using either conducting polymers as e.g. poly(3,4-ethylenedioxythiophene) (PEDOT) or using Iridium Oxide (IrOx) targeting both the chemical and the topographical approach to improve the electrode performance.

These modification techniques altogether result in a reduction of the electrode impedance and accordingly enable the use of small implantable electrodes with superior electrical characteristics compared to unmodified equivalents. However, the techniques used to modify the electrodes are typically time and cost intensive, especially if a cleanroom processes is required. Furthermore, alteration steps that are integrated into the overall fabrication process of certain electrodes cannot easily be applied to other types of electrodes and (individual) single site modification is not always possible or comes at unreasonably high effort and costs. Electrode coatings like IrOx or PEDOT have additionally been reported to be brittle or prone to delaminate, further restricting their use [23, 24].

In this study, we target these deficiencies by introducing a simple add-on functionalization process for cheap, fast and universal impedance reduction of microelectrodes by growing a three dimensional nanofibrous coating herein referred to as Pt-nanograss. A wet chemical process, inspired by the work of Sun et al. (2008) [25], was used to deposit the nanostructured Pt layer on top of conventional smooth Pt electrodes in either a passive or an electrochemically accelerated active deposition approach for improving the electrochemical properties of neural microelectrodes. This modification process resulted in an astonishing enlargement of the electrochemical surface area and consequently a substantial reduction in impedance with more than factor 60 compared to non-modified samples. The grass like appearance was revealed by high resolution scanning electron microscopy. The effect on the electrochemical properties of the microelectrode was evaluated by a multitude of electro-analytical methods chosen to target the qualities considered to be most essential for stable and selective recording and stimulation of neurons. A direct comparison to state of the art materials like IrOx or PEDOT revealed competitive performance for the Pt-nanograss modified electrodes in all categories tested. In terms of impedance the nanograss in fact outperformed both these materials. Furthermore, the chemical reduction process employed does not require any specific control parameters or cleanroom environment, making our process applicable to virtually any kind of available probe or multi electrode array. In comparison to the platinum black deposition, all used chemicals for the Pt-grass formation are free from cytotoxic components. The cost-efficiency and simplicity of the Pt-nanograss formation, combined with its (chemical) biocompatibility and high stability, accordingly offers tremendous potential for improving the electrical performance of small electrodes.

2. Materials and methods

2.1 Test samples

Flexible polyimide based probes carrying platinum microelectrodes, fabricated as described in [26], were used as test platform for the applied modification processes and further served as reference samples in their unmodified state (Fig. 1). All electrodes were manufactured with a sputtered Pt-layer as basic metallization (300nm thick, sandwiched between 5 µm polyimide on each side) and featured electrode sites with diameters ranging from 5µm to 500 µm. Sputter deposition was performed on a Leybold Univex 500 sputter device (Oerlikon Leybold Vacuum GmbH, Cologne, Germany) at 300 W RF-power.

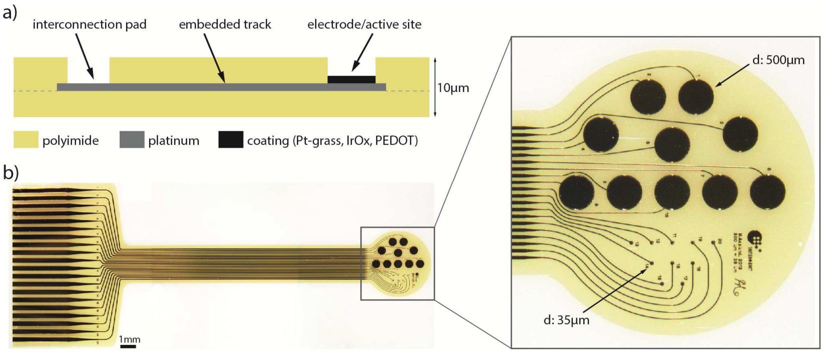

Figure 1: Design schematic (a) and electrode layout (b) of the flexible polyimide probes used as substrate for depositing and evaluating the Pt-nanograss coating.

2.2 Deposition of the Pt-nanograss

The platinum nanograss was deposited in a chemical reduction reaction using an aqueous solution of 2.5mM H_2PtCl_6 and 1.5mM formic acid (HCOOH), purchased from Sigma and used without further purification. For the passive deposition approach, the solution was filled into a vial with the electrode mounted vertically in the solution. Deposition was carried out at room temperature for 48 hours until the reduction reaction was complete, indicated by a colour change of the solution from yellow to clear.

For the active deposition process, a three electrode electrochemical setup was used. The electrode receiving the Pt-nanograss coating was thereby connected as working electrode and a stainless steel as well as an Ag/AgCl electrode were used as counter and respectively reference electrode. Pt-grass formation was conducted potentiostatically from the same composition of the H_2PtCl_6 solution as described above at a potential of -0.1 V vs Ag/AgCl for 300 s using a PGSTAT302N potentiostat (Metrohm, Filderstadt, Germany). After formation of the Pt-grass, the electrodes were rinsed with water and dried at room temperature.

2.3 Deposition of IrOx and PEDOT for reference measurements

Reference samples, carrying either IrOx or PEDOT coatings, were fabricated in parallel to the Pt-nanograss deposition on probes of the same design using standard deposition protocols. The IrOx deposition was integrated in the cleanroom process of the polyimide probe. Sputtering was performed using a Leybold Univex 500 sputter device (Oerlikon Leybold Vacuum GmbH, Cologne, Germany) and was introduced subsequent to the sputtering of the Pt substrate electrode. Thereby an IrOx layer of 800 nm in thickness was realized on top of the active electrode sites. The PEDOT film deposition was performed as previously described in [27] using an electro-polymerization process with polystyrenesulfonate (NaPSS) as counter-ion. The process results in an adherent coating confined to the active electrode site, often referred to as PEDOT:PSS. A polymer layer of 800 nm thickness was realized. This process is well in line with standard PEDOT electrodeposition protocols described in the literature.

2.4 Electrochemical analysis:

The electrochemical characteristics of Pt-nanograss modified electrodes, in comparison to IrOx, PEDOT and bare Pt, were assessed by a range of electrochemical methods. Complex impedance ($\underline{Z}$) was evaluated by electrochemical impedance spectroscopy (EIS). Charge storage capacity (CSC) was estimated by cyclic voltammetry (CV) and charge injection capacity (CIC) was evaluated by a pulse based stress test as described in [28, 29], herein referred to as pulse testing.

EIS and CV measurements were performed in a conventional three electrode setup using the test material as working electrode. A stainless steel sheet (2 cm^2) and an Ag/AgCl electrode served as counter and reference electrode while saline solution (phosphate buffered saline, PBS) at a concentration of 0.1M was used as electrolyte. Vertex potentials for CV were chosen according to the water window for Pt (-0.6 V to 0.9 V, 0.1 V/s sweep rate) and kept constant during all measurements to facilitate comparison between the different materials. CSC is here defined as the area enclosed within one CV-cycle.

Impedance measurements were performed in a frequency range of 0.1 Hz to 10 kHz with a sinusoidal excitation signal of 10 mV. For further analysis data was fitted with an R(RC) equivalent circuit model using the software Nova (Metrohm, Filderstadt, Germany). The model was used to extract the double-layer capacitance at the electrode/electrolyte interface (see Fig. 2). Additionally, the cutoff-frequency, determined by the transition of the impedance from a capacitive behaviour (45° slope) to a resistive behaviour (no slope) with a phase angle of -45° in the impedance magnitude and phase plot, respectively, was identified to compare pre- and post-deposition properties.

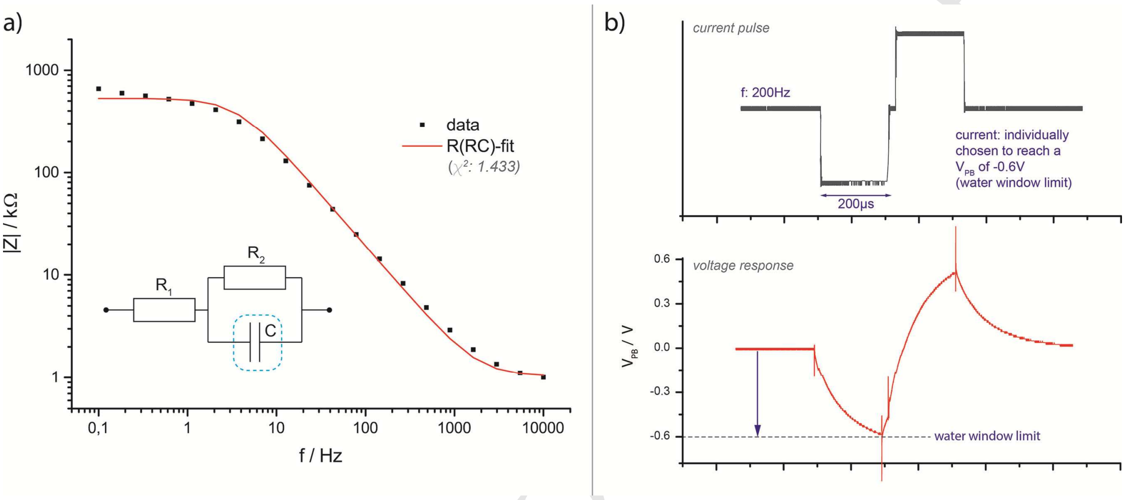

Figure 2: Electrochemical analysis methods. a) EIS measurement showing the impedance data fitting according to the R(RC) equivalent circuit model. b) Current signal and corresponding voltage drop at the electrode (V_{PB}) (after subtraction of the IR-drop) as used for the pulse-testing.

Pulse-testing was done with a PlexStim electrical stimulator (Plexon Inc., Dallas, USA) in order to assess the maximum charge injection capacity and evaluate the long-term performance of the coating. For measuring the charge injection, a biphasic charge balanced rectangular current-pulse of 200µs pulse duration, and a frequency of 200 Hz, was applied while the current density was subsequently increased until the measured voltage drop at the phase boundary (V_{PB}) reached the water window at a value of -0.6 V (see Fig. 2). Therefore, the ohmic voltage drop (“IR-drop”) over the electrolyte was subtracted. Long-time pulsing of the Pt-nanograss was done with a biphasic rectangular (cathodic first) current pulse of 200 µs pulse duration and 1 mA current amplitude at a frequency of 200 Hz. Pulse durations were chosen according to previously reported values for evaluation of the electrochemical properties of neural electrodes [29, 30]. The pulse widths corresponds to typical nerve excitation properties in the range of the chronaxy at which the energy to elicit nerve excitation by rectangular current pulses is at minimum. The applied charge of 200 nC/phase is thereby 10 times higher compared to previously reported parameters for stability-evaluation of microelectrodes [28] and is consequently considered to provide stability information regarding the long-term behaviour of the electrode. The voltage drop at the phase boundary was monitored and used to evaluate the stability of the coating over time. A total of 240 M pulses was applied over a test period of 2 weeks.

2.5 Imaging of the probes

Scanning electron microscopy (SEM) and Focussed Ion Beam (FIB) imaging methods were used to study the topography as well as the cross sectional structure of the Pt-nanograss coatings. SEM pictures were taken at a Nova NanoSEM (FEI, Hillsboro, USA) with a nominal maximum resolution of 1 nm. In total, 16 samples were investigated by SEM in top view as well as under 30° and 45° tilted angles. FIB was done on a Strata 400 STEM (FEI) at KNMF (KIT, Karlsruhe, Germany) where 10 samples were examined. The graphs displayed here show representative pictures for SEM and FIB imaging.

2.6 Cell studies

Potential toxicity of the Pt-nanograss was assessed by means of an elution test according to the guidelines in the standard ISO 10993-5 and using the human neuroblastoma cell line SH-SY5Y as model. For this analysis, large area samples were fabricated by depositing Pt nanograss on single pieces of a platinized silicon wafer. Sterile culture medium was exposed to the Pt-grass surfaces at three different concentrations, 1.25 cm^2/ml, 2 cm^2/ml and 3.2 cm^2/ml, for 24 h at 37 °C. Elutions exposed to the same amount, temperature and time of Ag/AgCl electrodes (E-244, scienceproducts) were used as a positive control and ordinary culture medium was used as a negative control. Three 96-well plates were prepared with 4 wells per concentration. Cells were resuspended in their respective elution and added to each well at a concentration of 20 000 cells per 0.2 ml elution and well. Viability was evaluated at test start (2 h exposure), after 24 h exposure and finally after 72 h exposure, using the 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [31]. At each evaluation point MTT was added to all wells of one plate which was subsequently incubated for 4 h at 37 °C. After the addition of 200 µl of dimethyl sulfoxide (DMSO) to each well, and further two hours of incubation, absorbance at 595 nm was measured using an ELISA microplate reader (Enspire, PerkinElmer GmbH, Rodgau, Germany) and used as proxy for the number of living cells in each well.

3. Results

A fast and simple process for the realization of platinum nano-structures has been investigated in this work with respect to modification of neural microelectrodes yielding an electrical impedance that was found lower than for any of the generally considered low-impedance-materials like IrOx or PEDOT. Following results briefly summarize the process findings before focussing on the electrical performance with respect to relevant measures for neural electrodes.

3.1 Deposition process

Two different deposition approaches were employed: a passive approach, were growth is governed solely by diffusion processes and an electrochemically active deposition process were growth is accelerated by the presence of an electric field. Flexible polyimide electrodes featuring individual platinum sites of either 35 µm or 500 µm diameter were characterized by means of CV and EIS both before and after they were subjected to the Pt-nanograss add-on process. The electrode sites thereby showed a black coating revealing the successful formation of the Pt-nanograss on the sputtered platinum. Since nanofibers were found to grow unspecifically also on the insulating surfaces, a post-deposition cleaning step was introduced to avoid short circuits and ensure that the coating was confined only to the individual electrode sites. A process comprising the use of ultra-sonication in a water bath for 30min (contact free method) was found efficient. As faster alternative, it was found that the insulation could be mechanically cleaned by wiping the electrode with a wet tissue given that the individual electrode sites are not in level with the polyimide insulation but protected in trenches approximately 5µm in depth. (Fig. 3b). The nano-grass layer on the metallic sites was thereby not affected by any of the cleaning procedures and proved to be stable and well adherent to the sputtered platinum layer underneath.

In contrast to the passive Pt-grass process, the active deposition of the Pt-grass employing the electrochemical reduction mechanism resulted in clearly defined local Pt-grass formation only on the connected electrode sites. Neither the non-connected metallic surfaces nor the polyimide substrate showed any sign of contamination as can be seen in Fig. 3c. It was possible to perform simultaneous active depositions by short-circuiting several electrode sites before connecting them to the working electrode of the potentiostat. The yielded Pt-grass formation was homogenous over all connected sites and showed no colour differences even for simultaneous deposition on differently sized electrode sites (5 µm to 500 µm diameter) as could be verified with an optical microscope (DM4000, Leica Mikrosysteme, Wetzlar, Germany).

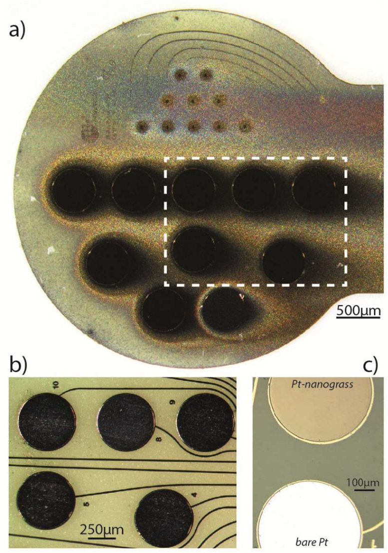

Figure 3: Optical images of passively deposited Pt-nanograss on a polyimide probe before (a) and after (b) the mechanical cleaning procedure. The probe in (c) shows two neighbouring electrode sites with and without actively deposited Pt-grass on the upper and lower site respectively.

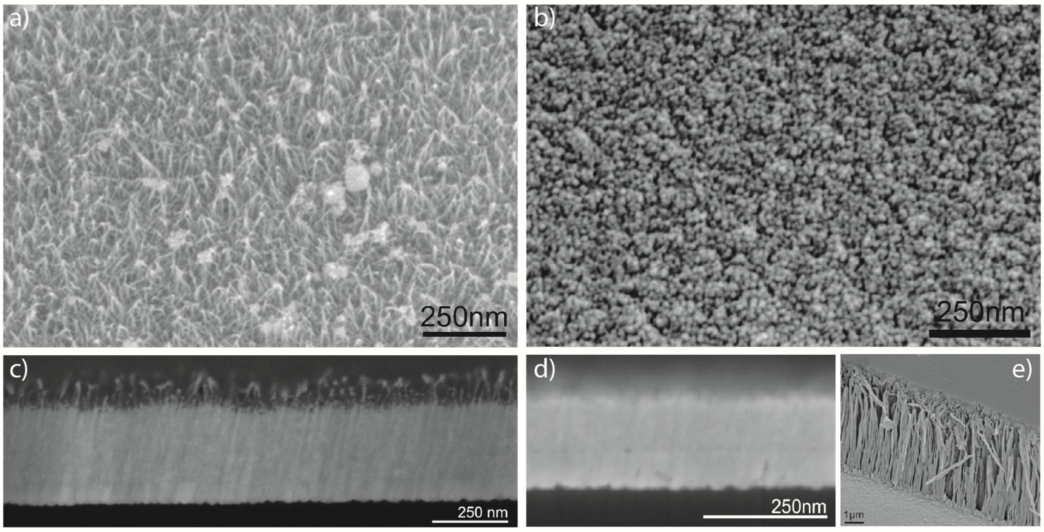

Figure 4: SEM images showing the topography and the cross-section of passively deposited Pt-nanograss (a,c) in comparison to the actively formed coating (b,d). The close-up in (e) shows the recessed metallization plane (bottom, with Pt-grass) and the polyimide side walls. The fibrous texture is known to be the typical structure of

an RIE edge in polyimide. The comparison between the lower Pt-grass coated and the upper insulating plane reveals that there is no unspecific coverage of the former with grass like structures.

3.2 Structure evaluation

In order to examine the roughness increase and compare the topography of passively and actively deposited Pt-grass, samples of both types were closely examined using SEM and FIB imaging techniques. Representative micrographs are given in Fig. 4 showing passively formed Pt-grass on the left (Fig. 4a, c) and an actively deposited layer on the right (Fig. 4b, d and e). A clear difference can be seen from both the topography and the cross sectional view between the two deposition mechanisms, revealing a grass-like structure with an aspect ratio of 13.7 (±1.1) for the passive process and a grain-like structure (aspect ratio: 2.1 ±0.1) for the active process. Aspect ratios were determined by image analysis of the cross-section in seven FIB images. Despite this difference, both types of Pt-grass samples result in a substantial roughness increase compared to the sputtered Pt for non-modified electrodes (bare Pt not shown). A close integration of the Pt-grass to the sputtered platinum could be verified for both process alternatives as no voids or defect sites could be identified in the cross sectional images. Fig. 4e provides a detail of the actively deposited sample showing the side wall of the circular trench in the polyimide (fibrous polyimide structure as a consequence of the RIE-etching process) where the lower plane represents the metallization level (including the Pt-grass) and the upper plane shows the polyimide or respectively the substrate surface. The difference in the structure of these two planes confirmed the previously reported observation that no Pt-grass was formed next to the metal sites during the active Pt-grass deposition.

3.3 Electrochemical characterization

CV and EIS measurements were performed before and after the Pt-grass modification process in order to evaluate the effect of the nano-grass on the electrical performance of the electrode. Fig. 5a represents the impedance recording of a 35 µm diameter electrode without (black) and with the Pt-nanograss (blue) showing a significant decrease of the impedance of almost 2 orders of magnitude following the nanograss modification. The cutoff-frequency could be reduced from >10 kHz to 300 Hz as indicated in Fig. 5a.

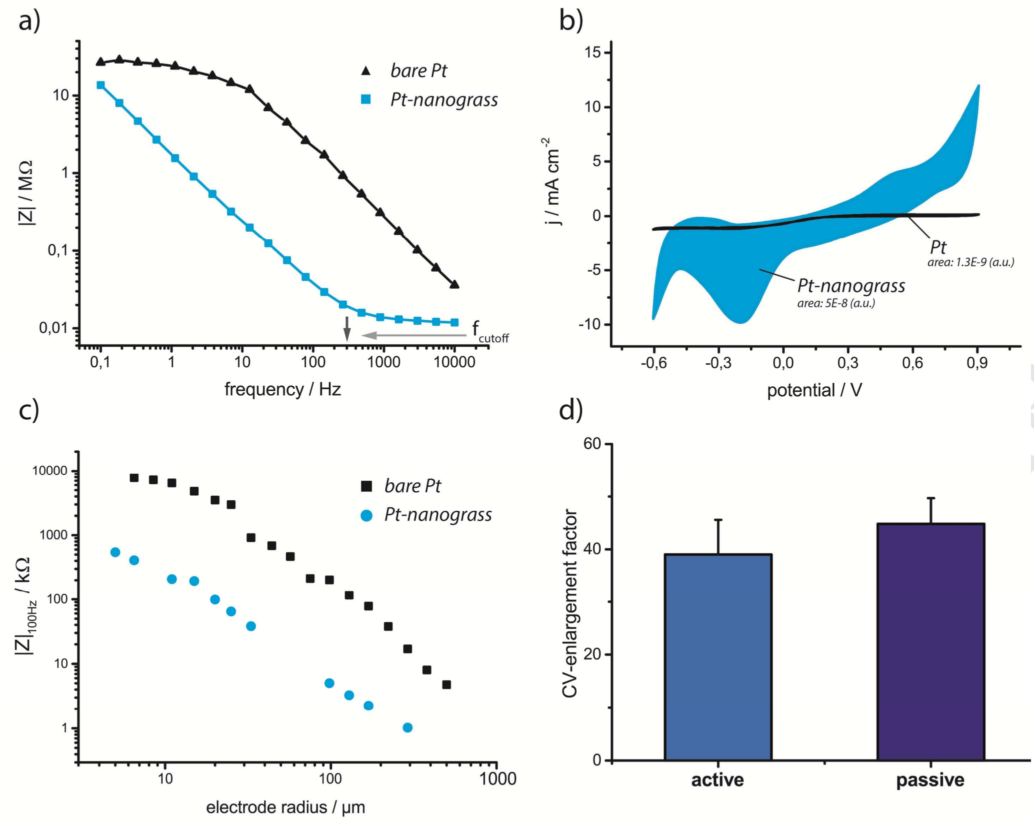

Figure 5: EIS (a) and CV-measurements (b) on 35µm diameter electrode spots carrying the passive Pt-grass (blue squares) in comparison to sputtered Pt (black triangles). The impedance reduction was found to be homogenous over a large size range (c). The CSC, extracted from the area under the CV-curves, shows comparable improvement for actively and passively deposited Pt-grass with respect to bare Pt (d). n=7.

In Fig. 5c, the impedance reduction, based on the deposition of Pt-nanograss (employing the passive approach), is shown for a frequency of 100 Hz over a selection of differently sized electrodes in the range of 5 µm to 500 µm. Thereby the impedance characteristic of the bare Platinum as well as the Pt-grass modified electrodes with respect to the overall electrode size could be approximated with a linear trend-line in the double logarithmic plot. These two trend-lines showed the same slope and only differed by an offset factor which confirms that the impedance reduction effect by the Pt-grass deposition is size-independent.

In accordance with the low impedance recordings, the CV measurements given in Fig. 5b, showed a dramatic increase in the charge delivery capacity (CDC) for the modified electrode.

The CV-enlargement factor, given by the area under the CV for the modified electrode normed to the non-modified state (sputtered Pt), was used to evaluate the difference between actively and passively deposited Pt-grass. According to Fig. 5d, this factor was found comparable for both processes, showing an overall increase of ~40 times for electrodes regardless of which deposition approach that was used for forming the Pt-nanograss.

3.4 Comparison to state of the art materials

Polyimide based electrodes featuring either sputtered platinum, IrOx or PEDOT:PSS as conducting sites were compared to the herein proposed Pt-nanograss in the following disciplines: impedance (Fig. 6a); CV enlargement factor (Fig. 6b); capacity increase (Fig. 6c) and CIC (Fig. 6d). Enlargement factors are given with respect to sputtered Pt as untreated state.

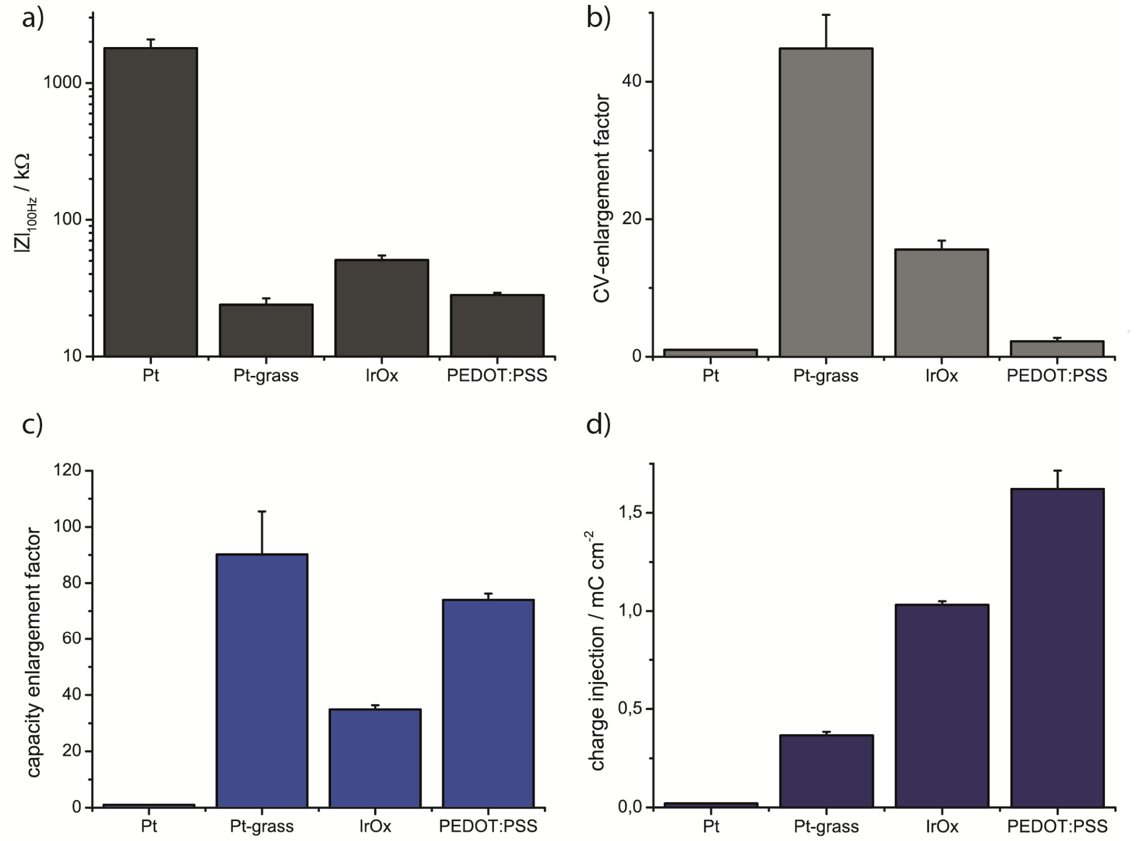

Figure 6: Comparison of Pt-grass coated electrodes (passive process) with IrOx and PEDOT electrodes of equal dimensions using EIS (a), CV (b), capacity enlargement (c) and CIC (d) as reference value. Enlargement factors are given with respect to bare Pt. Measurements are for n=5 with standard deviation as indicated in graphs.

The impedance at 100 Hz for the Pt-grass samples was thereby found to be the lowest in comparison to all other materials with a difference of almost two orders of magnitude to the bare Pt-surface. Similarly, the Pt-grass was found to provide the highest CDC value amongst the tested materials showing a CV-enlargement of ~40 as compared to < 20 for IrOx and < 5 for the PEDOT coating. The capacity enlargement factor, determined via impedance data fitting according to Fig. 2, displayed the highest increment for the Pt-grass modified electrode, even though the difference to the conducting polymer was less apparent as seen for the CV-enlargement. Only in the CIC, the Pt-grass was found not to outperform the competing materials but showed the smallest improvement. Nevertheless, the CIC for the Pt-grass was substantially higher than when measured on the bare Pt surface.

The stability of the Pt-nanograss was assessed by applying a rectangular current pulse with 200 nC/phase at a frequency of 200Hz for two weeks in saline solution. Thereby the potential at the phase boundary was found to decrease only by 2.7 % over 240 M applied pulses. Subsequent optical microscopy confirmed that the pulsed sites looked essentially equal to the un-pulsed state after two weeks of testing, displaying no signs of delamination or other macroscopic changes as seen by 100x magnification.

3.5 In-vitro cell studies

Non-toxicity of the nanograss coating was confirmed by an elution testing spanning over different concentration levels. As can be seen from Tab. 1, there was no significant difference between the negative control and the Pt-grass, indicating that no toxic substances were released from the Pt-nanograss. In contrast, cells that were exposed to eluents from Ag/AgCl electrodes (positive controls) showed clear toxicity in the assay.

Table 1: Cell viability for different eluent-concentrations of Pt-grass samples at various exposure times. Average over 4 wells with standard deviation is shown.

	2h	***24h***	***72h***
1.25 cm^2/ml	94 (±6)%	85 (±10) %	55 (±9) %
2.0 cm^2/ml	83 (±3) %	69 (±5) %	69 (±4) %
3.2 cm^2/ml	80 (±5) %	68 (±5) %	106 (±9) %
pos contr.	9 (±0) %	7 (±1) %	3 (±0) %
neg contr.	100 %	100 %	100 %

4. Discussion

The electrochemical characteristics of implantable micro-electrodes influence decisively the quality of recordings from neural interfaces and determine crucial limits for stimulation of neuronal tissue with respect to biological as well as material safety aspects. One key measure is the electrical impedance, which has a strong dependency on the size of the electrodes in a way that smaller electrodes feature significantly higher impedance values than larger ones. This leads to low signal to noise ratios and restricted stimulation possibilities so that selective communication with neurons requires a trade-off between size and electrical performance. In order to overcome this problem, various strategies have been proposed to increase the surface roughness of an electrode to minimize its impedance. However, these techniques add significant costs and work load to the overall fabrication process of an electrode so that practical applicability is limited.

In this study we introduce a new method in terms of an add-on functionalization process to increase the electrode roughness at significantly reduced costs and effort compared to previously described alternatives. The herein proposed Pt-nanograss was thereby found to provide excellent electrical properties as electrode coating material, being capable of reducing the impedance of an electrode by almost two orders of magnitude. This practically enables the usage of much smaller electrode sites with higher selectivity for neural recordings or allows the transfer of significantly larger amounts of charge over the electrode interface for stimulation of neurons under electrochemically safe conditions. In practice, a 200 µm diameter electrode site (bare Pt) can be replaced by a 20 µm electrode carrying the nanograss coating without changing the electrode impedance. Thereby only 1% of the initial geometrical area is consumed on the probe which enables both the more selective communication with neurons as well as increasing the number of electrode sites per given area on the probe for higher spatial resolution.

The comparison to the commonly used materials PEDOT and IrOx revealed similar or superior properties for the Pt-nanograss. Hence, the herein proposed add-on functionalization offers a suitable alternative for boosting the performance of small electrodes whereas the commonly known and widely accepted platinum is retained as metallization material. This aspect is specifically interesting if platinum is already used for the basic metallization of the electrode since bimetallic corrosion effects can thus be eliminated. Furthermore, the deposition at room temperature enables a stress-free film growth and hence does not involve thermally introduced strain-forces to the probe. It is worthwhile mentioning here that the electrochemical characteristics of conducting polymer films are highly depending on the used monomer and counter ion, the polymerization method, the film thickness and the oxidation state. The herein analysed PEDOT:PSS coating accordingly only serves as reference point for the classification of the Pt-nanograss performance and does not necessarily stand for the best possible representative for the class of conducting polymers.

The chemical deposition of the Pt-nanograss can be applied to potentially any kind of electrode type independent of the substrate material, shape, size and amount of contact sides, depicting a rather universal process. Modification of single individual sites is straight forward, as well as simultaneous deposition on multiple electrode sites on one electrode, or batch processing of multiple different electrodes. Parallel or individual site modification can be done with the same setup and accordingly comes without further effort and required time in contrast to the cleanroom processes that are e.g. required for individual IrOx depositions. The active process can be performed in a few minutes (300s actual deposition time) and accordingly offers a very fast modification of an electrode. The passive process in contrast requires a process time of two days. Nevertheless it does not require a specific device and the actual work load is limited to a few minutes for immersing and subsequently cleaning the electrode. Unspecific coverage, leading to short-circuit of the entire electrode, could only be observed for the passively deposited Pt-nanograss. The proposed cleaning strategies using either a wipe or exposing the electrode to ultrasonication proved to be efficient for removing the excess Pt-grass. At the same time, both cleaning-processes are sufficiently simple and fast so they do not interfere with the overall target of providing a simple add-on process for impedance reduction. Excessive wiping and exposure to several hours of sonication furthermore revealed excellent adhesion of the Pt-grass to the electrode sites which could be confirmed optically and electrically by means of impedance measurements.

In contrast to platinum black, involving lead components [15] during the fabrication, the Pt-nanograss as described in our work does not require such hazardous substances. Accordingly, there are no elution products expected which could have a potentially toxic effect on cells. This is confirmed by the biocompatibility test performed for different concentrations and exposure times. A toxic material would lead to clear correlation of reduced number of viable cells with increased surface areas exposed and with prolonged exposure time, something we did not see in our measurement. We therefore conclude that our material does not elute toxic by-products which is a first step for showing its relevance also from the biological perspective.

The test format used here, quantifying only the number of living cells, is sensitive to differences in initial seeding density, especially for the longest time frame of 72 h, suggesting that a weak negative effect on cell viability and proliferation could be masked by overall high variability of the test. It should however be noted that the exposed surface area studied is extreme (3.2 cm^2) to be compared to the 32000 smaller area of a microelectrode of 100 x 100 µm². It is clear that the topographical profile of the surface is beneficial in terms of electrical properties. Whether it also has an influence on the cellular adhesion to the surface remains to be investigated since there is no conclusive result in the literature stating what the optimal topography would be from this perspective. In a couple of recent studies, gold nanoparticle coated surfaces were studied with regard to their ability to preferentially anchor neurons instead of astrocytes and to mitigate the proliferation of microglial cells [32, 33]. The conclusion was that nanostructures do indeed influence which cells that adhere and that the proportion of neurons in comparison to astrocytes were significantly higher on the nanostructured surfaces in comparison to the flat ones. Whether this is true also for the feature shape and size presented here and, furthermore, is an effect that translates from in vitro to in vivo models is not yet clarified.

Although active and passively deposited Pt-nanograss shows similar electrochemical properties, the structure is clearly different resulting in an aspect ratio that is seven times larger for the passively fabricated structures than for the electrodeposited ones. The reason for this effect was not further investigated since the electrochemical performance was of main interest from the electrode perspective. Nevertheless, we believe that the accelerated nucleation in the active process is the driving mechanism behind the different structures. This observation is in good agreement with a study from Lee et al 2007 [34] describing the controlled growth of Pt-nanowires from hexachloroplatinic acid. By slowing down the reduction rates they were able to form uniform nanowires with similar appearance compared to our Pt-nanograss which is also formed at slow reduction rates in contrast to the active process.

In summary we introduced a nanostructured Pt coating for improving the electrochemical performance of neural microelectrodes with respect to recording or stimulation purposes. A simple and accelerated process has been described yielding an impedance that is lower than for any other material known to this community. In fact it is possible to reduce the electrode area to 1% of the untreated electrode site and yet retain equal impedance. This enables substantially improved spatial resolution in neural recordings. The high CSC in combination with strong adhesion further show the potential for use in chronic stimulation and consequently demonstrate the relevance of the herein proposed Pt-nanograss for improving neural electrodes.

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References

[1] Ordonez J, Schuettler M, Boehler C, Boretius T, Stieglitz T. Thin films and microelectrode arrays for neuroprosthetics. MRS Bulletin. 2012;37:590-8.
[2] Epstein LJ, Palmieri M. Managing Chronic Pain With Spinal Cord Stimulation. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine. 2012;79:123-32.
[3] Thompson DM, Koppes AN, Hardy JG, Schmidt CE. Electrical Stimuli in the Central Nervous System Microenvironment. Annual review of biomedical engineering. 2014;16:397-430.
[4] Holtzheimer PE, Mayberg HS. Deep Brain Stimulation for Psychiatric Disorders. Annual Review of Neuroscience. 2011;34:289-307.
[5] Raspopovic S, Capogrosso M, Petrini FM, Bonizzato M, Rigosa J, Di Pino G, et al. Restoring Natural Sensory Feedback in Real-Time Bidirectional Hand Prostheses. Science Translational Medicine. 2014;6:222ra19.
[6] Guenther T, Lovell NH, Suaning GJ. Bionic vision: system architectures: a review. Expert Rev Med Devices. 2012;9:33-48.
[7] Plachta DT, Gierthmuehlen M, Cota O, Espinosa N, Boeser F, Herrera TC, et al. Blood pressure control with selective vagal nerve stimulation and minimal side effects. Journal of neural engineering. 2014;11:036011.
[8] Stieglitz T, Rubehn B, Henle C, Kisban S, Herwik S, Ruther P, et al. Brain–computer interfaces: an overview of the hardware to record neural signals from the cortex. In: Joost Verhaagen EMHIHJWABBGJB, Dick FS, editors. Progress in Brain Research: Elsevier; 2009. p. 297-315.

[9] Homer ML, Nurmikko AV, Donoghue JP, Hochberg LR. Implants and Decoding for Intracortical Brain Computer Interfaces. Annual review of biomedical engineering. 2013;15:383-405.

[10] Hughes MP, Bustamante K, Banks DJ, Ewins DJ. Effects of electrode size on the performance of neural recording microelectrodes. Microtechnologies in Medicine and Biology, 1st Annual International, Conference On 20002000. p. 220-3.

[11] Green RA, Hassarati RT, Bouchinet L, Lee CS, Cheong GLM, Yu JF, et al. Substrate dependent stability of conducting polymer coatings on medical electrodes. Biomaterials. 2012;33:5875-86.

[12] Boretius T, Jurzinsky T, Koehler C, Kerzenmacher S, Hillebrecht H, Stieglitz T. High-porous platinum electrodes for functional electrical stimulation. Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2011;2011:5404-7.

[13] Green RA, Matteucci PB, Dodds CWD, Palmer J, Dueck WF, Hassarati RT, et al. Laser patterning of platinum electrodes for safe neurostimulation. Journal of neural engineering. 2014;11:056017.

[14] Park S, Song YJ, Boo H, Han J-H, Chung TD. Arrayed hybrid nanoporous Pt pillars. Electrochemistry Communications. 2009;11:2225-8.

[15] Marrese CA. Preparation of strongly adherent platinum black coatings. Analytical Chemistry. 1987;59:217-8.

[16] Desai SA, Rolston JD, Guo L, Potter SM. Improving Impedance of Implantable Microwire Multi-Electrode Arrays by Ultrasonic Electroplating of Durable Platinum Black. Frontiers in Neuroengineering. 2010;3:5.

[17] Tang R, Pei W, Chen S, Zhao H, Chen Y, Han Y, et al. Fabrication of strongly adherent platinum black coatings on microelectrodes array. Sci China Inf Sci. 2014;57:1-10.

[18] Robinson DA. The electrical properties of metal microelectrodes. Proceedings of the IEEE. 1968;56:1065-71.

[19] Schuettler M, Doerge T, Wien SL, Becker S, Staiger A, Hanauer M, et al. Cytotoxicity of Platinum Black. 10th Annual Conference of the International Functional Electrical Stimulation Society. Montreal, Canada: IFESS_2005; 2005.

[20] Zhou DM. Platinum electrode and method for manufacturing the same. Google Patents; 2005.

[21] Cui X, Lee VA, Raphael Y, Wiler JA, Hetke JF, Anderson DJ, et al. Surface modification of neural recording electrodes with conducting polymer/biomolecule blends. Journal of Biomedical Materials Research. 2001;56:261-72.

[22] Cogan SF, Plante TD, Ehrlich J. Sputtered iridium oxide films (SIROFs) for low-impedance neural stimulation and recording electrodes. Engineering in Medicine and Biology Society, 2004 IEMBS '04 26th Annual International Conference of the IEEE2004. p. 4153-6.

[23] Thaning EM, Asplund MLM, Nyberg TA, Inganäs OW, von Holst H. Stability of poly(3,4-ethylene dioxythiophene) materials intended for implants. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2010;93B:407-15.

[24] Aregueta-Robles UA, Woolley AJ, Poole-Warren LA, Lovell NH, Green RA. Organic electrode coatings for next-generation neural interfaces. Frontiers in Neuroengineering. 2014;7:15.

[25] Sun S, Jaouen F, Dodelet J-P. Controlled Growth of Pt Nanowires on Carbon Nanospheres and Their Enhanced Performance as Electrocatalysts in PEM Fuel Cells. Advanced Materials. 2008;20:3900-4.

[26] Stieglitz T, Beutel Hr, Schuettler M, Meyer JU. Micromachined, Polyimide-Based Devices for Flexible Neural Interfaces. Biomedical microdevices. 2000;2:283-94.

[27] Boehler C, Asplund M. A detailed insight into drug delivery from PEDOT based on analytical methods: effects and side-effects. Journal of Biomedical Materials Research Part A. 2014:n/a-n/a.

[28] Boretius T, Schuettler M, Stieglitz T. On the stability of poly-ethylenedioxythiopene as coating material for active neural implants. Artificial organs. 2011;35:245-8.

[29] Rose TL, Robblee LS. Electrical stimulation with Pt electrodes. VIII. Electrochemically safe charge injection limits with 0.2 ms pulses (neuronal application). Biomedical Engineering, IEEE Transactions on. 1990;37:1118-20.

[30] Schuettler M. Electrochemical Properties of Platinum Electrodes in Vitro: Comparison of Six Different Surface Qualities. Engineering in Medicine and Biology Society, 2007 IEEE EMBS 2007 29th Annual International Conference of the IEEE2007. p. 186-9.

[31] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983;65:55-63.

[32] Chapman CA, Chen H, Stamou M, Biener J, Biener MM, Lein P, et al. Nanoporous gold as a neural interface coating: effects of topography, surface chemistry, and feature size. ACS applied materials & interfaces. 2015;7.

[33] Tan YH, Terrill SE, Paranjape GS, Stine KJ, Nichols MR. The influence of gold surface texture on microglia morphology and activation. Biomaterials Science. 2014;2:110-20.

[34] Lee EP, Peng Z, Cate DM, Yang H, Campbell CT, Xia Y. Growing Pt Nanowires as a Densely Packed Array on Metal Gauze. Journal of the American Chemical Society. 2007;129:10634-5.