Nanostructured Electrodes for In Vitro Electrical Stimulation Platforms

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Introduction: The significant burden associated with neural disorders requires urgent measures to expedite research and the development of new therapeutics. Electrical stimulation is a promising but still relatively underexplored treatment[1]. We believe that There is a lack of suitable tools for conducting high-throughput studies of neural electrical stimulation, encompassing cell anatomy and electrophysiological aspects[2]. Moreover, it remains unclear which combination of parameters (stimulation regimen) is effective in various applications. This lack of clarity arises from an incomplete understanding of the biophysical mechanisms governing the transfer of electrical signals to the biological environment.

To address these challenges, we have developed a testing platform that combines nanostructured platinum electrodes with a microgrooved-compartmentalized cell culture module. Additionally, we have fabricated and characterized the electrochemical properties of the nanostructured pt electrodes[3]. We studied the influence of nanostructuration on the electrical double layer that dictates the signal attenuation, and charge injection. Finally, we measured the influence of implementing nano-roughness on charge injection capacity of Pt electrodes.

Methodology: Nanocolumnar Pt electrodes (NC) obtained by electron beam evaporation of Pt in glancing angle (tilted) configuration on custom cut glass substrates. The growth rate will be fixed at 0.8 Å/s and the tilt angle at 80°. The morphology of nanostructures will be assessed by means of scanning electron microscopy, Figure 1 (top, left). Electrical impedance spectroscopy (EIS) measured using Metrohm Autolab Potentiostat AUT204.FRA32M, using a dummy set up. We used culture medium (DMEM w/o phenol red w/o serum w/o pyruvate) as electrolytes. A miniature Ag/AgCl electrode were used as the reference electrode in 3-electrode measurements. 10 mV was used as applied voltage, in a frequency range of 1 to 10^5 Hz, and Nyquist and Bode plots were obtained to extract impedance data. Using computational simulation, the corresponding equivalent circuit, was extracted to be used for simulation and calculation of signal attenuation. Cyclic voltammetry (CV) was done using the same setup, applying a voltage range of -0.5 to +0.9 V at a scan rate of 100 mV/s. The current/voltage plots were generated for each sample and charge injection capacity calculated using the area under the cathodic peak associated with the reduction reaction during the cathodic sweep.

The microgrooved-compartmentalized cell culture module was fabricated using standard UV and soft lithography techniques, Figure 2. Initially, metallic masks were prepared. A two-step photolithography process was employed to create a master mold in silicon (Si) for defining microgrooves measuring 500 µm in length, 10 µm in width (step 1) as well as compartments (step 2). In the first step, a 2-inch Si wafer was spin-coated with SU-8 resist in the appropriate formulation to achieve a layer thickness of approximately 15 µm for the grooves. After a soft bake following the manufacturer's instructions, the resist was exposed to UV light using the mask. Subsequent development and hard baking revealed the channel array mold on the Si wafer. Subsequently, a second layer of SU-8 resist with different properties was applied to achieve a thickness of around 100 µm, which was suitable for defining the pads, through the same UV lithography protocol. Those pads used as a guide to place silicone cell culture inserts. The final chamber was created from polydimethylsiloxane (PDMS, Sylgard 184 Dow Corning) by casting and curing the elastomer around silicone cell culture inserts.

Results and Discussions: We demonstrated that Z is significantly smaller at lower frequencies for NC electrodes compared to the Pt thin films (TF), Figure 1 (top, right). The NC effective capacitance (~ 536 µF) is also larger than that of TF (~ 5.5 µF) due to its increased electrochemically active area. We developed a model based on equivalent circuit obtained from the EIS and tested several stimulation protocols, compared the delivered signal distortion in NC and TF. Figure1 (bottom right), demonstrates an example of such simulation; an anodic pulse of 1 ms and 800 mV amplitude applied and the corresponding current delivered by NC and TF was measured. The delivered current signal was significantly attenuated when TF is used due to small double lyre capacitance, compared to NC. Charge storage capacity of Pt increased at least 3 time due to nanostructuration, Figure 1 (bottom, left).

Conclusions: We have developed a platform that combines nanostructured Pt electrodes with a microgrooved-compartmentalized cell culture module, for electrical stimulation of neural cells. We demonstrated that nanostructuration significantly improves electrochemical properties of the bioelectrodes, including charge storage capacity. Moreover, nanostructuration has positive effect on conserving the delivered signal. We also, established a methodology for simulating stimualtion conditions before experimental work. Future work will be focused on culturing neural cells in this platform, to expand their axonal compartment within the grooves, and testing the effect of electrical stimulation on growth and survival of the axons.

Acknowledgments

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References

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Figures

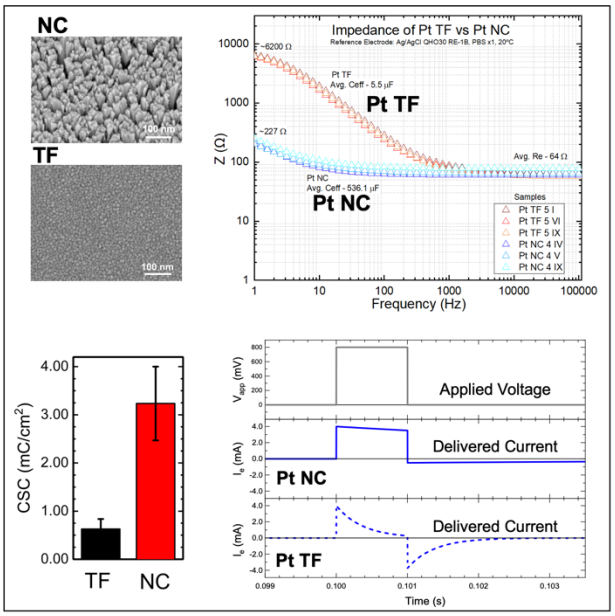

Figure 1. Electrodes and electrochemical characterizations. Top, left: Scanning electron microscopy images of nanocolumnar (NC) and thin film (TF) Pt electrodes. Top, right: Bode plot of Pt TF and Pt NC demonstrating the electrical impedance. Bottom, left: charge injection capacity. Bottom right: Simulation the delivered current by NC and TF in result of a 1 ms pulse at 800 mV applied voltage.

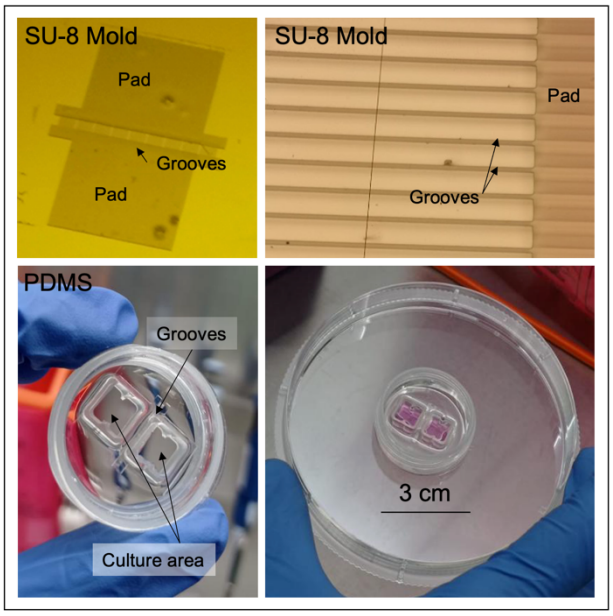

Figure 2. The microgrooved-compartmentalized cell culture module. Top, SU 8 mold. Bottom, PDMS final platform contain culture area and microgrooves.