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Neural Cell Response to Nanostructured Biosensor Surfaces

Zs. Bérces[a,b*], Á. Horváth[a], A. Jády[c], A. Pongrácz[a], E. Madarász[c], Z. Fekete[a]

[a] Research Center for Natural Sciences, HAS, P.O.Box 49, H-1525 Budapest, Hungary
[b] Pázmány Péter Catholic University, P.O. Box 278, H-1083 Budapest, Hungary
[c] Institute of Experimental Medicine, HAS, P.O.Box 67, H-1450, Budapest, Hungary

Abstract

In our work we investigate the interaction of cells and nanotextured surfaces as a model of implanted device surface and living tissue interaction. We developed a maskless nanostructuring method, which can be integrated into our neural biosensor fabrication process. Morphology of the fabricated nanograss was characterised using SEM. The nanorods are 520-800nm in height and their density is 18-70/μm^2. Electrochemical impedance spectroscopy and contact angles of different surfaces were measured. The specific surface area is 30 times larger than the reference. The contact-angle can be tuned. The samples will be tested in viability and adhesion assays using neural cell cultures.

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Keywords: nanostructuring, nanograss fabrication, nanosurface characterisation, biocompatibility, neural implant

1. Introduction

Cells in their natural environment interact with nanoscale structures like the extracellular matrix and its proteins. Based on biomimetic consideration, creating nanopatterned implant surfaces promises better cell adhesion and therefore better biocompatibility [1, 2]. In case of fibroblasts and osteoblasts it's already demonstrated that surfaces with specific surface roughness parameters show better cell adhesion [3]. On the other hand, there are only a few results in the case of neural and glial cells. Later on many groups investigated neural cell adhesion on

* Corresponding author. Tel.: +36-1- 3922614
E-mail address: berces@mfa.kfki.hu

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nanostructured biosurfaces. Some of them used porous silicon [4] or etched Si surfaces with nanometer scale structures [5–6]. In other cases, they used different materials, such as GaP [7] or polymers [8].

By modulating the specific surface area, wetting and nano-pattern regularity of the nanostructured samples, several groups published better neural cell adhesion and viability on nanostructured surfaces compared to the smooth references in the past few years. In 5 days long experiments, rat neural cells were observed to migrate to nanostructured parts of the sample based on the work of Y.W. Fan et al [9]. Glial cells play an emphatic role in the answer to injuries such as an electrode implantation. In 1997, Turner et al. showed lower astrocyte adhesion on nanostructured samples compared to smooth ones using immortalized cell line. In the same article, the authors presented that primer neural and glial cells react the other way around [1]. In conclusion the results in this field are rather controversial and for sufficient conclusions more systematic measurement series would be needed.

Our work aims to synthesize bioimplant surfaces with nanoscale patterns using novel combination of micro- and nanomachining techniques [10]. The proposed maskless nanopatterning method can easily be integrated into the fabrication process of neural microelectrodes. The expected results are envisioned to minimize the immune response of the neural tissue to the surface of the implanted microelectrodes and thus enable efficient functionality in long-term experiments.

2. Materials and Methods

2.1 Sample fabrication

During the fabrication process first, 500 nm thick thermal oxide is grown on a 4” (100) oriented Si wafer. Then 1000 nm poly-Si is deposited in a Tempress LPCVD chamber. The micropatterning of the black-Si is performed by photolithography. Nanopattern formation is carried out by deep reactive ion etching (DRIE) at cryogenic temperature in an Oxford Plasmalab 100 chamber. After black-Si formation platinum sputter deposition is performed and followed by a second patterning step using lift-off process. Fig.1 shows the schematic process flow of the fabrication method.

The advantages of the process are that the nanostructuring is maskless and it can fully be integrated into an implantable Si microelectrode fabrication process.

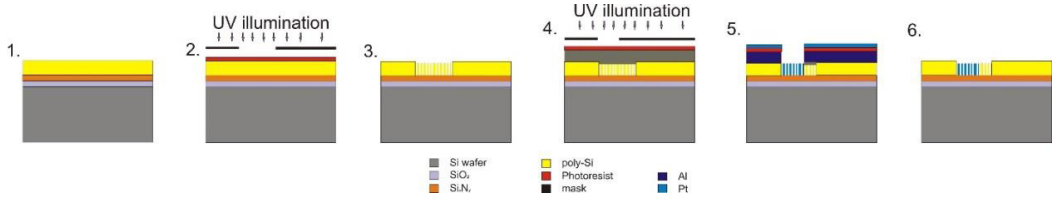

Fig. 1. Schematic process flow of the sample fabrication method

2.2. Characterization of surface morphology and electrochemical measurements

The effect of different etching parameters and platinum layer thickness was investigated by SEM. The pillar height and density parameters of the fabricated samples were also extracted from micrographs.The specific surface area of the nanostructured and platinized samples were recovered from cyclic voltammetry measurements. Electrochemical impedance spectroscopy was carried out to show the impedance reduction, which also refers to surface area enhancement. Since wetting of several surface morphologies apparently influences cell adhesion [11], it was characterized by contact angle measurements. We also examined samples with surface adhesive proteins, usually used for neural cell culturing. This way, misinterpretation of in vitro results due to possible planarization effects is minimized. Cell adhesion protein coating was carried out in the Research Institute for Experimental Medicine, HAS. The proteins were PLL (Poly –L-lysine) and AK-c(RGDfC). PLL is an artificial protein for neural cell culturing. AK-c is a synthetic adhesive polypeptide [12] especially for neural progenitors. To forecast surface

biocompatibility, adhesion and viability of NE-4C cell line will be investigated on our chips with different surface morphology by quantitative colorimetric assays.

3. Results

3.1. Surface morphology

The morphological parameters of the samples (pillar density, pillar height) were derived from scanning electron micrographs. The nanopillars are between 520-800 nm in height, and their density is 18-70/μm^2 depending on the fabrication parameters of the DRIE process. The AK-c(RGDfC) protein is supposed to cover the pillars in a monomolecular layer while PLL covers the surface in a thicker layer. Representative SEM pictures of both coated and uncoated samples can be seen on Fig. 2.

3.2. Electrochemical properties

Impedance yield in case of both uncoated and protein coated samples was also measured. The expected impedance reduction can be seen on the results of the EIS measurements (Fig. 3.A) In the case of uncoated nanostructured samples, the specific surface area derived from CV measurements is 30 times larger. (See fig.3. B) The CV curves on protein coated samples were also measured. While the AK monolayer coating shows no change in impedance, a significant impedance increasing was measured in the case of PLL.

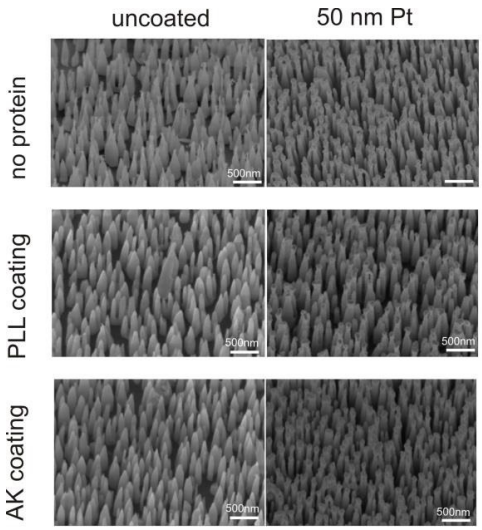

Fig. 2. Representative SEM images of protein coated nanostructures and non-coated reference samples. In the case of PLL coatings pillars became bulkier, in the case of AK only a thin layer can be seen. Scale bar is 500 nm.

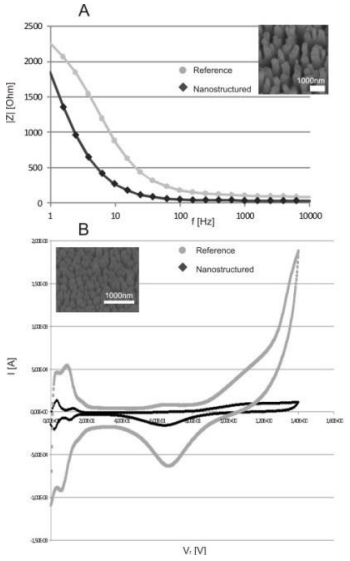

Fig. 3. Representative curves of electrochemical measurements: A) Electrochemical impedance spectroscopy, B) Cyclic voltammetry

3.3. Contact angles

Contact angles are found to be tunable, and it depends on the black silicon surface morphology, the surface material namely whether is coated by platinum, and also on the thickness of the platinum layer because of this properties influence on surface morphology. A representative change in contact angle can be seen on Fig. 4.

However more systematic investigation on the above properties' influence on contact angle is needed and will be investigated in the near future.

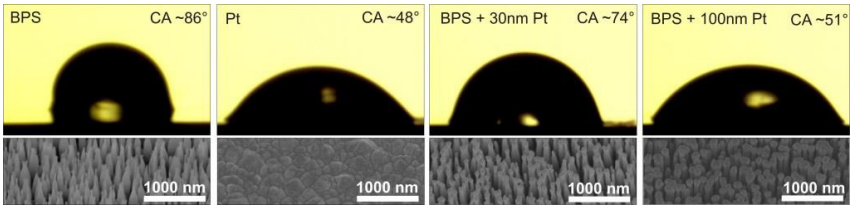

Fig. 4. Effect of surface nanostructuring and metallization of BPS surfaces on static contact angle

4. Conclusion

Based on our preliminary studies, the nanopattern morphology of our chips are tunable. The surface area growth is significant compared to the Pt surface currently used as electrode contact site. The specific surface area growth can be measured by impedance reduction which is an additional advantage. The adhesion proteins do not planarise nanopatterns, however, impedance growth is detected as the samples have been coated. Samples will be investigated with and without these proteins in cellular assays with NE-4C brain stem cell line in MTT and methylene blue staining assays.

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