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Attachment of primary mouse astroglial cells on neural implant surfaces

Zs. Bérces[a,b], B. Csernyus[b], H. Liliom[c], K. Schlett[c,d], D. Pinke[a], P. Lőw[e], Á. Horváth[b], Z. Fekete[b], A. Pongrácz[b,*]

[a]*Faculty of Information Technology, Pázmány Péter Catholic University, 50/A Práter u., H-1083, Budapest, Hungary*
[b]*MTA EK NAP B Research Group for Implantable Microsystems, 29-33 Konkoly-Thege út., H-1121, Budapest, Hungary*
[c]*Department of Physiology and Neurobiology, Eotvos University, 1/C Pázmány P. sétány, H-1117, Budapest, Hungary*
[d]*MTA ELTE NAP B Neuronal Cell Biology Research Group, 1/C Pázmány P. sétány, H-1117, Budapest, Hungary*
[e]*Department of Anatomy, Cell and Developmental Biology, Eotvos University, 1/C Pázmány P. sétány, H-1117, Budapest, Hungary*

Abstract

In vitro micro- and nanofabricated test chips were used to investigate mouse primary cortical astroglial cell reactions to different surfaces of a multichannel neural microelectrode implant. The following surface types were fabricated by MEMS technology and characterized by scanning electron microscopy: poly-Si, Pt, nanostructured Si and nanostructured Pt. Survival of primary cortical mouse astroglial cells was analysed by fluorescent microscopy 24 hours after seeding. Our results show that the nanostructured surfaces are not toxic to the primary mouse astroglial cells.

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Keywords: NeuroMEMS, neural implant, BCI, gliosis, cell-surface interaction, primary astroglial cells

1. Introduction

The development of electrical recording and stimulating implantable neural electrodes as well as the exploration of their clinical effects and usage in different neural dysfunctions (e.g. Parkinson's disease, Tourette syndrome, essential tremor) are rapidly growing scientific areas [1], [2], [3].

* Corresponding author. Tel.: +36-1-3922222 ext:2614; fax: +36-1-3922226.
E-mail address: pongracz@mfa.kfki.hu

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However, physical insertion of a neural microelectrode implant into brain tissue causes local injury and triggers defence reactions of the central nervous system (CNS) [4]. The so-called Foreign Body Response (FBR) alters the physiochemical environment and the function of the surrounding tissue [5]: glial scar formation and neural loss take place [6], which leads to sensing inaccuracy [7], to instability [8] and very often to failure of device functionality [9]. Detailed understanding of FBR and its effect on physiological recordings is still a big challenge, which can modify the appropriate interpretation of in vivo recordings.

It is well known that the micro or nanostructure surfaces of artificial biomedical materials play a key role in the behaviour of the attached cell [10-13]. As nanostructured environment is naturally present in the ECM (extracellular matrix) and on the cell membranes of the neighbouring cells in the CNS, one might assume that nanofabricated implant surface can improve the biocompatibility of the device. The toxicity of nanostructured surfaces, however, is still under debate.

By using micro- and nanofabrication techniques, our aim is to develop implant surfaces with nanometre-range patterns which would delay the negative tissue responses to the implanted electrode, resulting in improved neural implants with long-term efficiency.

Our current work focuses on the toxicity study of poly-Si nanopillars with or without Pt coating. Survival of mouse primary cortical astroglial cells on Si and Pt implant surfaces was studied 24 hours after seeding, by staining the nuclei with a fluorescent dye.

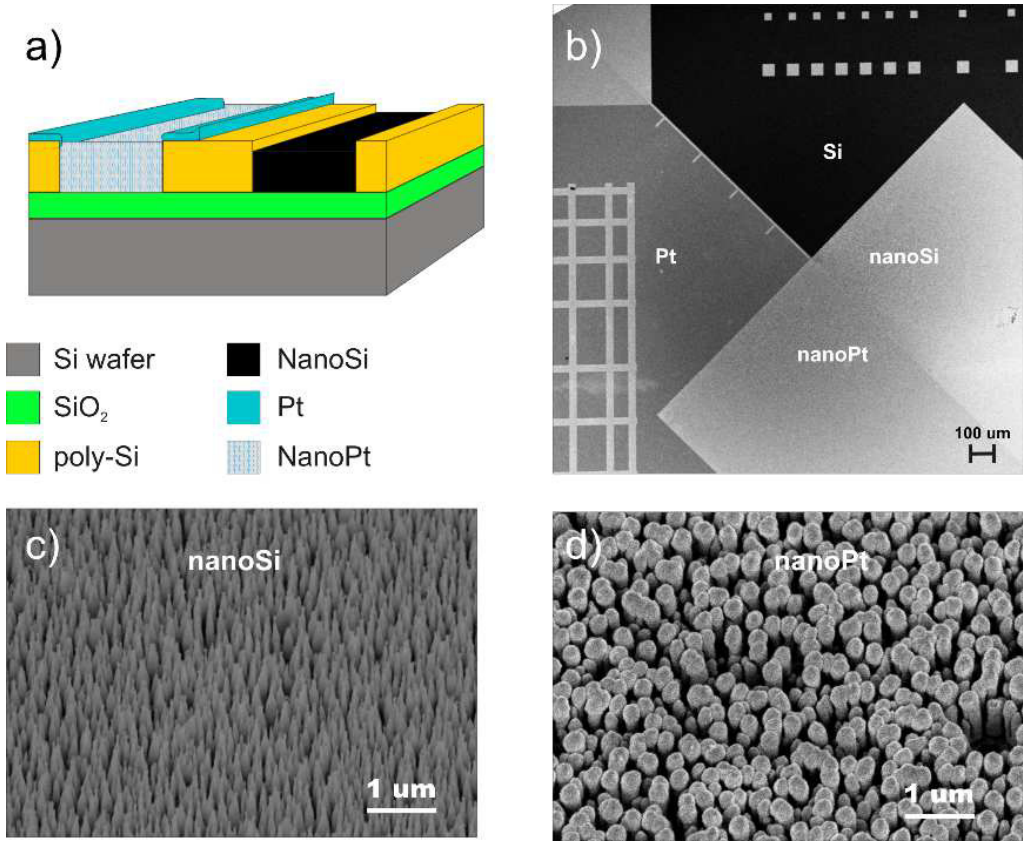

Fig. 1. (a) Schematic cross sectional view of an in vitro test chip (b) SEM image of a test chip with four different surfaces: polycrystalline Si, nanostructured Si, Pt, and nanostructured Pt. (c) 10° tilted SEM image of nanostructured Si (d) 30° tilted SEM image of nanostructured Pt

2. .Experimental

2.1. Nanostructured surfaces

The investigated nanotopographies were made by large-area, maskless, cryogenic plasma etching and subsequent thin film deposition, a technology which can be integrated into the manufacturing process of a multichannel neural microelectrode [14]. We designed and fabricated in vitro test chips containing individual regions of Si, nanostructured Si, Pt, and nanostructured Pt surfaces (Fig. 1). The height of the nanopillars was between 520-800 nm with a density of 18-70 pillars/μm^2, depending on the fabrication parameters of the reactive ion etching process [14]. Fig. 1(b) and (c) show representative SEM images of nanostructured Si and Pt covered surfaces.

2.2. Cell culture and fluorescent microscopy

Microchips were sterilized at 180°C for 4 hours and then placed in a 24-well culture plate without any further surface treatment. $5x10^4$ primary mouse astroglial cells, obtained from neonatal pups and re-seeded twice before plating, were seeded in each well and were kept in high glucose DMEM media supplemented with 10% fetal calf serum at 37°C and 5% CO_2. After 24 hours, cultures were fixed with 4% paraformaldehyde (20 min, RT). Fixated microchips were placed on a microscope slide and were covered with Mowiol containing DAPI (4',6-diamidino-2-phenylindole) to visualize cell nuclei.

Samples were investigated by a Zeiss Axio Observer Z1 inverted fluorescence microscope equipped with a Zeiss Colibri illumination system. Whole-chip images were captured by a 10x objective and an AxioCam MRm camera using the mosaic-type image stitching module of the AxioVision software.

3. Results and discussion

Survival of primary mouse astroglial cells on the nanostructured Si and nanostructured Pt surfaces was compared to cell attachment on flat Si and Pt surfaces. Fig. 2(a) and (b) show the cell nuclei 24 hours after plating on a whole microchip and on an enlarged nano-Pt/flat Pt region. Astrocyte density on each investigated surface was normalized to the adjacent reference flat Si surface of the same chip, next to the nanostructured Si region in order to minimize the nonuniformity of the cell seeding. Normalized cell densities are shown on Fig. 2(c). Nanostructured surfaces had similar cell nuclei density compared to the flat surfaces.

Based on the cell counts, the investigated nanostructured Si and Pt surfaces are not toxic to the primary mouse astroglial cells. Further studies are on their way to characterize the effect of Si and Pt nanopillars on the attachment, spreading and proliferation of astrocytes.

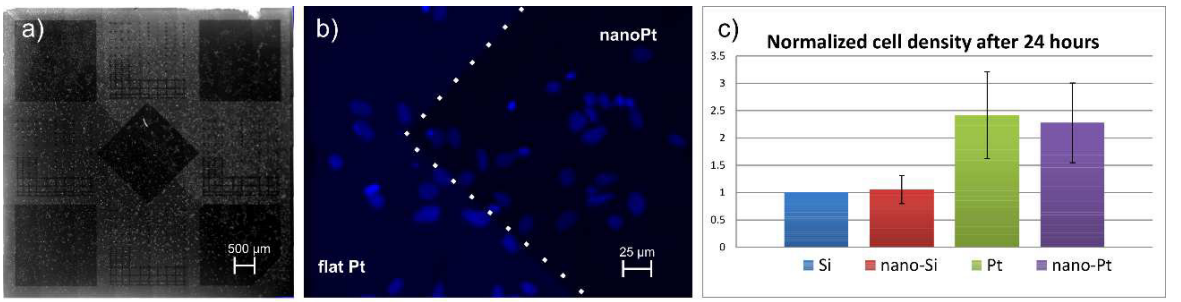

Fig. 2. (a) Overview of primary astroglial cell culture seeded onto a test chip with nanostructured and flat Si surfaces with or without Pt coverage. Nuclei of the cells are visualized by DAPI staining (white signal). (b) Fluorescent image of primary glial cells on nanostructured and flat Pt. Blue: DAPI positive cell nuclei; (c) Normalised cell density on the four investigated surfaces (Si, nanoSi, Pt, nanoPt) 24h after seeding. No cell density difference was observed between the flat and nanostructured surfaces indicating that nanopillars are not toxic to primary mouse astrocytes.

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