Micron 92 (2017) 39–42

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Short communication

How to image cell adhesion on soft polymers?

Silke Seyock, Vanessa Maybeck, Andreas Offenhäusser*

Institute of Complex Systems (ICS-8/PGI-8), Forschungszentrum Jülich, 52428 Jülich, Germany

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 7 November 2016
Accepted 7 November 2016
Available online 9 November 2016

Keywords:
Focused ion beam
Cell interface
PDMS
Elastomers

ABSTRACT

Here, we present a method to investigate cell adhesion on soft, non-conducting polymers that are implant candidate materials. Neuronal cells were grown on two elastomers (polydimethylsiloxane (PDMS) and Ecoflex®) and prepared for electron microscopy. The samples were treated with osmium tetroxide (OsO_4) and uranylacetate (UrAc). Best results can be achieved when the polymers were coated with a thin iridium layer before the cell culture. This was done to emphasize the usage of soft polymers as supports for implant electrodes. A good contrast and the adhesion of the cells on soft polymers could be visualized.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The state of the art to treat neuronal degeneration like Parkinson's disease is deep brain stimulation (DBS). Most electrodes for DBS are made of polyurethane and platinum-iridium electrodes (Anon, 2016a). Polyurethane's Young's modulus is between 10 MPa and 1000 MPa (Anon, 2016b). It is more flexible than metals and not only protects the brain from direct contact with the hard wires connecting the electrodes but also electrically insulates these wires. Though it alleviates symptoms, electrode implantation causes a direct inflammatory response and later a chronic response from the body (Leach et al., 2010). One approach to reduce this response is to use softer support materials that can adapt better to soft tissue (Young's modulus 1 kPa to 180 kPa (Clatz et al., 2005; Schiavone et al., 2009). This also improves close contact between devices and cells. A general trend towards soft implant materials can be observed in the literature, increasing by two orders of magnitude since 1980. This increased use of soft materials raises new challenges in investigating how neurons interact with the substrate. Not only for brain implants but also for other implants, like the cochlear implant, it is useful to investigate softer electrode materials.

Neuronal cell culture is used to validate implant materials. The focus of this study is how the interface between neurons and soft materials (PDMS and Ecoflex®) can be studied. PDMS, for example, has a known biocompatibility and is already used in implant tests (Vaysse et al., 2015). Electron microscopy techniques are used, including focused ion beam (FIB) milling to view how cells adhere to soft substrates. The major problem is that the polymers are mostly non-conductive and difficult to cut and image using a FIB. They exhibit high charging effects and react with the ion beam (Moon et al., 2007a, 2007b). To overcome these problems, we present a staining protocol (as can be seen in Fig. 1a) for the polymers to increase contrast and stability.

2. Methods

2.1. Sample preparation

The silicone elastomer PDMS (Sylgard 184 Dow Corning) was mixed 1:10 (curing agent: base). The two components of the platinum-catalyzed silicones Ecoflex 5® and Ecoflex 0–10® (both Smooth-on) were mixed 1:1. The PDMS samples were cured for 4 h at 60 °C, Ecoflex 5® for 5 min at room temperature, and Ecoflex 0–10® for 4 h at room temperature. After curing all samples were sterilized for 30 min with UV and cut into small blocks.

2.2. Sample coating

Before cell culture the samples were micro contact printed with poly-D-lysine (0.01 mg/ml), extracellular cell matrix (0.0924 mg/ml), and gelatin (0.2 mg/ml) in Gey's balanced salt solution as described in (Albers et al., 2015). Briefly, PDMS stamps

* Corresponding author.
E-mail address: a.offenhauesser@fz-juelich.de (A. Offenhäusser).

http://dx.doi.org/10.1016/j.micron.2016.11.002
0968-4328/© 2016 Elsevier Ltd. All rights reserved.

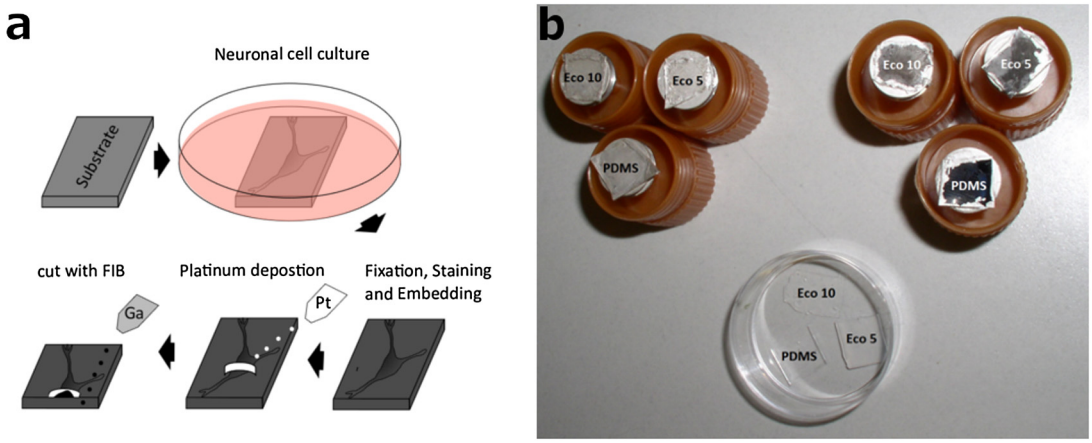

Fig. 1. (a) Schematic of sample production: neuronal cells are cultured on the substrate, then fixed, stained, and embedded. After platinum deposition the sample is cut using a FIB. (b) Overview of PDMS, Ecoflex 5® and Ecoflex 0–10® samples, bottom: samples before treatment, left top: samples after glutaraldehyde treatment, right top: samples with glutaraldehyde treatment, OsO_4 and UrAc staining.

were incubated for 20 min in the solution on ice. Stamps were then dried with nitrogen and placed onto the samples for 20 min.

2.3. Cell culture

Primary cortical neurons from E18 Wistar rats were seeded on the samples at 260 cells per mm^2 in 1 ml of Neurobasal Medium (Life Technologies, supplemented with 1% B27 (Gibco), 0.05 mM L-glutamine, and 50 μg/ml gentamicin). One hour after seeding, the medium was changed. The cells were incubated at 5% CO_2, 100% humidity, at 37 °C for 15 days and fed twice per week by exchanging half the medium.

2.4. Staining

Samples were washed with phosphate-buffered saline (PBS) and fixed for 10 min with 3.2% glutaraldehyde in PBS. After washing with PBS and MilliQ, they were treated with 1% OsO_4 in cacodylate buffer (Morphisto) for 2 h on ice. Samples were washed with MilliQ and left for 30 min in 1% tannic acid (Electron Microcopy Sciences) in MilliQ. Post fixation was done overnight in 2% UrAc (Polyscience) in Bidest. After washing again with MilliQ, the samples were stepped to 100% ethanol as described in (Czeschik et al., 2015). To preserve cells for SEM, they were embedded in epoxy-resin by stepping from pure ethanol to pure resin as described previously (Belu et al., 2016).

2.5. FIB cuts

The samples were glued to a SEM stub using silver glue (Plano GmbH). To protect the cells from the ion and electron beams the samples were sputtered for 30 s at 15 mA with platinum (a 1-1.5 μm thick layer). The samples were cut with 30 kV and 0.79 nA or 9.3 nA, polished with 30 kV and 0.79 nA, and imaged at 3 kV, with a current of 21 pA to 1.4 nA using a backscattered electron detector. An electron beam scan of 10 μm^2 with 3 kV and 5.5 nA was applied to the samples without cells.

All experiments were performed with the approval of the Landesumweltamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany (number 84-02.04.2015.A173). If not stated otherwise, all chemicals were purchased from Sigma Aldrich.

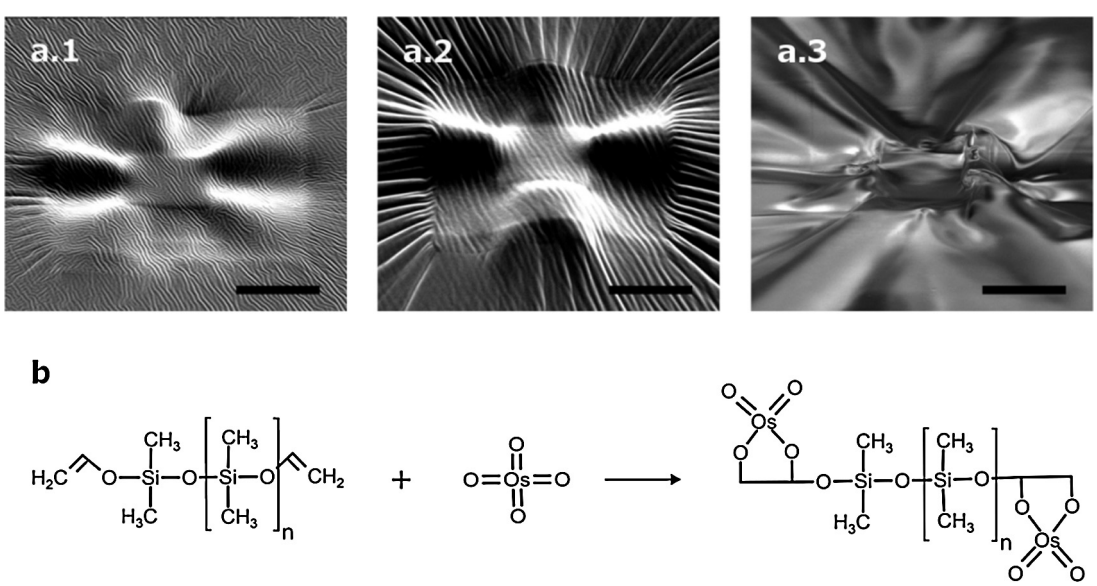

Fig. 2. (a) Reaction of sputtered samples without cells, after electron beam imaging, scale bars 10 μm (a.1) PDMS (a.2) Ecoflex 5® (a.3) Ecoflex 0–10®. (b) Reaction equation of PDMS with OsO_4, OsO_4 binds to the double bonds of PDMS.

Download English Version:

https://daneshyari.com/en/article/5457075

Download Persian Version:

https://daneshyari.com/article/5457075

Daneshyari.com