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Interaction of glia with a compliant, microstructured silicone surface

Ivan R. Minev [a,b], Pouria Moshayedi [b], James W. Fawcett [b], Stéphanie P. Lacour [a,*]

[a] Ecole Polytechnique Fédérale de Lausanne (EPFL), Centre for Neuroprosthetics, STI | IMT/IBI | LSBI, CH-1015 Lausanne, Switzerland
[b] Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB2 0PY, UK

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ABSTRACT

Soft bioengineered surfaces offer a route towards modulating the tissue responses to chronically implanted devices and may enhance their functionality. In this communication we fabricate microtopographically rich and mechanically compliant silicone surfaces for use in soft neural interfaces. We observe the interaction of primary rat microglia and astroglia with arrays of tall and short (4.7 and 0.5 μm) vertically oriented polydimethylsiloxane (PDMS) micropillars and a flat PDMS surface in vitro. With the pillar size and spacing that we use (1.3 μm diameter and 1.6 μm edge to edge), glia are found to engulf and bend tall pillars. The cytoskeleton of cells adhering to the pillar arrays lacks actin stress fibers; instead we observe actin ring formations around individual pillars. Tall, but not short pillar arrays are inhibitory to migration and spreading for both microglia and astrocytes. When compared to a flat PDMS surface and short pillar arrays, tall micropillar arrays cause nearly a 2-fold decrease in proliferation rates for both cell types. The antimitotic properties of tall pillar arrays may be useful for reducing the density of the glial capsule around brain-implanted devices.

1. Introduction

Penetration of a foreign body into the brain, e.g. a neural electrode, leads to neurodegeneration and neuroinflammation [1]. Once the acute inflammatory response declines, a chronic response is observed. Injured neurons die in the vicinity of the implant while glial scar encapsulation forms, limiting efficient neuron–electrode coupling. The inflammatory capsule is mainly composed of activated microglia, astrocytes, extracellular matrix proteins and vasculature.

To improve the chronic integration of neural implants in the brain, various biological, biochemical or electroactive coatings have already been evaluated [2–4]. These are designed to accommodate the differences in biofunctionality between the man-made electrode implant and the surrounding neuronal cells. Only recently, the topography of the implant and the mechanical properties of the implant's materials have been incorporated as valuable parameters for long-term neural implant design [5,6].

To this end, implant materials and architectures are evolving to incorporate bioengineering strategies for promoting tissue regeneration without scarring. For example, flexible and compliant implants promise to reduce shear stress at the implant–tissue interface [7,8]. The assembly of electrodes in situ by polymerization of conductive polymers in between living neurons may reduce initial insertion trauma and bring neurons closer to electrodes [9]. The introduction of soft gel coatings may bridge the brain–electrode mechanical mismatch and facilitate controlled release of molecules promoting neuronal regeneration [10]. Implants with surfaces mimicking the extracellular matrix microtopography may help to organize tissue morphology at the implant surface and manage the glial response [11–13]. Dense nanowire or conducting nanotube arrays can improve the current injection capability of flexible electrodes and mechanically stabilize the interface [14,15].

We are pursuing the development of mechanically compliant neural electrode implants using elastomeric rubber materials [16,17] and microstructuring of the implant surfaces. Dense arrays of vertically oriented elastomer micropillars are engineered at the implant surface giving the "illusion" of a mechanically softer surface despite a bulk material orders of magnitude stiffer than the neuronal cells [18].

As a first step towards the development of such soft neural electrodes, this paper evaluates in vitro the short-term response of primary glia to arrays of "bendy" silicone micropillars (diameter 1.3 μm, height 4.7 μm). We compare these to cells cultured on a surface containing only raised bumps of the same size and spacing (diameter 1.5 μm, height 0.5 μm) and a flat silicone surface. We concentrate on glia since they vastly outnumber neurons in the brain and are the main elements of the brain tissue reaction to an implanted foreign body [19]. Microglia originate from blood monocytes and are the resident macrophages of the brain; following injury, they migrate to the injury site within hours [5,20,21]. Concomitantly, microglia experience a phenotype transformation

* Corresponding author. Tel.: +41 21 69 31181.
E-mail address: stephanie.lacour@epfl.ch (S.P. Lacour).

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from their quiescent state to amoeboid microglia (more compact with no mesh of thin processes), capable of phagocytosis, undergoing increased cell proliferation, migration and hypertrophy [22]. The initial microglial reaction is followed by activation of astrocytes. This leads to the formation of a scar where newly formed and hypertrophic astrocytes overlap (intermingle), forming gap junctions to create a tight meshwork of hyperfilamentous processes that may be mechanically prohibitive for axon regeneration [23].

Here, we show that the glial cells are sensitive to substrate topography and stiffness in vitro.

2. Materials and methods

2.1. Pillar fabrication

Tall and short pillars were initially made on chips of silicon wafer coated with SU-8 photoresist (2000 series, Microchem Corp.) patterned using standard UV photolithography. The SU-8 microstrutures were used to make a negative polydimethylsiloxane (PDMS) mold containing holes. A droplet of uncured PDMS was then sandwiched between the floor of a culture well (35 mm glass-bottom culture wells from MatTek Corp.) and the mold containing the holes. By applying gentle pressure, the uncured PDMS mixture was forced to fill the holes. A flat stamp was used to fabricate a featureless control PDMS surface. Dishes were then cured for 48 h at 70 °C and the molds carefully peeled off from the glass bottom. Following mold release, tall pillars were found to collapse on each other into bunches. To resuspend them in their ordered vertical positions, dishes were filled with ethanol and sonicated. Without allowing the pillar arrays to dry, the dishes were sterilized with 70% ethanol and washed with sterile deionized water. The substrates were then treated with aqueous solution of poly-D-lysine (150–300 kDa, 20 μg ml^{-1}, 5 ml per well), followed by incubation with a red tracer dye for pillar visualization (Vybrant DiI, Molecular Probes, 5 μl ml^{-1} for 1 h), thoroughly washed and incubated with 5 ml of cell culture medium (Dulbecco's Modified Eagle's Medium, supplemented with 10% fetal calf serum and 1% penicillin–streptomycin). Hereinafter, we refer to the latter as standard medium.

2.2. Cell cultures

All animal experiments were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986, institutional and project license guidelines. Microglia and astrocyte cultures were prepared according to established protocols [24]. Briefly, cortices from (P0–P2) Sprague–Dawley rats were explanted, minced, triturated and cultured in poly-D-lysine-coated flasks filled with standard medium. Mixed glial cultures prepared in this way were allowed to reach confluence before cells were harvested for experiments. In order to separate microglia from astrocytes, flasks containing mixed cultures were shaken for 40 min inside a shaker incubator performing a circular motion. The supernatant from each flask, containing dislodged microglia and OPCs was transferred to an uncoated bacteriological Petri dish and incubated for 1 h. This allowed microglia to attach to the bottom surface of the petri dish, leaving non-adherent OPCs and debris in suspension. Adherent microglia were dislodged by incubating with 5 ml 0.1% trypsin–EDTA (Gibco) for 5–10 min. To inhibit trypsin, 10 ml of standard medium was added, and the mixture centrifuged for 5 min at 2000 rpm. The cell pellet was then resuspended in 1 ml of standard medium in preparation for plating. To obtain pure astrocyte cultures, flasks containing mixed cortical culture were shaken for at least 12 h. Flasks were washed three times with PBS, which together with the shaking resulted in a smooth astrocyte monolayer. Astrocytes were trypsinized inside the flask, centrifuged and resuspended in 1 ml of standard medium in preparation for plating.

Plating was performed by taking an aliquot of cell suspension and placing it in culture wells that were preincubated with 5 ml of standard medium and contained the PDMS test substrates at their bottoms. The final cell density in the wells was 0.2–0.4×10^6 cells per well (200–400 cells mm^{-2}) for both monocultures.

Time-lapse movies using phase-contrast microscopy were obtained for live cells 24 h after plating. Cells were incubated with an antibody that labels focal adhesion complexes (HMβ1-1, Bio Legend) and the interactions of live cells with the pillar matrix were imaged with a spinning disk confocal microscope (Olympus IX 70 using a 100× oil immersion objective). The actin cytoskeleton of fixed cells was visualized with phalloidin (conjugated with Alexa Fluor 488, Molecular Probes), and cell nuclei with Hoechst 33342 (Sigma–Aldrich). Dividing nuclei were labeled with a mitotic marker (Ki67, Vector Laboratories) using Alexa Fluor 488 from Molecular Probes as a secondary antibody.

3. Results

3.1. Force exerted by glial cells on PDMS micropillars

The PDMS pillars and flat control surface produced in this study are illustrated in Fig. 1a–c. Tall pillars are 4.71 ± 0.09 μm tall, 1.28 ± 0.03 μm in diameter and have 1.60 ± 0.09 μm edge-to-edge spacing. Short pillars are 0.50 ± 0.04 μm tall, 1.50 ± 0.03 μm in diameter and have 1.41 ± 0.06 μm edge-to-edge spacing.

We first observed that live glia interact with the matrix by deflecting tall pillars as illustrated in Fig. 1d; (for microglia see Supplementary Fig. 1). Cells have been incubated with an antibody to highlight focal adhesion complexes, hence delimiting the cell boundaries; the pillars are visualized with a dye absorbed on the PDMS surface. The image in Fig. 1e is a confocal slice coincident with the tops of pillars. It illustrates that the largest deflections occurred at the cell periphery where increased concentration of focal adhesions was also observed. It further illustrates that forces are directed towards the center of the cell.

In order to obtain an estimate of the forces that cells exert on individual pillars, we observe the deflection of the pillar top and use the theory of bending beams for distributed loads. We assume that cells penetrate to the base of the pillars and that the actin sheet exerts a constant force density (force per unit length) along the pillar, and that forces are normal to the long axis of the pillar. The total force F on a pillar is therefore given by:

$$F = \frac{3\pi E r^4}{l^3}\Delta x \tag{1}$$

where E is the elasticity modulus of PDMS (we use a value of 1 MPa), r is the radius of a pillar, l is the pillar length, and Δx is the deflection of the pillar top [25].

The highest force observed on an individual pillar is 13 ± 3 nN for microglia and 24 ± 3 nN for astrocytes (pillar touching its closest neighbor). Most pillars, however, experience significantly smaller forces with more than half of the pillars being either undeflected, or experiencing forces below the detection limit of 3 nN (Fig. 1f).

3.2. Glial cell mobility on PDMS micropillars

The actin cortex of cells adhering to the three test surfaces is markedly different. The actin cortex of microglia attached to the