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The relationship between glial cell mechanosensitivity and foreign body reactions in the central nervous system☆

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ABSTRACT

Devices implanted into the body become encapsulated due to a foreign body reaction. In the central nervous system (CNS), this can lead to loss of functionality in electrodes used to treat disorders. Around CNS implants, glial cells are activated, undergo gliosis and ultimately encapsulate the electrodes. The primary cause of this reaction is unknown. Here we show that the mechanical mismatch between nervous tissue and electrodes activates glial cells. Both primary rat microglial cells and astrocytes responded to increasing the contact stiffness from physiological values ($G' \sim 100$ Pa) to shear moduli $G' \geq 10$ kPa by changes in morphology and upregulation of inflammatory genes and proteins. Upon implantation of composite foreign bodies into rat brains, foreign body reactions were significantly enhanced around their stiff portions *in vivo*. Our results indicate that CNS glial cells respond to mechanical cues, and suggest that adapting the surface stiffness of neural implants to that of nervous tissue could minimize adverse reactions and improve biocompatibility.

1. Introduction

Implantation of medical devices leads to a local foreign body reaction (FBR), which can cause local and systemic problems. In this process, implants are encapsulated by reactive tissue, which in the central nervous system (CNS) consists mainly of activated glial cells – microglia and astrocytes – surrounded by extracellular matrix. Glial cells make up about half of the cell population in the brain. Microglial cells are the resident immune cells of the CNS and the first line of defense, whereas astrocytes, which assume a multitude of functions, are the most abundant glial cells in the brain. The reactive process, which starts with the activation of glial cells, can damage local neurons, and the subsequent dendritic retraction and neuronal death may contribute to a gradual decline in the function of implanted electrodes [1–3].

Glial cell activation is characterized by hypertrophy (increase in volume and processes), proliferation (increase in cell number), and inflammatory reactions [4,5]. After subsidence of an acute injury response, implanted foreign bodies become chronically surrounded by activated microglial cells [6], which release proinflammatory and immunoregulatory substances [7], followed by a layer of reactive astrocytes [8] with an increased production of intermediate filaments (particularly glial fibrillary acidic protein, GFAP) [4,5]. The resulting glial scar may have a toxic effect on local neurons and acts as a physical barrier around the implant [6], thus insulating it from the remaining neurons and, in case of an electrode, detrimentally increasing its impedance [9].

The universal occurrence of the FBR is not well correlated with the chemical nature of the implant [10], which is generally selected to be inert. This poses the question about the trigger of an FBR. Importantly, CNS cells not only respond to chemical but also to mechanical signals (i.e., they are mechanosensitive). For example, most neuronal and glial cell types adapt their morphology and cytoskeletal composition to the stiffness of their surrounding *in vitro* [11–17].

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CNS tissue belongs to the softest tissues in nature [17], and neural implants are usually orders of magnitude stiffer than the physiological cell environment. Hence, it seemed possible that local cells respond to a mismatch of mechanical compliance between nervous tissue and the implant. To pursue this idea, we exposed microglia and astrocytes, the main contributors to FBRs in the CNS, to materials of different stiffness but same chemical properties and tested their morphological and inflammatory responses to these mechanical signals *in vitro* and *in vivo*.

2. Materials and methods

2.1. Cell cultures

Compliant culture substrates were fabricated from polyacrylamide as described previously [13] and functionalized with poly-D-lysine solution (PDL; Sigma) (see Supporting information for details and Fig. S5 for control measurements of rheological properties and PDL coating). All animal experiments were conducted in accordance with the United Kingdom Animals (Scientific Procedure) Act of 1986 and institutional guidelines. Cell cultures were prepared from neonatal Sprague–Dawley rat cerebral cortices as previously described [18]. 50,000 astrocytes or 100,000 microglia suspended in 300 μl culture medium were added onto each gel. All *in vitro* experiments were done after one day in culture.

2.2. Quantitative morphometry of glial cells

Phase contrast images of cells (72–344 cells per gel in 5–8 fields of view, 3 cultures per cell type) were taken. According to changes in morphology, four morphological categories were defined for microglia and five for astrocytes and arbitrary scores of 1 (for the round cell shape) to 4 or 5 (for the most spread cell shape) were assigned to each category [13] (Fig. 1).

2.3. Immunocytochemistry

Cells were fixed in 4% paraformaldehyde (PFA) for 10 min and washed in PBS three times for 10 min. Cells were then treated with 10% normal goat serum (Sigma) in PBS containing 0.1% Triton-X100 (Sigma; PBS-TX100) for 1 h. For IL-1β, the blocking step was performed with normal donkey serum (Stratech). Primary antibodies (or cocktails of primary antibodies; for details see Supporting information) were prepared in proper dilutions in PBS-TX100 and added to the cells. Cells were incubated for 2 h at room temperature, or alternatively overnight at 4 °C, washed, and incubated with appropriate secondary antibodies. After 1 h incubation at room temperature, cells were washed and mounted with FluorSave mounting reagent.

Image stacks were obtained with a confocal laser scanning microscope (Leica SP2 or Zeiss 510Meta). For Figs. 2 and 3, maximum projections were generated using ImageJ (NIH). In Fig. 2, gray levels were adjusted with Adobe Photoshop, settings for the same channels were kept constant for all samples.

2.4. Genome-wide profile of gene expression

Total RNA was extracted using RNeasy MinElute Cleanup Kit (QIAGEN) following the manufacturer's protocol. RNA quality and quantity was assessed using an RNA Pico Chip kit (RNA 6000 Pico Chip Kit, Agilent Technologies); genomic DNA was removed using the Ambion® TURBO DNA-*free*™ DNase Treatment (Ambion) kit. RNase activity was prohibited by implementing 20 Units of SUPERase-In (Ambion), and RNA quality and quantity evaluated again. Equal RNA quantities were processed with WT-Ovation™ Pico RNA Amplification System (NuGEN).

Sense-Target cDNA was generated, fragmented, biotinylated and hybridized onto Affymetrix Rat Gene 1.0 ST microarrays. The hybridized probe arrays were stained with streptavidin phycoerythrin conjugate and scanned using an Affymetrix GeneChip 7G scanner. Raw image data were converted to CEL using Affymetrix GeneChip Operating Software (GCOS). All downstream analysis of microarray data was performed using GeneSpring GX 11 (Agilent). CEL files were used for both the robust multiarray average (RMA) [19] and Probe Logarithmic Intensity Error estimation (PLIER) analyses [20], and expression values on the chip were normalized to the chip's 50th percentile. Each independent experiment included three arrays from three biological replicates. Statistical analysis was performed using a one-sample Student's *t*-test, which was applied to the mean of each normalized value against the baseline value of 1. Genes regulated differently by more than 1.3-fold from the control condition with a $P < 0.05$ were considered significant. Only genes which met the above criteria using RMA and PLIER were examined and shown in Tables S1, S2, S8 and S9.

Functional analyses were performed (Tables S3, S4, S10 and S11) using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems Inc., USA; www.ingenuity.com) to identify the biological functions and diseases that were most significant to the data set (Tables S5, S6, S12 and S13). The significance of the association between the data set and the pathway was measured by two means: (1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway is displayed and (2) Fisher's exact test was used to calculate a *P* value to determine the probability that the association between the genes in the data set and the pathway is due to chance

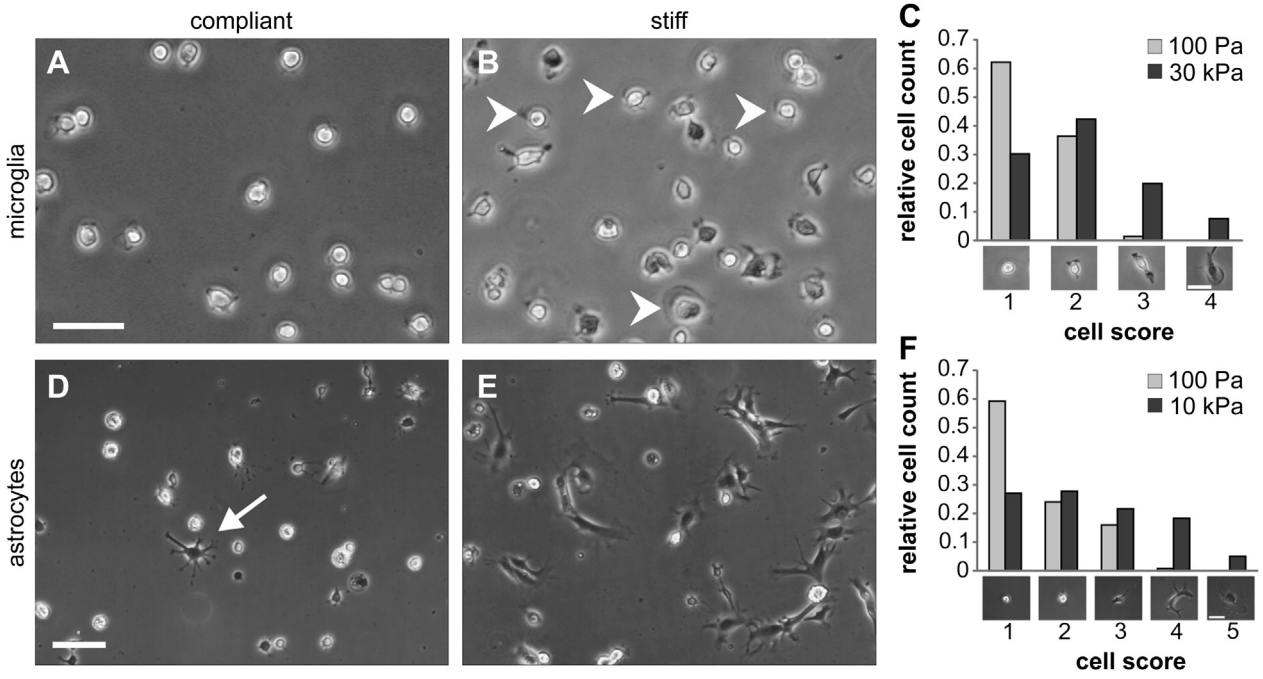

Fig. 1. Glial cell morphology depends on substrate stiffness. Both primary microglial cells (A–C) and astrocytes (D–F) change their morphology in response to the stiffness of the substrate. (A, D) On compliant substrates mimicking brain tissue elasticity ($G' = 100$ Pa) both cell types showed spherical morphologies. Moreover, astrocytes occasionally showed star-like morphologies, resembling their *in vivo* shape (arrow in D). (B, E) On stiffer substrates ($G' = 30$ kPa and 10 kPa for microglia and astrocytes, respectively), glial cells spread significantly more and extended several processes. An activated phenotype was frequently observed on stiff gels (arrowheads in B). Scale bars: 50 μm. (C, F) A quantitative cell shape analysis (score according to cell morphology [13]) confirmed that morphological changes in microglia and astrocytes cultured on stiff substrates were significant ($P_{microglia}$ = 1E-30 ($N_{stiff} = 447$; $N_{soft} = 418$); $P_{astrocytes}$ = 2E-39 ($N_{stiff} = 550$; $N_{soft} = 625$); Mann–Whitney test). Scale bars: 30 μm.