Journal of Neuroscience Methods 217 (2013) 17–25

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

Examining the inflammatory response to nanopatterned polydimethylsiloxane using organotypic brain slice methods

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HIGHLIGHTS

- Nanopatterning of PDMS is used as a method for reducing the inflammatory response of glial cells.
- Unique feature of this study is using *in vitro* brain slice cultures to depict the native response.
- Cells cultured with the nanopatterned PDMS pins had a decrease of GFAP, TNFα and IL-1β gene expression.
- By day 7, all 4 markers tested were lower with nanopatterned pins compared to non-patterned pins.
- Results suggest nanopatterning influences cell morphology and some of the molecular signals.

ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form 23 April 2013
Accepted 25 April 2013

Keywords:
Polydimethylsiloxane (PDMS)
Organotypic brain slices
Neural electrode
Nanopatterning
Reactive astrocytes

ABSTRACT

A long-term effect of chronically implanted neural electrodes is the formation of a glial scar made up of reactive astrocytes, microglia and the matrix proteins they generate. Studies have shown glial fibrillary acidic protein (GFAP) and cytokines interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNFα), and transforming growth factor beta 1 (TGFβ1) are involved with the initial and modulation phases of reactive astrogliosis. In the present study, nanopatterning of polydimethylsiloxane (PDMS) was attempted as a method for reducing the inflammatory response of glial cells. A unique feature of this study is the use of *in vitro* brain slice cultures (organotypic cultures) in order to more accurately depict the native response. The aim of the study was to determine whether nanotopography could reduce inflammatory signals typically resultant from neural electrode implantation. Specifically, observation of cell alignment and surveillance of GFAP, IL-1β, TNFα, and TGFβ1 gene expression around the PDMS pins was performed. Results of this study confirm nanopatterning not only influences cell morphology, but some of the molecular signals as well. These results collectively indicate nanopatterning improves the biocompatibility of PDMS by reducing inflammatory markers such as GFAP, IL-1β, TGFβ1 and TNFα compared to the non-patterned PDMS pins.

1. Introduction

A long-term effect of chronically implanted neural electrodes is the formation of a glial scar made up of reactive astrocytes, microglia and the matrix proteins they generate (Polikov et al., 2005; Seil and Webster, 2008). The initial response to implanted electrodes is an acute neuroinflammation mediated by microglia and subsequent astrocyte reactivity, which leads to astrogliosis (Rao et al., 2012a; Sofroniew and Vinters, 2010). Studies have shown that a variety of cytokines are involved with the initial and modulation phases of reactive astrogliosis (Farina et al., 2007; John et al., 2003; Liberto et al., 2004; Rao et al., 2012a). These cytokines include interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNFα), and transforming growth factor beta 1 (TGFβ1). Astrocytes express receptors to these signaling molecules and their binding results in an astrocytic reactive response (Farina et al., 2007; John et al., 2003; Liberto et al., 2004; Rao et al., 2012a). In addition to receiving these signals, astrocytes also generate and release cytokines such as IL-1β and TNFα which promote neurotoxicity whereas TGFβ1 acts as a neuroprotective (Farina et al., 2007; John et al., 2003; Liberto et al., 2004; Rao et al., 2012a). Another known marker for activated astrocytes is an upregulation of intermediate

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0165-0270/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2013.04.023

filament, glial fibrillary acidic protein (GFAP) (Gervasi et al., 2008; Polikov et al., 2005, 2006; Szarowski et al., 2003; Turner et al., 1999). IL-1β, TNFα, and TGFβ1 can be expressed by both microglia and astrocytes (Farina et al., 2007; John et al., 2003; Liberto et al., 2004; Rao et al., 2012a). The acute neuroinflammatory response is typically short-lived and unlikely to be harmful to neuronal survival (Glass et al., 2010; Rao et al., 2012a; Yoles et al., 2001). The acute response can be resolved by a negative feedback mechanism resulting in reduced levels of IL-1β and TNFα and increased levels of TGFβ1 (Liberto et al., 2004; Moolwaney and Igwe, 2005; Rao et al., 2012a). Acute neuroinflammation is considered beneficial to the central nervous system (CNS), because it prevents further tissue destruction and contributes to repairing the damaged tissue (Liberto et al., 2004; Rao et al., 2012a). Although the acute response is considered favorable, if not resolved, it will transform into a chronic response thus rendering in the formation of a glial scar around the implanted electrode. Therefore, the chronic presence of the electrode results in glial scarring around the implantation site which consequently impedes the electrode signal. One of the goals of neural electrode design is to develop novel strategies which minimize this acute neuroinflammation.

Current scientific strategies to inhibit the initiation of glial scarring around a chronic device range from altering the geometry, roughness, size, shape and materials of the device (Grill et al., 2009; Kotov et al., 2009; Kotzar et al., 2002; Szarowski et al., 2003). In order to properly design a successful neural electrode, biomedical engineers strive to understand the cellular environment. *In vivo* conditions comprise of cells living in the extracellular matrix (ECM) meshwork with a three-dimensional and high aspect ratio topographical textures on a micron and nano scale (Kriparamanan et al., 2006; Millet et al., 2010; Wu et al., 2006). This 3-D environment provides cells the topographical cues required for them to differentiate and perform their specific functions. Studies have shown that surfaces which mimic the nanotopography of the natural environment *in vivo* result in an improved biocompatible response (Curtis et al., 2004; Ding et al., 2010; Ereifej et al., 2012; Kotov et al., 2009; Millet et al., 2010; Zervantonakis et al., 2011). Nanotopography has been demonstrated to affect the cells' morphology, alignment, adhesion, proliferation, gene expression profiles and even prevent biofouling and contamination on the material's surface (Curtis, 2005; Curtis et al., 2004; Das et al., 2008; Fok-Seang et al., 1998; Kriparamanan et al., 2006). Topography affects gene expression of cytokines, growth factors, signaling molecules, and cytoskeleton-linked molecules (Kriparamanan et al., 2006). Studies utilizing nanotopography with neural electrode materials have primarily focused on surface alterations of the active sites on the conducting material (Brunetti et al., 2010; Gobbels et al., 2010; Negi et al., 2010). Negi et al. studied how increasing the surface area available for neuronal contact would improve the spatial resolution and selectivity (Negi et al., 2010). The area of electrode insulating material that is exposed to tissue is much greater compared to the area of conducting material, therefore, the material properties of the electrode insulating material may have more of an effect on cellular response (Rao et al., 2012b). Currently, there are very few studies examining the effect nanotopography have on glial cells, specifically on the reduction of the glial cells inflammatory response (Kotov et al., 2009; Pennisi et al., 2009, 2011).

In the present study, nanopatterning of polydimethylsiloxane (PDMS) was attempted as a method for reducing the inflammatory response of glial cells. Previous studies have investigated the biocompatibility of neural electrode biomaterials (Ereifej et al., 2011, 2012). Poly(methyl methacrylate) (PMMA) was shown to be biocompatible with astrocytes and could efficiently be nanopatterned, however, its lack of flexibility is not optimal for a rolling methodology. PDMS elastomer is a standard material that is commonly used when investigating topographical surface modifications due to its optimal physical properties. PDMS is an inexpensive, hydrophobic polymer with fairly low surface energy and thermal stability (Berdichevsky et al., 2010; Choi et al., 2009; Makamba et al., 2003; Millet et al., 2007; Zervantonakis et al., 2011). PDMS provides easy release from the patterned substrate mold during processing which gives it the advantage of rapid prototyping capabilities and the ability to fabricate complex patterns down to the nanometer scale (Choi et al., 2009; Makamba et al., 2003; Millet et al., 2007; Zervantonakis et al., 2011). Moreover, devices fabricated with PDMS have been useful for biological studies due to their ease of fabrication, high gas permeability, optical transparency, and low water permeability of the structures formed from this material (David and Duffy, 1999; Makamba et al., 2003; Millet et al., 2007; Samuel and Sia, 2003; Merkel et al., 2000).

PDMS has been widely used in biomedical applications since it is non-toxic and biocompatible with the surrounding tissue (Chou et al., 2012; Guo and Deweerth, 2009; Lacour et al., 2010; Ochoa et al., 2013; Rao et al., 2012b). Moreover, PDMS has elasticity similar to *in vivo* tissue thus rendering it an optimal biomaterial choice for neural electrode devices (Lacour et al., 2010; Lotters et al., 1997; Rao et al., 2012b). Recently, PDMS devices have been incorporated as structural components for patterning neuronal growth in culture and studying the neural circuit physiology (Berdichevsky et al., 2009, 2010; Hanson et al., 2009; Millet et al., 2007). Compared to other polymers used in micro-electro-mechanical systems (MEMS) fabricated neural electrodes, such as polyimide, parylene and PMMA, PDMS is better suited due to its elasticity, mechanical strength, ease of fabrication, and biocompatible compliance with tissue and cells (Chou et al., 2012; Guo and Deweerth, 2009; Lacour et al., 2010; Ochoa et al., 2013; Rao et al., 2012b).

A unique feature of this study is the use of *in vitro* brain slice cultures (organotypic cultures) in order to more accurately depict the native response. Organotypic cultures are used due to their ability to retain circuit architecture, connectivity and physiology specific to their *in vivo* origin (Berdichevsky et al., 2009; Davids et al., 2002; Morin et al., 2006). Therefore, this culture system has many advantages over the traditional monoculture *in vitro* biocompatibility testing methods (Berdichevsky et al., 2009; Morin et al., 2006). The organotypic culture resembles the *in vivo* model better than dissociated monocultures (Cavaliere and Matute, 2011). Organotypic cultures are able to preserve neuron–glia interactions, maintain tight contact between individual cells and extracellular matrix molecules, support tissue-specific transport and ion diffusion systems (Cavaliere and Matute, 2011).

The aim of the present study was to determine whether nanotopography can reduce inflammatory signals typically resultant from neural electrode implantation. The uniqueness of this *in vitro* model lies with the assumption that the slicing of the organotypic brain tissue elicits a similar inflammatory cascade found after neural electrode insertion. Previous data was utilized in determining the nanopattern feature dimensions for this study. The 3600 patterned PMMA surface (277 nm width and 200 nm depth) was determined to elicit less of an inflammatory response as compared to the other patterned and non-patterned surfaces (Ereifej et al., 2012). The surface instigated cell alignment along the nanopattern, less protein adsorption, less cell adhesion, proliferation and viability, inhibition of GFAP and MAP2k1 compared to all other substrates tested (Ereifej et al., 2012). PDMS was spin coated on nanopatterned substrates, peeled and rolled into cylindrical pins in order to comply with the 3D geometry of neural electrodes. Thereafter, PDMS pins were placed in organotypic cultures for glial cell examination. Specifically, observation of cell alignment and surveillance of GFAP, IL-1β, TNFα, and TGFβ1 gene expression around the PDMS