Characterization of dorsal root ganglion neurons cultured on silicon micro-pillar substrates for high-resolution electrophysiological recordings

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Introduction

The dorsal root ganglion (DRG) contains pseudounipolar neurons that convey sensory information from the periphery to the central nervous system. Therefore, an understanding of electrophysiology these neurons is crucial and allow researchers to study communication between neurons. The development of measurement techniques for the study of electrophysiological properties of cells enable single-cell recording. With advanced micro-electrode arrays (MEAs) based on integrated complementary metal oxide semiconductor, any neuron grown over custom arrays can be recorded at high spatio-temporal resolution.
Our study focus on characterization of DRG neurons cultured on various types of silicon micro-pillar substrates (MPS), as directly related to the design of MEAs. We expect that substrate topography support normal DRG neuron growth and enhance neurite alignment. At the same time we expect a greater presence of neurons in certain areas of MEAs, which could be useful for subsequent electrophysiological studies.

Objectives

To investigate the influence of micro-pillar substrate architecture on distribution and morphology of main types of DRG neurons and to compare DRG neuron growth patterns between MEAs and glass surfaces

Methods

DRG neurons harvesting – from adult male Sprague Dawley rats

Culture of DRG neurons – dissociation in trypsin/liberase solution, plating on MPS and glass coverslips and cultured for 1, 3 and 7 day *in vitro* (DIV)

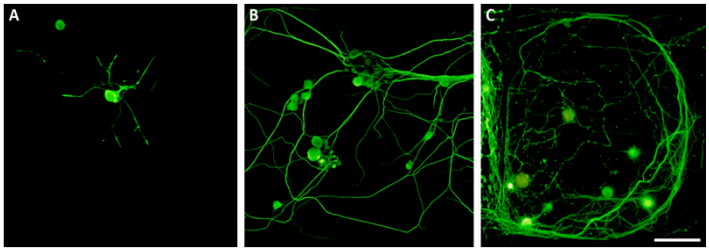
Immunocitochemistry and cell imaging – using markers for different DRG neuronal subtypes: NeuN, N52, IB4, CGRP

Counting DRG neurons on MPS and coverslips – using flourescent microscope

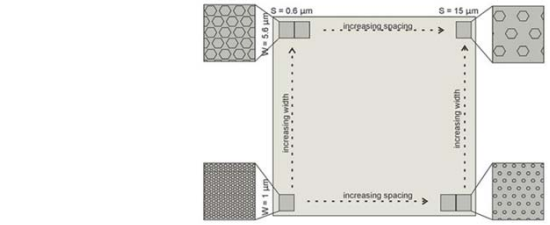
Morphometric analysis of DRG neurons – using ImageJ software (NIH)

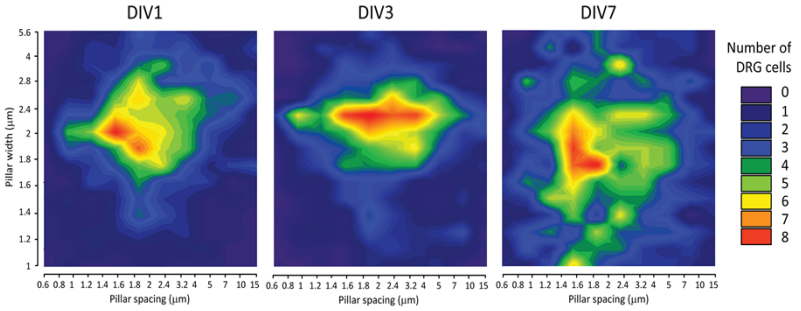
Results

Neuronal growth on MPS after DIV1, DIV3 and DIV7

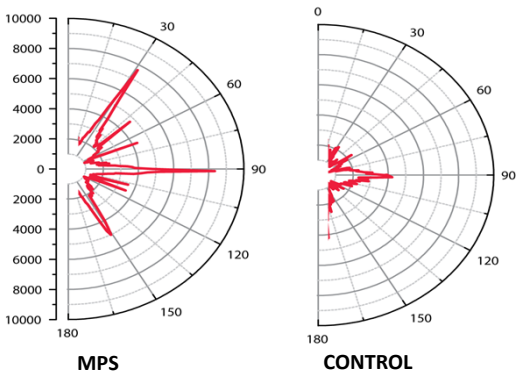


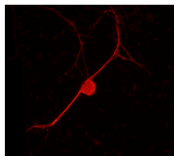
Micro-pillars with different geometries influence distribution of DRG neurons





Specific micro-pillar spacing elicits directional DRG neurite orientation compared to control glass surfaces





Conclusion

Specific areas of the MPS provide better enviroment for DRG neurons growth and enhance specific pattern of neurite aligment.

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