Characterization of dorsal root ganglion neurons cultured on silicon micro-pillar substrates

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Aim of Investigation: In recent years, numerous studies have noted that substrate topography has a significant influence on neuronal morphology, but the extent of morphological changes depends on the type of neurons as well as geometry and physical properties of the substrate. Our study focuses on characterization of DRG neurons cultured on silicon micro-pillar substrates (MPS), as directly related to the design of micro-electrode arrays (MEAs).

Methods: The study was conducted on Sprague-Dawley neonatal and male adult rats. The research plan was approved by the Ethical Committee of the University of Split. DRG neurons were cultured on MPS and glass coverslips until 7th day *in vitro.* Specific neuronal subtypes were identified by immunofluorescence using markers for: neurofilament 200 (N52), isolectin B4 (IB4) and calcitonin gene-related peptide (CGRP). DRG neuronal distribution and morphology were determined on MPS areas with different widths (1–5.6 μm) and spacings (0.6–15 μm). Morphometric analysis, including alignment and length of the neurite, was carried out with the use of ImageJ Fast Fourier Transform (FFT) - Oval Profile plugin and NeuronJ plugin.

Results: We showed that MPS provide a permissive environment for growth of adult and neonatal DRG neurons, equally well as control glass surfaces. Better alignment and length of the neurites was observed on MPS relative to the control glass surfaces, for both adult and neonatal neurons. On MPS areas of particular spacing-range (0.6–1.4 μm), more DRG neurons were present; neurites were longer and more aligned. Furthermore, MPS architecture influences growth directionality of all main DRG neuronal subtypes: large myelinated neurons, peptidergic and nonpeptidergic neurons. In the above mentioned micro-pillar spacing, neurites preferentially oriented along three directional axes at 30°, 90° and 150°. We also noticed that further increase in spacing between the pillars on MPS reduce the topographic guidance and orientation of all DRG neuronal subtypes.

Conclusion: Our results indicate that micro-pillar substrate topography affect the morphology of DRG neurons. The knowledge gained by this research will allow us and other researchers in this field of interest to fabricate MEA with precisely defined physical features for successful electrophysiological recordings of DRG neurons.

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