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

Repić, T.[1], Madirazza K.[2], Bektur E.[3], Sapunar, D. [1]

[1]Laboratory for Pain Research, School of Medicine, University of Split, Croatia

[2]Speech and Hearing Research Laboratory, School of Medicine, University of Split, Croatia

[3]Histology and Embryology Department, School of Medicine, Eskisehir Osmangazi University, Turkey

Introduction

- Substrate topography has a significant influence on neuronal morphology *in vitro*.
- Morphological changes depends on the type of neurons as well as geometry and physical properties of the substrate.
- Aim of our study: Investigate the influence of silicon micro-pillar substrate (MPS) topography on the distribution and morphology (neurite length, number and directionality) of the main dorsal root ganglion (DRG) neuronal subtypes, both neonatal and adult ones.
- The knowledge gained by this research will allow us to fabricate micro-electrode arrays (MEAs) with precisely defined physical characteristics for successful electrophysiological recordings of DRG neurons with high spatio-temporal resolution.

Methods

Figure 1. Micro-pillar substrate (MPS) layout consist of:
- 150 areas with 3 µm high hexagonal pillars
- pillar width is from 1-5.6 µm --> horizontal bins
- pillar spacing is from 0.6-15 µm --> vertical bins

Culture of DRG neurons:
- from adult and neonatal Sprague-Dawley rats
- seeding on experimental MPS and control glass coverslips
- culturing for 1, 3 and 7 day *in vitro* (DIV)

Immunofluorescence and cell imaging:
- specific DRG neuronal subtypes were identified by simultaneous staining of NeuN and N52 or IB4 or CGRP

Analysis of DRG neuronal distribution on MPS:
- using SigmaPlot software

Quantification of neurite alignment:
- using Fast Fourier Transform (FFT) algorithm of NIH-ImageJ software supported by the "Oval Profile" plug-in

Measurement of neurite number and length:
- "NeuronJ" plug-in of NIH-ImageJ software

Conclusion

Specific geometrical features (pillar spacing from 0.6-3.2 µm) of silicon micro-pillar substrate topography significantly affect directionality, length and number of neurite outgrowths, and provide better environment for growth of DRG neurons, both neonatal and adult ones, compared to control glass surfaces.

Results

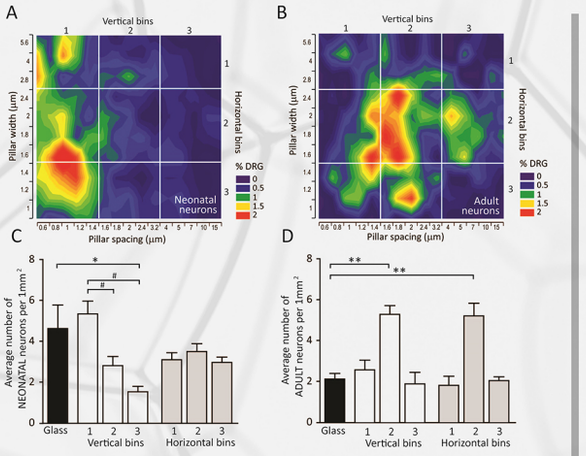

Figure 2. MPS topography influences the distribution of DRG neurons in culture: Surface plots (A,B) and average number (C,D) of neonatal (A,C) and adult (B,D) DRG neurons --> difference in their preferential distribution is observed.

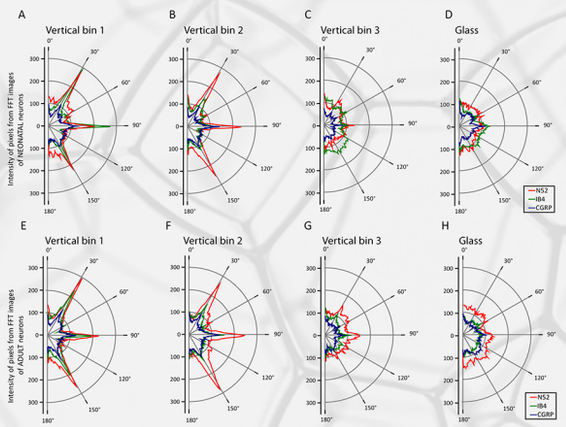

Figure 4. MPS topography elicits directional growth of DRG neurites: FFT analysis of neonatal (A-D) and adult (E-H) neurite outgrowths --> existence of topographical guidance in vertical bins 1 and 2 (pillar spacing: 0.6-3.2 µm) is observed compared to vertical bin 3 (pillar spacing: 4-15 µm) and control glass surface.

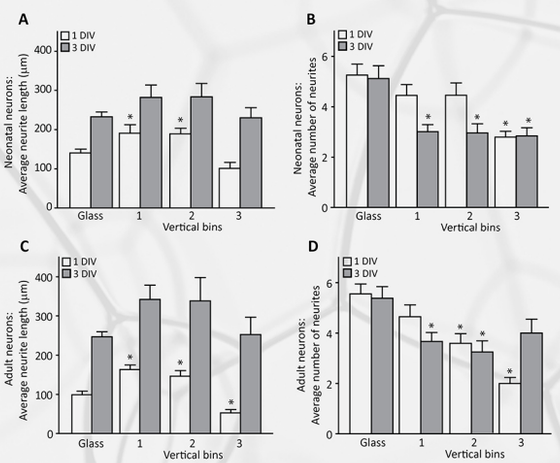

Figure 3. MPS topography affects the length and number of DRG neurites:
Average neurite length (A,C) and neurite number (B,D) of neonatal (A,B) and adult (C,D) DRG neurons --> MPS provides favorable environment for growth of neurons with lower number of longer neurites.

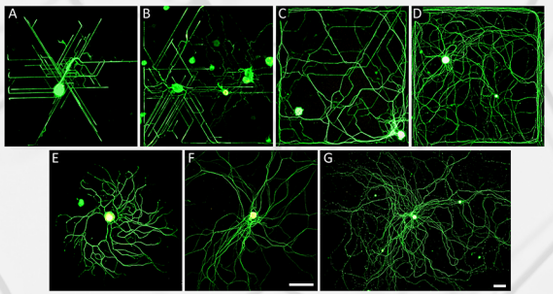

Figure 5. Examples of immunostained DRG neurons on:
MPS vertical bin 1 (A), bin 2 (B-C), bin 3 (D) and control glass (E-G) are showing the same results as on Fig. 4.

tihana.repic@mefst.hr

Funded by the Croatian Foundation for Science (HRZZ); Grant No. 4126