Guiding Neuronal Responses: Impact of Micro- and Nano-Scale Topographies on Neurite Growth and Morphology

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Surface topographies influence neurite outgrowth, morphology, and network formation. While most studies use simple patterns to guide neuronal alignment, or employ microfluidic channels to confine axonal growth[1,2], the effects of topographical cues in guiding complex neuronal interconnectivity remains underexplored. This work investigates diverse micro- and nano-patterned surfaces to develop biomimetic platforms for precise control of neuronal growth and connectivity, with applications in tissue engineering and regenerative medicine.

Silicon stamps with various micro- and nano-patterns were fabricated via photolithography and nanoimprint lithography, transferred onto polydimethylsiloxane, and coated with poly-D-lysine and laminin. Primary cortical neurons with density of 11,300 cells/cm^2 were cultured on these platforms for 27 days *in vitro*, monitored, fixed, and fluorescently labeled to assess their responses to various physical cues.

Figure 1 illustrates dendritic growth and morphology on flat, nanograting, nanohole, and nanopillar surfaces. Nanograting promoted dense dendritic branching and network formation, while nanoholes and nanopillars supported more isolated neuronal growth, aiding single-neuron visualization.

Micrograting patterns significantly influence neuronal growth direction, but the effects of high aspect ratio gratings remain unclear. Here, 10 μm wide, 20 μm pitch, and 50 μm deep microgrooves with horizontal and vertical orientations were studied. Figure 2 shows neurons primarily grew on the ridges, with limited axons and dendrites extending down the sidewalls or across the microgrooves. This resulted in neurons to extend along the ridges instead of spreading across the microgrooves.

When neurons were seeded on the bottom surface of 10 μm wide, 30 μm pitch, and 50 μm high microchannels, they extended to the sidewalls and reached the top of the ridges. Fluorescent images showed that axons grew along the microchannel sidewalls, as shown in Fig. 3(a). Cell bodies were found inside and outside of the microchannels as shown in Fig. 3(b).

These findings provide insights for designing guidance structures to precisely control neuron distribution, alignment, and connectivity using micro- and nano-topographies.

[1] F. Milos, A. Belu, D. Mayer, V. Maybeck, and A. Offenhäusser, "Polymer nanopillars induce increased paxillin adhesion assembly and promote axon growth in primary cortical neurons," *Adv. Biol.* 5, 2000248 (2021).
[2] M. Liu, A. Wu, J. Liu, H.-W. Huang, Y. Li, Q. Shi, Q. Huang, and H. Wang, "Arched microfluidic channel for the promotion of axonal growth performance," *iScience* 27, 110885 (2024).

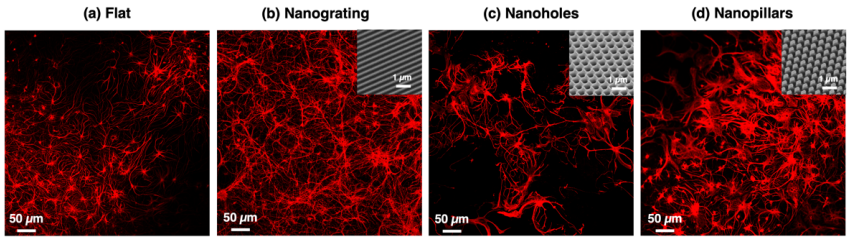

Figure 1: Dendritic growth and morphology. Dendrites were labeled with marker MAP2 (red). (a) Flat surface. (b) Nanograting with 150 nm width, 400 nm pitch, and 100 nm depth. (c) Nanoholes with 220 nm diameter (dia.), 550 nm pitch, and 500 nm depth arranged in hexagonal pattern. (d) Nanopillars with 220 nm dia., 550 nm pitch, 500 nm depth. Nanograting enhanced dense dendritic branching and network formation, whereas nanoholes and nanopillars facilitated isolated neuronal growth for single-neuron visualization.

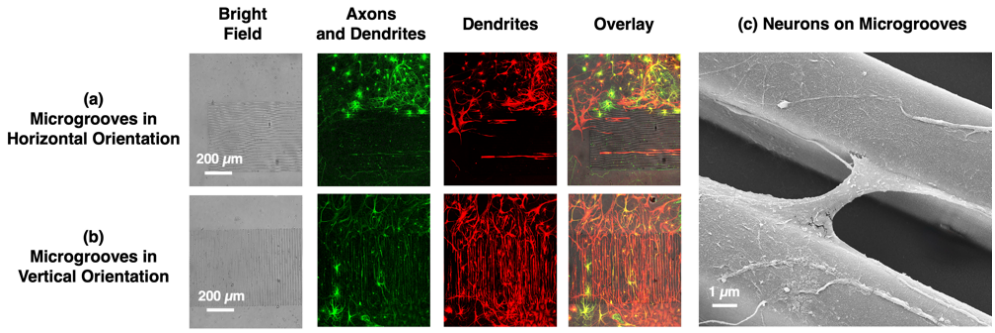

Figure 2: Axonal and dendritic growth on microgrooves with width of 10 μm, pitch of 20 μm, and height of 50 μm. Neurons were labeled with Beta III Tubulin (green; marking axons and dendrites) and MAP2 (red; marking dendrites). Microgrooves were oriented in (a) horizonal and (b) vertical directions to investigate neuronal responses. (c) Neuronal growth on microgrooves. Neurons grew on ridges instead of extending down into microgrooves.

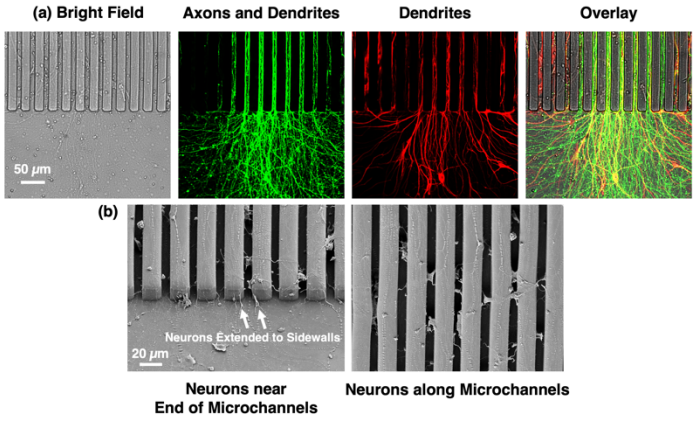

Figure 3: Neurite growth and connectivity in 10 μm wide, 30 μm pitch, and 50 μm high microchannels. (a) Neurons were labeled with Beta III Tubulin (green; marking axons and dendrites) and MAP2 (red; marking dendrites). Neurites grew along the microchannels. (b) Neuronal connectivity along and across microchannels. Neurons extended to microchannel sidewalls.