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Photopolymerized microfeatures for directed spiral ganglion neurite and Schwann cell growth

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ABSTRACT

Cochlear implants (CIs) provide auditory perception to individuals with severe hearing impairment. However, their ability to encode complex auditory stimuli is limited due, in part, to poor spatial resolution caused by electrical current spread in the inner ear. Directing nerve cell processes towards target electrodes may reduce the problematic current spread and improve stimulatory specificity. In this work, photopolymerization was used to fabricate micro- and nano-patterned methacrylate polymers to probe the extent of spiral ganglion neuron (SGN) neurite and Schwann cell (SGSC) contact guidance based on variations in substrate topographical cues. Micropatterned substrates are formed in a rapid, single-step reaction by selectively blocking light with photomasks which have parallel line-space gratings with periodicities of 10–100 μm. Channel amplitudes of 250 nm–10 μm are generated by modulating UV exposure time, light intensity, and photoinitiator concentration. Gradual transitions are observed between ridges and grooves using scanning electron and atomic force microscopy. The transitions stand in contrast to vertical features generated via etching lithographic techniques. Alignment of neural elements increases significantly with increasing feature amplitude and constant periodicity, as well as with decreasing periodicity and constant amplitude. SGN neurite alignment strongly correlates ($r = 0.93$) with maximum feature slope. Multiple neuronal and glial types orient to the patterns with varying degrees of alignment. This work presents a method to fabricate gradually-sloping micropatterns for cellular contact guidance studies and demonstrates spatial control of inner ear neural elements in response to micro- and nano-scale surface topography.

1. Introduction

Neural prosthetics are intended to replace or substantially augment motor and sensory functions of neural pathways that have been lost or damaged due to physical trauma, disease, or genetics. Ongoing developments in the fields of neurobiology, materials science, and tissue engineering are enabling innovative device designs and modifications that may considerably expand the functional potential of such complex medical devices. However, much of this functional potential remains unrealized due to poor host tissue integration that significantly limits the performance of most neural prostheses [1].

In particular, neural prosthetic performance is limited by low spatial signal resolution at the neural–electrode interface [2,3]. Consequently, prostheses fail to recapitulate the intimate, precise interactions inherent to neural networks and therefore fail to provide precise motor or sensory stimulation. For example, visual resolution provided by retinal prostheses is limited to few sensory pixels, at least in part, by electrical signal overlap caused by spatial separation of stimulating electrodes from the target neurons in the retina [3]. Similarly, the cochlear implant (CI) enables basic auditory perception to individuals with severe hearing loss but provides limited tonal information due to comparable limitations in spatial signal control. For CIs, spatial limits to tonal fidelity occur due to electrical signal spread across the neural–electrode interface that excites neurons which are outside of the preferred area of stimulation. As a result, non-specific signaling causes CI patients to struggle with complex auditory stimuli such as music appreciation, voice comprehension in environments with noise, and voice

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intonations [4,5]. Furthermore, because the nervous system is generally dependent upon signaling that is location specific, analogous signal resolution challenges are expected for all devices that interact with the nervous system. Precise spatial signal control will, therefore, be critical to achieve significant performance improvements in next-generation neural-prosthetics.

To address spatial resolution challenges, significant research interest has focused on guiding sensory neurites to approach or even contact prosthesis electrodes [2,6–15]. Spatial proximity to stimulating electrodes would allow for lower stimulation thresholds that would reduce problematic signal overlap, enable higher stimulatory specificity, and perhaps lead to greater precision in both signal input and biological functional output. Recent research illustrates a variety of methods used to direct the outgrowth of regenerative neural processes, including aligned microfibers [16], parallel micro- and nano-channel morphology [17,18], axonal conduits [19], cyto-mimetic patterning [20], bioactive molecule patterning [21–24], diffusion gradients of chemo-attractants [25], and electrical fields [26]. However, the principle focus of many of these studies is to induce neurites, particularly those of the sciatic nerve, to optimally extend in one direction to bridge large gaps typical of nerve injuries. Using similar guidance techniques may also be advantageous to address spatial resolution challenges at the neural–prosthetic interface by enabling spatial control of regenerative sensory neurites specific to the prosthesis.

Among the various methods used to direct cell growth, controlling cell–material interactions based on surface topography, or contact guidance, is of particular interest due to the stability, reproducibility, and high degree of control over surface physical features via well-established micro- and nano-scale patterning techniques. Additionally, contact guidance is a versatile technique used to induce specific morphologies of multiple cell types, including epithelial cells [27], fibroblasts [28], stem cells [29], osteoblasts [30], as well as neuronal and glial cells [31]. In addition to controlling cell morphology, contact guidance has also been shown to regulate gene expression that may be advantageous for neural regeneration. For example, Schwann cells increase neurotrophin expression when cultured on microgrooved chitosan and poly(D,L-lactide) compared to those grown on smooth substrates [32]. Micropattern dimensions such as ridge width, groove depth, and pattern shape can be tuned to influence cellular outgrowth and spatial orientation as well [33,34]. Consequently, recent neural contact guidance studies inspire confidence that neuritic processes relevant to current or developing prosthetics, such as those of spiral ganglion neurons (SGNs) or retinal ganglion neurons, may be spatially oriented towards stimulating electrodes for increased signal specificity and enhanced prosthetic performance.

Accordingly, in this study, we show that photopolymerization enables facile and rapid generation of micro- and nano-patterned methacrylate substrates for contact guidance studies and also demonstrate the extent to which inner ear neural elements, namely SGN neurites and spiral ganglion Schwann cells (SGSCs), spatially orient to 3D topographical cues. We have previously reported that SGNs and SGSCs adhere to and survive on copolymer methacrylates similar to those used for this study [35]. Micropattern feature spacing is controlled using Ronchi rule optics with varied band-spacing as photomasks and feature amplitude is tuned by terminating the reaction at specific time increments to temporally arrest amplitudes as they develop throughout the reaction. Feature amplitude is also tuned by modulating photoinitiator concentration and UV light intensity. Gradually sloping features produced by this method stand in contrast to the majority of contact guidance studies that use lithographic procedures which produce features with defined vertical edges [32,36]. Variations in the extent of SGN neurite and SGSC alignment are demonstrated using gradually sloping, parallel ridge-groove patterns that have periodicities of 10–50 μm and amplitudes of 250 nm–8 μm. Alignment behavior of astrocytes (ACs) as well as neurites from dorsal root ganglion neurons (DRGNs), trigeminal neurons (TGNs), and cerebellar granular neurons (CGNs) serve as glial and neuronal comparisons, respectively. The extent of neurite alignment is also shown to strongly correlate with maximum feature slope.

2. Materials and methods

2.1. Glass slide pretreatment

Standard 2.54 cm × 7.62 cm glass microscope slides were functionalized with a methacrylated silicon bonding agent to prevent delamination of polymer substrates from the glass during sample characterization and cellular studies. The slides were first treated under vacuum with O_2 plasma for 3 min at 30 W RF power (PDC-001 Harrick Plasma Expanded Cleaner, Ithaca, NY). Immediately following removal from the plasma chamber, the slides were immersed in a 1/100 v/v solution of 3-(trimethoxysilyl)propyl methacrylate (Aldrich) and n-hexane (Aldrich) overnight in a covered container at room temperature (~21 °C). Upon removal, each slide was rinsed with fresh hexanes and allowed to dry in a fume hood before being placed in a sealed container. Functionalized slides were observed to have a slightly translucent appearance following the hexane rinse. The slides were immediately used as a substrate for polymerization when removed from the sealed container.

2.2. Micropatterned substrate fabrication

Monomer mixtures of 40 wt% hexyl methacrylate (HMA, Aldrich) and 59 wt% 1,6-hexanediol dimethacrylate (HDDMA, Aldrich) were prepared with 1 wt% of 2,2-dimethoxy-2-phenylacetophenone (DMPA, BASF) as the photoinitiator unless otherwise specified. A sample volume of 20 μl was pipetted onto the center of a functionalized glass slide and was subsequently covered with a 2.54 cm × 2.54 cm glass-chrome Ronchi rule photomask (Applied Image Inc., Rochester, NY) for patterned samples, or with a cut untreated glass slide of the same dimensions for unpatterned samples. Pre-polymer formulations spread evenly under the photomasks due to capillary forces. Polymer samples were cured with a high-pressure mercury vapor arc lamp (Omnicure S1500, Lumen Dynamics, Ontario, Canada) at a 365 nm light intensity of 16 mW/cm^2. The curing module had an 8 mm diameter liquid light guide that was equipped with an 8 mm aperture × 50 mm length beam homogenizing fused silica light pipe (Edmund Optics). A collimating lens (RLQ-1, Asahi Spectra) was attached to the end of the light guide. UV radiation was shuttered at specific times. Following polymerization, photomasks were removed from polymer surfaces and samples were washed with 95% ethanol to remove all residual monomer. Samples were allowed to air dry before use.

2.3. Micropattern characterization

2.3.1. White light interferometry

Micropattern periodicity and absolute channel amplitude were measured by white light interferometry (Dektak Wyko 1100 Optical Profiling System, Veeco). Channel amplitude was reported as the difference between the maximum ridge value and the adjacent minimum groove value. Average feature height was determined by measuring channel amplitude in nine different areas across each sample ($n = 3$ or more). Periodicity was measured as the distance between the highest points on adjacent ridges and was consistent with photomask band spacing. 2D profiles and 3D images were generated using *Vision* software associated with the instrument.

2.3.2. Scanning electron microscopy

Patterned polymer morphology was examined by scanning electron microscopy (SEM, S-4800, Hitachi). For top down images, patterned samples were mounted with the glass side down on aluminum SEM stubs using conductive silver paint. For cross-sectional images, glass substrates and patterned polymers were fractured and then mounted vertically on specimen stages. The SEM specimen stage was angled using an automated stage and software controls to capture angled cross-sectional images. Prior to examination by SEM, each polymer surface was sputter coated with gold. Electron accelerating voltage was set at 2 kV.

2.3.3. Atomic force microscopy

The slope between grooves and ridges was determined by atomic force microscopy (AFM, Asylum Atomic Force Microscope, Asylum Research). A microscope cantilever with a force constant of 46 N/m and a tuning frequency of 316.62 kHz was used. Samples were scanned at a rate of 5 μm/s with 512 points taken per scan line across 50 μm. *X* and *Y* position data were obtained from the instrument software from 2D profiles ($n = 3$) taken at different locations on pattern surfaces. Average and maximum slopes were calculated from profile data.