Published in final edited form as:

Otol Neurotol. 2018 January ; 39(1): 119–126. doi:10.1097/MAO.0000000000001622.

Photopolymerized microfeatures guide adult spiral ganglion and dorsal root ganglion neurite growth

Linjing Xu¹, Alison E. Seline¹, Braden Leigh², Mark Ramirez¹, C. Allan Guymon², and Marlan R. Hansen^{1,3}

¹Department of Otolaryngology-Head and Neck Surgery, University of Iowa, Iowa City, IA 52242

²Department of Chemical Engineering, University of Iowa, Iowa City, IA 52242

³Department of Neurosurgery, University of Iowa, Iowa City, IA 52242

Abstract

Hypothesis—Microtopographical patterns generated by photopolymerization of methacrylate polymer systems will direct growth of neurites from adult neurons, including spiral ganglion neurons (SGNs).

Background—Cochlear implants (CIs) provide hearing perception to patients with severe to profound hearing loss. However, their ability to encode complex auditory stimuli is limited due, in part, to poor spatial resolution caused by spread of the electrical currents in the inner ear. Directing the regrowth of SGN peripheral processes towards stimulating electrodes could help reduce current spread and improve spatial resolution provided by the CI. Previous work has demonstrated that micro- and nano-scale patterned surfaces precisely guide the growth of neurites from a variety of neonatal neurons including SGNs. Here we sought to determine the extent to which adult neurons likewise respond to these topographical surface features.

Methods—Photopolymerization was used to fabricate methacrylate polymer substrates with micropatterned surfaces of varying amplitudes and periodicities. Dissociated adult dorsal root ganglion neurons (DRGNs) and SGNs were cultured on these surfaces and the alignment of the neurite processes to the micropatterns was determined.

Results—Neurites from both adult DRGNs and SGNs significantly aligned to the patterned surfaces similar to their neonatal counterparts. Further DRGN and SGN neurite alignment increased as the amplitude of the microfeatures increased. Decreased pattern periodicity also improved neurite alignment.

Conclusion—Microscale surface topographic features direct the growth of adult SGN neurites. Topographical features could prove useful for guiding growth of SGN peripheral axons towards a CI electrode array.

Keywords

Photopolymerization; Micropatterning; Surface topography; Nerve regeneration; Neural prosthesis; Adult neurons

Introduction

Deafness typically results from loss of the sensory hair cells in the cochlea and this is followed by degeneration of the afferent spiral ganglion neurons (SGNs) themselves (1–3). Cochlear implants (CIs) replace the mechanosensory transduction function of hair cells, delivering direct electrical stimulation of the SGNs and thereby providing hearing sensation for deaf patients. Although, current CIs provide meaningful speech recognition in quiet, speech recognition significantly deteriorates in noisy environments and CI users are challenged by other complex listening tasks such as music appreciation (4,5). The inability of CIs to provide fine spectro-temporal cues likely contributes to the poor performance in these complex tasks (6). The limited spectral resolution provided by CIs arises from the interaction of nearby channels thereby reducing the number of independent perceptual channels (5,7). These channel interactions are due, at least in part, to the physical gap that separates the SGNs in the modiolus and the CI electrodes in the scala tympani (8).

Given these limitations, there is lively interest in improving the neural prosthesis interface by directing SGN axon regeneration to approach or even contact the stimulating electrodes (9–17). However, to be effective, such axonal regrowth would need to be highly organized, recapitulating the normal pattern of afferent innervation. If successful, such tissue engineering stands to dramatically enhance the neural prosthesis interface and increase the fidelity and number of independent channels provided by stimulating electrodes.

Strategies to guide regrowth of neurites include the use of patterned bioactive molecules such as laminin, fibronectin, or the EphA4-IgG-Fc-chimera, among others (16,18–25). Other studies used controlled gradients of soluble neurotrophic or chemoattractive/repulsive factors to guide neurite growth (26–30). In addition to these biochemical cues, cells respond to topographical features in the environment (31–33). Patterned topographies have emerged as another method to direct cellular patterning such as axon growth (17,34,35). Of particular relevance, recent work demonstrated that neurites from cultured neonatal SGN respond to topographical features that guide neurite growth (36–38). The extent to which adult SGNs, the target cells of the CI, likewise respond to topographical features remains unknown and is the focus of this work.

Methacrylate polymers are bioinert, nontoxic, and commonly used in a variety of clinical applications including: intraocular lens, contact lens, dental resins, cellular encapsulation, facial fillers, and bone cements (39). We recently used photopolymerization, i.e. the use of light to produce polymers, of methacrylate monomers to produce micro- and nano-scale patterned polymer platforms (38). These platforms proved highly effective in directing growth of neurites from a variety of neonatal neurons, including SGNs (38). Here we sought to determine the extent to which adult sensory neurons (dorsal root ganglion neurons, DRGNs) and SGNs would likewise respond to similar topographical guidance cues.

Materials and Methods

Photopolymerization to generate micropatterned methacrylate substrates

Physical unidirectional micro-grooved polymer patterns were created as previously described (38). 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 O₂ 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. The slides were immediately used as a substrate for polymerization when removed from the sealed container.

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. 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) with periodicities ranging from 10–50 µm for patterned samples, or with a cut untreated glass slide of the same dimensions for unpatterned samples. Polymer samples were cured with a high-pressure mercury vapor arc lamp (Omnicure S1500, Lumen Dynamics, Ontario, Canada) at a light intensity of 16 mW/cm² measured at 365 nm. 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 to control the polymerization rate and, hence, tune feature amplitude as previously described (38). Following polymerization, photomasks were removed from polymer surfaces and samples were washed with 95% ethanol to remove residual monomer. White light interferometry was used to characterize micropattern amplitude and periodicity as previously described (38). Prior to use in culture, patterned and unpatterned HMA:HDDMA substrates were coated with laminin (20 µg/ml) overnight at 4°C.

Scanning Electron Microscopy

The morphology of the polymers was examined by scanning electron microscopy (SEM, S-4800, Hitachi) as previously described.(38) Each polymer surface was sputter coated with gold. 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. Electron accelerating voltage was set at 2 kV.

Neuronal cultures

Primary dissociated cultures of adult dorsal root ganglia (DRG) and spiral ganglia were prepared from P21–25 Sprague Dawley rats. DRG were harvested by removing the spinal

column and then dissecting the lumbar and thoracic ganglia. SG were collected from isolated temporal bones as previously described (40). In this case the otic capsule bone is dense and was picked away from the modiolus and Rosenthal's canal. The lateral cochlear wall and sensory epithelium were removed piecemeal. Ganglia were collected and placed in an iced Hank's Balanced Salt Solution with calcium and magnesium until dissection was complete. Once all ganglia were collected, the HBSS solution was replaced with HBSS lacking calcium and magnesium with 0.25% trypsin and 0.2% collagenase. The ganglia were enzymatically digested for 20 minutes at 37°C. Following enzymatic digestion, 10% fetal bovine serum (FBS) was added to inactivate the trypsin. Once the tissue settled to the bottom of the vial the supernatant was removed and the tissue pellet was washed three times with culture media and then resuspended in culture media. Using 200 and 1000 µl plastic pipet tips, the ganglia were mechanically dissociated and 200–500 µl of the dissociated SG and DRG suspensions were added to each pre prepared slide. DRG cultures were maintained in Neurobasal-A medium with B-27 (Gibco) and nerve growth factor (NGF, 50 ng/ml, ThermoFisher Scientific). SG cultures were maintained in Dulbecco's Modified Eagle Medium supplemented with N2 additives, 5% FBS, neurotrophin-3 (NT-3, 50 ng/ml, Sigma–Aldrich) and brain derived neurotrophic factor (BDNF, 50 ng/ml, R&D Systems). The cultures were incubated in 6.5% CO₂ at 37°C for 48 h. The cultures were grown for 48 h to provide adequate time for neurites to establish a trajectory and allow for survival of the maximal number of neurons. A minimum of 3 separate cultures was used for each condition.

Immunostaining

The cultures were fixed after 48h by removing the old media from the slides and rinsing three times with phosphate buffered saline (PBS). This was followed by adding 1 ml of 4% paraformaldehyde to each slide and placing them at 4°C for 20 minutes. DRG and SG cultures were immunostained with anti-neurofilament 200 (NF200) antibodies (1:400, Sigma–Aldrich) to label neurons and their processes. Alexa 488 or Alexa 546 conjugated secondary antibodies (1:1000, Invitrogen) were used to detect primary antibody immunolabeling. Slides were cover slipped with ProLong Gold anti-fading reagent with DAPI (ThermoFisher Scientific).

Determination of Neurite Alignment

Digital epifluorescent images of the entire slide surface were captured on a Leica DMIRE2 microscope (Leica Microsystems, Bannockburn, IL) with Leica DFC350FX digital camera using the scanslide feature Metamorph software (Molecular Devices, Silicon Valley, CA). The pattern direction was set horizontally prior to image capture. Total neurite length was determined from digital images by measuring the longest process of each neurite using the measurement tool in Image J (NIH, Bethesda, MD) as previously described.(41) Neurite alignment was calculated as previously described (38,42). Briefly, alignment to the pattern was defined as a ratio of unaligned length per neurite length. The ratio is represented as $[(TL - AL)/TL]$, where TL is total neurite length and AL represents aligned length in the pattern direction. Aligned length (AL) was determined by measuring the distance from the neuronal cell body to the neurite terminus in a straight line in the direction of the micropattern. Alignment ratios close to zero represent neurites that closely follow the pattern. A wandering neurite, which does not strongly align to the pattern, has a high alignment ratio. To analyze

neurite alignment on unpatterned substrates, aligned distance was also measured in relation to the horizontal plane.

Statistic analysis

A Kruskal-Wallis one-way analysis of variance on ranks followed by post hoc Dunn's test was performed using Sigmaplot (Systat Software, Inc) to test for significance of differences in alignment ratio among the various conditions. The p-value was set at < 0.05 . Pearson correlation coefficient of alignment ratio and neurite length was likewise determined using SigmaPlot.

Results

Alignment of adult DRGNs align to micropatterned surfaces depends on feature amplitude and periodicity

Scanning electron microscopy (SEM) was used to demonstrate the patterned substrate morphology (Fig. 1). Repeating features are seen across the polymer surface (Fig. 1A). The transitions between the grooves and ridges of the patterns seen in cross section SEM are relatively smooth (Fig. 1B) similar to many native tissue environments rather than the sharp or abrupt features generated by other patterning techniques.

To determine the sensitivity of neurites from adult sensory neurons to various micropatterns we first used DRGNs as these are significantly more abundant than SGNs. Our labs have previously demonstrated robust growth of neonatal DRGNs and SGNs on the HMA:HDDMA polymer systems used in this study (38). Likewise adult DRGNs exhibited normal neural morphology with robust neurite growth on the HMA:HDDMA polymers (Fig. 2A–B).

To evaluate the influence of channel amplitude on the extent of adult DRGN neurite alignment, the cells were cultured on micropatterns with a constant periodicity of $50\text{ }\mu\text{m}$ but with varying channel amplitudes of 1, 3, and $8\text{ }\mu\text{m}$ (Fig. 2C). DRGN neurite alignment increased significantly with increasing channel depth. DRGN neurites had an average alignment ratio of 0.035 ± 0.017 (mean $\pm$ SEM) on unpatterned surfaces. This significantly decreases to 0.0247 ± 0.018 on substrates with a periodicity of $50\text{ }\mu\text{m}$ and amplitude of $1\text{ }\mu\text{m}$, indicating that adult DRGNs align their neurites in response to relatively shallow microfeatures. With increased channel depth, adult DRGN neurites became even more closely aligned to the micropattern with alignment ratios of 0.215 ± 0.017 and 0.146 ± 0.011 on $3\text{ }\mu\text{m}$ and $8\text{ }\mu\text{m}$ amplitude polymers respectively. Neurite alignment increased with increasing amplitude confirming that micropatterns with higher amplitudes (e.g. deeper grooves) provide more robust alignment compared to lower amplitude ($p < 0.05$, Kruskal-Wallis one way ANOVA on ranks with posthoc Dunn's test). Indeed the alignment ratio was approximately 50% lower on micropatterns with a channel amplitude of $8\text{ }\mu\text{m}$ compared to micropatterns with $1\text{ }\mu\text{m}$ amplitude channels. The low alignment ratio on $8\text{ }\mu\text{m}$ amplitude channels also indicates that the neurites strongly align to the pattern along their entire length as shown in figure 2B.

To determine the effect of feature frequency, or periodicity, on the extent of neurite alignment, adult DRGN neurite alignment was measured on patterns with periodicities of 10, 33.3, and 50 μm and a constant amplitude of 1 μm . (Fig 2D). Adult DRGN neurites became significantly more aligned as pattern periodicities decreased from 50–10 μm . In fact, DRGN neurite alignment on micropatterns with 10 μm periodicity and relatively shallow 1 μm amplitude pattern was significantly better (lower alignment ratio) than their alignment on a 50 μm periodicity and 8 μm deep amplitude pattern ($p<0.05$).

Neurites from adult SGNs strongly align to micropatterned surface features

Having confirmed that adult sensory neurons respond to the microtopographical features used in this study, we sought to determine whether such patterns would likewise guide the growth of neurites from adult SGNs. As the yield of neurons from adult SGNs is quite low, we limited the patterns to the extremes of those investigated with adult DRGNs. First, we compared adult SGN neurite alignment on unpatterned surfaces or patterns of 50 μm periodicity and channels amplitudes of either 1 or 8 μm (Fig. 3A–C). In the case of adult SGNs, channels of both amplitudes strongly induced neurite alignment, with significantly lower alignment ratios compared to unpatterned surfaces ($p<0.05$). Although there was a slight trend towards better alignment on patterns with higher amplitudes, this was not statistically significant. Similarly, adult SGN neurites demonstrated a slight, but not statistically significant, trend toward improved alignment on micropatterns with a periodicity of 10 μm compared to micropatterns with 50 μm periodicity when the channel amplitudes was constant at 1 μm . These results highlight the sensitivity of SGN neurites to topographical features in that even relatively weak micropatterns (50 μm periodicity, 1 μm amplitude) induce robust adult SGN neurite alignment. Interestingly, the adult SGN neurite alignment ratio was consistently lower compared to the alignment ratio of adult DRGN neurites for micropatterns of similar amplitudes and periodicities (Figs 2C,D and 3C,D).

We considered the possibility that longer neurites might yield lower alignment ratios. To explore this possibility we performed a Pearson correlation comparing neurite alignment and length for all neurites. Figure 4 presents these results as scatterplots. There was no significant correlation between neurite length and alignment ratio for either adult DRGN ($r^2=0.0116$) nor for adult SGNs ($r^2=0.0015$). Thus the ability of micropatterned surfaces to guide neurite alignment does not appear to depend on the length of a given neurite.

Discussion

Neural prostheses generally and cochlear implants in particular provide a significantly degraded signal compared to native neural circuits. This is due, in part, to the physical distance between the stimulating electrodes and the target neurons that they activate. Closing this physical gap either by moving the electrodes closer to the neurons (e.g. perimodiolar positioning) or by inducing organized nerve fiber regrowth towards the electrodes represent emerging strategies to improve the signal resolution provided by the prosthesis (8,13,17). One method that allows for stable, predictable cues that precisely guide neurite regeneration is to physically patterning the surface of nerve regeneration scaffolds; the overall motivation

for these studies was to establish the ability of adult SGNs to respond to such topographical guidance cues.

We have previously shown that micropatterned HMA:HDDMA substrates effectively guide regeneration of neonatal SGNs as well as other peripheral (trigeminal and DRG) neurons, central (cerebellar granular) neurons, and glial (Schwann cells, astrocytes and oligodendrocytes) (38). The results presented here confirm that adult neurons are likewise responsive to these micropatterned surfaces. Thus, micropatterned surfaces may provide effective cues to precisely guide the regeneration of adult neural processes towards a desired target, such as a stimulating electrode.

These results confirm that increasing channel amplitude or feature frequency tends to improve alignment of neurites from adult neurons as is the case with neonatal neurons (38). Prior work with neonatal SGNs demonstrated that the neurite alignment ratio asymptotically approached unity for patterns with a periodicity of 50 μm and an amplitude of $\sim 8 \mu\text{m}$. To allow for comparison to this prior work, we limited the highest amplitudes to 8 μm in this study (38). Adult DRGN neurite alignment improves as amplitude increases with constant periodicity and also improves as periodicity decreases with constant amplitude. In both cases, the slope grade that transitions between grooves and ridges also increases (38). Indeed, we have previously shown that maximum feature slope strongly correlates with neurite alignment and that feature slope appears to be a stronger predictor of alignment than either periodicity or amplitude.(38) Apparently, neurite alignment in response to physical cues may depend more strongly on feature contrast than on absolute feature height. Interestingly, a growth cone is approximately 5 μm in diameter but features as small as 250 nm induce robust guidance of neonatal SGN neurites when maximum feature slope is sufficiently steep (38). This trend towards improved alignment with increased amplitude or decreased periodicity was more evident in adult DRGNs compared to SGNs. One possible reason for this observation is that neurite alignment was already near maximum for adult SGNs on surfaces with relatively shallow slopes so that further increases in channel amplitude or decreases in frequency did not significantly improve alignment.

Several factors may contribute to the strong alignment observed on 1 μm amplitude samples with frequent features. Specifically, patterns that have smaller periodicities present more opportunities for the growth cone of a regenerating neurite to encounter a physical feature and orient to the pattern direction over a given distance compared to patterns with larger periodicities. Moreover, as discussed above, decreasing pattern periodicity while maintaining channel amplitude increases the slope between minimum groove depths and the adjacent maximum ridge heights.

In addition to feature periodicity and amplitude, alignment of neonatal SGN neurites also depends on the stiffness of the patterned substrate. Recently it was shown that neonatal SGN neurite alignment significantly increases with increasing material modulus for two different methacrylate polymer systems (43). Interestingly, neonatal SGN neurites respond to material stiffness cues that are orders of magnitude higher (GPa) than what is typically encountered in native neural environments (kPa) (43).

While SGN neurites can precisely track the unidirectional patterns used in these studies, they appear less facile at tracking multidirectional turning cues. For example, when neonatal SGN cultures are plated on micropatterns that consist of repeating 90° angles turns, they tend to follow a horizontal trajectory and fail to precisely track the sharp 90° turns. Thus, they are substantially influenced by, but do not consistently track, multidirectional patterns (44).

When considering the use of micropatterns to guide neurites towards a neural prosthesis, alignment along the entire length of a neurite may be crucial to preserve signal fidelity by limiting interaction with adjacent neurites since unaligned regions of a neurite are more likely to encounter and interact with other neurites. Furthermore, to limit inaccurate spatial signaling, it may be necessary to maintain tight control over a population of tens to thousands of neurites within a specific region. While wandering neurites may align to the pattern throughout some of their length, a significant portion may not track in the pattern direction, which could ultimately lead to a loss of stimulatory specificity and resolution. Accordingly, microtopography surfaces that will be used to control neurite alignment should be designed to quickly orient and maintain spatial control of regenerating processes along their entire length.

The interaction of glial cells with a surface represents another important consideration in design of platforms to guide neurites towards a neural prosthesis. Schwann cells strongly support and influence SGN neurite regeneration such that nearly all SGN neurites remain in close contact with a neighboring Schwann cells (45–47). Further, regenerated neurites will likely need to be myelinated to provide optimal processing of signals from a stimulating prosthesis. To this end, neonatal spiral ganglion Schwann cells have been shown to align themselves to the same micropatterned surfaces as those used in this study (38,48) and remain closely approximately to regenerating SGN neurites (37). Although we did not measure the alignment of adult spiral ganglion Schwann cells in this study, it seems likely that denervated adult Schwann cells will respond in a similar manner as neonatal Schwann cells since the adult Schwann cells, including those in the spiral ganglion, revert to an immature phenotype when they lose contact with an axon (49–51).

Recent studies have begun to elucidate the intracellular signaling mechanisms that allow neurons and other cells to sense and respond to physical features. Neurites tend to grow within the grooved features of a microchannel (44). When a growth cone interacts with the raised slope of the ridge, TrpV1 channels sense the transition and activate intracellular signaling cascades involving Ca^{2+} and cyclic nucleotide secondary messengers and ultimately leading to the activation of RhoA GTPase and Rho associated kinase (48,52). Interestingly, these intracellular signaling events are similar to those activated by chemorepulsive molecules that repel or arrest growth cone extension. Taken together, these observations suggest that the raised features in the micropatterned surfaces function similar to chemorepulsive cues to direct neurite growth.

Several hurdles remain to design a suitable neural platform that supports and directs SGN fiber growth towards cochlear implant electrodes. The biomaterials used in cochlear implants induce a vigorous fibrous response that encapsulates the electrode array (53). In

addition to negatively influencing impedances and cochlear implant outcomes, this capsule may limit the proximity to which neurites can be guided to electrode arrays as currently designed. In addition to incorporating guidance cues as envisioned in this study, next generation prostheses may need to be constructed from significantly different biomaterials and include electrode designs that limit inflammatory and fibrous responses (13,17). A second challenge is providing stimuli to induce regrowth of neurites and several studies have sought to identify suitable stimuli (28,54). Yet another challenge will be to provide a “stop” signal once a neurite has reached an appropriate target.

Conclusions

Photopolymerization is a useful and facile method to fabricate neural regeneration substrates with varied micropatterned topography. Similar to their neonatal counterparts, neurites from adult DRGNs and SGNs track and align themselves to micropatterned surfaces. Alignment of DRGN neurites increases significantly with increasing feature amplitude as well as with decreasing periodicity whereas adult SGN neurites align strongly even to patterns with relative shallow amplitudes or widely spaced features. Topographically patterned substrates may prove useful for precisely guiding neurites from SGNs into close proximity of a stimulating electrode in an effort to improve the tonal resolution provided by CIs.

Acknowledgments

Support: R01DC012578, P30DC010362, and T35HL748535

References

1. Alam SA, Robinson BK, Huang J, et al. Prosurvival and proapoptotic intracellular signaling in rat spiral ganglion neurons in vivo after the loss of hair cells. *The Journal of comparative neurology*. 2007; 503:832–52. [PubMed: 17570507]
2. Leake PA, Hradek GT. Cochlear pathology of long term neomycin induced deafness in cats. *Hear Res*. 1988; 33:11–33. [PubMed: 3372368]
3. Spoendlin H. Retrograde degeneration of the cochlear nerve. *Acta Otolaryngol*. 1975; 79:266–75. [PubMed: 1094788]
4. Wilson BS, Dorman MF. Cochlear implants: a remarkable past and a brilliant future. *Hear Res*. 2008; 242:3–21. [PubMed: 18616994]
5. Shannon RV, Fu QJ, Galvin J 3rd. The number of spectral channels required for speech recognition depends on the difficulty of the listening situation. *Acta Otolaryngol Suppl*. 2004:50–4.
6. Wilson BS, Dorman MF. The surprising performance of present-day cochlear implants. *IEEE Trans Biomed Eng*. 2007; 54:969–72. [PubMed: 17554816]
7. Rubinstein JT. How cochlear implants encode speech. *Current opinion in otolaryngology & head and neck surgery*. 2004; 12:444–8. [PubMed: 15377959]
8. Roche JP, Hansen MR. On the Horizon: Cochlear Implant Technology. *Otolaryngol Clin North Am*. 2015; 48:1097–116. [PubMed: 26443490]
9. Brors D, Aletsee C, Schwager K, et al. Interaction of spiral ganglion neuron processes with alloplastic materials in vitro(1). *Hear Res*. 2002; 167:110–21. [PubMed: 12117535]
10. Evans AJ, Thompson BC, Wallace GG, et al. Promoting neurite outgrowth from spiral ganglion neuron explants using polypyrrole/BDNF-coated electrodes. *Journal of biomedical materials research Part A*. 2009; 91:241–50. [PubMed: 18814235]
11. Miller JM, Le Prell CG, Prieskorn DM, et al. Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral

- processes: effects of brain-derived neurotrophic factor and fibroblast growth factor. *J Neurosci Res.* 2007; 85:1959–69. [PubMed: 17492794]
12. Pettingill LN, Richardson RT, Wise AK, et al. Neurotrophic factors and neural prostheses: potential clinical applications based upon findings in the auditory system. *IEEE Trans Biomed Eng.* 2007; 54:1138–48. [PubMed: 17551571]
13. Richardson RT, Wise AK, Thompson BC, et al. Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons. *Biomaterials.* 2009; 30:2614–24. [PubMed: 19178943]
14. Roehm PC, Hansen MR. Strategies to preserve or regenerate spiral ganglion neurons. *Current opinion in otolaryngology & head and neck surgery.* 2005; 13:294–300. [PubMed: 16160524]
15. O’Leary SJ, Richardson RR, McDermott HJ. Principles of design and biological approaches for improving the selectivity of cochlear implant electrodes. *J Neural Eng.* 2009; 6:055002. [PubMed: 19721188]
16. Frick C, Muller M, Wank U, et al. Biofunctionalized peptide-based hydrogels provide permissive scaffolds to attract neurite outgrowth from spiral ganglion neurons. *Colloids Surf B Biointerfaces.* 2017; 149:105–14. [PubMed: 27736723]
17. Cai Y, Edin F, Jin Z, et al. Strategy towards independent electrical stimulation from cochlear implants: Guided auditory neuron growth on topographically modified nanocrystalline diamond. *Acta Biomater.* 2016; 31:211–20. [PubMed: 26593784]
18. Branch DW, Corey JM, Weyhenmeyer JA, et al. Microstamp patterns of biomolecules for high-resolution neuronal networks. *Med Biol Eng Comput.* 1998; 36:135–41. [PubMed: 9614762]
19. Branch DW, Wheeler BC, Brewer GJ, et al. Long-term maintenance of patterns of hippocampal pyramidal cells on substrates of polyethylene glycol and microstamped polylysine. *IEEE Trans Biomed Eng.* 2000; 47:290–300. [PubMed: 10743770]
20. Gustavsson P, Johansson F, Kanje M, et al. Neurite guidance on protein micropatterns generated by a piezoelectric microdispenser. *Biomaterials.* 2007; 28:1141–51. [PubMed: 17109955]
21. Miller C, Jeftinija S, Mallapragada S. Micropatterned Schwann cell-seeded biodegradable polymer substrates significantly enhance neurite alignment and outgrowth. *Tissue Eng.* 2001; 7:705–15. [PubMed: 11749728]
22. Oliva AA Jr, James CD, Kingman CE, et al. Patterning axonal guidance molecules using a novel strategy for microcontact printing. *Neurochem Res.* 2003; 28:1639–48. [PubMed: 14584818]
23. Schmalenberg KE, Uhrich KE. Micropatterned polymer substrates control alignment of proliferating Schwann cells to direct neuronal regeneration. *Biomaterials.* 2005; 26:1423–30. [PubMed: 15482830]
24. Brors D, Bodmer D, Pak K, et al. EphA4 provides repulsive signals to developing cochlear ganglion neurites mediated through ephrin-B2 and -B3. *The Journal of comparative neurology.* 2003; 462:90–100. [PubMed: 12761826]
25. Evans AR, Euteneuer S, Chavez E, et al. Laminin and fibronectin modulate inner ear spiral ganglion neurite outgrowth in an in vitro alternate choice assay. *Developmental neurobiology.* 2007
26. Altschuler RA, Cho Y, Ylikoski J, et al. Rescue and regrowth of sensory nerves following deafferentation by neurotrophic factors. *Ann N Y Acad Sci.* 1999; 884:305–11. [PubMed: 10842602]
27. Dazert S, Kim D, Luo L, et al. Focal delivery of fibroblast growth factor-1 by transfected cells induces spiral ganglion neurite targeting in vitro. *Journal of cellular physiology.* 1998; 177:123–9. [PubMed: 9731752]
28. Wise AK, Hume CR, Flynn BO, et al. Effects of localized neurotrophin gene expression on spiral ganglion neuron resprouting in the deafened cochlea. *Molecular therapy : the journal of the American Society of Gene Therapy.* 2010; 18:1111–22. [PubMed: 20216530]
29. Wise AK, Richardson R, Hardman J, et al. Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. *The Journal of comparative neurology.* 2005; 487:147–65. [PubMed: 15880560]

30. Wittig JH Jr, Ryan AF, Asbeck PM. A reusable microfluidic plate with alternate-choice architecture for assessing growth preference in tissue culture. *J Neurosci Methods*. 2005; 144:79–89. [PubMed: 15848242]
31. Curtis AS, Gadegaard N, Dalby MJ, et al. Cells react to nanoscale order and symmetry in their surroundings. *IEEE Trans Nanobioscience*. 2004; 3:61–5. [PubMed: 15382646]
32. Dalby MJ. Topographically induced direct cell mechanotransduction. *Medical engineering & physics*. 2005; 27:730–42. [PubMed: 15921949]
33. Dalby MJ, Childs S, Riehle MO, et al. Fibroblast reaction to island topography: changes in cytoskeleton and morphology with time. *Biomaterials*. 2003; 24:927–35. [PubMed: 12504513]
34. Fozdar DY, Lee JY, Schmidt CE, et al. Selective axonal growth of embryonic hippocampal neurons according to topographic features of various sizes and shapes. *Int J Nanomedicine*. 2010; 6:45–57. [PubMed: 21289981]
35. Koppes RA, Park S, Hood T, et al. Thermally drawn fibers as nerve guidance scaffolds. *Biomaterials*. 2016; 81:27–35. [PubMed: 26717246]
36. Mattotti M, Micholt L, Braeken D, et al. Characterization of spiral ganglion neurons cultured on silicon micro-pillar substrates for new auditory neuro-electronic interfaces. *J Neural Eng*. 2015; 12:026001. [PubMed: 25627109]
37. Clarke JC, Tuft BW, Clinger JD, et al. Micropatterned methacrylate polymers direct spiral ganglion neurite and Schwann cell growth. *Hear Res*. 2011; 278:96–105. [PubMed: 21616131]
38. Tuft BW, Li S, Xu L, et al. Photopolymerized microfeatures for directed spiral ganglion neurite and Schwann cell growth. *Biomaterials*. 2013; 34:42–54. [PubMed: 23069708]
39. Ratner, B., Hoffman, A., Schoen, F., et al. *Biomaterials Science*. 2. 2004. An introduction to Materials in Medicine; p. 10-11.
40. Hansen MR, Zha XM, Bok J, et al. Multiple distinct signal pathways, including an autocrine neurotrophic mechanism, contribute to the survival-promoting effect of depolarization on spiral ganglion neurons in vitro. *J Neurosci*. 2001; 21:2256–67. [PubMed: 11264301]
41. Roehm PC, Xu N, Woodson EA, et al. Membrane depolarization inhibits spiral ganglion neurite growth via activation of multiple types of voltage sensitive calcium channels and calpain. *Molecular and cellular neurosciences*. 2008; 37:376–87. [PubMed: 18055215]
42. Cheng EL, Leigh B, Guymon CA, et al. Quantifying Spiral Ganglion Neurite and Schwann Behavior on Micropatterned Polymer Substrates. *Methods Mol Biol*. 2016; 1427:305–18. [PubMed: 27259935]
43. Tuft BW, Zhang L, Xu L, et al. Material stiffness effects on neurite alignment to photopolymerized micropatterns. *Biomacromolecules*. 2014; 15:3717–27. [PubMed: 25211120]
44. Tuft BW, Xu L, White SP, et al. Neural Pathfinding on Uni- and Multidirectional Photopolymerized Micropatterns. *ACS applied materials & interfaces*. 2014
45. Whitton DS, Tieu D, Grover M, et al. Spontaneous association of glial cells with regrowing neurites in mixed cultures of dissociated spiral ganglia. *Neuroscience*. 2009; 161:227–35. [PubMed: 19324078]
46. Jeon EJ, Xu N, Xu L, et al. Influence of central glia on spiral ganglion neuron neurite growth. *Neuroscience*. 2011; 177:321–34. [PubMed: 21241783]
47. Hansen MR, Vijapurkar U, Koland JG, et al. Reciprocal signaling between spiral ganglion neurons and Schwann cells involves neuregulin and neurotrophins. *Hear Res*. 2001; 161:87–98. [PubMed: 11744285]
48. Li S, Tuft BW, Xu L, et al. Microtopographical features generated by photopolymerization recruit RhoA/ROCK through TRPV1 to direct cell and neurite growth. *Biomaterials*. 2015; 53:95–106. [PubMed: 25890710]
49. Cheng E, Hansen MR. Schwannomas provide insight into the role of p75(NTR) and merlin in Schwann cells following nerve injury and during regeneration. *Neural Regen Res*. 2016; 11:73–4. [PubMed: 26981085]
50. Ahmad I, Fernando A, Gurgel R, et al. Merlin status regulates p75 expression and apoptotic signaling in Schwann cells following nerve injury. *Neurobiol Dis*. 2015; 82:114–22. [PubMed: 26057084]

51. Provenzano MJ, Minner SA, Zander K, et al. p75(NTR) expression and nuclear localization of p75(NTR) intracellular domain in spiral ganglion Schwann cells following deafness correlate with cell proliferation. *Molecular and cellular neurosciences*. 2011; 47:306–15. [PubMed: 21658451]
52. Li S, Tuft B, Xu L, et al. Intracellular calcium and cyclic nucleotide levels modulate neurite guidance by microtopographical substrate features. *J Biomed Mater Res A*. 2016; 104:2037–48. [PubMed: 27062708]
53. Ishai R, Herrmann BS, Nadol JB Jr, et al. The pattern and degree of capsular fibrous sheaths surrounding cochlear electrode arrays. *Hear Res*. 2017; 348:44–53. [PubMed: 28216124]
54. Shibata SB, Cortez SR, Beyer LA, et al. Transgenic BDNF induces nerve fiber regrowth into the auditory epithelium in deaf cochleae. *Experimental neurology*. 2010; 223:464–72. [PubMed: 20109446]

Figure 1. Representative SEM images of micropatterned HMA:HDDMA polymers. Top down (A) and cross-sectional (B) SEM images of a patterned polymer substrate. Gradual transition between groove and ridge feature is indicated by the arrow.

Figure 2.

Alignment of adult DRGNs align to micropatterned surfaces depends on feature amplitude and periodicity. **A&B.** Representative epifluorescent images of adult DRGN cultures on (A) an unpatterned or (B) a micropatterned (amplitude 1 μm , periodicity 10 μm) HMA:HDDMA polymer substrate. The cultures are labeled with anti-neurofilament (NF200) antibody (red). Scale bar=100 μm . **C.** Average neurite alignment ratio for adult DRGNs on unpatterned (UP) substrates or patterned substrates with channel amplitudes of 1, 3 or 8 μm and a constant periodicity of 50 μm . **D.** Average neurite alignment ratio for adult DRGNs on unpatterned (UP) substrates or patterned substrates with periodicities of 50, 33 or 10 μm and a constant channel amplitude of 1 μm . Numbers within the bars represent the total number of neurites analyzed. Error bars present standard error. * $p<0.05$ by Kruskal-Wallis one way ANOVA on ranks with post hoc Dunn's test.

Figure 3.

Adult SGNs strongly align to micropatterned surfaces. **A&B.** Representative epifluorescent images of adult SGN cultures on (A) an unpatterned or (B) a micropatterned (amplitude 1 μm , periodicity 50 μm) HMA:HDDMA polymer substrate. The cultures are labeled with anti-neurofilament (NF200) antibody (red). Scale bar=100 μm . **C.** Average neurite alignment ratio for adult SGNs on unpatterned (UP) substrates or patterned substrates with channel amplitudes of 1 or 8 μm and a constant periodicity of 50 μm . **D.** Average neurite alignment ratio for adult SGNs on unpatterned (UP) substrates or patterned substrates with periodicities of 50 or 10 μm and a constant channel amplitude of 1 μm . Numbers within the bars represent the total number of neurites analyzed. Error bars present standard error.

* $p < 0.05$ by Kruskal-Wallis one way ANOVA on ranks with post hoc Dunn's test.

Figure 4.

Neurite alignment does not correlate with neurite length. **A&B.** Scatterplots of neurite alignment ratio and neurite length from (A) adult DRGNs or (B) adult SGNs. There was no significant correlation between neurite alignment ratio and neurite length for either type of neuron.