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Promoting Neuron Adhesion and Growth

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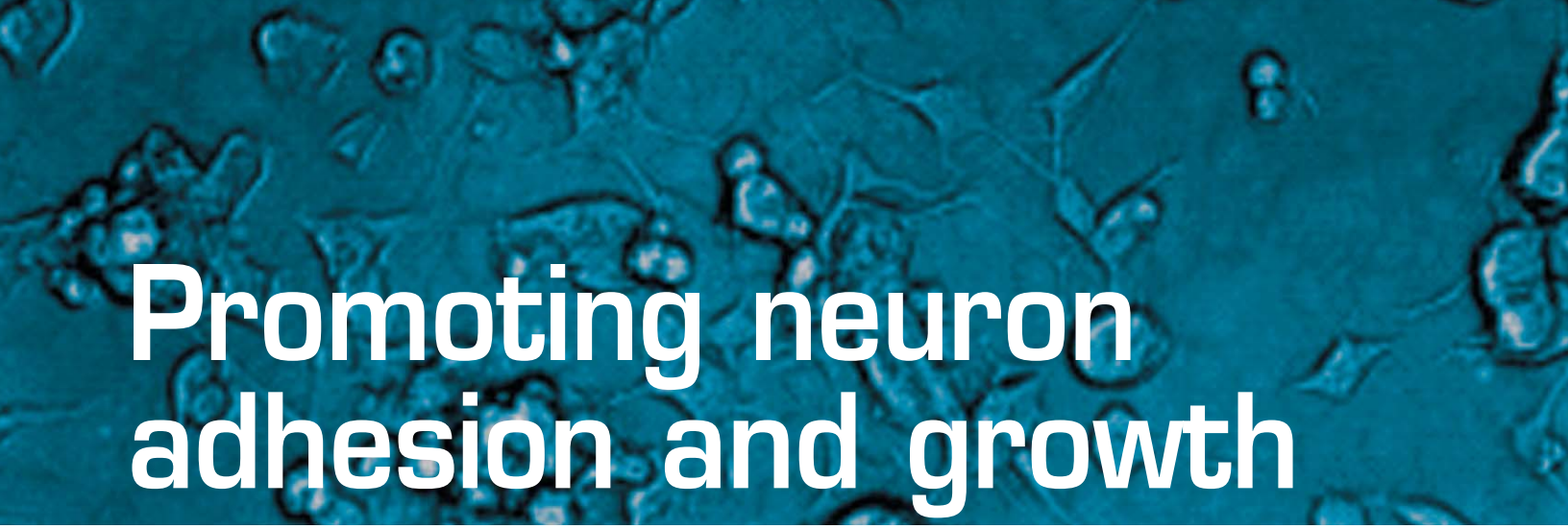

Promoting neuron adhesion and growth

During nervous system development, the extracellular matrix (ECM) plays a pivotal role offering anchorage points to maturing neurons and neurites, as well as a permissive environment for tissue formation. Thus enhancement of cell adhesion is often an important criterion when designing biomaterials for neural tissue engineering. In addition to functionalizing biomaterials with ECM-derived cell adhesive molecules, there is emerging evidence that indicates the surface topography, stiffness, and electrical properties play an important role in neuron adhesion and neurite outgrowth. We describe recent developments in biomaterials modification for simulating the microenvironment in order to promote neuron adhesion and growth, as well as to encourage nerve regeneration after injury or disease.

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Many biomaterials can be designed as three-dimensional scaffolds or hydrogels and further engineered to support neuron survival, adhesion, proliferation, and guidance. The biodegradability and biocompatibility of these materials are important design criteria for promoting nerve regeneration in the injured nervous system (NS). A wide variety of materials, synthetic or natural, have been explored in the past, and have been recently reviewed[1–4]. Here we focus on recently developed materials modification strategies where the chemical, physical, and mechanical microenvironment is engineered to stimulate neuron adhesion and neurite outgrowth. For example, scaffold designs incorporating topographical features, physicochemical properties, and methods to deliver soluble factors can potentially recapitulate the *in vivo* microenvironment. We discuss the importance of the extracellular matrix (ECM), not only in terms of the factors required by neurons for survival and adhesion, but also the mechanical microenvironment, which has been largely neglected until recently.

Cell migration and axonal guidance

Cell migration is an essential early step in the development of the vertebrate NS. These primitive cells are guided to the appropriate targets through a complicated mix of surface-bound and soluble

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cues. Immature neurons arise in germinal layers and migrate to their destinations in the NS, where they extend axons and dendrites and form synaptic connections. Surface and soluble factors are instrumental in guiding axons toward their targets. A clear understanding of these factors is vital in order to design and implement biomaterials for neural tissue engineering.

Extracellular matrix molecules

The ECM is a complex mixture of proteins and polysaccharides that occupies the interstitial space in tissues. Cells secrete and facilitate the organization of these ECM constituents. In turn, the assembled ECM plays a vital role in regulating cell behavior throughout development and adulthood, providing anchorage and mechanical buffering, aiding intercellular communication, and segregating different tissues. The influential role of the ECM is evident during NS development, where the ECM shapes progenitor cell migration and differentiation, as well as guiding how maturing neurons extend new axons.

Laminins serve as a key component of the ECM and offer binding sites for self-polymerization, cells, and other ECM macromolecules. To date, 15 laminins have been characterized that share a similar trimer structure generated from five α, four β, and three γ chains[5]. In the NS, laminins help form basement membranes that facilitate neuronal and glial cell interactions. It has been demonstrated that laminin is required for neuronal migration in the developing cerebellum[6,7]. Laminin contains several binding motifs that interact predominantly with cell-surface integrins (primarily $\alpha_1\beta_1$ and $\alpha_6\beta_1$)[8–10], but also shows association with α-distroglycan[11,12]. Jacques *et al.*[13] have demonstrated that neural precursor migration on laminin can be significantly inhibited by blocking the $\alpha_6\beta_1$ integrin. Studies with embryonic stem cells have observed that lack of β_1 integrin blocks basement membrane formation and the expression of laminin protein[14]. It is clear that laminin provides essential attachment points enabling axons to extend and exert forces on the ECM[15–17].

Proteoglycans are important ECM molecules that have been implicated in neuronal and axonal guidance. These highly negatively charged molecules consist of a core protein with numerous glycosaminoglycan (GAG) side chains and are grouped into two major classes: heparin sulfate proteoglycans (HSPGs) and chondroitin sulfate proteoglycans (CSPGs). Several studies have demonstrated that either exogenous addition of HSPG or enzymatic removal of HSPG leads to axonal guidance defects during development[18–20]. This has led to findings that demonstrate a functional association between HSPGs and several secreted and transmembrane proteins. As a result, the role of HSPGs in guidance appears to be tied to sequestering slit, netrin, and semaphorin proteins (for a thorough review, see elsewhere[21]). The influence of CSPG on guidance is not as well understood as that of HSPG. However, it is clear that CSPG also has a potent inhibitory effect on neuron guidance. Studies have demonstrated that CSPG makes up part of the glial scar that effectively halts central nervous system (CNS) axonal regeneration, and leads to abnormal axonal growth cones[22]. Recent findings have shown that, similar to HSPGs, CSPGs also functionally associate with semphorins and inhibit axonal extension[23]. There has been little focus on the use of proteoglycans in neural tissue engineering strategies because of their inhibitory activity in the NS.

Soluble factors

The neurotrophin (NT) family of growth factors is the most widely studied of the extracellular signals that influence neuronal development and guidance. Of the NTs, nerve growth factor (NGF), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF) have been the most thoroughly investigated. NGF and NT-3 promote axonal growth from sensory neurons through activation of their corresponding tyrosine kinase (Trk) receptors, TrkA[24–26] and TrkC[27,28], respectively. BDNF is known to enhance survival and promote axon growth from retinal ganglion cells and hippocampal neurons via its receptor, TrkB[29]. Studies have shown that NGF encourages axonal extension *in vitro*[30,31] and leads to axonal growth *in vivo*[32]. NGF has even been shown to overcome axon growth inhibition by CSPG in dorsal root ganglion (DRG) neurons[33]. Similar to NGF, NT-3 is known to enhance the growth of axons *in vivo* significantly for both peripheral and central neurons[34–37]. BDNF has been shown to play an important role in the regulation of synapse structure and function, especially in glutamatergic synapses[38]. It has also been demonstrated that BDNF and NT-3 significantly influence axon path-finding, as well as aiding axonal regeneration in rats following spinal cord injury[39,40]. Moreover, during development these NTs direct the path-finding of maturing axons to their innervating targets by a combination of long-range attractive and repulsive cues[41]. The functions and importance of these NTs in the NS have recently been reviewed[42]. Recent studies also provide evidence that basic fibroblast growth factor (bFGF-2) and glial cell line-derived neurotrophic factor (GDNF) may play a role in facilitating nerve regeneration in the peripheral nervous system (PNS) and CNS. Both growth factors have been shown to influence neurons, Schwann cells, and oligodendrocytes toward axonal growth and remyelination following injury[43,44]. Ciliary neurotrophic factor (CNTF) has been shown to act primarily on neurons as a survival factor following injury in the PNS[45]. Thus far, CNTF has not demonstrated any functional or regenerative benefits for nerve repair[46], however, it may show synergistic effects with other NTs[47].

The ECM and soluble factors help to shape development of the NS and continue to play an important role in maintenance of the adult NS. Pioneering studies have explored the roles of ECM molecules and soluble factors in development and disease in the NS. Tissue engineering and regenerative medicine often look to developmental biology for clues to aid in the development of strategies for promoting tissue regeneration after disease or traumatic injury (Fig. 1). These studies have steered engineering techniques toward creating and modifying biomaterials to mimic the ECM to control cell adhesion,

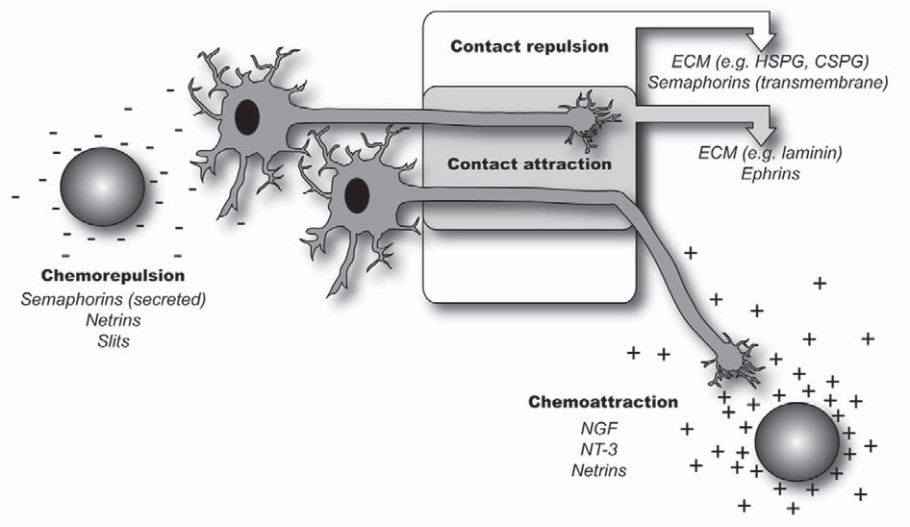

Fig. 1 The ECM, through a combination of contact-mediated (short-range cues) and soluble (long-range cues) factors, guides the axons of maturing neurons to their innervating targets during development. While the attractive cues 'pull' on the axons, the repulsive cues 'push' axons along the path toward their proper targets.

survival, and growth. These techniques can also be used to develop tools to help study fundamental processes in biology, such as neuron retrograde signaling or the effects of substrate stiffness on neuron attachment and survival.

Topographical features of biomaterials to control cell adhesion, survival, and growth

Patterning techniques: soft lithography to create surface topography

Early studies aimed to control nerve outgrowth using cell-adhesive peptides derived from laminin or fibronectin. These peptides can be patterned in specific regions on chemically functionalized glass surfaces, while non-cell-adhesive poly(ethylene glycol) (PEG) or other molecules can be patterned in other regions[48,49]. Fig. 2 shows this technique applied to hippocampal neurons cultured on a surface with alternating patterns of cell-adhesive peptides and PEG. Neurons only adhere to, and extend neurites on, surfaces patterned with the cell-adhesive peptide. Recent advances in microcontact printing and soft lithography techniques allow more precise microtopographical features to be created for control of axon growth at the micro- and nanoscale. Poly(dimethyl siloxane) (PDMS) is the biocompatible, but nondegradable, material most commonly used for fabricating topographical features by soft lithography[50].

While chemical cues are important for neuron adhesion and axon guidance, research has demonstrated that topographical features are also important in guiding axon growth and growth cone path-finding[50–53]. For example, Li and Folch[52] have shown that embryonic mouse cortical neurons turn at the edge of a groove between 22 μm and 69 μm deep, but will grow up or down a shallow groove 2–5 μm deep. While the depth of the microgroove affects axonal growth, Mahoney *et al.*[54] have demonstrated that the width of microchannels,

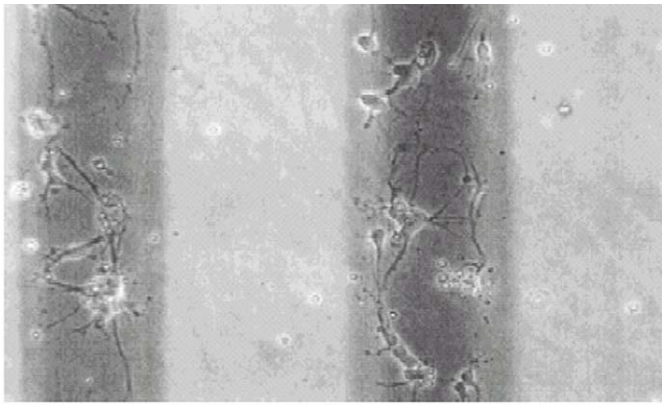

Fig. 2 Hippocampal neurons cultured on a polymeric film surface with alternating patterns of PEG (light) and CGYIGSR (a cell-adhesive peptide derived from laminin) self-assembled monolayers on Au (dark) after four days of culture. Neurons only grow and extend neurites on regions modified with cell-adhesive peptides. Original magnification is 200x. (Reprinted with permission from[48]. © 1998 John Wiley & Sons, Inc.)

from 20–60 μm in size, also affects the orientation of neurite outgrowth. Neurites tend to grow parallel to the channel wall in narrow microchannels, but perpendicular to the channel wall in wider microchannels (40–60 μm), where neurites grow until they reach the channel wall. Advances in soft lithography have also allowed the creation of topographical features on the nanometer scale by using electron beam lithography. Johansson *et al.*[53] have shown that axons from mouse sympathetic and sensory neurons display contact guidance on nanopatterned poly(methyl methacrylate) (PMMA) surfaces without coating the surfaces with cell-adhesive proteins or peptides. The preference of axons to grow on ridge edges and elevations, rather than in grooves (Fig. 3), suggests that these nano- and microtopographical features can be incorporated into tissue-engineering design strategies to provide contact guidance for nerve regeneration.

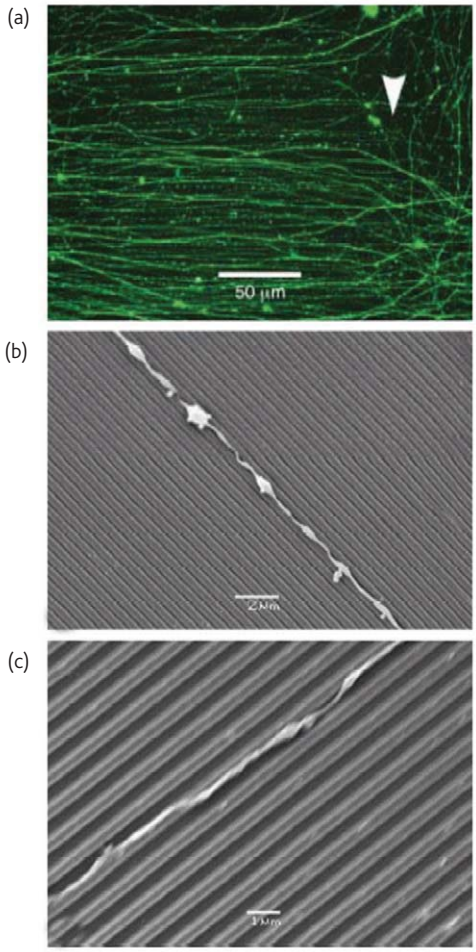

Fig. 3 (a) Axons align with a horizontally imprinted pattern of 200 nm width and 400 nm pitch. The arrow indicates the border of the pattern. (b,c) Scanning electron micrographs show that axons prefer to grow along the ridge edges and not in the grooves when the dimensions of the imprinted patterns are (b) 100 nm width and 500 nm pitch and (c) 400 nm width and 800 nm pitch. (Reprinted with permission from[53]. © 2006 Elsevier Ltd.)

Guidance tubes, fibrous matrices, and cellular co-cultures

The three-dimensional structure of biomaterial scaffolds plays a significant role in neuron adhesion, as well as neurite outgrowth and orientation. As a result, nerve guidance channels (NGCs) and, more recently, fibrous matrices and cell templates have been investigated extensively for use as nerve guidance substrates. NGCs have been the subject of rigorous research aimed at promoting nerve regeneration in both the PNS and CNS, and have built on the successful regrowth of short (~10 mm) nerve injuries in the periphery.

A recent study shows that dual concentration gradients of laminin- and NGF-loaded lipid microtubules in agarose hydrogels (an anisotropic scaffold) embedded in a polysulfone NGC promote the regeneration of a transected rat sciatic nerve over a large gap of 20 mm[55]. Interestingly, the total number of myelinated axons and axon density were higher in the anisotropic scaffold than the autologous nerve graft,

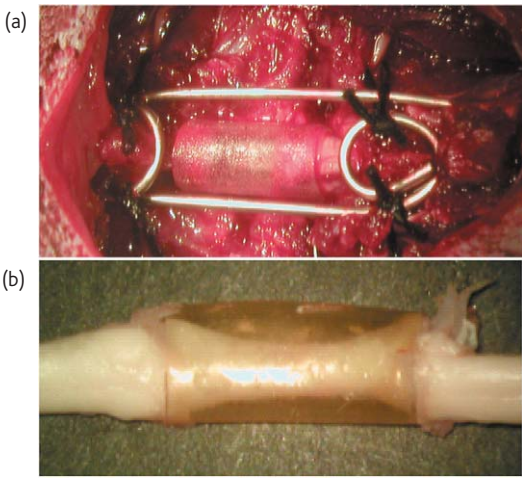

Fig. 4 (a) Dorsal view of transected spinal cord stumps placed within a transparent chitosan channel. The distance between stumps is approximately 3.5 mm. (b) A thick tissue bridge formed 14 weeks post-transplantation when brain-derived neural stem/progenitor cells have been implanted[59].

but both had similar functional recovery. ECM hydrogels have been shown to improve peripheral nerve regeneration over long distances, and Deister *et al.*[56] have recently demonstrated that co-gels of collagen I and laminin are optimal for promoting neurite length and overall volume.

While the initial strategy of developing NGCs was to bridge long gaps between nerve stumps, there is increasing evidence that suggests filling NGCs with growth factors[57] or seeding with support (and/or stem) cells can promote superior axon growth into these channels for potential application in spinal cord repair[58]. Recent studies have shown that neural stem/progenitor cells (NSPCs) delivered within a chitosan NGC can promote regeneration of a thick tissue bridge in a rat after a complete spinal cord injury (Fig. 4)[59,60]. Prior to culturing the NSPCs, chitosan tubes were coated with laminin to improve cell adhesion; *in vivo* these NGCs enhance cell survival (which is normally very poor when cells are delivered alone) and facilitate differentiation predominantly to oligodendrocytes and astrocytes[59]. Hence, NGCs not only serve as a support bridge between the two stumps of the spinal cord, but also protect the transplanted cells from the hostile environment following spinal cord injury[60]. In this study, differentiated NPSCs may have secreted factors that attract host tissue and axons into the construct although this was not quantified. Moreover, recent findings also indicate that resident precursor cells proliferate and differentiate into oligodendrocytes and astrocytes following CNS injury to promote tissue repair[61].

Surface topography provides spatial and physical cues to the growth cone, obviating the need for a cell-adhesive surface. Creating a scaffold that mimics the *in vivo* three-dimensional architecture of the ECM is relevant for tissue regeneration, as cell-matrix interactions are a vital component to cell survival, differentiation, and proliferation. The fibrous protein structure of the ECM ranges from 50–500 nm[62,63]. The

high surface-area-to-volume ratio offered by nanofiber scaffolds is believed to enhance cell adhesion, survival, and proliferation[64].

Three techniques have been developed to synthesize nanofibrous scaffolds, namely electrospinning, self-assembly, and phase separation. These approaches have recently been reviewed elsewhere[65]. This review primarily focuses on studies that have used electrospinning, a common technique used for the fabrication of micro- and nanofibers.

Electrospinning techniques allow the formation of nanofibrous structures and scaffolds that partially mimic the physical cues and microenvironment of the ECM, thus directly influencing cell migration, as well as axonal growth and guidance. Relative to simple NGCs, electrospun scaffolds provide increased surface area in conjunction with improved surface topography, which can enhance regeneration. As a result, micro- and nanofibers have become an attractive option in neural tissue engineering as they provide a large surface area for tissue growth in addition to providing spatial cues to growing axons.

Fig. 5 shows images of highly aligned and random nanofibers synthesized by electrospinning synthetic polymers. Studies have demonstrated that the speed of neurite growth is increased on highly aligned fibers compared with those that are randomly arranged[66,67]. In addition, axon growth from sympathetic neurons derived from the PNS are guided lengthwise and influenced by fiber diameter[68]. Wen and Tresco[69] have demonstrated that the filament diameter at the cellular and subcellular levels (5 μm and 30 μm) produces highly directional and robust neurite outgrowth in sympathetic neurons. Interestingly, when nylon microfibers are embedded in agarose gels, axons are able to extend along the full length of the fibers. However, in the absence of the fibers, axons are unable to extend into the gels because agarose is inherently nonadhesive to the cells[70]. Building on this idea, Seidlits *et al.*[71] have shown that three-dimensional microstructures can be 'written' directly in hydrogel substrates by spatially activating photoreactive moieties using multiphoton excitation at the focal point of a pulsed laser. Besides providing directional cues and promoting neurite outgrowth using fibers, electrospun poly(ε-caprolactone) nanofibers have been shown to direct NSPC differentiation into primarily oligodendrocytes[72].

A primary goal of biomaterial modification is to mimic the ECM to create a microenvironment that stimulates neuron adhesion and neurite outgrowth. Another way to achieve this goal (other than through biomaterial modification) is to co-culture with cells from either the vasculature, such as endothelial cells, or NS, such as Schwann cells and oligodendrocytes, which have been shown to improve neuron adhesion, neurite outgrowth, and to myelinate axons[73,74]. Schwann cells can support functional axonal regeneration and have been shown to release trophic factors that are important for axon myelination[58,74,75]. Moreover, co-cultures of Schwann cells with sympathetic neurons in magnetically aligned collagen gel rods show that Schwann cells are highly associated with the elongating neurites and form axially aligned chords similar in morphology to the Bands of Büngner[76]. Thus, glial co-culture can provide stimuli to enhance tissue growth and could help improve other regenerative medicine strategies.

Biomaterials modification for nerve adhesion, growth, and guidance

Peptides and extracellular matrix proteins

It is well known that ECM proteins such as laminin, collagen, and fibronectin play important roles in cell adhesion, growth, and tissue remodeling[77]. Extensive research has focused on grafting whole proteins or specific active sequences of these proteins to biomaterial surfaces in order to enhance neuron adhesion and growth[55,78]. The most widely studied sequences derived from the ECM are CDPGYIGSR, GQAASIKVAV, GRGDS, and PHSRN. When tethered to nonadhesive or moderately adhesive substrates, the peptide-modified surfaces show significantly improved neuron adhesion. Other active peptide sequences derived from the ECM, and their associated functions, have been reviewed recently[79].

When specific cell-adhesive peptides are presented in a spatially controlled manner, neurite outgrowth can be limited to only those areas[80–82] or volumes when presented in three dimensions[80,83]. Fig. 6 shows sympathetic neurons growing into a three-dimensional GRGDS-peptide channel created inside an agarose hydrogel. Moreover, when these peptides are presented as a concentration gradient, neurites

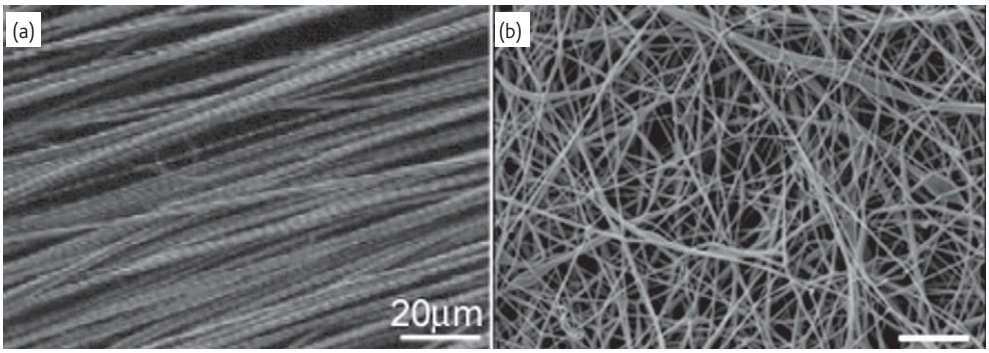

Fig. 5 (a) Scanning electron micrograph of aligned poly-L-lactate fibers. (Reprinted with permission from[67]. © 2007 John Wiley & Sons, Inc.) (b) Scanning electron micrograph of random poly(ε-caprolactone) nanofibers produced by electrospinning. (Reprinted with permission from[72]. © 2008 Brill.)

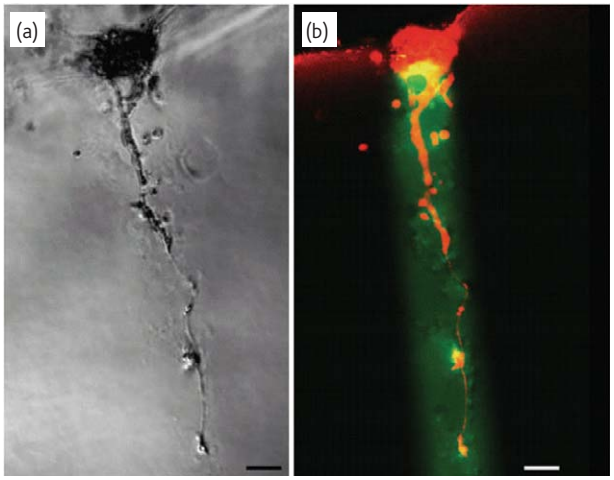

Fig. 6 Primary rat dorsal root ganglia neurons (DRGs) plated on three-dimensional patterned, GRGDS oligopeptide-modified, 0.5 wt% agarose gels. DRG neurons grow within GRGDS-oligopeptide-modified agarose channels only, not in surrounding volumes. The DRG cluster on top of the channel shows that the DRGs have migrated to the GRGDS channel and extended neurites into the peptide-channel as viewed by: (a) brightfield microscopy, and (b) confocal fluorescent microscopy, where the channel is green (fluorescein-labeled oligopeptide) and the cells are red (cytoskeletal F-actin rhodamine–phalloidin stain), confirming cell migration into the oligopeptide-modified channel. Scale bars: 100 μm. (Reprinter with permission from[80]. © 2004 Nature Publishing Group.)

are guided toward the higher end of the concentration gradient[84,85]. Although peptide modification can be finely tuned, longer peptide sequences are often required because the short peptide sequence does not provide the proper conformation to investigate the effects of protein-cell interaction on neuron adhesion and neurite outgrowth. Thus, substrate modification with whole proteins is also important to understand mechanisms of cell function, which impact design strategies for neural tissue engineering.

Substrate stiffness (elasticity)

Substrate stiffness, or the intrinsic elasticity of the matrix, is currently emerging as an important physical factor influencing cell response[86]. This discovery first came to light from *in vitro* studies with epithelial cells and fibroblasts cultured on collagen-coated gels of varied stiffness. This work reveals that substrate stiffness affects DNA incorporation and apoptosis in these cells[87]. Although the molecular pathways are yet to be characterized, it has been shown that myocytes, neurons, astrocytes, and mesenchymal stem cells (MSCs) sense substrate stiffness[86,88–94]. These studies highlight the fact that substrate stiffness is important to anchorage-dependent cells. Studies that have examined neuronal and glial responses to substrate elasticity are of particular interest to CNS regeneration. Neurons have been shown to prefer weaker gels for neurite extension in three dimensions[89]. Another study has shown that neurons do survive on gels of varied stiffness; however, astrocytes will not survive on softer substrates, even with proper media supplementation[90]. This study also demonstrates that weaker polyacrylamide gels enhance neurite branching, thus offering enhanced potential for forming synaptic connections.

A study by Georges *et al*.[93] shows that cortical neurons and astrocytes respond differently to substrates of varied stiffness. Astrocytes show disorganized cytoskeletons and uncharacteristic morphologies on soft fibrin-coated polyacrylamide gels, compared with astrocytes cultured on stiffer substrates. Neurons show similar responses on soft and stiff gels in terms of axon extension. Cultures of mixed cell populations demonstrate that neurons have enhanced attachment and growth on softer gels, whereas astrocyte growth is suppressed. On laminin-coated tissue culture plastic (very stiff) surfaces, astrocytes typically overgrow neurons. More recent work by Engler *et al*.[92] demonstrates that substrate elasticity can guide lineage specification and phenotype commitment of adult MSCs. Polyacrylamide gels were created at stiffnesses that mimic brain (Young's elastic modulus, E_Y = 0.1–1 kPa), muscle (E_Y = 8–17 kPa), and collagenous bone (E_Y = 25–40 kPa) and coated with 1 μg/cm^2 type I collagen. Immunohistochemistry and gene expression microarray profiling reveals that the soft matrices are neurogenic, stiffer matrices are myogenic, and comparatively rigid surfaces are osteogenic. Previous studies have demonstrated that substrate elasticity can guide primary and stem cells toward desired phenotypes and functions. Cells derived from the NS seem to be sensitive to matrix stiffness; tissue-engineering scaffolds should incorporate this knowledge to achieve the desired responses.

Electrically conducting polymers

The inherent nature of neurons is to transmit electrochemical signals throughout the NS and, as a result, they are highly influenced by electrical stimuli. Moreover, many ECM materials such as collagen, tissues rich in GAGs, and mineralized bone have been shown to exhibit piezoelectric effects, or the generation of electrical charges resulting from an applied mechanical stress on the tissue[95]. Initial research demonstrated that an applied electric field can accelerate neurite outgrowth and influence neurite orientation[96,97]. Fig. 7 shows enhanced neurite outgrowth from PC12 cells cultured on a conductive polymer surface after electrical stimulation. Consequently, these findings served as an impetus to engineer conducting polymers that are capable of stimulating neurite outgrowth to promote nerve regeneration.

Polymers such as polypyrrole (PPy) are attractive materials because of their inherent electrically conductive properties, relatively easy preparation, flexibility in altering surface characteristics, *in vitro* cytocompatibility, and *in vivo* biocompatibility[98,99]. One limitation with these conducting polymers is their inability to biodegrade. There are only a handful of studies that have attempted to overcome this problem[100–102]. In one study, conducting regions of the polymer were tethered together by biodegradable ester linkers[100]. A recent review describes different conducting polymers, their synthesis, and applications in tissue engineering, biosensors, and neural probes[99].

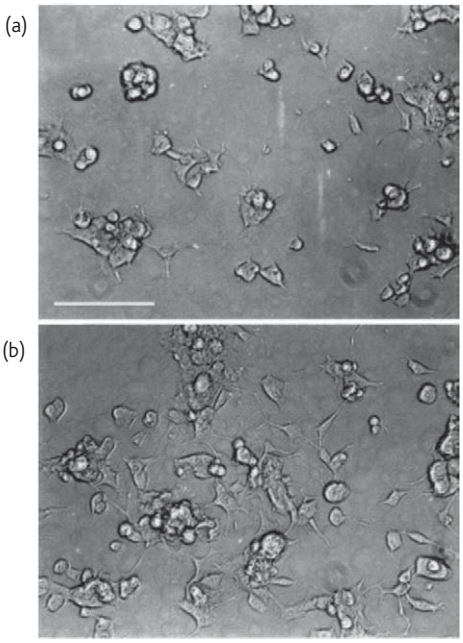

Fig. 7 PC12 cell differentiation on poly(styrene sulfonate)-doped polypyrrole (PPy) in (a) the absence and (b) presence of electrical stimulation (100 mV) across the polymer film. Neurite outgrowth in PC12 cells is enhanced upon electrical stimulation. Images were acquired 24 hrs after stimulation. Scale bar: 100 μm. (Reprinted with permission from[98]. © 1996 Materials Research Society.)

One advantage of using PPy is that it can be electropolymerized with other reagents to balance charges, control conductivity, wettability, and surface properties. However, the choice of reagent can lower the conductivity of PPy. Recent studies have shown that grafting cell-adhesive peptides, functional proteins, or growth factors to PPy surfaces does not significantly alter the conductivity of PPy and, most importantly, enhances cell interfacing[103,104]. Gomez and Schmidt[104] created a bioactive PPy surface where NGF is photochemically tethered. Upon electrical stimulation, and with immobilized NGF, cortical neurons have greater neurite outgrowth compared with no electrical stimulation. The electrical properties of conducting polymers and their versatility in modification make these materials attractive for neural tissue engineering applications.

Conclusions and future directions

The principal goal of regenerative medicine is to promote tissue regeneration and healing after injury or disease. This can be achieved through the delivery of cells and/or factors in a tissue-engineered scaffold designed to provide a biomimetic microenvironment conducive to cell adhesion, proliferation, differentiation, and host tissue integration. Most biomaterial scaffolds are biodegradable, biocompatible, and provide a temporary niche for cell-replacement strategies. The microenvironment of this niche can be tailored for neural tissue engineering with the following strategies: chemical factors, such as those of the ECM, including both haptotactic (contact-mediated) and chemotactic (diffusible/gradient) cues; physical factors, such as topographical features to guide cell growth; mechanical factors, such as the modulus of the material to influence cell differentiation; and electrical factors to influence orientation and performance. These elements are combined in the tissue-engineering strategy where the biomaterial provides these factors within a three-dimensional construct in order to guide and promote cell survival and function in the regeneration of tissues.

While a variety of neural tissue engineering strategies have been developed and discussed in this review, it is likely that a combination of these strategies is necessary to achieve functional nerve regeneration after traumatic injury or disease. Since support cells are known to secrete ECM and soluble factors important to neurons, biomaterials that can provide a niche for multiple cell types are advantageous. Scaffolds that include cells in the supporting matrix can take advantage of the factors secreted from these cells to promote greater survival/differentiation and host tissue integration. These supporting cells can also be engineered to produce desired proteins or factors required for enhancing neuron survival, adhesion, and neurite outgrowth.

Future tissue-engineering and biomaterials strategies are likely to include scaffolds that incorporate multiple, synergistic stimuli in order to further enhance regeneration after injury or disease. In order to promote regeneration, stem cell strategies are likely to be incorporated into biomaterials design strategies: either endogenous stem cells could be stimulated through the delivery of the appropriate factors and stimuli[105,106], or exogenous stem cells could be transplanted within biomaterial-based scaffolds. Neural tissue engineering strategies will need to focus on overcoming inhibitory factors, such as proteoglycans, in order to promote nerve regeneration *in vivo* effectively[107]. Ultimately, the goal of recreating the niche is to promote repair in sites that would otherwise not heal. With the NS, the goal is to advance these technologies to facilitate regeneration of both the PNS and CNS after injury or disease. mt

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