Neural interactions with materials

Paul Donald Dalton [1, 2], Joerg Mey [3]

[1]Deutsche Wollforschungsinstitut, Pauwelstr. 8 Aachen, Germany 52056, [2]Med-X Research Institute, Shanghai Jiao Tong University, China, [3]Institute of Biology II, RWTH-Aachen, Kopernikusstr. 16, Germany

TABLE OF CONTENTS

1. ABSTRACT

This article focuses on cell-material interactions of neurons in the context of nerve regeneration and tissue engineering applications. In this review, the cellular and molecular environment of neurons is described in both the uninjured and injured tissue. The interactions between specific materials and neurons is explained, in addition to heterogeneous substrates and guiding scaffolds that are used experimentally.

2. INTRODUCTION

In mammals, axonal regeneration with recovery of functions is only observed after lesions of peripheral nerves (PN; 1). Although neurite sprouting and even axonal growth occurs after spinal cord or brain injury, successful connections beyond the lesion-induced scar are typically not observed within the central nervous system (CNS). In addition, a phase of secondary degeneration ensues, which is even more damaging than the primary lesion. In contrast
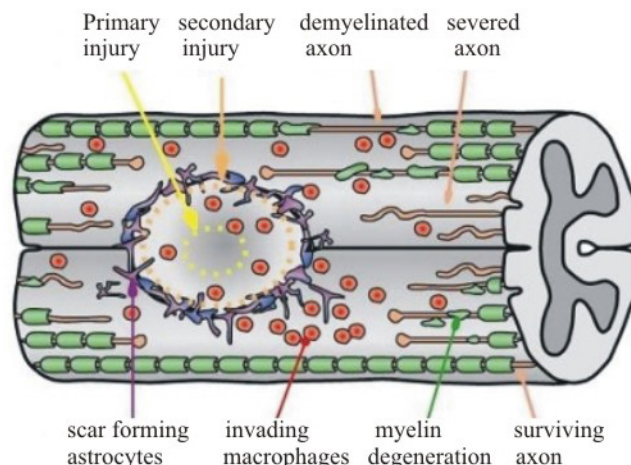
Figure 1. Physiological consequences after spinal cord contusion. Drawing by Sven Möllers (©Sven Möllers, with permission).

to peripheral nerve injury, CNS lesions therefore cause widespread neuronal death. Except for an anti-inflammatory treatment with methylprednisolone, no pharmacological treatment of spinal cord injury exists in the clinic (2). The different prospects of regeneration are believed to be largely due to different cellular and molecular components of the environment of PN and CNS and less to internal properties of the corresponding neurons: For illustration, motoneurons are located within the ventral horn of the spinal cord, i.e. CNS, yet their axons regrow in an injured peripheral nerve. Sensory neurons, on the other hand, are located in dorsal root ganglia, i.e. PNS, yet after transection of the dorsal roots their axons will not enter into the spinal cord. The use of materials in the nervous system is defined by these paradigms, and materials that influence the regenerative and inflammatory nature after injury are particularly relevant for therapeutic approaches.

3. INTERACTIONS BETWEEN NEURONS AND THEIR ENVIRONMENT *IN VIVO*

3.1. Physiological processes after PNS- and CNS-lesions

The three main problems after CNS injury are: 1- development of a growth inhibitory glial scar, 2- secondary neuronal and glial degeneration as a delayed consequence of the direct injury, and 3- the failure of axonal regeneration in white matter tracts of the adult spinal cord and brain (Figure 1). Responding to this challenge, most attention has been spent either on strategies to overcome the inhibitory barrier of the glial scar or to promote the growth of axon collaterals to compensate permanently severed connections (3). Another recent approach has been to modify inflammatory reactions in order to limit the secondary degeneration (2).

In the absence of these impediments, the main problem in the PNS remains in the reconnection of severed nerves that are separated by a large injury gap. Here the therapeutic approach demands the implantation of artificial structures that guide naturally regenerating axons. To investigate physiological processes after PNS and CNS

injury, a number of *in vivo* models are being used, most often with the rat as the favoured species (Table 1).

3.1.1. Inflammatory reaction

The consequences of spinal cord injury go beyond the disruption of the directly injured neurons and fibres. In fact, the area of secondary oligodendroglial and neuronal degeneration is far larger than the region of immediate lesion. This protracted damage is due to a disruption of blood supply and a cascade of inflammatory events largely affected by resident microglia, hematogenous neutrophils and macrophages. Depending on the type and extent of injury, the contribution of blood-derived cells to this inflammation is substantial, particularly, when the trauma causes a physical disruption of the blood brain barrier (4-6). Neutrophils are the protagonists in the immediate inflammatory response within 24 hrs after injury (7). They are followed by a second wave of macrophage/microglia activation after two days and, even later, by the activation of astrocytes (4, 8-9). By releasing reactive oxygen species it is mostly neutrophils and macrophages that cause tissue destruction. Therefore, a partial inhibition of this mechanism, e.g. with the steroid methylprednisolone, is intended in medical treatment of spinal cord injury (2, 10-11). Microglia cells also release excitatory neurotoxins such as quinolinic acid and glutamate (12). In addition, microglia and macrophages attack and phagocytose injured neurons, oligodendrocytes and the resulting debris of myelin and neurons (6, 13).

Another deleterious factor after various kinds of CNS injury is a massive cellular depolarization, which is classi-cally attributed to an increase in extracellular K^+ and release of glutamate (Glu) (14). The original cause of this is the interruption of blood supply and consequent deficit in ATP production within the cells. Excitotoxicity is often attributed to a strong increase in Glu and other physiological signals like NO or arachidonic acid, which result in a pathological increase of the intracellular Ca^{2+} concentration in neurons (15). This then activates proteases, lipases and peroxidases which may lead to necrotic cell death. Alternatively, apoptotic pathways are

Neural interactions with materials

Table 1. Frequently used animal models for the investigation of nerve regeneration *in vivo*

PNS	Sciatic nerve	crush or transection, investigation of spinal roots, dorsal root ganglia, spinal cord motor neurons; implantation of PN autografts or artificial nerve guides, behavioural analysis of hindlimb motor control
PNS/CNS	Facial nerve	transection, investigation of neurons in the nucleus of the facial nerve
PNS	Cavernous nerve	crush, transection, transplantations; investigation of major pelvic ganglion, physiological analysis of penile erection
CNS	Spinal cord	contusion injury, compression injury, hemisection, electrolytic injury; major models for molecular and cellular analysis of CNS injury, behavioural analysis of movement
CNS	Optic nerve	crush, transection; investigation of retinal ganglion cells, major model for transplantation of peripheral nerves into the CNS; physiological and behavioural analysis of visual functions
CNS/PNS	Other fibre tracts and nerves	e.g. : postcommissural fornix, bulbectomy, various stab wounds of the cerebral cortex; auditory nerve, lingual nerve transection
CNS	Excitotoxic lesions	6-OH-DA, MPTP, quinolinic, kainic, ibotenic and quisqualic acid, NMDA, cholinergic toxin AF64 and other toxins, investigation of neurodegenerative disorders, e.g. Huntington's disease (GABAergic neurons), Parkinson's disease (dopaminergic neurons), Alzheimer (cholinergic neurons); implantation of stem cells
CNS	Ischemia	middle cerebral artery occlusion (stroke model), occlusion of thoracic descending aorta (spinal cord ischemia/reperfusion)

Approaches with transgenic animals and various models for neurodegenerative and neuropsychiatric diseases are not listed.

believed to be involved, also triggered by the excess of intracellular Ca^{2+} (16).

After peripheral nerve injury the debris scavenging function is fulfilled by macrophages, and since the clearance of myelin debris is a necessary requirement for axonal regeneration, it has been suggested that a different inflammatory reaction and faster removal of inhibitory substances account for the better regenerative properties of the PNS (17-18). In both instances a large number of cytokines are synthesized, with stabilizing and with damaging effects on neuronal connections (19-23).

3.1.2. Axonal growth
Peripheral nerve injury causes Wallerian degeneration of the distal nerve segments. At the lesion site, the axotomized neurons form growth cones, followed by sprouting of neurites. Since the affected nerve cells are situated within the ventral spinal cord (motor neurons), dorsal root ganglia (sensory neurons) or paravertebral sympathetic chain ganglia (autonomic neurons), fibre growth always progresses towards the periphery. When contact remains between the proximal and distal nerve stump and even when tissue connection is provided by a muscle or a variety of implants, the regenerating axons can elongate over long distances into the degenerating peripheral nerve, which provides the most favourable substrate for this process. Neurosurgeons take advantage of these physiological properties, and more than 200,000 peripheral nerve repair procedures are annually performed in the US alone (24). In the last decade many intracellular signals that regulate or concur with axonal regeneration have been elucidated (25). Since this review focuses on interactions between cells and their environment, these cascades will not be discussed here.

Despite reports by Tello and others early in the 20th century that neurons of the adult mammalian CNS can regenerate their axons when confronted with a growth permissive environment (26), it long remained the prevalent dogma that nerve cells of brain or spinal cord lose their regenerative ability during development (1, 27). However, in the 1980s Albert Aguayo and coworkers in Montreal demonstrated that axons from spinal cord neurons

and retinal ganglion cells regenerate into implanted peripheral nerve grafts (28-29). Since then, the general conviction holds the growth inhibitory environment responsible for the absence of axonal growth after CNS injury rather than intrinsic properties of the neurons (27). Within the spinal cord or in fibre tracts of the brain, lesions are followed by the formation of growth cones and local axonal sprouting. This limited growth response is not sustained, presumably because the regenerating neurites encounter inhibitory signals within the gliotic scar and white matter myelin (30-31). It is important to keep in mind, though, that the physiology of CNS neurons does change during maturation, such that these cells cannot be easily induced to regenerate axons or dendrites, even when confronted with the same growth promoting stimuli as embryonic neurons (3, 32-33).

3.1.3. Formation of the glial scar
Astrocytes and oligodendrocytes are the predominant macroglial cells in the CNS. Like CNS neurons they derive from the neuroepithelial lineage and are absent in peripheral nerves. In response to transection, contusion or compression injury of the spinal cord, astrocytes are activated. Within several days, depending on the type of injury, they form a glial scar, which results in an impenetrable barrier for growing axons (1, 34). This scar consists of the astrocytes themselves and of extracellular matrix (ECM) material, deposited by astrocytes and by proliferating fibroblasts. The astrocytes trigger a physiological stop signal in axons that make contact (35), and the scar itself forms a physical and molecular obstacle to fibre growth (3-4, 36-37). Although this property appears to be detrimental when seen in the light of functional recovery, the scar protects the integrity of the tissue by sealing it off from the external environment, e.g. from possible infections. The scar also contributes to the healing process by pulling wound margins closer together and by increased revascularization (38).

3.1.4. Neuroglial interactions
In addition to scar formation, activated astrocytes secrete a number of signals that have positive as well as negative effects on neurite survival, the maintenance of synaptic contacts and on axonal growth (39). Depending on

Table 2. Molecular environment after traumatic injury of spinal cord and peripheral nerves

Functions	Molecules	Cellular sources
proinflammatory cytokines	**IL-1-alpha, IL-1-beta, IL-6,** IL-12, **TNF-alpha, IFN-gamma**, MCSF	**macrophages, microglia**, lymphocytes, astrocytes
other proinflammatory factors	arachidonic acid/prostaglandins, NO, glutamate, extracellular K$^+$,	**macrophages, neurons**, astrocytes
neurotrophins and cytokines that are neuroprotective or promote axonal regeneration	**NGF, BDNF, NT-3**, NT-4/5, **CNTF, GDNF, LIF,** CT-1, PDGF, IGF-1	**Schwann cells, neurons, astrocytes,** endoneurium, microglia
anti-inflammatory cytokines or neuroprotective effects	VEGF, FGF-1, FGF-2, **TGF-beta1**, TGF-beta2, IL-10	endothelial cells, glia, macrophages
extracellular matrix molecules that support axonal regeneration	**laminins**, fibronectin, collagen I/III, collagen IV; receptors: alpha-1-beta-6 and other integrins	Schwann cells, fibroblasts
extracellular matrix molecules that restrict axonal growth	chondroitin-, heparan-, keratan sulphate proteoglycans (**CSPG,** HSPG, KSPG), NG-2, tenascin C, collagen IV	macrophages, microglia, oligodendrocyte precursors, astrocytes
myelin inhibitory molecules	**Nogo**, myelin associated glycoprotein (**MAG**), oligodendro-cyte myelin glycoprotein (**Omgp**), ephrin B3	oligodendrocytes
chemorepellents that act on axonal growth cones	Semaphorins 3A-F, 4A-G, 5A, 5B, 6A-C, 7A; ephrins	neurons, astrocytes
chemokines	MCP-1, MIP-1-alpha, RANTES and others	Schwann cells, macrophages,
hormones, retinoids	T3, T4; sex hormones, glucocorticoids; retinoic acid	circulation; meninges, oligodendrocyte precursors
cell surface proteins that mediate neuroglial inter-action	**N-CAM**, PSA-N-CAM, **cadherins**, L-1, TAG1/axonin-1, **neuregulin**, ErbB2/B3, sonic hedgehog, desert hedgehog	glia, neurons
cell surface receptors that mediate immune responses	ICAM-1, B7-1, MHC-I, MHC-II, LFA-1, complement receptors, integrins: alpha-M-beta-2, alpha-4-beta-1, alpha-L-beta-2, alpha-X-beta-2, Fc-gamma receptor	lymphocytes, macrophages/microglia

Molecules are listed with their predominant function. Many have secondary and often competing effects; for instance, cytokines of the TGF-beta family can also be proinflammatory and IL-6 neuroprotective. Collagen is used as a scaffold for regenerating axons, but collagen IV also forms a basis for the growth repellent glial scar. Factors with particular relevance, as gathered from a high density of publications, are in bold type.

their state of differentiation, the interaction between astrocytes and neurons can be conducive to neurite outgrowth because they provide trophic support, energy substrates, and remove excess glutamate and free radicals (see below; 40).

Oligodendrocytes form the myelin sheaths in the CNS. Their contribution to traumatic reactions has not been investigated as extensively as that of astrocytes. Most importantly, the inner sheaths of myelin contain a number of growth inhibitory molecules (Table 2; 31,41). Axonal growth cones, when exposed to these molecules, collapse and do not continue to grow. While the secretion of injury-related signals by oligodendrocytes seems less important than that of astrocytes or microglia, recent investigations suggest that they, too, play a role in this context (42-43).

Schwann cells (SC) originate from the neural crest. Their main function is the myelination of peripheral nerves. Although the PNS myelin is as inhibitory to axonal growth as CNS myelin, SC secrete a basal lamina that remains present during Wallerian degeneration and provides an excellent growth substrate for elongating axons. In addition, SC are a source of neurotrophic molecules (44-45). Interactions between SC and neurons are thus both neuroprotective and conducive to axonal regeneration (46). This is also true for olfactory ensheathing cells (OEC), the myelinating glia of the olfactory tract (47). SC and OEC have been used as cellular implants in many approaches to induce regeneration or limit secondary degeneration after CNS injury (47-50).

3.2. The cellular environment
3.2.1. Microglia and macrophages in the CNS
Unless a major insult or infection with disruption of the blood brain barrier allows lymphocytes to enter the CNS, the microglia provides immunoprotection of the

tissue. In contrast to the macroglial cell types described above, microglia belongs to the monocyte lineage of mesodermal origin. In non-injured CNS the resting microglia has long ramified processes that are oriented parallel to nerve fibres in the white matter and display a stellate morphology in the grey matter. Upon injury, infection, ischemia or autoimmune-mediated inflammation, microglia undergo a rapid transformation to an activated condition. This is characterized by an increase in the size of the cell body, a retraction of distal and thickening of proximal branches (4, 6, 51). They can further be converted into a phagocytotic state and are then mor-phologically indistinguishable from hematogenous macrophages, which appear in regions where the blood brain barrier is disrupted. As mentioned above, microglia and macrophages are largely responsible for the inflammatory reaction. As such they express IgG, receptors for Fc-gamma and complement fragments, the alpha-M-beta2-integrin and ICAM1, a cell surface receptor for integrins that mediate adhesion to granulocytes, lymphocytes and other microglia cells (52). During various stages of activation, additional integrins, cell adhesion molecules, chemokine receptors, MHC class I and MHC class II are expressed, and a number of cytokines are secreted (Table 2). When lymphocytes enter the CNS, they produce an array of microglia activating cytokines, particularly IFN-gamma (6, 53). Other circulating pro-inflammatory molecules that diffuse into the CNS compartment and regulate microglia/macrophage activity include TNF-alpha, IL-1 and chemokines. The second major monocyte-related cell type in the CNS are perivascular macrophages located between the neural parenchyma and the vascular endothelial cells. A related function and similar position between endothelial cells and basal membrane have meningeal macrophages. Both phagocytotic cell types are immunoreactive for ED2, macrophage scavenger receptor, and MHC-II (54).
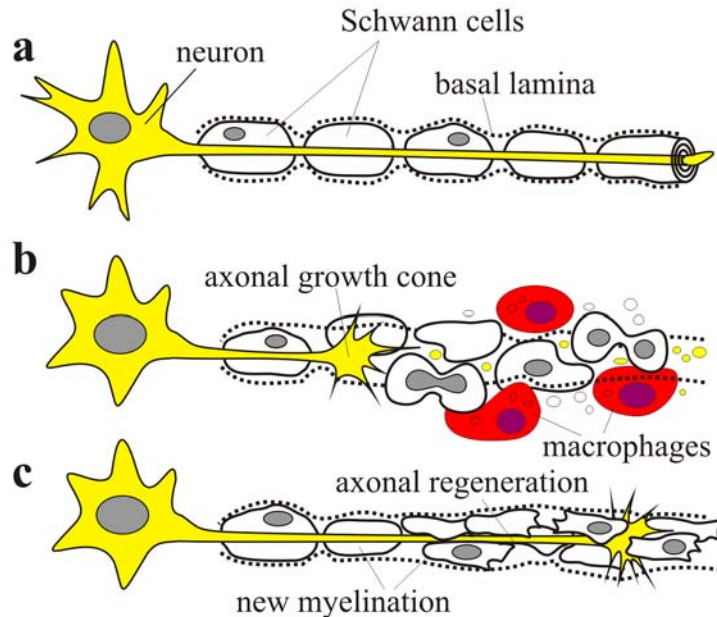
Figure 2. Physiological consequences after peripheral nerve injury. a Most neurons whose fibres run through peripheral nerves are located in the ventral spinal cord (motoneurons), dorsal root ganglia (sensory neurons) or paravertebral sympathetic chain ganglia (autonomic neurons). Their axons are insulated by Schwann cells (SC), larger, fast conducting fibres are myelinated. SCs are surrounded by a basal lamina. b Wallerian degeneration: hematogenous macrophages phagocytose debris from degenerating axons and myelin. The remaining basal lamina provides a growth substrate for axons. The neuron is metabolically challenged and forms a growth cone at the lesion site. Schwann cells dedifferentiate and proliferate. **c** Axonal regeneration, weeks after injury. Schwann cells re-differentiate, ensheath and subsequently re-myelinate the elongated fibres (©Jörg Mey, redrawn after ref. 67).

3.2.2. Macrophages in peripheral nerves

While peripheral nerves and spinal roots contain a fair proportion of resident monocytes, additional hematogenous macrophages are recruited after PN injury (Figure 2). Their phagocytotic activity is of crucial importance for the removal of myelin debris and products of neuronal degeneration, and this is a prerequisite for successful axonal regeneration (20-21, 55). Several populations of macrophages have been identified in the PNS. They include ED1/ED2 positive endoneurial macrophages which show a transient expression of MHC-II after injury. These resident cells are related to the perivascular macrophages of the brain (54, 56). They are replaced by bone marrow-derived cells from the blood with a turnover half life of less than three months in the rat (57). A large number of MHC-II positive cells that are negative for ED1 and ED2 enter peripheral nerves and spinal roots after injury, and yet another population of ED1 positive macrophages are also blood-derived (56). The invasion of macrophages involves loose contact with vascular endothelial cells, then integrin-mediated adhesion, perception of chemoattractants, penetration of endothelium and basal lamina and differentiation into tissue macrophages (21, 58).

With respect to the preservation of tissue integrity and axon regeneration, the role of macrophages seems ambivalent: although their activity is quite necessary for axonal regeneration in peripheral nerves, in the CNS the influence of microglia/macrophages is more often considered destructive because of their role in immune-mediated neuropathies (21, 59, 62), and because better axonal growth is observed in the spinal cord of mice with a reduced inflammatory response (10, 60). Similarly, the application of the microglia inhibitory tripeptide Thr-Lys-Pro after optic nerve injury prolonged survival of retinal ganglion cells and supported axonal regeneration in peripheral nerve grafts (13, 61).

3.2.3. Astrocytes

Astrocytes are the most numerous macroglia cells of the CNS. They perform a number of essential tasks in normal physiology of the nervous system, including K^+-buffering, regulation of the blood-brain-barrier, neurotransmitter recycling and anaplerotic reactions of which the neurons themselves are incapable. Various forms of CNS injury activate astrocytes. This activation is characterized by an increase in metabolism, cellular hypertrophy, and a marked increase in expression of intermediate filaments glial fibrillary acidic protein (GFAP), nestin and vimentin. In line with their task of securing homeostasis of the nervous tissue, the main function of astrocytes after injury appears to be the formation of the glial scar, thereby insulating the healthy tissue from uncontrollable processes in the damaged area. Unfortunately, this scar presents a major obstacle for regenerating axons (36, 63). On the other hand, the astrocytes' metabolic reaction represents an adaptive response to protect other brain cells from energy depletion, free radicals, ammonia and Ca^{2+} overload (40).

Two types of astrogliosis can be distinguished, anisomorphic and isomorphic gliosis. At the site of tissue disruption after an open injury anisomorphic gliosis is observed. This entails astrocyte proliferation, hypertrophy and scarring (64). In isomorphic gliosis, astrocytes are activated but retain a stellate morphology. This is the response to a milder insult where the blood brain barrier remains intact or at a distance from the injury. It is less dramatic, transient and is associated with improved functional recovery (40, 65). A paracrine release of growth related factors (Table 2) constitutes an important beneficial influence of astrocytes for the long-term survival of affected neurons (66).

3.2.4. Olfactory ensheathing cells and Schwann cells

Schwann cells myelinate axons in peripheral nerves, and OEC, which comprise a number of histo-chemically identifiable subtypes, perform this task in the olfactory tract and bulb (47). Although the olfactory bulb belongs to the CNS, this tissue and its glial cells share important characteristics of the peripheral nervous system. In mammals, receptor cells in the sensory epithelium project an axon into the olfactory bulb, where synapses are formed within the glomeruli. Fast turnover of olfactory receptor cells throughout adult life requires constant axonal growth and synaptic plasticity in the olfactory bulb. Though OEC share some metabolic properties with astrocytes, this physiological situation implies that they support rather than restrict axonal growth. Indeed, Schwann cells and OEC promote axonal regeneration and remyelinate regenerated nerve fibres in various lesion models (47, 67).

Following a peripheral nerve crush, the loss of contact to viable axons causes dedifferentiation and proliferation of the Schwann cells. These activated Schwann cells supply a number of trophic and chemotactic signals that are crucial for macrophage activation as well as for neuronal survival, the initiation of neurite outgrowth at the site of injury and axon regeneration into the distal segment of the nerve (68-69). As soon as nerve axons grow toward their target, neuroglial interactions induce Schwann cells to re-differentiate and to re-myelinate the newly formed fibres (67).

This growth promoting activity of Schwann cells is illustrated by the fact that the inhibitory properties of the dorsal root entry zone correlate with the proximal border of the Schwann cell domain. It is important to note that intact peripheral nerves, similarly to CNS fibre tracts, do not support axon regeneration (70-71). Therefore, a switch of the mature Schwann cells to a growth-promoting state seems to be necessary for axon elongation within peripheral nerves. While the spinal cord does not normally contain Schwann cells, following compression, contusion or electrolytic lesions that cause oligodendroglial cell death and demyelination, a massive infiltration of Schwann cells also occurs into spinal cord tissue (46, 72). The cells accumulate along and around lesioned fibre tracts and ensheath axons. Since this invasion and replacement of myelin, which appears to be triggered by microglial signals, develops rather late, 3-4 weeks after injury (72), it may constitute a long-term repair mechanism.

3.2.5. Oligodendrocytes, meninges and synantocytes

By providing the axonal myelin sheath, oligodendrocytes are necessary for the normal propagation of electrical activity in the CNS. With respect to regeneration, three aspects of the interactions between oligodendrocytes and neurons deserve particular attention: 1) During the first two days following spinal cord injury, oligodendrocytes are particularly prone to apoptotic cell death (73). This is accompanied by degradation of myelin sheaths, which compromises nerve cell functions directly, and affects neurons indirectly because de-myelination renders them vulnerable to microglial attack and phagocytosis (74-75). 2) The growth-inhibitory properties of CNS myelin prevent plastic changes and compensatory regeneration from fibre tracts not directly affected by the lesion. Responsible for this are a number of molecules within the oligodendrocytic membranes, which in the non-lesioned CNS probably serve to restrict uncontrolled sprouting (76). The most important candidates are Nogo-66, myelin associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (Omgp; 31, 72) and ephrinB3 (77). 3) On the positive side, oligodendroglial signals support survival of injured neurons. This property has long escaped scientific attention, but nonetheless, secreted molecules from oligodendrocytes, including glial-derived neurotrophic factor (GDNF; 78), insulin-like growth factor-I (IGF-I; 79), leukemia inhibitory factor (LIF; 80) and neurotrophins (81), are likely to be neuroprotective.

The movement of meningeal cells into the damaged spinal cord creates an impediment for axon re-generation because these cells are also components of the glial scar (34). However, meningeal cells can release growth factors, which may explain why implantation of meningeal fibroblasts into injured rat spinal cord promotes axonal growth (82). Meningeal cells influence the proliferation and maturation of neuroblasts (83) and synthesize bone morphogenetic proteins (BMPs; 84), basic fibroblast growth factor (FGF-2), transforming growth factor beta (TGF-beta; 85) and insulin-like growth factor-II (IGF-II; 86). Recently, retinoic acid (RA) was shown to be synthesized by meninges, oligodendrocytes and, induced after spinal cord injury, by a subpopulation of NG2 expressing cells (43). NG2 positive cells resemble protoplasmic astrocytes. They include mainly oli-godendrocyte precursors but may also be able to generate neurons (87). They have been characterized as a novel form of glia, referred to as synantocytes (88) or polydendrocytes (89). A subpopulation seems to be particularly relevant in the context of this review because they proliferate after injury and are responsible for the increased production of extracellular signals (42-43, 90-91). These cells may be inhibitory to axonal growth given that this is a property of the NG2 epitope (92), although it is not clear whether NG2 prevents axon growth in the injured spinal cord (93).

3.3. The molecular environment
3.3.1. Neurotrophins

The degenerating distal stump of an injured peripheral nerve is not only able to guide axonal elongation within but has an additional chemoattractive quality

directed at growing neurites. This tropic influence seems to be effective only within a short distance, however (94). Molecules that are secreted in a paracrine fashion within the lesioned tissue, be it a peripheral nerve or the CNS, trigger the major events associated with axonal regeneration: In peripheral nerves, neuregulins are secreted by axons and provide crucial signals to the Schwann cells to control their state of differentiation in general and myelination in particular. Cytokines are important mediators of the local inflammatory reaction immediately after injury, while neurotrophins and the GDNF-family of cytokines protect injured neurons and transform injured peripheral nerves into a growth promoting substrate (Table 2; 95).

The neurotrophins are a family of peptides that support survival, axonal growth and differentiation in a variety of neuronal populations. With the discovery of the activity of nerve growth factor (NGF) in the 1950s, they are historically the first family of neurotrophic molecules, and because of their potent effects on neurons, the related scientific literature is quite overwhelming. In March 2008, a Medline search on "NGF" alone revealed almost 10,000 publications. In mammals, the neurotrophin family comprises NGF, brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3 and NT-4/5. The peptides act as noncovalent homodimers and bind to two classes of receptors, the Trk-family of receptor tyrosine kinases and p75, a member of the tumour necrosis factor-alpha (TNF-alpha) receptor family (96). Their signal transduction pathways have been the subject of many reviews (e.g. 97-99). Their main functions are support of neuronal survival during development, guidance of axonal growth and the regulation of synaptic plasticity (97, 100). All factors and receptors show marked changes in expression after lesions in CNS and PNS and are involved in various disorders (98, 101-104). Consequently, neurotrophins are one class of molecules that was soon explored in clinical studies to treat neurodegenerating diseases – albeit with questionable results so far (105-106).

Related functions are served by the GDNF family. It consists of four members, GDNF, neurturin, persephin and artemin. They signal through a receptor complex which consists of a high affinity binding subunit (GFR-alpha) coupled to the signal transduction unit RET. Different ligand binding subunits exist, which confer specificity to the system: GFR-alpha1 (binds GDNF), GFR-alpha2 (neurturin), GFR-alpha3 (persephin) and GFR-alpha4 (artemin; 107-108). Peripheral nerve lesions cause a marked increase in the expression of GFR-alpha1, GFR-alpha3, a decrease in GFR-alpha2 and little change in RET (109). GDNF is a survival factor for mesencephalic dopaminergic neurons and motoneurons, and the other members of the family promote the survival of sympathetic and other peripheral neurons (97, 108).

3.3.2. Cytokines and chemokines

Within hours after injury, an increase of the inflammatory cytokines interleukin (IL)-1-alpha and tumour necrosis factor (TNF)-alpha is observed, rapidly followed by IL-1-beta and IL-6 (95, 110-111). In a neuroscience context, peptides of the IL-6 type are referred to as neuropoietic cytokines or neurocytokines. Their members signal via the common receptor gp130 plus one or two specific ligand binding receptors. Ligand binding causes phosphorylation of the transmembrane protein gp130, recruitment of Janus kinases, phosphorylation of the STAT transcription factors and the activation of other intracellular signal transduction chains including the ras/raf-MAP kinase pathway (112). The neuropoietic cytokines are particularly important for neuronal regeneration because, on one hand, they exert neurotrophic functions (CNTF, LIF, IL-6, CT-1), and on the other hand are important mediators of the inflammatory reactions after injury (IL-6, IL-11, MCSF). Their receptors are expressed by neurons and glial cells of the central and peripheral nervous system. There are specific differences in the activation of the Jak/STAT signalling pathway after lesions in CNS and PNS (113).

The transforming growth factor beta (TGF-beta) family has been implicated in the induction of Schwann cell proliferation, production of extracellular matrix and neurotrophin synthesis as well as synthesis or repression of cell adhesion molecules (114). TGF-beta1 is important as an anti-inflammatory cytokine. Two types of TGF-Beta transmembrane receptors with serine/threonine kinase activities exist (T-betaR-I and T-betaR-II). After ligand binding they form a stable ternary complex and phosphorylate Smad2/Smad3 transcription factors. Gene activity is then activated by complexes of phosphorylated Smad2/Smad3 with Smad4 molecules (115). In addition, TGF-beta binds to the extracellular matrix (67, 104, 111).

Since neuroprotective activity was reported for erythropoietin (EPO) 10 years ago, EPO has come to be seen as a critical component of a generalized system of local tissue protection involving multiple mechanisms (116). Research is now under way to develop and test variants, for instance carbamylated EPO, that do not have hematopoietic side effects for the treatment of brain ischemia and injury.

Vascular endothelial growth factor (VEGF) is a secreted homodimeric heparin binding glycoprotein, whose best known functions are as mitogen and attractant for vascular endothelial cells (117). Four transmembrane receptors for VEGF are known: fms-like tyrosine kinase-1 (flt-1, VEGF-R1), foetal liver kinase-1 (flk-1, VEGF-R2), flt-4 (VEGF-R3) and neuropilin (NP, which also binds to semaphorin 3A). The tyrosine kinases flt-1 is found in neurons, endothelial cells in the brain, astrocytes and microglia cells. Endothelial cells and neurons also express flk-1. In addition to important functions for the cerebral vasculature, VEGF and its receptors are neuroprotective under ischemic conditions and various brain lesions, which may be related to glial activation and blood supply (117). The cytokine enhances proliferation of astrocytes and microglia (118), but direct neuroprotective effects of VEGF without vascular and glial involvement have also been reported (119).

Chemokines are small peptides (<10 kDa) that are secreted locally and attract lymphocytes and

macrophages towards the focus of an inflammatory process. They activate a family of G-protein coupled transmembrane receptors. A variety of chemokines and chemokine receptors are expressed in the nervous system (120). During Wallerian degeneration the chemokines macrophage inflammatory protein 1-alpha (MIP1-alpha) and monocyte chemoattractive protein 1 (MCP-1) as well as the cytokines TGF-beta-1 and IL-1-beta are involved in macrophage recruitment (21). Especially MCP-1 is strongly upregulated early following sciatic nerve lesion and remains elevated for a long time (121). After spinal cord hemisections MCP-1 and MIP1-alpha are also induced but not as much as in the periphery (122). Another chemokine, RANTES (Regulated upon Activation Normal T cell Expressed and Secreted), showed a lesion-induced increase in Schwann cells and endothelial cells, but the changes in RANTES expression are lower, and its function has not yet been resolved (121-123).

3.3.3. Hormones and retinoic acid

A number of hormone receptors are expressed in PNS and CNS tissue, including receptors for thyroid hormone, growth hormone and retinoid receptors. Not many experiments have been performed to explore whether differential activation of these receptors is involved in the responses to injury or may be used to experimentally promote regeneration. Hormones reach target tissues via the circulation, and therefore the local response involves regulation of receptors rather than hormone production (124-125). Thyroid hormones (T3, T4) support survival and neurite outgrowth from rat sensory neurons, their receptors are regulated by Schwann cell-axon contact, and local treatment with T3 increases sciatic nerve regeneration *in vivo* (124). Growth hormone has also been reported to improve nerve regeneration, for instance after cavernous nerve injury (126-127). The use of corticosteroids to limit the inflammatory reaction has already been mentioned (11, 128). It was shown that the rate of axonal regeneration and motor neuron survival following axotomy correlates with the level of circulating testosterone (129-130).

Similar to steroids or thyroid hormone, retinoic acid (RA) binds to receptors of the nuclear receptor superfamily, thereby acting as a transcriptional activator, but in contrast to hormones it is synthesized locally. In the nervous system, RA regulates neuronal and glial differentiation and controls the expression of a number of intercellular mediators that participate in nerve regeneration (131), suggesting that this is also involved in traumatic events after injury (132-133). This is demonstrated by recent experiments with sciatic nerve lesions and spinal cord contusion injury. In both tissues, the RA synthesizing retinaldehyde dehydrogenase-2 is expressed, and its activity was found to increase after spinal cord contusion injury (43). In the PNS, lesions cause RA-induced gene transcription, intracellular translocation of retinoid receptors and increased transcription of cellular retinoid binding proteins and retinoid receptors (134-135). Activation of the receptor RAR-beta appears to be responsible for neurotrophic effects of RA (32, 136). While the physiological role of RA in the injured nervous system is still under investigation (137), studies have recently been performed to support nerve regeneration with RA or RAR-beta *in vivo* (138-140).

3.3.4. Extracellular matrix

With respect to nerve regeneration two components of the extracellular matrix (ECM) are of primary importance: basement membranes and the glial scar. In the PNS, the remaining basement membranes of degenerating nerve segments provide the superior growth substrate for axons. De-differentiated Schwann cells that promote axonal regeneration are aligned inside the basal lamina tubes, the so-called bands of Bungner. After crush or transection these basal lamina tubes persist in the distal nerve stump and provide an important scaffold for regrowing axons (141). Experiments with many of the various ECM molecules demonstrate that the ability to support fibre growth is largely due to laminin. Laminins are not only crucial for axonal regeneration but also for Schwann cell differentiation and myelination in the injured PNS (142). Therefore, laminins are frequently used as a coating for experimental growth substrates (see 4.1.). Common and highly abundant ECM proteins are the collagens (143). Collagen is secreted by fibroblasts, endothelial cells and astrocytes and also has a positive influence on fibre growth, even within the CNS (144).

In the CNS the astrogliotic scar poses the main barrier for regeneration. The molecular substrate for this inhibition seems to be heparan sulphate proteoglycans and chondroitin sulphate proteoglycans (CSPG; 3, 63). These ECM molecules consist of a protein core linked by four sugar moieties to a sulphated glucosaminoglycan (GAG) chain of repeating disaccharide units. Astrocytes produce four classes of proteoglycans. These are heparan-, dermatan-, keratan- and chondroitin sulphate proteoglycans (145). The expression of a number of CSPG, e.g. aggrecan, brevican, neurocan, NG2, phosphacan, increases after spinal cord lesions. They were found to restrict axonal growth (92). While inhibitory proteoglycans are exclusively expressed in neurons in the non-lesioned tissue, their main source after injury are reactive fibrous astrocytes. The presence of inhibitory CSPG seems to be a major cause for the failure of regenerating axons to penetrate the gliotic scar. In cases where axonal regeneration does take place as in the embryonic spinal cord or in cold blooded animals there is no or very little upregulation of CSPG after injury (145). In addition, the ECM of gliotic scars contains collagen IV, glycoproteins laminin, fibronectin, keratan sulphate proteoglycans, tenascin C, and thrombospondin (146).

4. INTERACTIONS BETWEEN NEURONS AND MATERIALS *IN VITRO*

4.1. Biologically derived materials

While numerous important molecules in the natural environment of the neuron have been identified, only a few have been investigated with tissue engineering approaches. Measurable outcomes of *in vitro* investigations for tissue engineering applications typically focus on adhesion and neurite outgrowth (i.e. length of neurites). The ECM is therefore of particular importance, and many
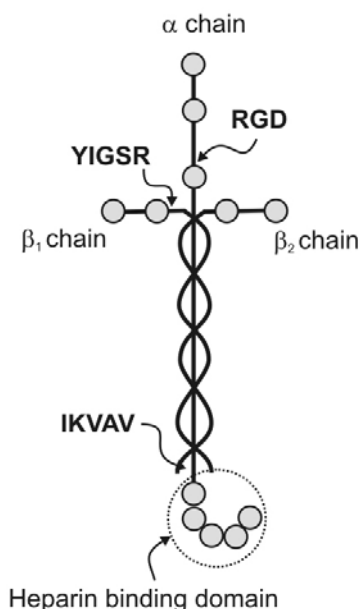
Figure 3. Structure of laminin-1 and selected well-known domains and binding sites. The sites that bind with integrin receptors are indicated; however the RGD site (which is well known for integrin binding) is not accessible until proteolytic cleavage of the adjacent domain.

components of the basal lamina tubes provide robust growth of neurons and neurites *in vitro*.

4.1.1. Laminin

Many peptide sequences investigated for neuron/material interactions are derived from various bioactive domains of laminin, a basement membrane glycoprotein. The family of laminins all have high molecular weights, ranging from under 500 kDa to nearly 1 000 kDa and forms a "cross-shaped" molecule (Figure 3). Fifteen laminin heterotrimers have been identified in mammals, and each isoform consists of three polypeptide chains; one alpha, one beta and one gamma chain. Laminin-1, appears to be the major laminin expressed during embryogenesis (147) and consists of alpha-1, beta-1 and gamma-1 chains with individual molecular weights of approximately 400 kDa, 210 kDa and 200 kDa respectively (148). In total, five alpha, three beta and three gamma polypeptide chains have been identified in mammals. Laminin provides multiple biological signals to a variety of cells including the promotion of adhesion, neurite outgrowth, cell spreading, cell growth, tumour metastasis and collagenase IV secretion. While the sequential adsorption of PLL/laminin onto polystyrene culture wells is an important positive control for *in vitro* culture, and rigorously supports axonal growth, laminin is difficult to isolate, process and incorporate into a functional *in vivo* material. Therefore, peptide sequences from the laminin chain have been widely used for delivering bioactivity to tissue engineering devices for the nervous system.

The well-investigated cell adhesive sequence of RGD (peptide fragments such as Arg-Gly-Asp will be described in the shortened form i.e. RGD) is present in the alpha chain of laminin and is reviewed elsewhere (149). While there are regions of laminin which are integrin binding, these are not simple peptide motifs but significant sections of the protein. The RGD sequence in laminin, however, is accessible only to cells after proteolytic degradation of the adjacent domain (150). *In vitro*, the neurite extension has been found to be dependant on the concentration of RGD, and excessive RGD peptides incorporated with another ECM, fibrin, have shown to reduce the extent of neurite outgrowth (151).

The peptide sequence of YIGSR, however, can induce greater neurite lengths than RGD (152) and is widely used as a ligand for *in vitro* applications (153-154). YIGSR, located on the beta-1 chain (Figure 3), interacts through a 67 kDa laminin binding protein on the cell membrane. The amide-terminated arginine (YIGSR-NH$_2$) has twice the bioactivity of YIGSR-COOH, and similar activity to CDPGYIGSR-COOH, the extended form of the peptide (155). Adhesion of various cell types on synthetic materials is possible through adsorption of YIGSR-modified molecules to the surface, or via chemical binding (156-157).

Another notable peptide sequence of laminin is IKVAV, located on the C-terminal end of the long arm of the alpha-1 chain (Figure 3). This sequence promotes cell adhesion, neurite outgrowth, experimental metastasis, collagenase IV activity, angiogenesis, plasminogen activator activation, cell growth, tumour growth and differentiation of progenitor cells (147, 158-159). A 110 kDa membrane-associated laminin binding receptor binds to IKVAV (160-161).

Combinations of peptides can demonstrate negative, additive and synergistic effects on the neurite length of neuronal cultures (151). For example, in modified fibrin matrices, the combination of RGD and IKVAV reduces neurite lengths compared to either peptide used alone. Conversely, the synergistic effect of the peptide combinations of RGD, YIGSR, IKVAV and RNIAEIIKDI peptides in functionalizing fibrin induces neurite extension greater than the individual effects of the peptides combined. The combination of two particular peptides – YIGSR and IKVAV – has been used to promote robust adhesion and neurite outgrowth on fluorinated polymer surfaces (162). Although the active peptide sequences aforementioned are effective at promoting neuron adhesion and axonal growth, the use of extended peptides (i.e. CDPGYIGSR and CQAASIKVAV) further improves the cell response to equivalent levels to that achieved with laminin-modified surfaces (154). It is likely that the extended peptide results in a more 'natural' configuration of the active sequence. Spacer groups, which do not contribute to the bioactivity, also increase the extent of neurite outgrowth by allowing more conformations of the active peptide (162).

4.1.2. Collagen

The many types of collagen are described elsewhere – a Table of collagen types and distributions is
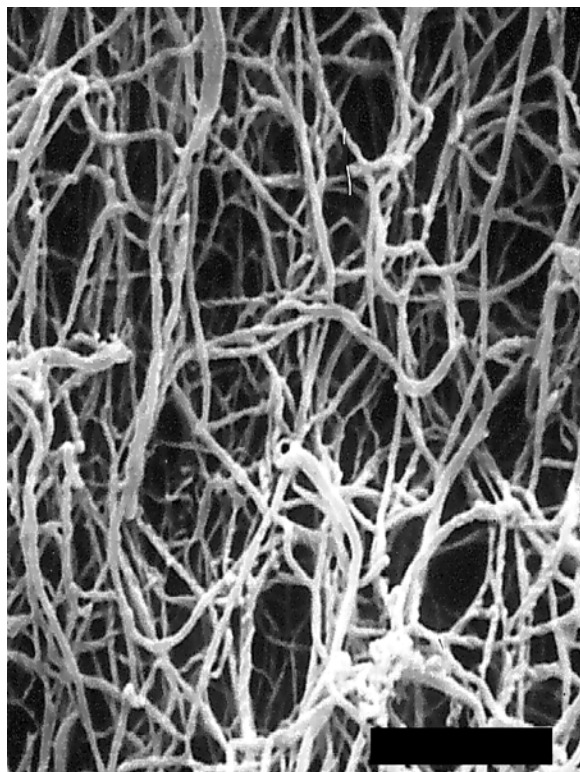
Figure 4. Magnetically-oriented matrices are possible when diamagnetic or paramagnetic molecules are gelled in a high strength magnetic field (c.a. 4-10T). A scanning electron microscope image of collagen in the presence of a magnetic field is shown. Image is provided courtesy of Professor Xavier Navarro of Universitat Autónoma de Barcelona, Spain. The scale bar for (A) is 2 microns.

well-summarized in Gelse *et al.* (143) Collagen IV performs comparatively better than other forms of collagen *in vitro*, with longer neurites from DRGs observed (163). Collagen IV is non-fibrillar and is present in basement membranes. Collagen is also a diamagnetic anisotropic molecule and can be oriented during matrix formation in high strength magnetic fields (Figure 4). Collagen gels formed in 9.4 Tesla (T) had oriented fibrils and promotes improved *in vitro* guidance of DRG neurites (164). This improved regeneration is also reflected for *in vivo* studies (165).

Collagen I has been successfully used as a matrix for cell entrapment and can be adsorbed onto TCP surfaces as a control for robust neurite outgrowth. When growth through a matrix is desired, the concentration should be low enough to permit this. Concentrations between 1.0 and 2.0 mg/ml have been used and are identified as optimum values for the matrices used for *in vivo* PNS experiments (166-167). A commercially available Vitrogen 100™, containing 95-98% collagen I, and 2-5% collagen III is often used in encapsulation or neurite outgrowth studies. The *in vivo* performance of collagen as a regenerative matrix in the PNS is excellent (167) and improved with growth factors such as FGF (168). Chondroitin-6-sulphate has also been

combined with collagen and used as a matrix for nerve guidance channels. Introduced as low concentrations, the collagen-GAG matrix induced peripheral nerve regeneration to the level of the autograft (169). The introduction of 2% chondroitin-6-sulphate to collagen increases the regeneration capacity of the matrix, and may be due to reduced diffusion of growth factors with GAG binding sites. Such a matrix should therefore only promote regeneration in the presence of diffusible growth factors.

Collagen nerve guides have demonstrated regenerative capacities for both the spinal cord, peripheral nerve and dorsal roots (170-172). Axons penetrate into the lumen of collagen nerve guides in all instances, including the fully transected spinal cord.

4.1.3. Fibrin

Fibrin is spontaneously formed in many examples of wound healing, and is a temporary matrix (1-2 weeks) prior to remodelling and ECM formation. A fibrin bridge is formed within nerve guides used in the PNS, and is the ECM upon which regenerating axons and Schwann cells migrate. Commercial fibrin precursors contain two components; one of fibrinogen, plasma fibronectin, factor XIII, plasminogen, aprotinin, human albumin and the second consisting of thrombin. The fibrinogen is polymerized with the addition of thrombin, while factor XIII takes part in crosslinking. The eventual morphology of fibrin is greatly influence by a number of factors including the Ca^{2+} concentration. The resulting hydrogel is a matrix with high water content, and is of particular interest in the spinal cord, where fibrin demonstrates regeneration (173-174). The introduction of fibrin to fully transected hydrogel nerve guidance channels resulted in significant brain-stem labelling from retrograde tracing.

Biologically active peptides can be introduced into fibrin matrices, by the synthesis of peptides containing Factor XIII in one domain, and the bioactive peptide in another (151). In such experiments, the peptide sequences of RGD, YIGSR, IKVAV, RNIAEIIKDI or N-cadherin were synthesized with a substrate for Factor XIII (NQEQVSP). The functionalized fibrin matrices were formed around entire rat DRGs, and neurite outgrowth was compared with non-functionalized fibrin. Increased neurite length was observed from YIGSR, IKVAV or RNIAEIIKDI modified fibrin with increasing peptide concentration, while RGD and N-cadherin-modified fibrin increased neurite length, but decreased upon higher concentrations.

Heparin-modified fibrin can significantly retard the diffusion of nerve growth factor (NGF), so as to deliver neurotrophins locally to the invading neurites (175). A low-affinity heparin binding domain is present on BDNF and NT-3, and therefore neurotrophin diffusion through a heparin-modified fibrin is slowed. When heparin is present at concentrations much higher than the growth factor, DRG neurite extension is enhanced two-fold. When the growth factors are not delayed by the heparin, the extended neurites are not influenced.
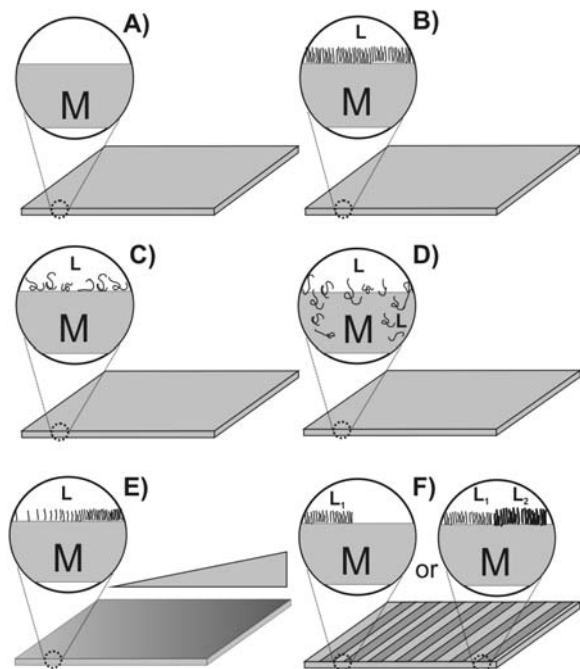
Figure 5. Schematics of various 2D substrates depicting A) unmodified substrates of material M; B) covalently attached ligands (L) to material surface (e.g. 162); C) adsorbed ligands onto material surface (e.g. 189); D) entrapped ligands within material (e.g. 163); E) ligands bound to the surface in a linear gradient (215) and F) patterned substrates with either the material or a second ligand as one of the regions (e.g. 237).

Fibrin, like collagen, can be oriented with high magnetic fields during matrix formation. The concentration of Ca^{2+} is also critical on whether the resulting fibrin matrix is of an oriented nature. Higher concentrations were strongly oriented by magnetic fields in the order of 4-10T (176). Various growth factors have accompanied fibrin for *in vivo* applications (174, 177).

4.1.4. Matrigel™

Matrigel™ is a commercially available basement membrane solution originating from mice and containing predominantly laminin, but also heparin sulphate proteoglycans, entactin, nidogen and growth factors and is available at a concentration of 12 mg/ml. When diluted to 4 mg/ml, Matrigel™ supports excellent regeneration when used in the PNS similar to collagen (167). Magnetically oriented scaffolds can be formed when the field is applied during its gelation, similar to collagen and fibrin. *In vivo* PNS studies of magnetically aligned Matrigel™ or collagen demonstrate superior regeneration to their non-aligned forms (178).

Many successful cell transplantation strategies for the spinal cord have been pioneered with Matrigel™ (179-181). Improved axon penetration results when Matrigel™ is combined with cellular transplantation; when used alone the brainstem labelling is lower in the fully transected spinal cord when compared directly with other matrices, such as fibrin (174). Due to its origin from a sarcoma cell line, the use of Matrigel™ for clinical applications is problematic.

4.1.5. Fibronectin

Fibronectin contains RGD sequences that are well-known for interacting with integrin receptors on the cell membrane. Fibronectin (FN), which has binding sites for collagen, is soluble in plasma, while in ECM components, it adopts a fibrillar structure. FN is particularly susceptible to chain transformations due to shear-induced mechanisms and the resulting oriented substrates support neurite growth both *in vitro* and *in vivo* (182-183). The configuration in which a protein is adsorbed onto a surface is critical for controlling cell behaviour and depends on the substrate for adsorption (Figure 5c). Proteins may be denatured during adsorption; there may be a tendency for proteins to unfold to allow further binding with the surface (184). FN's biological activity has been found to be influenced by the substrate type and whether it is co adsorbed with another protein (185-186). Surface-induced conformational effects are prevalent, and this affects the exposure of the cell-adhesive domains of the protein (187). Synthetic polymers with FN functionalities also adsorb readily with bioefficacy onto surfaces (187).

The reported effects of fibronectin on neurite growth, however, can be contradictory. Proteolytically obtained fragments of FN influenced the neurite outgrowth of PNS and CNS in contrasting manners, including the growth, then retraction of CNS neurons when exposed to 80 to 125 kDa fragments of FN (188) while DRG outgrowth is maintained. Adsorbed fibronectin on TCP resulted in NGF-primed PC12 cells extending few axons and having a rounded shape (189), and neurite outgrowth of embryonic retinal cells is lower when compared to laminin (190). The addition of FN also inhibited the aggregation of PC12 neurons encapsulated by collagen gels (166). In contrast, when FN is entrapped within pHEMA, the neurites of DRGs are extended to a similar length as with collagen III (163). Fibronectin mats and conduits have also performed very well for *in vivo* applications; both in the PNS and spinal cord (182-183). Interestingly, fibronectin has been implicated in impairing the ability of oligidendrocytes to remyelinate CNS axons (191).

4.1.6. Agarose

Agarose is polysaccharide extracted from seaweed, and will form physical gels after dissolution in water and cooling. Agarose has been primarily used for performing three dimensional (3D) *in vitro* culture (192-193). While agarose gels can be chemically modified for superior neurite outgrowth, pure agarose gels will also support the extension of neurites (193). *In vitro* culture models for the glial scar have also been developed based upon layered agarose hydrogels, with and without chemical modification. A difference in the elastic modulus of agarose is sufficient as a barrier for DRGs while when a chondroitin sulphate-modified agarose is formed, the growth of DRG neurites was significantly restricted at the interface (194).
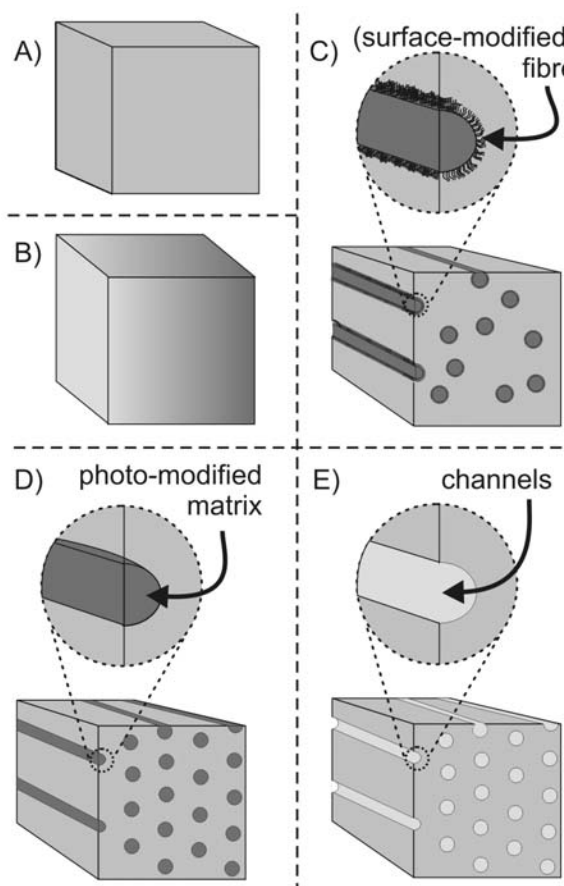
Figure 6. Examples of various three-dimensional structures for tissue engineering of the nervous system. Matrices that are uniform in structure (A) have been widely investigated (e.g. 166). Gradients of molecules formed within three-dimensional structures (B) are a model for axonal guidance (e.g. 195-196), while fibres infiltrated or coated with a matrix induce robust regeneration (C) (e.g. 240). Discrete regions of bioactive matrix (D) guide axons (e.g. 199, 257), while matrices with channels (E) also promote guided regeneration (e.g. 248).

Agarose has also been investigated as a material for creating linear concentration gradients for 3D culture systems (195-196). Chemical gradients in the nervous system have been attributed to induce either axonal guidance or repulsion. For example, point sources of netrin-1 have been found to exert both attractive and repulsive effects on the guidance on growth cones, depending on the levels of cAMP (197). Extracellular Ca^{2+} levels affected the turning response of growth cones when exposed to point sources of BDNF or NT-3 (198). Single point delivery of molecules, however, results in an exponential decay of the concentration with distance. Using agarose gels, a well-defined *linear* concentration gradient can be generated by the Fickian diffusion of molecules through the material (Figure 6b). Such experiments provide a constant gradient of molecules to the growth cone, irrespective of distance from the source. Combinations of growth factors can extend the effective maximum guidance distance, which is the

distance the axon can be directed prior to growth cone receptor saturation (196).

Agarose gels modified with CDPGYIGSR results in improved DRG neurite growth compared to agarose (153). The modification of agarose with photolabile groups permits the coupling of biological molecules in discrete regions. With photolithographical techniques and a modified agarose, lasers can be focused to locally modify agarose with bioactive functionalities. The end result is 200 microns diameter chemical channels of GRGD-modified agarose suspended in agarose that guide neurites in 3D culture as depicted in Figure 6d (199).

4.1.7. Alginate

Alginate is a polyanionic copolymer with repeat units of mannuronic and glucuronic sugar residues and will crosslink upon exposure to divalent cations such as Ca^{2+}. It has attracted interest as an injectable controlled release hydrogel (200) and as a tissue engineering scaffold for non-neuronal cells (201-202). Alginate is predominantly derived from brown algae and has been used as a matrix for hippocampus-derived neurospheres (203). Thermally reversible properties can be also induced by incorporating methylcellulose into alginate (204). Alginate, when freeze-dried, supports axonal regrowth in the spinal cord (205) and the peripheral nerve (206), and can deliver FGF (207). Non-tubular substrates of alginate for large gap *in vivo* peripheral nerve regeneration have also been fabricated and perform well (208).

4.2. Synthetic and biohydrid materials

Neuron adhesion naturally results through specific receptor-ligand interactions with ECM proteins, such as laminin, collagen, FN and proteoglycans. Synthetic materials are devoid of such bioactive molecules, so in many instances the surfaces are biologically modified with various techniques; primarily chemical conjugation, physical adsorption, or entrapment of bioactive molecules. The use of bioactive peptide sequences from laminin is increasingly used to instigate a specific cell-material interaction. Peptides may be readily bound to surfaces using conjugation chemistry techniques (also referred to as coupling reactions) that require reactive chemical functionalities (often termed functional handles) on the material. Hermanson (209) comprehensively provides further insights into the conjugation reactions available, and a range of heterofunctional crosslinking molecules are commercially available for this purpose. Peptide surfaces are also typically more robust during ethanol sterilization, while proteins can denature more readily.

4.2.1. Puramatrix™

Puramatrix™ is a commercially available self-assembling peptide solution that is intended for applications in three-dimensional culture. Consisting of amino acid sequences, the peptide matrices described by Zhang *et al.* (210), Semino *et al.* (211) *a*nd Holmes *et al.* (212) were developed into a commercial product. The transparent, high water content matrix can be used to encapsulate cells (213), however cell adhesion is non
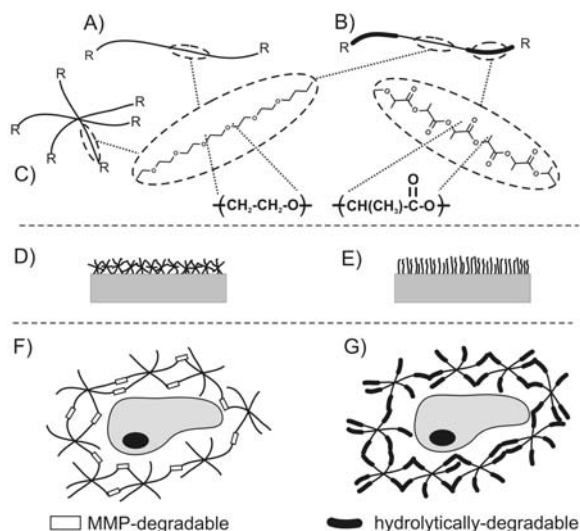
Figure 7. Schematics of selected forms of poly (ethylene glycol) (PEG). The chemical structure of A) linear PEG, B) a poly (ethylene glycol-co-lactide) block copolymer and C) a six-armed star PEG. The end functionality (R) is typically the only practical species of this polymer. Linear PEG can be introduced on the surface (D); however star PEG-modified surfaces (E) demonstrate improved resistance to protein adhesion (224). Cells can be encapsulated by star-PEGs using Michael-Type Addition reactions (F) using matrix metalloproteinases (MMP) (226-227). Alternatively, nerve cells can be encapsulated by a polymerizing a methacrylate-functionalized multi-arm PEG-co-PLA block copolymer (G) (228). *Note: these schematics are NOT to scale and the number of arms on the star PEGs can vary.*

integrin related. Alternatively, hippocampal slice cultures with a peptide scaffold entrap migrating hippocampal neurons (211). NGF-primed PC12 cells also extend neurites into Puramatrix™ (212).

4.2.2. Poly (2-hydroxyethyl methacrylate)

Bioactive proteins can be entrapped within a material during processing; however, such processing should not denature or damage the encapsulated protein. Entrapped ligands, such as NGF, can be added to poly (2-hydroxyethyl methacrylate) (pHEMA) sheets during aqueous-solution polymerization (Figure 5d). The NGF is entrapped, and influences PC12 behaviour in a similar manner to that of soluble NGF; i.e. neurites are extended when NGF is entrapped in pHEMA. The entrapment of NGF in this instance appears to be quite effective, as approximately 80% of proteins are entrapped, as determined through extraction of I^{125} labelled NGF (214). In similar entrapment experiments, the GAGs heparin, chondroitin sulphate and hyaluronic acid were ineffective as substrates for neurite outgrowth (163).

As previously mentioned, gradients of soluble molecules are known to be of great significance to guide axons, and a minimum concentration gradient is required for this. Surface-bound gradients also can guide neurites (Figure 5e). A device developed for manufacturing linear gradients in electrophoresis hydrogels can be adopted for entrapping concentration gradients of large molecules within hydrogels (215). With such gradient makers, NGF has been entrapped within pHEMA as a linear gradient, and guided neurites from PC12 cells along the axis of increasing concentration. The neurites of the PC12 cells were noticeably thicker than when soluble NGF is used, and this may be due to interactions between NGF and the high-affinity TrkA receptor. When bound to NGF, the TrkA receptor is known to increase F-actin polymerization (216) and, as the TrkA receptor is continuously activated by the bound NGF molecule, it was hypothesized that excessive F-actin production resulted in the thick neurites observed (215).

PHEMA-based nerve guides, like collagen nerve guides, support axonal regeneration in the fully transected spinal cord of the rat (172, 217). The penetration of NF200-labelled axons into the nerve guides, or the number of retrograde-labelled axons can be quantified and compared with various matrices. The inclusion of fibrin into pHEMA-based nerve guides resulted in greater retrograde-labelling when compared to collagen, Matrigel or methylcellulose (174). The pHEMA-based nerve guidance channels have a similar elastic modulus to the spinal cord (218-219).

4.2.3. Poly (alpha-hydroxy acids)

The poly (alpha-hydroxy acids), representing a class of polymers widely used for biomedical applications such as sutures and drug release applications (i.e. PGA, PLGA, PDLA, PLLA and PCL), are hydrolytically degradable compounds. Similar to PEG, the functional handles in poly (alpha-hydroxy acids) for conjugation with peptides are limited. Unlike PEG, the poly (alpha-hydroxy acids) are insoluble in water, and processing is typically performed in the melt or in a non-aqueous solvent. The attachment of PC12 cells onto PCL is poor, except when laminin is adsorbed onto the surface (220). Cylinders of poly (DLA-co-GA) their degradation products did not affect neurons beyond what is obtained normally after injury, and nerve guides and oriented scaffolds demonstrate *in vivo* regenerative properties with cell transplantation into the spinal cord (221-222).

4.2.4. Polyethylene glycol

Polyethylene glycol (PEG) (Figure 7) is a particularly unique synthetic polymer, which has been exploited for the PEGylation of many proteins (223). PEG-modified surfaces are non-adhesive to cells and reduce protein adsorption, and are widely used for cell adhesive/non-adhesive patterning strategies. As the backbone of PEG has no functional handles for conjugation, reactions typically occur through the end-groups of the chain (Figure 7a-c). A range of end-functionalities, including isocyanate, acrylate and vinyl sulfone can be introduced and reacted together, rendering a crosslinked hydrogel or matrix. Surfaces modified with multi-armed PEG (Figure 7d) have shown improved cell/protein resistance compared to linear PEG (Figure 7e) (224). Linear PEG substrates are only stable for periods up to approximately a month, before losing their nonadhesive properties (225), while the longevity of multi-armed PEG surfaces is still unknown.

Michael addition reactions, in particular, provide a methodology where chemical crosslinking of PEG can occur in the presence of cells, without loss in cell vitality (Figure 7f). Homogeneous or heterofunctional conjugation agents crosslink PEG into a 3D encapsulating matrix, and has been investigated for non-neuronal cell types. The conjugation or crosslinking reagents may also contain biofunctionalities or be synthesized degradable by MMPs (226-227). PEG can also be rendered degradable by incorporating a poly (alpha-hydroxy acid) into the backbone (PLA or PGA), forming a block copolymer (Figure 7b). The endgroups are methacrylate moieties, which can be polymerized to form a hydrogel (Figure 7g). Nerve cells, when encapsulated within such systems, maintain vitality and extend their processes at different rates depending on the hydrolytically degradable back-bone of the PEG macromer (228).

4.2.5. Poly (pyrrole)

Oxidized poly (pyrrole) (PPy) is an electrically conducting polymer with surface charges, and is of interest for neural applications, as electromagnetic fields can influence the guidance of neurons *in vitro* and *in vivo*.

In the absence of an electric field, neurites extend from PC12 cells and chick sciatic nerve implants to similar lengths on PPy as on TCP. The neurite length of the PC12 cells was even greater on PPy surfaces than TCP when an electric field was applied (229). Dissociated cortical neurons also demonstrated excellent adherence to PPy *in vitro*. The electrical stimulation of PPy after FN adsorption influenced the length of extended neurites (230). *In vivo* implants of PPy were considered biocompatible when inserted into the cerebral cortex (231) and PPy nerve guides for peripheral nerve regeneration have been implanted (232).

4.3. Heterogeneous materials, scaffolds and oriented matrices

Patterned substrates for neurons have been primarily developed for two purposes; as neurite guidance systems for neural networks, or as selective substrate experiments. In tissue engineering applications, the guidance of neurons in organs is of interest and this is reflected in the resulting scaffolds that support 3D neurite outgrowth. The *in vivo* environment is one of three-dimensions, where neurons are in contact with other cells, and different ECMs surrounds the cell body and axons. Transforming well defined, patterned 2D substrates into 3D constructs, however, is technically challenging and not trivial. Oriented scaffolds can be formed with a range of methodologies, but developing 3D materials with discrete regions of bioactive molecules is still problematic and a challenge for neural tissue engineering.

4.3.1. Patterned surfaces

The guidance of neurites is the most important aspect of material/neuron interactions, and heterogeneous surfaces (Figure 5f) are used to assess the contact-mediated aspects of growth as the growth cone will respond to both surface-bound and soluble molecules. Patterned surfaces for neurite guidance can be achieved with various

techniques, and has over thirty years of history (233). One of the first studies of patterned DRG adhesion and growth was performed by Letourneau (234), who demonstrated that the growth cones of the growing neurites were able to preferentially decide between two materials. Patterning is common with adhesive/non adhesive surface combinations; such an experiment shows that while guidance of neurites results from laminin/BSA patterns, the length of the neurites was significantly shorter than a uniformly modified laminin (235). The adhesion of dissociated cortical neurons on patterns of ECM on polystyrene was significantly improved with the mixing of PLL to the adhesive ECM (236).

Patterned substrates that effectively guided neurites are not limited to adhesive/non-adhesive configurations. Avci *et al.* (237) demonstrated the efficacy of patterned DM-GRASP/laminin and laminin – both of which are adhesive substrates for neurite extension. DM-GRASP is a CAM which plays a role in the developmental visual system, regarding axonal growth and navigation in the embryonic retina. In this instance, the tracts of DM-GRASP in the chick retina that assist in the guidance of retinal ganglion cells during embryogenesis also result in neurite guidance on patterned substrates with both regions being neuron-adhesive.

Gradients of surface-bound IKVAV, deposited along pattern lengths also demonstrated preferential growth of axons (238). The majority of growth cones turned and migrated up the gradient, and slowed down at patterned intersections. Once the choice of path was made by the growth cone, the velocity of outgrowth returned to the previous rates.

4.3.2. Fibres

Fibres are known to guide axons along their length, when their diameter is c.a. 250 microns or lower. An excellent overview regarding the effect of micron-sized fibres on neurite guidance is Smeal *et al.* (239). Fibres are particularly interesting guidance substrates, as they are a simple and inexpensive approach to creating a three-dimensional guiding system. The classical experiment of guiding neurites with fibres is to deposit a fibre onto a flat substrate, and determine the effect of neurite guidance. Fibres have shown maximum axon lengths with diameters between 35 and 20 microns, however smaller diameters have been more difficult to reproducibly achieve with mechanically drawn fibres. Alginate- and fibronectin-coated poly-ß-hydroxybutyrate (PHB) fibres have also been inserted after a second injury to the spinal cord (240) (Figure 6c).

Until the recent interest in electrospinning, the generation of perfectly symmetrical, oriented sub-micron fibres has been problematic. The generation of nano-grooved surfaces has been demonstrated through the manufacture of a template, and then using the moulding template to create channels onto the surface. Neurite alignment is significantly along the long axis of the grooves (241). Densely deposited, oriented PLLA electrospun fibres with diameters down to 300 nm, induce growth along the

Neural interactions with materials

Table. 3. A selection of biomolecules/materials with an effect on neurons

| Molecules or biomolecules	Observations			Effects / Observations (e.g., cell behaviour)	Refs
	in vivo	*in vitro*			
PLL/Laminin		2D		Robust neurite growth	standard procedure for *in vitro* assays
Laminin		2D		PC12s with NGF longer neurites than without NGF	189
P (L-ornithine)/myelin		2D		Inhibitory surface for neurite growth	256
BSA on TCP		2D		NGF-primed PC12s – failed adherence	189
YIGSR or IKVAV		2D		E18 Hippocampal –cell adhesion	162
YIGSR/IKVAV (50:50)		2D		E18 Hippocampal – synergistic effect on neurites length	162
CDPGYIGSR/ CQAASIKVAV (50:50)			3D	Good neurite outgrowth: DRG	154
GGGGGGYIGSR or GGGGGGIKVAV		2D		E18 Hippocampal – longer neurites than YIGSR or IKVAV	162
Collagen Type IV		2D		Strong Neurite outgrowth DRG	163
Collagen Type I or III		2D		Neurite outgrowth DRG	163
Collagen Type I (1mg/ml) Collagen Type I + laminin Collagen Type I + FN			3D	PC12 – aggregates grow to same extent	166
Magnetically oriented Type I Collagen (2mg/ml)	*in vivo*			Improved PN regeneration	165
Magnetically oriented Type I Collagen (2mg/ml)			3D	Improved neurite outgrowth: E11 DRGs	164
Empty collagen NGs in full SCI transection	*in vivo*			Axon penetration into NG observed	172
Fibrin in P (AN-VC) NG	*in vivo*			Rat dorsal nerves – increase in axons	151
Fibrin in pHEMA NG	*in vivo*			Transected rat SC – retrograde labelling	174
beta-NGF in heparin-modified fibrin			3D	DRG - Neurite extension increased up to 100%	175
BDNF in heparin-modified fibrin			3D	DRG - Neurite extension increased up to 80%	175
NT-3 in heparin-modified fibrin			3D	DRG – Neurite length unaffected	175
Magnetically oriented fibrin (1-5 mg/ml)			3D	DRG – oriented neurite outgrowth	176
RNIAEIIKDI- or YIGSR- or IKVAV-modified fibrin			3D	DRG – increased neurite outgrowth with concentration	151
RGD- or N-Cadherin-modified fibrin			3D	DRG – longer neurites but, decreased with concentration	151
RNIAEIIKDI, RGD, YIGSR & IKVAV modified fibrin			3D	DRG –Synergy increase neurite length	151
RNIAEIIKDI, RGD, YIGSR & IKVAV modified fibrin in P (AN-VC) NGs	*in vivo*			Rat dorsal nerves – 3-fold increase in axons.	151
Chondroitin sulphate, heparin or HA- modified pHEMA		2D		Poor neurite outgrowth DRG	163
Collagen-GAG filled NG	*in vivo*			Increased regeneration over collagen	169
Matrigel™ in pHEMA NG	*in vivo*			Transected rat SC – no retrograde labelling	174
Magnetically aligned Matrigel™	*in vivo*			Improved PNS regeneration over non-aligned form	178
Schwann cell or OEC transplants in Matrigel™ and P (AN-VC) NGs	*in vivo*			Penetration of axons into NG after SC full/hemi-section	180-181
Fibronectin conduits and mats in SC and PNS	*In vivo*			Supports regeneration	183
Agarose			3D	Supports DRG neurite outgrowth	193
CDPGYIGSR-modified agarose			3D	E9 DRG – Increased neurite outgrowth	153
RGD; RGD/agarose			3D	Neurite outgrowth DRG	199, 257
NGF gradients in agarose			3D	PC12 & DRG min. gradient of 133 ng/ml/mm	195-196
NGF/NT-3 gradients in agarose			3D	DRG min. gradient of 80 ng/ml/mm	196
Alginate			3D	Supports hippocamal-derived neurospheres	193; 203
Freeze-dried alginate	*In vivo*			Supports axonal regrowth in SC	205
Poly-*b*-hydroxybutyrate NGs coated with alginate and FN and Schwann cells	*in vivo*			Reduced SC cavitation	240
Puramatrix			3D	Extension of PC-12 neurites into matrix	212
Puramatrix in slice culture			3D	Entrapment of Hippocampal neurons	211
beta-NGF in pHEMA		2D		Strong Neurite outgrowth DRG	163
NGF gradients in pHEMA		2D		PC12 Guided neurite outgrowth with bound gradient	215
Empty P (AN-VC) NGs in full SCI transection	*in vivo*			No axon penetration observed	181
Empty pHEMA-MMA NGs in full SCI transection	*in vivo*			Axon penetration into NG observed	217
Methylcellulose in pHEMA NG	*in vivo*			Transected rat SC – retrograde labelling	174
Poly (pyrrole)		2D		Increased neurite length in electric field than TCP	229
Poly (pyrrole)	*in vivo*			Integration into cerebral cortex; PNS regeneration	231-232
Poly-L-lysine (Pattern)		2D		Hippocampal – guidance of neurites	258
DM-GRASP/Laminin (on PLL/glass) (Pattern)		2D		RGCs – long axon growth, fast speed.	237
DM-GRASP/laminin and laminin (Pattern)		2D		DM-GRASP/laminin	237
CDPGYIGSR/CQAASIKVAV on PTFE fibres (diameter=30 microns)		2D		DRG adhesion and neurite guidance	259
PLL/laminin on PP fibres		2D		DRG guidance below c.a. 250 m	239
FN-F108 on PP fibres (diameter = 75 microns)		2D		DRG adhesion and neurite guidance	187
Low-pressure moulded PLGA foams	*in vivo*			supports axons with seeded Schwann cells	248
Freeze channelled PVA-coated PLLA in Dioxane			3D	DRG survival, neuritogenesis and cell penetration	252
Freeze channelled PLGA (50/50), 75%; PLL-PLGA (50/50) 25% in Dioxane/water	*in vivo*			Seeded neural stem cells improved function in hemisection SCI	255
Freeze channelled PDLA/P (DLA-PEG) (90/10)	*in vivo*			quantified growth of axons in SC implant	249
Freeze channelled agarose in water	*in vivo*			Scaffolds are well integrated into SC	250
Freeze channelled PLLA in benzene or dioxane; PLGA (85/15 or 75/25) in benzene				Continuous, oriented pores are formed	260
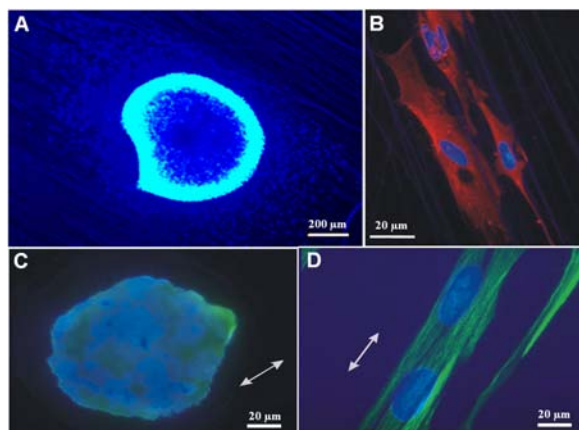
Figure 8. Electrospun fibres as substrates for cell guidance. (A) Cells from chick DRGs migrate *in vitro* on oriented electrospun nanofibers, as are (B) dissociated Schwann cells. The chemistry of the fibre is important in determining cell morphology as is seen with fibroblasts adhering on (C) PCL electrospun fibres and (D) PCL/collagen electrospun fibres with identical diameters. The arrows indicate the direction of the oriented nanofibers. Image modified from Schnell *et al.* (244).

long axis of the fibre (242). Electrospinning results in the manufacture of micro to nano scaled fibres, with excellent fibre symmetry, and promises to extend the classical "fibre and flat substrate" experiment to nano-scale diameters. Centimetre length nanofibres can be made with dual collection systems (243).

Such oriented nanofibers of PCL and collagen/PCL blends have been adhered to a chemically reactive starPEG substrate that continues to react with water to form a non-adhesive substrate (244). Such substrates induce stretched cellular morphologies to a range of cells, including neurons, Schwann cells, olfactory ensheathing cells, astrocytes and fibroblasts (Figure 8). When dorsal root ganglions are cut in half and placed upon these oriented nanofiber substrates, there is a an oriented migration of Schwann cells initially, then neurites, out of the DRG explant. While fibres may provide similar guidance to that of substrate patterning, the opportunities for incorporation of fibres into 3D structures is greater than patterned surfaces.

4.3.3. Three dimensional structures

An increasing number of *in vitro* investigations have taken place in 3D cultures, where cells more closely mimic their *in vivo* behaviour (245). Whereas on flat surfaces cell spreading is atypical for good surfaces, cells tend to aggregate when suspended within 3D gels (166). Proliferation or cell migration results in an increase of aggregate diameters, which can be easily measured with tumourigenic cell lines such as PC12 cells. The materials used in three-dimensional cell culture typically have high water content, and attempt to mimic the ECM environment that cells experience in the natural state. Obtaining the relevant mechanical properties, molecular recognition through the use of peptides, proteolytic degradation, and growth-factor binding domains are examples of properties

that a future matrix for three-dimensional studies may possess (246).

Three-dimensional *in vitro* culture is inconvenient and more expensive than traditional two-dimensional cell culture, but may recreate the native environment of the cell. In particular the separation of the neuron cell body and the axon is interesting, as the cell bodies of neurons are situated in the dorsal roots or in the spinal cord, the peripheral nerve environment primarily involves interactions between axons (not the cell body of the neuron) and glia and their surrounding ECM.

4.3.4. Channelled scaffolds

In a manner similar to the inherent guidance properties of fibres, the use of channels also results in the directed growth of neurites and is illustrated in Figure 6e. One simple technique involves the suspension of linear polymeric fibres into a liquid that is subsequently crosslinked, followed by the dissolution of the fibre with a suitable solvent (247). Another approach involves the low-pressure injection moulding of PLGA foams, resulting in a scaffold that supports neurite outgrowth when delivered with Schwann cells *in vivo* (248). Such approaches can be applied to a variety of materials, essentially converting isotropic materials into scaffolds with guidance properties (154). The surface modification of such channels significantly increases the neurite growth along the length both *in vitro* and *in vivo*.

4.3.5. Isothermal crystallization

The forces associated with crystal formation have been harnessed to generate oriented pores that are cm in length within polymer solutions and hydrogels. Controlled uniaxial freezing is an increasing popular technique for inexpensive oriented pore formation and can be applied to polyesters and hydrogels (249-251). *In vitro* experiments demonstrate DRG neurite and cell penetration into poly (vinyl alcohol) coated scaffolds PLLA (252), and PEG-PLA copolymers (253). Similar invasion of axons into oriented scaffolds results *in vivo*, and experiments using such freeze-channelled structures have been used as a substrate for neural stem cell and genetically modified Schwann cell transplantation into the spinal cord (254-255).

5. CONCLUSION

Many developments in material science have greatly advanced the available tools for *in vivo* applications. However, given the complex interactions between neurons, glia and the immune system *in vivo* it is important to note that tissue engineering has predominantly encompassed only limited areas of neuroscience. For example, the induction of neurite outgrowth and nerve regeneration with neurotrophic factors and the use of ECM proteins/peptides has attracted the most attention, while materials for neuroprotection strategies has been so far limited. Similarly, the interactions between biomaterials and glia need to be further understood.

Tissue engineering strategies have the capability of delivering multi-therapeutic strategies for the nervous

system, through improving transplanted cell survival, providing a substrate for growth and controlled delivery of beneficial molecules. As materials demonstrate their capacity to deliver therapeutic strategies to the nervous system, a wider range of approaches will be encompassed by this technology.

6. REFERENCES

1. S. Ramón y Cajal: Estudios sobre la degeneración y regeneración del sistema nervioso. *Imprenta hihos de Nicolás Moya,* Madrid (1914)

2. M.G. Fehlings, D.C. Baptiste: Current status of clinical trials for acute spinal cord injury. *Injury* 36, S-B113-S-B122 (2005)

3. J.W. Fawcett: Overcoming inhibition in the damaged spinal cord. *J.Neurotrauma* 23, 371-383 (2006)

4. W. Gomes-Leal, D.J. Corkill, M.A. Freire, C.W. Picanço-Diniz, V.H. Perry: Astrocytosis, microglia activation, oligodendrocyte degeneration, and pyknosis following acute spinal cord injury. *Exp.Neurol.* 190, 456-467 (2004)

5. P.G. Popovich: Alterations in immune cell phenotype and function after experimental spinal cord injury. *J.Neurotrauma* 18, 954-966 (2001)

6. G. Raivich, M. Bohatschek, C.U.A. Kloss, A. Werner, L.L. Jones, G.W. Kreutzberg: Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res.Rev.* 30, 77-105 (1999)

7. J.C. Fleming, M.D. Norenberg, D.A. Ramsay, G.A. Dekaban, A.E. Marcillo, A.D. Saenz, M. Paswuale-Styles, W.D. Dietrich, L.C. Weaver: The cellular inflammatory response in human spinal cords after injury. *Brain* 129, 3249-3269 (2006)

8. I. Dusart, M.E. Schwab: Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur.J.Neurosci.* 6, 712-724 (1994)

9. S.L. Carlson, M.E. Parrish, J.E. Springer, K. Doty, L. Dossett: Acute inflammatory response in spinal cord following impact injury. *Exp.Neurol.* 151, 77-88 (1998)

10. R. Gonzalez, J. Glaser, M.T. Liu, T.E. Lane, H.S. Keirstead: Reducing inflammation decreases secondary degeneration and functional deficit after spinal cord injury. *Exp.Neurol.* 184, 456-463 (2003)

11. W. Young, J. Kume-Kick, S. Constantini: Glucocorticoid therapy of spinal cord injury. *Ann.N.Y.Acad.Sci.* 743, 241-265 (1994)

12. P.G. Popovich, J.F. Reinhard Jr, E.M. Flanagan, B.T. Stokes: Elevation of the neurotoxin quinolinic acid occurs following spinal cord trauma. *Brain Res.* 633, 348-352 (1994)

13. S. Thanos, J. Mey: Type-specific stabilization and target-dependent survival of regenerating ganglion cells in the retina of adult rats. *J.Neurosci.* 15, 1057-1079 (1995)

14. H. Kager, W.J. Wadman, G.G. Somjen: Conditions for the triggering of spreading depression studied with computer simulations. *J.Neurophysiol.* 88, 2700-2712 (2002)

15. C. Arango-Davila, M. Escobar-Betancourt, G.P. Cardona-Gómez, H. Pimienta-Jiménez: Fisiopatología de la isquemia cerebral focal: aspectos básicos y proyección a la clínica. *Rev.Neurol.* 39, 156-165 (2004)

16. G. Denson, M. Fujikawa: Confusion between neuronal apoptosis and activation of programmed cell death mechnisms in acute becrotic insults. *Trends Neurosci.* 23, 410-411 (2000)

17. O. Lazarov-Spiegler, O. Rapalino, G. Agranow, M. Schwartz: Restricted inflammatory reaction in the CNS: a key impediment to axonal regeneration? *Mol.Med.Today* 4, 337-342 (1998)

18. M. Schwartz, G. Moalem, R. Leibowitz-Amit, I.R. Cohen: Innate and adaptive immune responses can be beneficial for CNS repair. *Trends Neurosci.* 22, 295-299 (1999)

19. P.E. Batchelor, M.J. Porritt, P. Martinello, C.L. Parish, G.T. Liberatore, G.A. Donnan, D.W. Howells: Macrophages and microglia produce local trophic gradients that stimulate axonal sprouting toward but not beyond the wound edge. *Mol.Cell.Neurosci.* 21, 436-453 (2004)

20. S. Jander, F. Lausberg, G. Stoll: Differential recruitment of CD8+ macrophages during Wallerian degeneration in the peripheral and central nervous system. *Brain Pathol.* 11, 27-38 (2001)

21. R. Kiefer, B.C. Kieseier, G. Stoll, H.P. Hartung: The role of macrophages in immune-mediated damage to the peripheral nervous system. *Progr.Neurobiol.* 64, 109-127 (2001)

22. S. Lacroix, L. Chang, S. Rose-John, M.H. Tuszynski: Delivery of hyper-interleukin-6 to the injured spinal cord increases neutrophil and macrophage infiltration and inhibits axonal growth. *J.Comp.Neurol.* 454, 213-228 (2002)

23. Y. Li, Y. Sauvé, D. Li, R.D. Lund, G. Raisman: Transplanted olfactory ensheathing cells promote regeneration of cut adult rat optic nerve axons. *J.Neurosci.* 23, 7783-7788 (2003)

24. A.C. Lee, V.M. Yu, J.B.I. Lowe, M.J. Brenner, D.A. Hunter, S.E. Mackinnon, S.E. Sakiyama-Elbert: Controlled

release of nerve growth factor enhances sciatic nerve regeneration. *Exp.Neurol.* 184, 295-303 (2003)

25. M. Makwana, G. Raivich: Molecular mechanisms in successful peripheral regeneration. *FEBS J.* 272, 2628-2638 (2005)

26. J.F. Tello: La influencia del neurotropismo en la regeneración de los centros nerviosos. *Trab.Lab.Invest.Biol.* 9, 123-159 (1911)

27. P.J. Horner, F.H. Gage: Regenerating the damaged central nervous system. *Nature* 407, 963-970 (2000)

28. S. David, A. Aguayo: Axonal regeneration into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science* 214, 931-933 (1981)

29. M. Vidal-Sanz, G.M. Bray, M.P. Villégaz-Pérez, S. Thanos, A. Aguayo: Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. *J.Neurosci.* 7, 2894-2909 (1987)

30. S. David, S. Lacroix: Molecular approaches to spinal cord repair. *Ann.Rev.Neurosci.* 26, 411-440 (2003)

31. M.T. Filbin: Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nat.Rev.Neurosci.* 4, 1-11 (2003)

32. J. Corcoran, P.L. So, R.D. Barber, K.J. Vincent, N.D. Mazarakis, K.A. Mitrophanous, S.M. Kingsman, M. Maden: Retinoic acid receptor beta2 and neurite outgrowth in the adult mouse spinal cord *in vitro*. *J.Cell Sci.* 115, 3779-3786 (2002)

33. J. Mey, S. Thanos: Ontogenetic changes in the regenerative ability of chick retinal ganglion cells as revealed by organ explants. *Cell Tissue Res.* 264, 347-355 (1991)

34. J.W. Fawcett, R.A. Asher: The glial scar and central nervous system repair. *Brain Res.Bull.* 39, 377-391 (1999)

35. F.J. Liuzzi, R.J. Lasek: Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway. *Science* 237, 642-645 (1987)

36. P. Bovolenta, I. Fernaud-Espinosa, R. Méndez-Otero, M. Nieto-Sampredro: Neurite outgrowth inhibitor of gliotic brain tissue. Mode of action and cellular localization, studied with specific monoclonal antibodies. *Eur.J.Neurosci.* 9, 977-989 (1997)

37. T.G. Bush, N. Puvanachandra, C.H. Horner, A. Polito, T. Ostenfeld, C.N. Svendsen, L. Mucke, M.H. Johnson, M.V. Sofroniew: Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron* 23, 297-308 (1999)

38. C.C. Stichel, H.W. Müller: The CNS lesion scar: new vistas on an old regeneration barrier. *Cell Tissue Res.* 294, 1-9 (1998)

39. J.R. Faulkner, J.E. Herrmann, M.J. Woo, K.E. Tansey, N.B. Doan, M.V. Sofroniew: Reactive astrocytes protect tisue and preserve function after spinal cord injury. *J.Neurosci.* 24, 2143-2155 (2004)

40. C.M. Liberto, P.J. Albrecht, L.M. Herx, V.W. Yong, S.W. Levison: Pro-regenerative properties of cytokine-activated astrocytes. *J.Neurochem.* 89, 1092-1100 (2004)

41. M.S. Chen, A.B. Huber, M.E. van der Haar, M. Frank, L. Schnell, A.A. Spillmann, F. Christ, M.E. Schwab: Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* 403, 434-439 (2000)

42. D.M. McTigue, P. Wei, B.T. Stokes: Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. *J.Neurosci.* 21, 3392-3400 (2001)

43. J. Mey, D. Morasutti, G. Brook, R.H. Liu, Y.P. Zhang, G. Koopmans, P. McCaffery: Retinoic acid synthesis by a population of NG-2 positive cells in the injured spinal cord. *Eur.J.Neurosci.* 21, 1555-1568 (2005)

44. F. Bajrovic, M. Srpcic, J. Sketelj: Schwann cell dependence of regenerating rat sensory neurons is inversely related to the quality of axon growth substratum. *J.Neuropathol.Exp.Neurol.* 60, 65-74 (2001)

45. I. Kohama, K.L. Lankford, J. Preiningerova, F.A. White, T.L. Vollmer, J.D. Kocsis: Transplantation of cyropreserved adult human Schwann cells enhances axonal conduction in demyelinated spinal cord. *J.Neurosci.* 21, 944-950 (2001)

46. G.A. Brook, D. Plate, R. Franzen, D. Martin, G. Moonen, J. Schoenen, A.B. Schmitt, J. Noth, W. Nacimiento: Spontaneous longitudinally orientated aconal regeneration is asociated with the Schwann cell framework within the lesion site following spinal cord compression injury of the rat. *J.Neurosci.Res.* 53, 51-65 (1998)

47. S.C. Barnett, L. Chang: Olfactory ensheathing cells and CNS repair: going solo or in need of a friend? *Trends Neurosci.* 27, 54-60 (2004)

48. R.J.M. Franklin, S.C. Barnett: Olfactory ensheathing cells and CNS regeneration: the sweet smell of success? *Neuron* 28, 15-18 (2000)

49. G. Raisman: Olfactory ensheathing cells - another miracle cure for spinal cord injury? *Nat.Rev.Neurosci.* 2, 369-375 (2001)

50. D.K. Resnick, C. Cechvala, Y. Yan, B.P. Witwer, D. Sun, S. Zhang: Adult olfactory ensheathing cell

transplantation for acute spinal cord injury. *J.Neurotrauma* 20, 279-285 (2003)

51. S. Thanos, C. Pavlidis, J. Mey, H.-J. Thiel: Specific transcellular staining of microglia in the adult rat after experimentally induced degeneration of carbocyanine-filled retinal ganglion cells. *Exp.Eye Res.* 55, 101-117 (1992)

52. A. Werner, C.U.A. Kloss, J. Walter, G.W. Kreutzberg, G. Raivich: Intercellular adhesion molecule-1 (ICAM-1) in the mouse facial motor nucleus after axonal injury and during regeneration. *J.Neurocytol.* 27, 219-232 (1998)

53. G. Raivich, M. Bohatschek, A. Werner, L.L. Jones, M. Galiano, C.U.A. Kloss, X.Z. Zhu, K. Pfeffer, Z.Q. Liu: Lymphocyte infiltration in the injured brain: role of proinflammatory cytokines. *J.Neurosci.Res.* 72, 726-733 (2003)

54. M.B. Graeber, W.J. Streit, G.W. Kreutzberg: Identity of ED2-positive perivascular cells in rat brain. *J.Neurosci.Res.* 22, 103-106 (1989)

55. H.S. Taskinen, T. Olsson, A. Bucht, M. Khademi, L. Svelander, M. Röyttä: Peripheral nerve injury induces endoneurial expression of IFN-gamma, IL-10 and TNF-alpha mRNA. *J.Neuroimmunol.* 102, 17-25 (2000)

56. P. Hu, E.M. McLachlan: Distinct functional types of macrophages in dorsal root ganglia and spinal nerves proximal to sciatic and spinal nerve transections in the rat. *Exp.Neurol.* 184, 590-605 (2003)

57. K. Vass, W.F. Hickey, R.E. Schmidt, H. Lassmann: Bone marrow-derived elements in the peripheral nervous system. *Lab.Invest.* 69, 275-282 (1993)

58. G.K. Tofaris, P.H. Patterson, K.R. Jessen, R. Mirsky: Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J.Neurosci.* 22, 6696-6703 (2002)

59. F. González-Scarano, G. Baltuch: Microglia as mediators of inflammatory and degenerative diseases. *Ann.Rev.Neurosci.* 22, 219-240 (1999)

60. M. Ma, P. Wei, T. Wei, R.M. Ransohoff, L.B. Jakeman: Enhanced axonal growth into a spinal cord contusion injury site in a strain of mouse (129X1/SvJ) with a diminished inflammatory response. *J.Comp.Neurol.* 474, 469-468 (2004)

61. S. Thanos, J. Mey, M. Wild: Treatment of the adult retina with microglia-suppressing factors retards axotomy-induced neuronal degradation and enhances axonal regeneration *in vivo* and *in vitro*. *J.Neurosci.* 13, 455-466 (1993)

62. L. Steinman, R. Martin, C. Bernard, P. Conlon, J.R. Oksenberg: Multiple sclerosis: Deeper understanding of its pathogenesis reveals new targets for therapy. *Ann.Rev.Neurosci.* 25, 491-505 (2000)

63. M. Nieto-Sampredro, J.E. Collazos-Castro, J.S. Taylor, G.Gudino-Cabrera, E. Verdú-Navarro, J.I. Pascual-Piédrola, R. Insausti-Serrano: Trauma en el sistema nervioso central y su reparación. *Rev.Neurol.* 35, 534-552 (2002)

64. J.L. Ridet, S.K. Malhotra, A. Privat, F.H. Gage: Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci.* 20, 570-577 (1997)

65. I. Fernaud-Espinosa, M. Nieto-Sampredro, P. Bovolenta: Differential activation of microglia and astrocytes in aniso- and isomorphic gliotic tissue. *Glia* 4, 277-291 (1993)

66. W.J. Streit, S.L. Semple-Rowland, S.D. Hurley, R.C. Miller, P.G. Popovich, B.T. Stokes: Cytokine mRNA profiles in contuse spinal cord and axotomized facial nucleus suggest a beneficial role for inflammation and gliosis. *Exp.Neurol.* 152, 74-87 (1998)

67. S.S. Scherer, J.L. Salzer: Axon-Schwann cell interactions during peripheral nerve degeneration and regeneration. In Jessen,K.R. & Richardson,W.D. (eds), *Glial cell development - basic principles and clinical relevance*. Oxford University Press, Oxford, pp. 299-330 (2001)

68. M.T. Filbin, J. Qiu, D. Cai : Glial influences on axonal regeneration. In Jessen,K.R. & Richardson,W.D. (eds), *Glial cell development - basic principles and clinical relevance*. Oxford University Press, Oxford, pp. 279-298 (2001)

69. R.P. Lisak, D. Skundric, B. Bealmear, S. Ragheb: The role of cytokines in Schwann cell damage, protection, and repair. *J.Infect.Dis.* 176, S173-S179 (1997)

70. K.S. Bedi, J. Winter, M. Berry, J. Cohen: Adult rat dorsal root ganglion neurons extend neurites on predegenerated but not on normal peripheral nerves *in vitro*. *Eur.J.Neurosci.* 4, 193-200 (1992)

71. M.C. Brown, E.R. Lunn, V.H. Perry: Poor growth of mammalian motor and sensory axons into intact proximal nerve stumps. *Eur.J.Neurosci.* 3, 1366-1369 (1991)

72. Y. Li, P.M. Field, G. Raisman: Death of oligodendrocytes and microglial phagocytosis of myelin precede immigration of Schwann cells into the spinal cord. *J.Neurocytol.* 28, 417-427 (1999)

73. S.L. Shuman, J.C. Bresnahan, M.S. Beattie: Apoptosis of microglia and oligodendrocytes after spinal cord contusion in rats. *J.Neurosci.Res.* 50, 798-808 (1997)

74. S. Casha, W.R. Yu, M.G. Fehlings: Oligodendroglial apoptosis occurs along degenerating axons and is

associated with FAS and p75 expression following spinal cord injury in the rat. *Neuroscience* 103, 203-218 (2001)

75. A. Minagar, E.G. Toledo, J.S. Alexander, R.E. Kelley: Pathogenesis of brain and spinal cord atrophy in multiple sclerosis. *J.Neuroimaging* 14, 5S-10S (2004)

76. F. Hu, S.M. Strittmatter: Regulating axon growth within the postnatal central nervous system. *Semin.Perinatol.* 28, 371-378 (2004)

77. M.D. Benson, M.I. Romero, M.E. Lush, Q.R. Lu, M. Henkemeyer, L.F. Parada: Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. *Proc.Natl.Acad.Sci.USA* 102, 11131-11136 (2005)

78. J. Strelau, K. Unsicker: GDNF family members and their receptors: expression and function in two oligodendroglial cell lines representing distinct stages of oligodendroglial development. *Glia* 26, 291-301 (1999)

79. A. Wilkins, S. Chandrean, A. Compston: A role for oligodendrocyte-derived IGF-1 in trophic support of cortical neurons. *Glia* 36, 48-57 (2001)

80. J. Mey, L. Henkes: Retinoic acid enhances leukemia inhibitory factor expression in OLN-93 oligodendrocytes. *Cell Tissue Res.* 310, 155-161 (2002)

81. X. Dai, L.D. Lercher, P.M. Clinton, Y. Du, D.L. Livingston, C. Vieira, L. Yang, M.M. Shen, C.F. Dreyfus: The trophic role of oligodendrocytes in the basal forebrain. *J.Neurosci.* 23, 5846-5853 (2005)

82. R. Franzen, D. Martin, A. Daloze, G. Moonen, J. Schoenen: Grafts of meningeal fibroblasts in adult rat spinal cord lesion promote axonal regrowth. *Neuroreport* 10, 1551-1556 (1999)

83. M. Sensenbrenner, J.C. Deloulme, C. Gensburger: Proliferation of neuronal precursor cells from the central nervous system in culture. *Rev.Neurosci.* 5, 43-53 (1994)

84. D.A. Charytoniuk, E. Traiffort, E. Pinard, O. Issertial, J. Seylaz, M. Ruat: Distribution of bone morphogenetic protein and bone morphogenetic protein receptor transcripts in the rodent nervous system and up-regulation of bone morphogenetic protein receptor type II in hippocampal dentate gyrus in a rat model of global cerebral ischemia. *Neuroscience* 100, 33-43 (2000)

85. R.W. Pelton, M.E. Dickinson, H.L. Moses, B.L. Hogan: *In situ* hybridization analysis of TGF beta3 RNA expression during mouse development: comparative studies with TGF beta1 and beta1. *Development* 110, 609-620 (1990)

86. K.A. Sullivan, E.L. Feldman: Immunohistochemical localization of insulin-like growth factor-II (IGF-II) and IGF-binding protein-2 during development of the rat brain. *Endocrinology* 135, 540-547 (1994)

87. A. Nishiyama, X.H. Lin, N. Giese, C.H. Heldin, W.B. Stallcup: Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. *J.Neurosci.Res.* 43, 299-314 (1996)

88. A.M. Butt, J. Kiff, P. Hubbard, M. Berry: Synantocytes: New functions for novel NG2 expressing glia. *J.Neurocytol.* 31, 551-565 (2002)

89. A. Nishiyama, M. Watanabe, Z. Yang, J. Bu: Identity, distribution, and development of polydendrocytes: NG2-expressing glial cells. *J.Neurocytol.* 31, 437-455 (2002)

90. L.L. Jones, Y. Yamaguchi, W.B. Stallcup, M.H. Tuszynski: NG2 is a major chondroitin sulfate proteoglyan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. *J.Neurosci.* 22, 2792-2803 (2002)

91. J.M. Levine: Increased Expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. *J.Neurosci.* 14, 4716-4730 (1994)

92. C.L. Dou, J.M. Levine: Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. *J.Neurosci.* 14, 7616-7628 (1994)

93. L.L. Jones, D. Sajed, M.H. Tuszynski: Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. *J.Neurosci.* 23, 9276-9288 (2003)

94. M.J. Politis, D. Ederle, P.S. Spencer: Tropism in nerve regeneration *in vivo*. Attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. *Brain Res.* 253, 1-12 (1982)

95. J.Y. Sheu, D.J. Kulhanek, F.P. Eckenstein: Differential patterns of ERK and STAT3 phosphorylation after sciatic nerve transection in the rat. *Exp.Neurol.* 166, 392-402 (2000)

96. N.Q. McDonald, M.V. Chao: Structural determinants of neuritrophin action. *J.Biol.Chem.* 270, 19669-19672 (2005)

97. J.G. Boyd, T. Gordon: Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol.Neurobiol.* 27, 277-324 (2003)

98. M.V. Chao, P. Casaccia-Bonnefil, B. Carter, A. Chittka, H. Kong, S.O. Yoon: Neurotrophin receptors: mediators of life and death. *Brain Res.Rev.* 26, 295-301 (2000)

99. W.J. Friedman, L.A. Greene: Neurotrophin signaling via trks and p75. *Exp.Cell Res.* 253, 131-142 (1999)

100. H. Thoenen: Neurotrophins and neuronal plasticity. *Science* 270, 593-598 (2000)

101. H.H. Althaus, C. Richter-Landsberg: Glial cells as targets and producers of neurotrophins. *Int.Rev.Cytol.* 197, 203-277 (2000)

102. B. Connor, M. Dragunow: The role of neuronal growth factors in neurodegenerative disorders of the human brain. *Brain Res.Rev.* 27, 1-39 (1998)

103. K.D. Dougherty, C.F. Dreyfus, I.B. Black: Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. *Neurobiol.Disease* 7, 574-585 (2000)

104. C. Gillen, C. Korfhage, H.W. Müller: Gene expression in nerve regeneration. *Neuroscientist* 3, 112-122 (1997)

105. F. Hefti: Neurotrophic factor therapy for nervous system degenerative diseases. *J.Neurobiol.* 25, 1418-1435 (1994)

106. H. Thoenen, M. Sendtner: Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. *Nat.Neurosci.Suppl.* 5, 1046-1050 (2002)

107. M.S. Airaksinen, M. Saarma: The GDNF family: signalling, biological functions and therapeutic value. *Nat.Rev.Neurosci.* 3, 383-394 (2002)

108. H. Sariola, M. Saarma: Novel functions and signalling pathways for GDNF. *J.Cell Sci.* 116 , 3855-3862 (2003)

109. D.L.H. Bennett, T.J. Boucher, M.P. Armanini, K.T. Poulsen, G.J. Michael, J.V. Priestley, H.S. Phillips, S.B. McMahon, D.L. Shelton: The glial cell line-derived neurotrophic factor family receptor components are differentially regulated within sensory neurons after nerve injury. *J.Neurosci.* 20, 427-437 (2000)

110. R. Lemke, M. Hartlage-Rübsamen, R. Schliebs: Differential injury-dependent glial expression of interleukins-1-Beta, alpha, and interleukin-6 in rat brain. *Glia* 27, 75-87 (1999)

111. M. Nakamura, R.A. Houghtling, L. MacArthur, B. Bayer, B.S. Bregman: Differences in cytokine gene expression profile between acute and secondary injury in adult rat spinal cord. *Exp.Neurol.* 184, 313-325 (2003)

112. P.C. Heinrich, I. Behrmann, G. Müller-Newen, F. Schaper, L. Graeve: Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem.J.* 334, 297-314 (1998)

113. F.W. Schwaiger, G. Hager, A.B. Schmitt, A. Horvat, G. Hager, R. Streif, C. Spitzer, S. Garnal, S. Breuer, G.A. Brook, W. Nacimiento, G.W. Kreutzberg: Peripheral but not central axotomy induces changes in Janus kinases (JAK) and signal transducers and activators of transcription (STAT). *Eur.J.Neurosci.* 12, 1165-1176 (2000)

114. B. Stark, T. Carlstedt, M. Risling: Distribution of TGF-beta, the TGF-beta type I receptor and the R-II recptor in peripheral nerves and mechanoreceptors; observations on changes after traumatic injury. *Brain Res.* 913, 47-56 (2001)

115. M. Reiss: Transforming growth factor-Beta and cancer: a love-hate relationship? *Oncol.Res.* 9, 447-457 (1997)

116. G. Grasso, A. Sfacteria, A. Cerami, M. Brines: Erythropoietin as a tissue-protective cytokine in brain injury: what do we know and where do we go? Neuroscientist 10, 93-98 (2004)

117. T. Yasuhara, T. Shingo, I. Data: The potential role of vascular endothelial growth factor in the central nervous system. *Rev.Neurosci.* 15, 293-307 (2004)

118. J.M. Krum, A. Khaibullina: Inhibition of endogenous VEGF impedes revascularization and astroglial proliferation: roles for VEGF in brain repair. *Exp.Neurol.* 181, 241-257 (2003)

119. J.M. Rosenstein, N. Mani, A. Khaibullina, J.M. Krum: Neurotrophic effects of vascular endothelial growth factor on organotypic cortical explants and primary cortical neurons. *J.Neurosci.* 23, 11036-11044 (2003)

120. V.C. Asensio, I.L. Campbell: Chemokines in the CNS. *Trends Neurosci.* 22, 504-512 (1999)

121. H.S. Taskinen, M. Röyttä: Increases expression of chemokines (MCP-1, KIP-1-alpha, RANTES) after peripheral nerve transection. *J.Peripheral Nervous System* 5, 75-81 (2000)

122. F.E. Perrin, S. Lacroix, M. Avilés-Trigueros, S. David: Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1-alpha and interleukin-1-Beta in Wallerian degeneration. *Brain* 128 , 854-866 (2005)

123. V. Appay, S. L. Rowland-Jones. RANTES: a versatile and controversial chemokine. *Trends Immunol.* 22, 83-87 (2001).

124. I. Barakat-Walter: Role of thyroid hormones and their receptors in peripheral nerve regeneration. *J.Neurobiol.* 40, 541-559 (1999)

125. D. García-Ovejero, S. Veiga, L.M. García-Segura, L.L. Doncarlos: Glial expression of estrogen and androgen receptors after rat brain injury. *J.Comp.Neurol.* 450, 256-271 (2002)

126. G.W. Jung, M. Spencer, T.F. Lue: Growth hormone enhynces regeneration of nitric oxide synthase-containing penile nerves after cavernous nerve neurotomy in rats. *J.Urol.* 160, 1899-1904 (1998)

127. J.R. Keast: The autonomic nerve supply of male sex organs - an important target of circulating androgens. *Behav.Brain Res.* 105, 81-92 (1999)

128. D.L. Behrmann, J.C. Bresnahan, M.S. Beattie: Modeling of acute spinal cord injury in the rat: neuroprotection and enhanced recovery with methylprednisolone, U-74006F and YM-14673. *Exp.Neurol.* 126, 61-75 (1994)

129. K.J. Jones: Androgenic enhancement of motor neuron regeneration. *Ann.N.Y.Acad.Sci.* 743, 141-161 (1994)

130.. K.J. Jones, S. Coers, P.D. Storer, L. Tanzer, N.B. Kindermann: Androgenic regulation of the central glia response following nerve damage. *J.Neurobiol.* 40, 560-573 (1999)

131. P. McCaffery, U.C. Dräger: Regulation of retinoic acid signaling in the embryonic nervous system: a master differentiation factor. *Cytokine Growth Factor Rev.* 11, 233-249 (2000)

132. J. Corcoran, M. Maden: Nerve growth factor acts via retinoic acid synthesis to stimulate neurite outgrowth. *Nat.Neurosci.* 2, 307-308 (1999)

133. J. Mey: A new target for CNS injury? The role of retinoic acid signaling after nerve lesions. *J.Neurobiol.* 66, 757-779 (2006)

134. N. Zhelyaznik, J. Mey: Expression of retinoic acid receptors and retinoid X receptors after sciatic nerve injury. *Neuroscience* 141, 1761-1774 (2006)

135. N. Zhelyaznik, K. Schrage, P. McCaffery, J. Mey: Activation of retinoic acid signaling after sciatic nerve injury: upregulation of cellular retinoid binding proteins. *Eur.J.Neurosci.* 18, 1033-1040 (2003)

136. J. Corcoran, B. Shroot, J. Pizzey, M. Maden: The role of retinoic acid receptors in neurite outgrowth from different populations of embryonic mouse dorsal root ganglia. *J.Cell Sci.* 113, 2567-2574 (2000)

137. K. Schrage, G. Koopmans, E.A. Joosten, J. Mey: Macrophages and neurons are targets of retinoic acid signaling after spinal cord contusion injury. *Eur.J.Neurosci.* 23, 285-295 (2006)

138. O. Arrieta, R. García-Navarrete, S. Zúñiga, G. Ordóñez, A. Ortiz, G. Palencia, D. Morales-Espinosa, N. Hernández-Pedro, J. Sotelo: Retinoic acid increases tissue and plasma contents of nerve growth factor and prevents neuropathy in diabetic mice. *Eur.J.Clin.Invest.* 35, 201-297 (2005)

139. M.O. Taha, M. Rosseto, M.M. Fraga, S.F. Mueller, D.J. Fagundes, N.F. Novo, A. Caricati-Neto: Effect of retinoic acid on tibial nerve regeneration after anastomosis in rats: histological and functional analysis. *Transpl.Proc.* 36, 404-408 (2004)

140. L.F. Wong, P.K. Yip, A. Battaglia, J. Grist, J. Corcoran, M. Maden, M. Azzouz, S.M. Kingsman, A.J. Kingsman, N.D. Mazarakis, S.B. McMahon: Retinoic acid receptor Beta-2 promotes functional regeneration of sensory axons in the spinal cord. *Nat.Neurosci.* 9, 243-250 (2006)

141. C. Ide, K. Tohyama, R. Yokota, T. Nitatori, S. Onodera: Schwann cell basal lamina and nerve regeneration. *Brain Res.* 288, 61-75 (1983)

142. Z.-L. Chen, S. Strickland: Laminin g1 is critical for Schwann cell differentiation, axon myelination, and regeneration in the peripheral nerve. *J.Cell Biol.* 163, 889-899 (2003)

143. K. Gelse, E. Pöschl, T. Aigner: Collagens - structure, function and biosynthesis. *Adv.Drug Del.Rev.* 55, 1531-1546 (2003)

144. V.J. Tom, C.M. Doller, A.T. Malouf, J. Silver: Astrocyte-associated fibronectin is critical for axonal regeneration in adult white matter. *J.Neurosci.* 24, 9282-9290 (2004)

145. J. Silver, J.H. Miller: Regeneration beyond the glial scar. *Nat.Rev.Neurosci.* 5, 146-156 (2004)

146. B. Chamak, V. Morandi, M. Mallat: Brain macrophages stimulate neurite outgrowth and regeneration by secreting thrombospondin. *J.Neurosci.Res.* 38, 221-233 (1994)

147. H. Colognato, P.D. Yurchenco: Form and function: The laminin family of heterotrimers. *Dev.Dynamics* 218, 213-234 (2000)

148. J. Engel, E. Odermatt, A. Engel, J.A. Madri, H. Furthmayr, H. Rohde, R. Timpl: Shapes, domain organizations and flexibility of laminin and fibronectin, two multifunctional proteins of the extracellular matrix. *J.Mol.Biol.* 150, 97-120 (1981)

149. U. Hersel, C. Dahmen, H. Kessler: RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 24, 4385-4415 (2003)

150. M. Aumailley, B. Gayraud: Structure and biological activity of the extracellular matrix. *J.Mol.Med.* 76, 253-265 (1998)

151. J.C. Schense, J. Bloch, P. Aebischer, J.A. Hubbell: Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. *Nat.Biotech.*18, 415-419 (2000)

152. J.C. Schense, J.A. Hubbell: Three-dimensional migration of neurites is mediated by adhesion site density and affinity. *J.Biol.Chem.* 275, 6813-6818 (2000)

153. M. Borkenhagen, J.F. Clémence, H. Sigrist, P. Aebischer: Three dimensional extracellular matrix

engineering in the nervous system. *J Biomed Mater Res* 40, 392-400 (1998)

154. T.T. Yu, M.S. Shoichet: Guided cell adhesion and outgrowth in peptide-modified channels for neural tissue engineering. *Biomaterials* 26, 1507-1514 (2005)

155. J. Graf, R.C. Ogle, F.A. Robey, M. Sasaki, G.R. Martin, Y. Yamada, H.K. Kleinman: A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67 000 laminin receptor. *Biochemistry* 26, 6896-6900 (1987)

156. S.P. Massia, S.S. Rao, J.A. Hubbell: Covalently immobilized laminin peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) supports cell spreading and co-localization of the 67-kilodalton laminin receptor with alpha-actinin and vinculin. *J Biol Chem* 268, 8053-8059 (1993)

157. J. Ranieri, R. Bellamkonda, E. Bekos, J. Gardella, H. Mathieu, L. Ruiz, P. Aebischer: Spatial control of neuronal cell attachment and differentiation on covalently patterned laminin oligopeptide substrates. *Int J Dev Neurosci* 725-735 (1994)

158. G.A. Silva, C. Czeisler, K.L. Niece, E. Beniash, D.A. Harrington, J.A. Kessler, S.I. Stupp: Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 303, 1352-1355 (2004)

159. K. Tashiro, G.C. Sephel, B. Weeks, M. Sasaki, G.R. Martin, H.K. Kleinman, Y. Yamada: A synthetic peptide containing the IKVAV sequence from the alpha chain of laminin mediates cell attachment, migration, and neurite outgrowth. *J Biol Chem* 264, 16174-16182 (1989)

160. H. Kleinman, B. Weeks, F. Cannon, T. Sweeney, G. Sephel, B. Clement, M. Zain, M. Olson, M. Judker, B. Burrous: Identification of a 110-kDa non-integrin cell surface laminin-binding protein which recognizes an alpha chain neurite promoting peptide. *Arch Biochem Biophys* 290, 320-325 (1991)

161. L. Luckenbill-Edds, C. Kaiser, T. Rodgers, D. Powell: Localization of 110 kDa receptor for laminin in brains of embryonic and postnatal mice. *Cell Tissue Res* 279, 371-377 (1995)

162. Y.W. Tong, M.S. Shoichet: Enhancing the neuronal interaction on fluoropolymer surfaces with mixed peptides or spacer group linkers. *Biomaterials* 22, 1029-1034 (2001)

163. S. Carbonetto, M.M. Gruver, D.C. Turner: Nerve fiber growth in culture on fibronectin, collagen and glycosaminoglycan substrates. *J Neurosci* 3, 2324-2335 (1983)

164. N. Dubey, P.C. Letourneau, R.T. Tranquillo: Guided neurite elongation and schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration. *Exp Neurol* 158, 338-350 (1999)

165. D. Ceballos, X. Navarro, N. Dubey, G. Wendelschafer-Crabb, W.R. Kennedy, R.T. Tranquillo: Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. *Exp.Neurol.* 158, 290-300 (1999)

166. S.P. Baldwin, C.E. Krewson, W.M. Saltzman: PC12 aggregation and neurite growth in gels of collagen, laminin and fibronectin. *Int J Dev Neurosci* 14, 351-364 (1996)

167. R.O. Labrador, M. Butý, X. Navarro: Influence of collagen and laminin gels concentration on nerve regeneration after resection and tube repair. *Exp Neurol* 149, 243-252 (1998)

168. R. Midha, C.A. Munro, P.D. Dalton, C.H. Tator, M.S. Shoichet: Growth factor enhancement of peripheral nerve regeneration through a novel synthetic hydrogel tube. *J Neurosurg* 99, 555-565 (2003)

169. L.J. Chamberlain, I.V. Yannas, H.P. Hsu, G. Strichartz, M. Spector: Collagen-GAG substrate enhances the quality of nerve regeneration through collagen tubes up to level of autograft. *Exp Neurol* 154, 315-329 (1998)

170. R.-Y. Liu, W.D. Snider: Different signaling pathways mediate regenerative versus developmental sensory axon growth. *J.Neurosci.* 21, RC164 (1-5) (2001)

171. S. Liu, G. Said, M. Tadie: Regrowth of the rostral spinal axons into the caudal ventral roots through a collagen tube implanted into hemisected adult rat spinal cord. *Neurosurgery* 49, 143-151 (2001)

172. M.H. Spilker, I.V. Yannas, S.K. Kostyk, T.V. Norregaard, H.P. Hsu, M. Spector: The effects of tubulation on healing and scar formation after transection of the adult rat spinal cord. *Restor Neurol Neurosci* 18, 23-38 (2001)

173. S.J. Taylor, J.W. McDonald, S.E. Sakiyama-Elbert: Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. *J Control Release* 98, 281-294 (2004)

174. E.C. Tsai, P.D. Dalton, C.H. Tator, M.S. Shoichet: Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. *Biomaterials* 27, 519-533 (2006)

175. S.E. Sakiyama-Elbert, J.A. Hubbell: Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. *J Control Release* 69, 149-158 (2000)

176. N. Dubey, P.C. Letourneau, R.T. Tranquillo: Neuronal contact guidance in magnetically aligned fibrin gels: effect of variation in gel mechano-structural properties. *Biomaterials* 1065-1075 (2001)

177. Q. Yin, G.Kemp, L.-G. Yu, S. Wagstaff, S. Frostick: Neurotrophin-4 delivered by fibrin glue promotes

peripheral nerve regeneration. *Muscle Nerve* 24, 345-351 (2001)

178. E. Verdu, R. Labrador, F. Rodriguez, D. Ceballos, J. Fores, X. Navarro: Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. *Restor Neurol Neurosci* 20, 169-179 (2002)

179. A. Ramón-Cueto, G.W. Plant, J. Avila, M.B. Bunge: Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J Neurosci* 18, 3803-3815 (1998)

180. X.M. Xu, A. Chen, V. Guénard, N. Kleitman, M.B. Bunge: Bridging Schwann cell transplants promote axonal regeneration from both the rostral and caudal stumps of transected adult rat spinal cord. *J Neurocytol* 26, 1-16 (1997)

181. X.M. Xu, V. Guénard, N. Kleitman, M.B. Bunge: Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. *J Comp Neurol* 351, 145-160 (1995)

182. J.B. Phillips, V.R. King, Z. Ward, R.A. Porter, J.V. Priestley, R.A. Brown: Fluid shear in viscous fibronectin gels allows aggregation of fibrous materials for CNS tissue engineering. *Biomaterials* 25, 2769-2779 (2004)

183. J. Priestley, M. Ramer, V. King, S. McMahon, R. Brown: Stimulating regeneration in the damaged spinal cord. *J Physiol Paris* 96, 123-133 (2002)

184. T.A. Horbett: Principles underlying the role of adsorbed plasma-proteins in blood interactions with foreign materials. *Cardiovasc Pathol* 2, S137-S148 Suppl (1993)

185. F. Grinnell, M.K. Feld: Adsorption characteristics of plasma fibronectin in relationship to biological activity. *J Biomed Mater Res* 15, 363-381 (1981)

186. F. Grinnell, M.K. Feld: Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody-binding and analyzed during cell-adhesion in serum-containing medium. *J Biol Chem* 257, 4888-4893 (1982)

187. R. Biran, K. Webb, M.D. Noble, P.A. Tresco: Surfactant-immobilized fibronectin enhances bioactivity and regulates sensory neurite outgrowth. *J Biomed Mater Res* 55, 1-12 (2001)

188. S. Rogers, J. McCarthy, S. Palm, L. Furcht, P. Letourneau: Neuron-specific interactions with two neurite-promoting fragments of fibronectin. *J Neurosci* 5, 369-378 (1985)

189. K.J. Tomaselli, C.H. Damsky, L.F. Reichardt: Interactions of a neural cell line (PC12) with laminin, collagen IV, and fibronectin: Identification of integrin-related glycoproteins involved in attachment and process outgrowth. *J Cell Biol* 105, 2347-2358 (1987)

190. D. Hall, K. Neugebauer, L. Reichardt: Embryonic neural retinal cell response to extracellular matrix proteins: developmental changes and effects of the cell substratum attachment antibody (CSAT). *J Cell Biol* 104, 623-634 (1987)

191. Z. Sisková, W. Baron, H. de Vries, D. Hoekstra: Fibronectin impedes "myelin" sheet-directed flow in oligodendrocytes: a role for a beta 1 integrin-mediated PKC signaling pathway in vesicular trafficking. *Mol Cell Neurosci* 33, 150-9 (2006)

192. A. Balgude, X. Yu, A. Szymanski, R. Bellamkonda: Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures. *Biomaterials* 22, 1077-1084 (2001)

193. R. Bellamkonda, J. Ranieri, N. Bouche, P. Aebischer: Hydrogel-based three-dimensional matrix for neural cells. *J Biomed Mater Res* 29, 663-671 (1995)

194. X. Yu, R. Bellamkonda: Dorsal root ganglia neurite extension is inhibited by mechanical and chondroitin sulphate-rich interfaces. *J Neurosci Res* 66, 303-310 (2001)

195. X. Cao, M.S. Shoichet: Defining the concentration gradient of nerve growth factor for guided neurite outgrowth. *Neuroscience* 103, 831-840 (2001)

196. X. Cao, M.S. Shoichet: Investigating the synergistic effect of combined neurotrophic factor concentration gradients to guide axonal growth. *Neuroscience* 122, 381-389 (2003)

197. G.L. Ming, H.J. Song, B. Berninger, C.E. Holt, M. Tessier-Lavigne: cAMP-dependent growth cone guidance by netrin-1. *Neuron* 19, 1225-1235 (1997)

198. H.J. Song, G.L. Ming, M.-M. Poo: cAMP-induced switching in turning direction of nerve growth cones. *Nature* 388, 275-279 (1997)

199. Y. Luo, M.S. Shoichet: A photolabile hydrogel for guided three dimensional cell growth and migration. *Nat Mater* 3, 249-253 (2004a)

200. R. Mumper, A. Hoffman, A. Puolakkainen, L. Bouchard, W. Gombotz: Calcium-alginate beads for the oral delivery of transforming growth factor- PI (TGF- p1) : stabilization of TGF- PI by the addition of polyacrylic acid within acid-treated beads. *J Control Release* 30, 241-251 (1994)

201. S. Chang, J. Rowley, G. Tobias, N. Genes, A. Roy, D. Mooney, C. Vacanti, L. Bonassar: Injection molding of chondrocyte/alginate constructs in the shape of facial implants. *J Biomed Mater Res* 55, 503-511 (2001)

202. J. Rowley, D. Mooney: Alginate type and RGD density control myoblast phenotype. *J Biomed Mater Res* 60, 217-223 (2002)

203. S. Wu, Y. Suzuki, M. Kitada, M. Kitaura, K. Kataoka, J. Takahashi, C. Ide, Y. Nishimura: Migration, integration, and differentiation of hippocampus-derived neurosphere cells after transplantation into injured rat spinal cord. *Neurosci Lett* 312, 173-176 (2001)

204. H.F. Liang, M.H. Hong, R.-M. Ho, C.-K. Chung, Y.-H. Lin, C.-H. Chen, H.-W. Sung: Novel method Using a Temperature-Sensitive Polymer (Methylcellulose) to Thermally Gel Aqueous Alginate as a pH-Sensitive Hydrogel. *Biomacromolecules* 5, 1917-1925 (2004)

205. Y. Suzuki, M. Kitaura, S. Wu, K. Kataoka, K. Endo, Y. Nishimura, C. Ide: Electrophysiological and horseradish peroxidase-tracing studies of nerve regeneration through alginate-filled gap in adult rat spinal cord. *Neurosci Lett* 318, 121-124 (2002)

206. K. Suzuki, Y. Suzuki, M. Tanihara, K. Ohnishi, T. Hashimoto, K. Endo, Y. Nishimura: Reconstruction of rat peripheral nerve gap without sutures using freeze-dried alginate gel. *J Biomed Mater Res* 49, 528-533 (2000)

207. M. Ohta, Y. Suzuki, H. Chou, N. Ishikawa, S. Suzuki, M. Tanihara, Y. Suzuki, Y. Mizushima, M. Dezawa, C. Ide: Novel heparin/alginate gel combined with basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. *J Biomed Mater Res* 71A, 661-668 (2004)

208. T. Hashimoto, Y. Suzuki, K. Suzuki, T. Nakashima, M. Tanihara, C. Ide: Review: Peripheral nerve regeneration using non-tubular alginate gel crosslinked with covalent bonds. *J Mater Sci Mat Med* 16, 503-509 (2005)

209. G.T. Hermanson: *Bioconjugate Techniques.* Academic Press (1996)

210. S. Zhang, T.C. Holmes, M. Diperssio, R.O. Hynes, X. Su, A. Rich: Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* 16, 1385 (1995)

211. C.E. Semino, J. Kasahara, Y. Hayashi, S. Zhang: Entrapment of migrating hippocampal neural cells in three-dimensional peptide nanofiber scaffold. *Tissue Engineering* 10, 643-655 (2004)

212. T.C. Holmes, S. Delacalle, X. Su, A. Rich, S. Zhang: Extensive neurite outgrowth and active neuronal synapses on peptide scaffolds. *PNAS* 97, 6278 (2000)

213. J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. Grodzinsky: Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: Implications for cartilage tissue repair. *PNAS* 99, 9996-10001 (2002)

214. S. Carbonetto, M.M. Gruver, D.C. Turner: Nerve fiber growth on defined hydrogel substrates. *Science* 216, 897-899 (1982)

215. T. Adams-Kapur, M.S. Shoichet: Immobilized concentration gradients of nerve growth factor guide neurite outgrowth. *J Biomed Mater Res* 68A, 235-243 (2004)

216. J. Kohn, R.S. Aloyz, J.G. Toma, M. Haak-Frendscho, F.D. Miller: Functionally anagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. *J Neurosci* 19, 5393-5408 (1999)

217. E.C. Tsai, P.D. Dalton, M.S. Shoichet, C.H. Tator: Synthetic hydrogel guidance channels facilitate regeneration of adult rat brainstem motor axons after complete spinal cord transection. *J.Neurotrauma* 21, 789-804 (2004)

218. T.K. Huang, G.L. Chang, H.S. Lin, F.R. Walter, L. Bunegin: Stress-strain relationship of the spinal cord of anesthetized cats. *J Biomechanics* 14, 269-276 (1988)

219. P.D. Dalton, L. Flynn, M.S. Shoichet: Manufacture of poly (2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes for use as nerve guidance channels. *Biomaterials* 23, 3843-3851 (2002)

220. R.L. Waddell, K.G. Marra, K.L. Collins, J.T. Leung, J.S. Doctor: Using PC12 cells to evaluate poly (caprolactone) and collagenous microcarriers for applications in nerve guide fabrication. *Biotechnol Prog* 19, 1767-1774 (2003)

221. S. Gautier, M. Oudega, M. Fragoso, P. Chapon, G. Plant, M. Bunge, J.-M. Parel: Poly (alpha-hydroxyacids) for application in the spinal cord: Resorbability and biocompatibility with adult rat Schwann cells and spinal cord. *J Biomed Mater Res* 42, 642-654 (1998)

222. M. Oudega, S. Gautier, P. Chapon, M. Fragoso, M. Bates, J.-M. Parel, M. Bunge: Axonal regeneration into Schwann cell grafts within resorbable poly (-hydroxyacid) guidance channels in the adult rat spinal cord. *Biomaterials* 22, 1125-1136 (2001)

223. M.J. Roberts, M.D. Bentley, J.M. Harris: Chemistry for peptide and protein PEGylation. *Adv Drug Del Rev* 54, 459-476 (2002)

224. J. Groll, Z. Ademovic, T. Ameringer, D. Klee, M. Moeller: Comparison of coatings from reactive star shaped PEG-stat-PPG prepolymers and grafted linear PEG for biological and medical applications. Biomacromolecules 6, 956-962 (2005)

225. D. Branch, B. Wheeler, G. Brewer, D. Leckband: Long-term stability of grafted polyethylene glycol surfaces for use with microstamped substrates in neuronal cell culture. *Biomaterials* 22, 1035-1047 (2001)

226. M.P. Lutolf, J.L. Lauer-Fields, H.G. Schmoekel, A.T. Metter, F.E. Weber, G.B. Fields, J.A. Hubbell: Synthetic matrix metalloproteinase-sensitive hydrogels for the

conduction of tissue regeneration: Engineering cell-invasion characteristics. *PNAS* 100, 5413-5418 (2003)

227. M.P. Lutolf, G.P. Raeber, A.H. Zisch, N. Tirelli, J.A. Hubbell: Cell-responsive synthetic hydrogels. *Adv Mater* 15, 888-892 (2003)

228. M.J. Mahoney, K.S. Anseth: Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. Biomaterials 27, 2265-2274 (2006)

229. C.E. Schmidt, V.R. Shastri, J.P. Vacanti, R. Langer: Stimulation of neurite outgrowth using an electrically conducting polymer. *PNAS* 94, 8948-8953 (1997)

230. A. Kotwal, C. Schmidt: Electrical stimulation alters protein adsorption and nerve cell interactions with electrically conducting biomaterials. *Biomaterials* 22, 1055-1064 (2001)

231. P.M. George, A.W. Lyckman, D.A. LaVan, A. Hegde, Y. Leung, R. Avasare, C. Testa, P.M. Alexander, R. Langer, M. Sur: Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. *Biomaterials* 26, 3511-3519 (2005)

232. S.J. Chen, D.Y. Wang, C.W. Yuan, X.D. Wang, P.Y. Zhang, X.S. Gu: Template synthesis of the polypyrrole tube and its bridging *in vivo* sciatic nerve regeneration. *J Mater Sci Lett* 19, 2157-2159 (2000)

233. J.M. Corey, E.L. Feldman: Substrate patterning: an emerging technology for the study of neuronal behavior. *Exp Neurol* 184, S89-S96 (2003)

234. P.C. Letourneau: Cell-to-substratum adhesion and guidance of axonal elongation. *Dev Biol*, 44 , 92-101 (1975)

235. H.-C. Tai, H.M. Buettner: Neurite outgrowth and growth cone morphology on micropatterned surfaces. *Biotechnol Prog* 14, 364-370 (1998)

236. A.K. Vogt, L. Lauer, W. Knoll, A. Offenhäusser: Micropatterned substrates for the growth of functional neuronal networks of defined geometry. *Biotechnol Prog* 19, 1562-1568 (2003)

237. H.X. Avci, P. Zelina, K. Thelen, G.E. Pollerberg: Role of cell adhesion molecule DM-GRASP in growth and orientation of retinal ganglion cell axons. *Dev Biol* 271, 291-305 (2004)

238. D. Adams, E. Kao, C. Hypolite, M. Distefano, W.-S. Hu, P. Letourneau: Growth cones turn and migrate up an immobolized gradient of the laminin IKVAV peptide. *J Neurobiol* 62, 134-147 (2005)

239. S.M. Smeal, R. Rabbitt, R. Biran, P.A. Tresco: Substrate curvature influences the direction of nerve outgrowth. *Ann Biomed Eng* 33, 376-382 (2005)

240. L.N. Novikov, L.N. Novikova, A. Mosahebi, M. Wiberg, G. Terenghi, J.-O. Kellerth: A novel biodegradable implant for neuronal rescue and regeneration after spinal cord injury. *Biomaterials* 23, 3369-3376 (2002)

241. C. Miller, S. Jeftinija, S. Mallapragada: Micropatterned Schwann cell-seeded biodegradable substrates significantly enhance neurite alignment and outgrowth. *Tissue Engineering* 7, 705-715 (2001)

242. R. Yang, R. Murugan, S. Wang, S. Ramakrishna: Electrospinning of nano/micro scale poly (L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials* 26, 2603-2610 (2005)

243. P.D. Dalton, D. Klee, M. Möller: Electrospinning with dual collection rings. *Polymer* 46, 611-614 (2005)

244. E. Schnell, K. Klinkhammer, S. Balzer, G. Brook, D. Klee, P.D. Dalton, J. Mey: Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-ε-caprolactone and a collagen/poly-ε-caprolactone blend. *Biomaterials* 28, 3012-3025 (2007)

245. S. Zhang: Beyond the Petri dish. *Nat Biotech* 22, 151-152 (2004)

246. J.A. Hubbell: Materials as morphogenetic guides in tissue engineering. *Current Opin Biotech* 14, 551-558 (2003)

247. L. Flynn, P.D. Dalton, M.S. Shoichet: Fiber templating of poly (2-hydroxyethyl methacrylate) for neural tissue engineering. *Biomaterials* 24, 4265-4272 (2003)

248. T. Hadlock, C. Sundback, D. Hunter, M. Cheney, J.P. Vacanti: A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. *Tissue Engineering* 6, 119-127 (2000)

249. S. Blacher, V. Maquet, F. Schils, D. Martin, J. Schoenen, G. Moonen, R. Jérôme, J.P. Pirard: Image analysis of the axonal ingrowth into poly (D,L-lactide) porous scaffolds in relation to the 3-D porous structure. *Biomaterials* 24, 1033-1040 (2003)

250. S. Stockols, M.H. Tuszynski: The fabrication and characterization of linearly oriented nerve guidance scaffolds for spinal cord injury. *Biomaterials* 25, 5839-5846 (2004)

251. S. Stockols, M. Tuszynski: Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. *Biomaterials* 27, 443-451 (2006)

252. V. Maquet, D. Martin, B. Malgrange, R. Franzen, J. Schoenen, G. Moonen, R. Jérôme: Peripheral nerve regeneration using bioresorbable macroporous polylactide scaffolds. *J Biomed Mater Res* 52, 639-651 (2000)

253. V. Maquet, D. Martin, F. Scholtes, R. Franzen, J. Schoenen, G. Moonen, R. Jérôme: Poly (D,L-lactide) foams modifed by poly (ethylene oxide)-block-poly (D,L-lactide) copolymers and a-FGF: *in vitro* and *in vivo* evaluation for spinal cord regeneration. *Biomaterials* 22, 1137-1146 (2001)

254. A. Hurtado, L. Moon, V. Maquet, B. Blits, R. Jérôme, M. Oudega: Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modifed to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord. *Biomaterials* 27, 430-442 (2006)

255. Y.D. Teng, E.B. Lavik, X. Qu, K.I. Park, J. Ourednik, D. Zurakowski, R. Langer, E.Y. Snyder: Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *PNAS* 99, 3024-3029 (2002)

256. G. Mukhopadhyay, P. Doherty, F.S. Walsh, P.R. Crocker, M.T. Filbin: A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. *Neuron* 13 , 757-767 (1994)

257. Y. Luo, M.S. Shoichet: Light-activated immobilization of biomolecules to agarose hydrogels for controlled cellular response. *Biomacromolecules* 5, 2315-2323 (2004)

258. J.M. Corey, B.C. Wheeler, G.J. Brewer: Compliance of hippocampal neurons to patterned substrate networks. *J Neurosci Res* 30, 300-307 (1991)

259. D. Shaw, M.S. Shoichet: Towards spinal cord injury repair strategies: peptide surface modification of expanded poly (tetrafluoroethylene) fibers for guided neurite outgrowth *in vitro*. *J Craniofacial Surg* 14, 308-316 (2003)

260. P.X. Ma, R. Zhang: Microtubular architecture of biodegradable polymer scaffolds. *J Biomed Mater Res* 56, 469-477 (2001)

Send correspondence to: Paul Donald. Dalton, Deutsche Wollforschungsinstitut, Pauwelstr. 8, Aachen, Germany 52056, Tel: 49 2418023343, Fax: 49 2418023301, E-mail: dalton@dwi.rwth-aachen.de