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Nanomaterials for Neural Tissue Engineering

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Abstract: This chapter reviews the use of strategies combining nanotechnology with other guidance cues (e.g., biomaterials, support cells, chemical factors, etc.) in neural tissue engineering. Nano-structure fabrication techniques and various nanomaterials in neural tissue engineering are presented.

Keywords: peripheral nerve regeneration, nerve guidance conduit, nerve growth factor, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, mesenchymal stem cells, embryonic stem cells, induced pluripotent stem cells, micropatterns, nanofibers, nanoparticles, nanomaterials, nanotubes, nanogels, neural regeneration, tissue engineering, electrospinning, biomaterials, self-assembly, conduits, degradable

1. Introduction to Neural Tissue Engineering

The major components of the nervous system are the central- (CNS) and peripheral nervous systems (PNS). The CNS consists of the brain and spinal cord, while the PNS is comprised of motor and sensory neurons that transfer impulses from and to the CNS, respectively, and enable communication between the CNS and other parts of the body. Following injury, damage to the nervous system may appear as demyelination of the nerves, nerve degeneration, scar tissue formation or a communication gap between neurons and/or support cells. As nerve degeneration progresses at the distal stump, this will likely result in serious, long term neurological deficits unless appropriate interventions are successfully implemented (Zhang et al., 2005a, Yucel et al., 2010). Unlike the CNS, the PNS has considerable endogenous regenerative capacity following injury in large part due to its glial cell components, the Schwann cells (SCs) (Horner and Gage,
The CNS also has glial support cells, the astrocytes and oligodendrocytes. The oligodendrocytes, like the peripheral SCs, form the insulating myelin sheath around the axons. However, unlike SCs, the oligodendrocytes hinder regeneration by the production of inhibitory molecules. Currently, several techniques are used to repair nerve lesions. Coaptation is one of the most common surgical procedures that use sutures to repair damaged nerves. The parts to be repaired are forcefully connected to each other by means of direct sutures. This technique is used for closing wounds, suturing lesions in severed nerves (Haastert et al., 2010) or treating bone fractures (Weinstein and Ralphs, 2004).

Other forms of therapy involve transplantation or grafting procedures. An example is an allograft wherein cells, tissues and organs are transplanted from donors of the same species. To prevent tissue rejection, both forms of transplantation require immunosuppression (IS) (Grinyo et al., 2010, Karabekmez et al., 2009), which sometimes causes infections and tumor formation in the recipient (Xiong et al., 2006, Han et al., 2012). Xenograft is the transplantation of tissue from a donor of a different species than the recipient. This procedure raises ethical concerns (Silva, 2006). In addition, the age between donor and host tissues must be considered, as tissues from different species age at different rates. The gold standard in transplantation therapy is autografting, wherein the patient’s own tissue is transplanted from one part of the body to another part of their body. It is the best approach for repairing nerve lesions, especially for short gaps (Siemionow and Brzezicki, 2009, Zheng and Cui). This procedure avoids ethical issues, reduces the likelihood of rejection and eliminates the need for IS. Drawbacks of this therapy include the need of multiple surgeries and donor site morbidity, and thus limit its widespread clinical use, especially for longer nerve gaps. However, recent advances in neural tissue engineering (NTE) show promise for neural regeneration.

Neural tissue engineering is an interdisciplinary field wherein medicine, engineering and life sciences work together to discover and develop alternatives for nerve regeneration (Langer and Vacanti, 1993). Various guidance cues are applied with engineering and clinical methods to repair nerve deficits and promote neural regeneration. These cues include use of support- and stem cells, trophic and growth factors, and biological and synthetic biomaterials. They are implemented on natural or artificial platforms called scaffolds. The scaffold design is crucial in
neural regeneration since these guidance cues can mimic the microenvironment of the extracellular matrix (ECM) and promote attachment, proliferation, growth, migration, differentiation and survival of the cells, and offer encouraging outcomes and useful alternatives.

Entubulized scaffolds are tubular devices with enhanced surface and structural properties that are used to enclose severed nerves and bridge nerve gaps, and offer interesting possibilities for therapeutic strategies (Dillon et al., 1998). These artificial nerve guidance conduits (NGCs) are made of biological or synthetic materials and consist of various types of fibers with tubes, channels or lumens of varying size. They can be combined with other guidance cues. These can be physical (e.g., nerve guidance conduits, patterned substrates, nano-scaffolds), chemical (e.g., neurotrophic or other growth factors), and cellular cues (e.g., SCs, stem cells, etc.) (Miller et al., 2001, Recknor et al., 2006). In combination with NGCs, their use has resulted in enhanced and oriented neural regeneration as well as restoration of function (Fig. 1). Studies have shown that use of NGCs with other guidance cues can be a favorable alternative to autologous nerve grafts (Sedaghati et al., 2011, Gu et al., 2011).

An ideal NGC must fulfill a number of requirements prior to its use for facilitated neural repair. Essential properties of an ideal NGC include:

1. Biocompatibility
2. Biodegradability
3. Low-toxicity
4. Infection resistance
5. Permeability
6. Porosity
7. Mechanical properties
8. Conductivity
9. Orientation
Biocompatibility plays a critical role in designing a nerve conduit. The scaffold material should be non-toxic, and not cause inflammation or swelling of tissues/cells at the transplant site. Biodegradable conduits reduce the number of surgeries needed to repair neural lesions. Conduits must maintain structural integrity before complete regeneration is achieved. The porosity and permeability of the NGC are crucial for maintaining mechanical strength and for the transfer of nutrients and metabolites.

The NGCs can be a platform for guidance cues, e.g., embedded growth factors, etc. to facilitate neural regeneration. The controlled and continuous release of such factors during the lifetime of the conduit is necessary to avoid critical problems such as inflammation, swelling and deterioration of surrounding tissues. Electric and magnetic cues may also stimulate enhanced regeneration. The conductivity of the NGC can be increased by using appropriate scaffold material or application of electrical cues; however, problems such as non-biodegradability of conductive polymers must be avoided.

**Fig. 1** Schematic diagram demonstrates the properties of an ideal nerve guidance conduit. Various guidance cues can be incorporated into NGCs to mimic the ECM. Reproduced with permission from (Marti et al., 2012).
Nanotechnology is the use of engineered materials on a nanometer (nm) scale. The sizes of these materials are generally between 1- and ~100 nm, and they are compatible with biological systems including regenerative neural tissue therapy. Nano-engineered structures, especially nanotubes (NTs) and nanofibers (NFs) imitate the structure of the extracellular matrix (ECM) and can mimic the environment of the nervous system. Recent and remarkable improvements in nanotechnology offer promising alternatives for neural regeneration studies. Nanotechnology can be used to engineer scaffolds with oriented NFs, NTs and other materials that are functionalized to serve as cell-binding domains or for the release of trophic- or growth factors. These scaffolds also facilitate nutrient- and oxygen diffusion, and provide topographical cues. Recent advances in nanotechnology, biomaterials and tissue engineering have improved the effectiveness of NGCs and enabled their use with a control at nano-scale to obtain enhanced and oriented (Fig. 2) neuroregeneration (Zhang and Webster, 2009).

In the next few sections, the use of nanotechnology combined with other guidance cues (e.g., biomaterials, support cells, chemical factors, etc.) in neural tissue engineering strategies is reviewed. Nano-structure fabrication techniques and nano-materials in NTE are presented. Applications and recent advances in research are also discussed.

Fig. 2 The schematic diagram shows growth and orientation of nerve cell fibers (A: Axons stained with anti-tau-protein, B: the nerve cell bodies stained with anti-MAP2 protein). Confocal microscope images of elongated axons
2. Nano-scaffold Design Techniques

The structure and geometry of the nano-structural scaffolds are very important to promote enhanced cell activity for neural regeneration. Thus, graft technology to produce these materials is critical to obtain optimum mechanical strength and surface properties for axon regeneration. Nano-structures made of several biomaterials have been shown to support axonal outgrowth. Several methods have been used to fabricate nano-structural scaffolds such as electrospinning, self-assembly and phase separation (Cunha et al., 2011).

a) Electrospinning

Electrospinning is a technique for producing NFs in micro-/nano-scale from natural and synthetic polymers or polymer composites. A solution of the starting material in an appropriate solvent is charged using a spinneret and a high voltage supply. This is done at a polymer concentration above the critical entanglement concentration, below which NFs are not produced. Under the influence of an electrical field, the surface tension of the polymer is overcome at the tip of the spinneret and the charged polymer jet is directed at a target, resulting in formation of a Taylor cone. Nanofibers emerging from spinneret are then collected in a parallel orientation using a rotating collector (Fig. 3). The latter can be a drum or a disk as well as a plate. The use of a stationary collector may result in randomly oriented NFs.
By electrospinning, fibers of nano- to micrometer size dimensions can be fabricated. Solution properties (i.e. concentration, conductivity, viscosity and elasticity) and process parameters (i.e. voltage, distance from target, needle identity, and temperature) can be altered to fabricate various types of fibers for various applications. The mechanical properties of the NFs (e.g., thickness, composition, surface area and porosity) are adjusted by controlling the solution and process parameters to optimize the alignment, stability and morphology (Leach et al., 2011b, Cunha et al., 2011). For different applications, the collection schemes can be varied as single ground, rotating single ground, horizontal ring, and in vitro onto cells (Ishaug-Riley et al., 1999). To overcome the disadvantages associated with the fiber thickness and random orientation, blends of different biomaterials can be used. Thus, various types of biological materials, synthetic biopolymers and composites have been used to manufacture NFs by electrospinning.

For neural regeneration, the features of the scaffolds are extremely critical to mimic the ECM and achieve successful repair. For example, electric fields used during fabrication enhance control of fiber alignment, which is significant for neural regeneration. Electrospun NFs have been shown to enhance and accelerate cellular activities such as proliferation, axon regeneration, growth and migration (Dong et al., 2006, Liu et al., 2012b) and have been used for neural tissue engineering applications (Kijenska et al., 2012, Wang et al., 2011a). Research to produce nano-
structural scaffolds with desired mechanical- and surface properties for nerve conduits using electrospinning is continuing.

b) Self-Assembly

Self-assembly (SA) is the reversible and spontaneous formation of a stable organized/ordered structure, from disordered elements through various weak interactions, e.g. non-covalent bonds and hydrophobic interactions. SA can occur at the molecular level and in the nano- and micro-scale. In nature, numerous types of SA processes are carried out. Amino acids, fatty acids, and other molecules come together to form self-assembled nano- or larger structures (Toksoz and Guler, 2009).

The SA process has been shown to occur with polypeptide sequences, and di- and triblock peptide amphiphilic copolymers. Porous NFs of 5-10 nm diameter fabricated by self-assembly from various biodegradable/biocompatible materials have the potential to mimic the 3-D microenvironment of the ECM and promote neural regeneration by enhancing cell attachment, proliferation, differentiation and migration. Amphiphilic oligopeptides composed of repeating units of hydrophilic and hydrophobic amino acids formed stable β-sheet structures in water and have been used to make scaffolds for neuronal regeneration (Zhang et al., 1995). Zhang et al. reported that with the addition of salts, these oligopeptides self-assembled into stable macroscopic structures of ordered filaments with porous enclosures, and supported cell attachment and synapse formation. Holmes et al. demonstrated the enhancement of neurite outgrowth with self-assembled peptide hydrogels (Holmes et al., 2000). Various macromolecules such as peptides have the ability to self-assemble and form ordered structures and nanomaterials due to several interactions between the components (Chang et al., 2008). Sur et al. designed a hybrid matrix consisting of collagen type I and a peptide amphiphile (PA). They produced homogenous NFs of 20-30 nm in diameter having the structural properties of the former and epitope-presenting ability of the latter components by self-assembly (Sur et al., 2012). The cellular density was shown to be manipulated by epitope concentration, which could be changed by PA concentration. Besides, controllable axon and dendrite growth were carried out as well as neuronal survival and maturation with epitope concentration adjustment. Furthermore, controllable axon and dendrite growth were carried out, as well as enhanced neuronal survival
and maturation with epitope concentration adjustment. In another recent study, Angeloni et al. used biodegradable bundles of PA-NFs (Fig. 4), which were fabricated by self-assembly and incorporated sonic hedgehog (SHH) protein molecules, for controlled delivery of the latter, which has an important role in cavernous nerve integrity (Angeloni et al., 2011).

![Fig. 4](image)

**Fig. 4** Structure of peptide-amphiphile (PA) used to form mono-domain noodle gels (a), molecular model of the PA molecule (b), nanofiber formation through self-assembly (c), nanofiber bundles assembled in a longitudinal alignment after noodle formation. Reproduced with permission from (Angeloni et al., 2011).

SA is viewed as an approach to change and control the functions of the scaffolds for neural repair (Holmes et al., 2000, Zhang et al., 2005b). However, self-assembled nanomaterials have limited mechanical strength and unfavorable degradation rates. Moreover, fabrication of these structures using this technique for large neural gaps is not very practical (Chang et al., 2008).

c) **Phase Separation**

Another technique for NF production is phase separation (PS). The rationale for phase separation is the physical incompatibility or immiscibility of the polymer and the solvent. Initially, the polymer is dissolved in a solvent and the resulting solution is kept at the gelation temperature to promote gel formation of the polymer phase. After this, the solvent can be extracted from the gel and a porous nanofibrous skeleton is recovered. There are various types of PS methods for preparing NFs, such as nonsolvent-, chemically- and thermally-induced phase separation (TIPS).
In TIPS, polymer-rich and solvent-rich phases are produced by reducing the temperature of the polymer solution. The solvent-rich phase is removed from the solution by extraction or simple evaporation, leaving behind a solid, skeletal-like polymer matrix with pores. The morphology of the pores depends on various factors like concentration of the polymer solution, solvent properties, PS temperature and type of solvent removal techniques. TIPS is classified into different methods depending on solvent freezing- and phase separation temperatures. If the former is lower than the phase separation temperature, then PS occurs when the solution is cooled to between the lower- and upper critical solution temperatures i.e. in the unstable region of the binary phase diagram. At this temperature, a stable morphological pore network forms in the polymer foam. This is called the liquid-liquid phase separation (LLPS) process. Several synthetic biodegradable polymer scaffolds have been formed using this method in solvents like tetrahydrofuran and dioxane/water etc. (Pavia et al., 2008, Nam and Park, 1999b, Hua et al., 2002).

In the solid-liquid phase separation (SLPS) process, the solvent freezing point is higher than the phase separation temperature of the solution, and the solvent freezes when temperature is reduced below this point. The polymer comes out of solution with a pore structure corresponding to the crystallization front of the solvent. Similar to LLPS, some of the synthetic biodegradable scaffolds have been produced using this method in solvent mixtures of dioxane and water (Nam and Park, 1999a). Microporous membranes of polypropylene, polyethylene etc. have been prepared using TIPS and SLPS (Lloyd et al., 1990).

3. Nano-structures

Nanomaterials of different morphologies, produced by electrospinning, phase separation and self-assembly, can enhance axonal outgrowth and facilitate neural regeneration. Most important of these nanomaterials are nanogels (NGs), nanoparticles (NPs) and, in particular, nanotubes (NTs) and nanofibers (NFs), as these can mimic the tubular structures and within cells and tissues (Gilmore et al., 2008).

a. Nanotubes
Nanotubes are cylindrical structures with diameters of ~1-100 nm (Jayatissa and Guo, 2009, Tran-Duc et al., 2011, Gallagher et al., 1993, Wang et al., 2004). They are capable of simulating various intracellular structures such as microtubules and axons, etc. and also can penetrate cell membranes. They have a very high tensile strength (i.e. high strength to volume ratio) (Yu et al., 2000, Demczyk et al., 2006). With respect to neural regeneration, NGCs with bundles of NTs may provide higher surface area compared to that of conduits alone (Fadel et al., 2008). Moreover, elongation in 1-D can provide a better support and orientation to axon regeneration (Huang et al., 2011, Liu et al., 2012a). Carbon NTs (CNTs) were tested and recommended for use as NGCs because of their favorable mechanical, elastic, electrical and rheological properties (Ashrafi and Hubert, 2006, Saether et al., 2003, Schulz et al., 2011). Graphene, the main constituent of CNTs, has been shown to promote neurite sprouting and outgrowth compared to tissue culture polystyrene substrates (Li et al., 2011). Various functional groups (e.g., carboxylic acid, ethylenediamine etc.) can be attached to CNTs to modify their chemical and electrical properties to promote controlled neuronal regeneration (Hu et al., 2004). It was reported that human embryonic stem cells differentiated into neural lineages more efficiently on silk-CNT scaffolds with higher expression levels of β-III tubulin and nestin (Chen et al., 2012). CNTs were also shown to promote formation of synaptic contacts and modulation of the cells’ plasticity (Cellot et al., 2011).

b. Nanofibers

Nanofibers or nanowires are similar to NTs in structure and functionality. They are porous cylinders oriented in 1-D with a high interconnectivity and high specific surface area. They can mimic the tubular structure associated with ECM like NTs. Various biomaterials have been used to make NFs using electrospinning (Dong et al., 2006, Meng et al., 2010, Yeganegi et al., 2010). NFs made of polyhydroxyalkanoate (PHA) were observed to promote neural stem cell attachment, synapse formations (synaptogenesis) and central nervous system regeneration (Xu et al., 2010). Chitosan NF mesh tubes immobilized with electrically-charged β-tricalcium phosphate (β-TCP) particles improved nerve regeneration by increasing axon density and area (Wang et al., 2010). Mammadov et al. used a synthetic peptide nanofiber to mimic the activity of laminin and heparan sulfate proteoglycans to enhance axonal growth and induce neuritogenesis, respectively. These self-assembled NF scaffolds were shown to be effective for neurite
outgrowth as well as surmounting the inhibitory effect of chondroitin sulfate proteoglycans (Mammadov et al., 2012). Electrospun-collagen NFs impregnated with neurotrophin-3 (NT-3) and chondroitinase ABC (ChABC) supported neuronal culture and neurite outgrowth for a longer period than bolus delivery of NT-3. These hold promise for nerve regeneration following spinal cord related injuries, as they provide topographical and biochemical cues and suppress the inhibitory activity during axonal regrowth in the CNS (Liu et al., 2012b).

c. **Nanoparticles**

Nanoparticles are tiny particles having at least one of dimension under 100 nm. NPs with lower melting points (Nanda, 2009), higher solar absorption capabilities (Edman Jonsson et al., 2011), and super paramagnetism (Mikhaylova et al., 2004) etc., are typically different from bulk materials. Due to this, NPs have been used in various industrial applications, e.g., electronics, biomedical engineering, optics etc. NPs have very high surface area-to-volume ratios resulting in high diffusion gradients (Soppimath et al., 2001) and enhanced released of substances such as drugs, proteins, peptides etc., from NPs to the surrounding environments.

Chitosan-heparin NPs impregnated with nerve growth factors were shown to improve nerve regeneration in mice and to function as a robust NP drug delivery system to the sciatic nerve (Gonçalves et al., 2012). Super-paramagnetic NPs functionalized with TrkB receptor antibodies were shown to be endocytosed into signaling endosomes by neurons which in turn promoted neurite outgrowth and activated TrkB dependent signaling (Steketee et al., 2011). Nano-silver embedded into collagen scaffolds coated with laminin and fibronectin were shown to increase axonal regeneration and number of the recovered nerves were comparable to an autologous nerve graft (Ding et al., 2011). Nerve regeneration was improved using microstructured polymer filaments in the form of nerve implants containing chitosan/siRNA NPs. These stable were rapidly internalized by cells and did not affect cell viability (Mittnacht et al., 2010). A nanostructured 2-D substrate comprising gold NPs attached to the surface of a cover glass via an adsorption system has been shown to improve neurite outgrowth of PC12 cells in the presence of electrical stimuli (Park et al., 2008). Such gold NPs can be used as suitable tools for nerve regeneration from neuronal cells.

d. **Nanogels**
Nanogels are among the more recent products to be used in nanotechnology. They are nano-scale hydrophilic cross-linked networks of biocompatible polymers and are mostly used for encapsulating drugs and as efficient drug delivery vehicles (Peng et al., 2012, Shidhaye et al., 2008). Nanogels are prepared using polymerization techniques such as free radical crosslinking (Sanson and Rieger, 2010), free radical precipitation (Blackburn and Lyon, 2008), nano-emulsion polymerization (Sasaki and Akiyoshi, 2010), etc. Porous scaffolds have been prepared from protein NGs (Zhu et al., 2011) which may be useful for NTE purposes as well. These gels have also been suggested as a promising system for delivering of drugs like oligonucleotides to the brain (Vinogradov et al., 2003).

4. Biomaterials for scaffold design

The success of conduit implantation for nerve regeneration depends on engrafting methods and materials as well as cellular activities such as survival, growth, proliferation and differentiation, and their synergistic use with trophic and growth factors. It is crucial to mimic the 3-D architecture of the microenvironment to achieve neural regeneration. Thus, an ideal NGC should possess aspects mimicking the ECM as a critical factor for neuroregeneration. For this purpose, the use of support cells and inducing chemicals on a nano/micro-platform made of appropriate biomaterials and ECM components is essential. NGCs mimicking the ECM can be used to control, direct and also modify the guidance cues. However, it is a challenging undertaking. As mentioned, biomaterials used for neural regeneration should fulfill requirements such as biodegradability, biocompatibility, porosity, and non-toxicity etc. Materials with these properties have been tested for neural regeneration (Table 1) (Deumens et al., 2010, Rutkowski et al., 2004, Schmidt and Leach, 2003, Blong et al., 2010). Candidates for this role are biological (biologically derived or natural) and synthetic materials. Combinations of these two types of materials have remarkable advantages due to the synergy obtained from the blended composites. Efforts to produce the ideal NGC incorporating ECM using different scaffold materials are likely to provide important benefits.

a. Biological materials
Nanofiber scaffolds made from biological (natural or biologically-derived) materials represent potential guidance cues to enhance nerve regeneration due to their ability to closely mimic the architecture of endoneurial tubes, which is a critical requirement for peripheral neuroregeneration (Biazar et al., 2010). Biological materials are derived, harvested or obtained from natural sources and have an advantage of presenting similar features to the host tissues or cells. The ECM is mainly a mixture of proteins and polysaccharides, i.e. collagen, proteoglycans, and glycosaminoglycans. Collagen, chitosan, laminin, fibrin, fibronectin and gelatin have been employed to produce scaffolds by the nano-design techniques for neural tissue engineering applications (Cunha et al., 2011).

Collagen is the main structural component of the ECM, and supports cellular activities and tissue regeneration. It is made of a triple helix and over 20 types of collagen have been identified, with type I being the most common isoform used for biomedical applications. High mechanical

<table>
<thead>
<tr>
<th>Type of Materials</th>
<th>Biomaterial Name</th>
<th>Nano-product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Collagen</td>
<td>NFs</td>
<td>(Timnak et al., 2011, Liu et al., 2012b, Wang et al., 2011b)</td>
</tr>
<tr>
<td></td>
<td>Chitosan</td>
<td>NF-tubes</td>
<td>(Wang et al., 2008)</td>
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<tr>
<td></td>
<td></td>
<td>NTs</td>
<td>(Wang et al., 2010)</td>
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<td></td>
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<td>NPs</td>
<td>(Goncalves et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>NFs</td>
<td>(Hu et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Fibrin</td>
<td>NGs</td>
<td>(Dubey et al., 2001)</td>
</tr>
<tr>
<td></td>
<td>Laminin</td>
<td>NFs</td>
<td>(Neal et al., 2009)</td>
</tr>
<tr>
<td>Synthetic</td>
<td>PLLA</td>
<td>NFs</td>
<td>(Leach et al., 2011b, Kakinoki et al., 2011, Prabhakaran et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>PLGA</td>
<td>NFs</td>
<td>(Bini et al., 2004, Panseri et al., 2008, Subramanian et al., 2011)</td>
</tr>
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<td></td>
<td></td>
<td>NPs</td>
<td>(Song et al., 2012)</td>
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<tr>
<td></td>
<td>PCL</td>
<td>NFs</td>
<td>(Jin et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>PPC</td>
<td>NFs</td>
<td>(Wang et al., 2011c)</td>
</tr>
<tr>
<td>Combined</td>
<td>P(LLA-CL)/Collagen</td>
<td>NF-Scaffold</td>
<td>(Ghasemi-Mobarakeh et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>SF/ P(LLA-CL)</td>
<td>NFs</td>
<td>(Wang et al., 2011a)</td>
</tr>
<tr>
<td></td>
<td>PAN-MA/Fibronectin</td>
<td>NFs</td>
<td>(Mukhatyar et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>SF/PLGA</td>
<td>NFs</td>
<td>(Li et al., 2012)</td>
</tr>
</tbody>
</table>

strength compared to other biological materials, good biocompatibility and biodegradability of this protein make it very attractive for NTE applications as well as other tissue engineering therapies. It can also be used with other biomaterials or cross-linked to chemicals to enhance its properties or enable secretion of trophic and growth factors (Liu et al., 2012b). Liu et al.
integrated NT-3 and ChABC onto electrospun collagen NFs. Nearly 50% crosslinking was obtained by microbial transglutaminase and a controlled release of the NT-3 was observed which promoted neurite outgrowth for a longer time than delivery of encapsulated NT-3. Heparin was found to be useful to prevent degradation of ChABC as well as to achieve a controlled NT-3 release. Wang et al. compared the proliferation of neural progenitor cells on aligned and randomly oriented electrospun NFs made of collagen. The authors found that aligned NFs caused faster expansion and a change in the cell cycle progression (Wang et al., 2011b). Liu et al. produced collagen NFs using a novel photochemical crosslinking method that prevented the denaturation of collagen (Liu et al., 2010). Clearly, the use of biofunctional NFs will grow since they supply biochemical cues besides topographical stimulation and support neural regeneration in the PNS, as well as the CNS. Timnak and co-workers used collagen and a common type of glycosaminoglycan to fabricate random and aligned electrospun-nanofibrous scaffolds with diameters ranging from 50 to 350 nm that mimics natural ECM. The biocompatibility of the scaffold was improved by crosslinking with genipin. Fiber diameters were adjusted with viscosity and flow rate of electrospinning solution, and aligned NFs were collected using a rotating collector. The authors stated the positive effect of the scaffold and fiber orientation on cell outgrowth (Timnak et al., 2011). Fast degradation rate and interaction with the integrin ECM receptors are possible disadvantages of collagen and need to be further studied to understand the mechanisms completely before its use in vivo (Khaing and Schmidt, 2012).

Chitosan is a linear polysaccharide and has numerous biomedical uses. It has been used for regeneration studies in various forms, i.e. fibers and sponges (Mi et al., 2001). Wang et al. produced electrospun nanofibrous tubes made of chitosan and tested for a sciatic nerve injury in rats. The recovery of sensory function was achieved with enhanced humoral permeation, cell attachment and migration using the chitosan nano/microfiber mesh tubes (Wang et al., 2008). Wang and co-workers tested the ability of electrical charge storage and the effect of β-TCP particles, which were immobilized on chitosan mesh NTs, on a sciatic nerve injury model in rats. Electrophysiological recovery was obtained as well as functional recovery of motor and sensory nerves with increased axon density and area with electrical polarization treatment (Wang et al., 2010). Goncalves et al. used a novel drug delivery method for a sciatic nerve injury. Chitosan was selected as scaffold material and heparin was bound to growth factors to form nanoparticles.
In vivo neural regeneration with sensorimotor performance was obtained in mice with the use of these safe nanomedicines (Gonçalves et al., 2012).

Silk is produced naturally by some insects and electrospun silk fibroin NF scaffolds are promising alternative materials for neural regeneration. They are favored due to their significant biocompatibility and mechanical properties (Altman et al., 2003). Hu et al. fabricated electrospun silk fibroin NFs and observed more ordered and aligned SCs with the extended processes with the use of these NFs (Hu et al., 2012).

Several natural materials have been used as nano-scaffolds for neural regeneration: Fibrinogen, fibrin, gelatin, elastin, laminin, hyaluronic acid, etc. (Khaing and Schmidt, 2012). They are shown to play critical roles in cell adhesion, growth, migration and differentiation as well as axonal outgrowth and neural regeneration. Biological materials have advantages such as higher biocompatibility, biodegradability and the ability to mimic the ECM compared to synthetic materials. However, they do pose some significant disadvantages, one is the potential for immunological and/or inflammatory induction because of them (Cunha et al., 2011). Other drawbacks include their weak mechanical properties compared to synthetic materials, and reduced amenability to chemical modification, which is indispensable for neural regeneration applications. Furthermore, variations in sources as well as inherent properties of the sources may produce unexpected responses following implantation. For these reasons, studies on modification techniques and homogeneity are needed to identify suitable biologically-derived materials for enhanced neural regeneration.

b. Synthetic materials

Synthetic materials have been used to mimic the ECM. Their controllable surface and structural properties are critical advantages for NGC fabrication. Biodegradable synthetic materials are favored to non-biodegradable ones since their uses eliminate the need for an additional surgery to remove the guidance conduit. Known degradation rates and mechanical properties with reproducibility favor the use of synthetic materials for neural regeneration applications. With their improved features, they are one of the main candidates for NTE conduits and they can be designed to overcome the problems such as immune response and fast degradation. However, lack of mimicking ECM and possible release of toxic chemicals with the degradation of the NGC material are main drawbacks. Like biological materials, synthetic materials have been tested as
scaffolding material for NGCs. They can be produced in various sizes including nano-scales using the techniques described earlier. These nano-structures made of various biodegradable polymers can be used as NGCs as well as integrated into NGCs. Nano-structured PLA (poly(lactic acid)), PGA (poly(glycolic acid), PLGA (poly(L-lactide-co-glycolide)), PCL (poly(caprolactone)), and PLCL (poly(lactic-co-caprolactone)) are among the most common synthetic materials for NTE applications (Corey et al., 2008, Wang et al., 2009).

PLA is an aliphatic polyester, present in two isoforms, D-(dextrorotary) (PDLA) and L-(levorotary) (PLLA) forms, and can also be in a mixed form composed of its two types. The L-form is preferred in biomedical applications since it is present in the body. The crystallinity depends on the form of PLA and is critical for the type of the biomedical use. Leach et al. fabricated highly aligned electrospun PLLA-NFs and observed that outgrowth of sensory and motor neurons were directed through these nano-scaffolds in vitro (Leach et al., 2011a). PLLA-NFs were modified with oligo- (D-lactic acid) bioactive-peptide conjugates and used for a sciatic nerve injury model in rats (Kakinoki et al., 2011). Kakinoki et al. stated that superior functional reinnervation was obtained with the modified PLLA nanofibrous NGC compared to silicone tube and PLLA conduits. Prabhakaran et al. blended two types of synthetic materials, PLLA and polyaniline to form NFs with fiber diameter of ~200 nm, and applied an electric field, which resulted in facilitated neurite outgrowth of neural stem cells (Prabhakaran et al., 2011).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Degradation product</th>
<th>Degradation period (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>L-Lactic acid</td>
<td>6-24</td>
<td>(Armentano et al., 2010, de Tayrac et al., 2008, Zilberman et al., 2005, Garlotta, 2001)</td>
</tr>
<tr>
<td>PGA</td>
<td>Glycolic acid</td>
<td>3-6</td>
<td>(Wen and Tresco, 2006, Armentano et al., 2010)</td>
</tr>
<tr>
<td>PLGA</td>
<td>D,L-Lactic &amp; Glycolic acids</td>
<td>3-12</td>
<td>(Lu et al., 2000, Armentano et al., 2010, Lu et al., 1999)</td>
</tr>
<tr>
<td>PCL</td>
<td>Caproic Acid</td>
<td>18-36</td>
<td>(Peña et al., 2006, Nair and Laurencin, 2006)</td>
</tr>
</tbody>
</table>

PGA is a similar polymer to PLA, except the repeated unit is glycolic acid instead of lactic acid. It is more hydrophilic and degrades faster than PLA (Table 2). To obtain a scaffold material with a desired biodegradability, the copolymer, PLGA can be formed by adjusting the ratios of lactic and glycolic acids. Since it is biodegradable and biocompatible, PLGA has been used in many
tissue engineering applications. Electrospun PLGA NFs were demonstrated to promote cellular activities and enhanced sciatic nerve regeneration in rat models (Panseri et al., 2008, Bini et al., 2004). Lee and co-workers coated PLGA electrospun NFs with polypyrrole to form electrically conductive nano-scaffolds. By applying electrical stimulation, growth and differentiation of rat PC12 cells were shown to be enhanced (Lee et al., 2009). In a recent study, Subramanian et al. manufactured uniaxially aligned electrospun PLGA nanofibrous scaffolds and demonstrated their ability to orient the direction of SCs and this yielded a better proliferation rate than random fibers (Subramanian et al., 2011). Song et al formed dual drug-loaded (PLGA)/mesoporous silica nanoparticles on an electrospun composite mat, with two model drugs (fluorescein (FLU) and rhodamine B (RHB)). The authors stated that most of the FLU was delivered rapidly, whereas RHB release was controlled by the nanoparticles (Song et al., 2012).

PCL is favorable due to its mechanical properties and slow degradation rate. PCL also has the ability of easy copolymerization with various polymers, i.e. PGA, PLA, collagen, which is very crucial to form a desired NGC scaffold. Jin et al. coated electrospun PLCL NFs with multi-walled carbon nanotubes (MWCNTs) and showed that they facilitated neurite outgrowth of rat dorsal root ganglia (DRG) neurons and focal adhesion kinase (FAK) expression of PC-12 cells (Jin et al., 2011). Wang and co-workers used poly(propylene carbonate) (PPC) to fabricate electrospun aligned nanofibrous scaffolds. They showed that the majority of neurite outgrowth and SC migration from DRG extended uni-directionally and parallel to the oriented fibers, while they were randomized on non-aligned fiber films (Wang et al., 2011c).

It is shown that synthetic materials are favored due to their advanced properties for NGC fabrication. Their easy modification and adjustable features for nano-scaffold designs make them one of the most important guidance cues for neural regeneration. However, combined use of synthetic biodegradable materials with the biological ones will overcome their main drawback and the resultant material can mimic closely the ECM.

c. Combined use of natural and synthetic materials

Combined use of natural and synthetic materials to produce NGCs has been pursued for the purpose of obtaining nano-structures that more closely mimic ECM and enhance nerve regeneration. Overcoming disadvantages of some biomaterials by using other types of biomaterials has resulted in well-developed NGCs. Ghasemi-Mobarak et al. blended PCL and
gelatin, and fabricated nanofibrous scaffolds. The authors reported aligned neurite outgrowth, enhanced neural differentiation and proliferation with the NFs (Ghasemi-Mobarakhe et al., 2008). Kijenska et al. produced electrospun nanofibrous scaffolds with an average diameter of ~250 nm from blends of poly (L-lactic acid)-co-poly(ε-caprolactone) (P(LLA-CL)), collagen I and collagen III, and investigated the effects of the composite material on cellular activities. Supported cell proliferation with an increase over 20% was achieved with the aligned composite scaffold compared to the polymer alone (Kijenska et al., 2012).

Using electrospinning (Fig. 5), Wang and co-workers combined (P(LLA-CL)) and silk fibroin (SF) to fabricate NFs used to treat 10 mm sciatic nerve lesions in rats. Functional recovery and oriented neural regeneration obtained with the composite, SF/P(LLA-CL) was greater than NGCs made of the synthetic polymer itself (Wang et al., 2011a). Fibronectin adsorption on aligned electrospun NFs made of poly-acrylonitrile methyl acrylate (PAN-MA) was shown to be superior compared to smooth films of the same polymer for SC cell migration and neurite outgrowth (Mukhatyar et al., 2011). In another recent study, Li et al. used electrospun NGCs made of PLGA/SF to bridge sciatic nerve gaps in rats and obtained results that were similar to those using to autologous nerve grafting (Li et al., 2012).

![SEM of aligned nanofibers](image)

**Fig. 5** The aligned NFs were unreeled from a drum collector onto a stainless steel bar and sealed with nylon monofilament suture stitches. Reproduced with permission from (Wang et al., 2011a).

It is apparent that the use of polymer blends consisting of natural and synthetic materials with nanotechnology provides promising advances for neural regeneration. Researchers are still working on developing the most efficient NGC to repair severe lesions with an enhanced and
oriented regeneration. The advantages of composites have prompted investigators to investigate combinations of various biological and synthetic materials for nano-designed conduits.

5. Drawbacks
Nanotechnology is currently one of the most promising and actively-pursued areas of research. However, besides the benefits, there may be unexpected drawbacks that will create challenges for scientists in the near future. At present, very little is known about the effects of nanomaterials on biological systems, public health and safety, and the environment.

In neural tissue engineering, not all of the findings on the use of nano-materials have been positive [45-49]. Multi-walled carbon NTs were found to retard the regenerative capacity of axons of DRG neurons without causing the death of cells (Wu et al., 2012). An electrically-conducting hydrogel containing single-walled carbon nanotubes (SWCNTs) was shown to reduce SC proliferation in a 2-D micro-environment without affecting cell viability. However, a similar hydrogel mimicking a 3-D environment seemed to have no significant effect on SC growth (Behan et al., 2011). Effects of SWCNTs were shown to decrease the DNA content of cells derived from avian embryonic spinal cord. Belyanskaya et al. showed that SWCNT reduced the number of glial cells in both PNS and the CNS, leading to a reduction of total sensory neurons and improved resting membrane potential of cultured DRG neurons as compared to controls (Belyanskaya et al., 2009).

Magnetic NPs have been used widely in biomedical studies. It was shown that iron oxide nanoparticles decreased the viability of cultured PC12 cells over time (Pisanic Ii et al., 2007). Moreover, Pisanic Ii et al. stated that increasing the NPs concentration caused detachment of the cells and decreased neurite and actin filament counts in mature PC12 cells compared to controls.

Nano-alumina, thought to be capable of crossing the blood-brain barrier, has been shown to decrease cell viability and promote loss of membrane integrity of neural stem cells at concentrations >100 μg/ml (Dong et al., 2011).

These drawbacks, along with those that cannot be predicted by the investigators as of yet, need to be overcome before the common use of nano-structures for neural regeneration are implemented in clinical settings. An increase in the number of in vivo studies using models which can mimic the human body are necessary before the implantation of NGCs. Promising results obtained up to now are encouraging researchers to investigate the most appropriate and safe biomaterial with the desired size ranges.
6. Conclusions and Future Directions

The cellular and chemical mechanisms essential for functionality of the nervous system are extremely complex and still not entirely understood. This in itself presents formidable challenges to neural tissue engineering efforts. Research to discover and implement neural regeneration strategies has been underway for several decades. Substantial improvements in neuroregeneration with functional recovery have been obtained using NGC implantation. However, technology for NGCs has not sufficiently advanced to allow them to completely replace grafts for their effectiveness, especially in cases of severe injuries and longer gaps. Advances in nanotechnology along with other NTE strategies have provided alternatives and implementation of guidance cues to facilitate healing of neural lesions. Several scaffold types have been used to make nano-NGCs from blends of natural or synthetic materials with the desired physical and chemical properties compatible with extracellular matrices. Their use has improved cell survival, adhesion, growth, proliferation, orientation and differentiation. Composites fabricated using the latest techniques in entubulization and bioengineering have produced encouraging results. Guidance cues are also critical to success, among them the use of specific cell types to maximize reinnervation and promote axonal growth to bridge nerve gaps. Growth factors have been combined with other guidance cues to facilitate nerve regeneration. Recent advances in tissue engineering have enabled the use of cells genetically modified to secrete specific growth factors. Thus, NGCs’ function is not only as guidance cues, but also as platforms for controlled release of therapeutic agents such as trophic factors and cellular constituents such as ECM molecules. Incorporation of these guidance cues may mimic the molecular and cellular components of the microenvironment. The use of nanotechnology offers many significant and attractive advantages; however, at the molecular level, it poses a number of challenges. Nano-engineered structures can be functionalized for specific use in neural regeneration and other applications. For example, nanoparticles are used in designing devices for the controlled release of medicines, while NFs and NTs can mimic the tubular structures of the ECM, a desirable feature in designing NGCs for the repair of neural lesions. However, improvements in specificity are still needed to enhance and expand their therapeutic potential for clinical treatments (Silva, 2006). In the case of NGCs,
the use of nano-structures for improved control of guidance cues offers potential for successful neuroregeneration therapies.

In spite of the progress made to date, more research on scaffold design and evaluation in vivo is still needed. This is a challenging undertaking, especially given the complexities of the nervous system. The ability to duplicate the microenvironment of the ECM with guidance cues must be evaluated in models that mimic the human body. Trials with animals larger than rodents will provide a better understanding of how regenerative therapies will perform in humans. The contributions of nanotechnology should be used for NGCs after overcoming the drawbacks and possible health risks. In spite of the complex challenges, nanotechnology, along with improved biomaterials, drug delivery capability and tissue engineering will provide alternative therapies with successful clinical applications.

7. Acknowledgement

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8. References:


