

● INVITED REVIEW

Nanotechnology and bio-functionalisation for peripheral nerve regeneration

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Abstract

There is a high clinical demand for new smart biomaterials, which stimulate neuronal cell proliferation, migration and increase cell-material interaction to facilitate nerve regeneration across these critical-sized defects. This article briefly reviews several up-to-date published studies using Arginine-Glycine-Aspartic acid peptide sequence, nanocomposite based on polyhedral oligomeric silsesquioxane nanoparticle and nanofibrous scaffolds as promising strategies to enhance peripheral nerve regeneration by influencing cellular behaviour such as attachment, spreading and proliferation. The aim is to establish the potent manipulations, which are simple and easy to employ in the clinical conditions for nerve regeneration and repair.

Key Words: nanomaterial; regenerative medicine; biomaterial; peptides; nerve regeneration; stem cells; nanotechnology

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Introduction

Annually more than half a million peripheral nerve injury cases are reported worldwide (Daly et al., 2012). Nerve autografting is the current 'gold standard' technique to repair a completely transected nerve with gap size larger than 30 mm. However, anaesthesia, denervation and numbness of the donor site, painful neuroma formation and time consuming operations are main limitations of this technique (Hood et al., 2009). In the last few years, numerous micro-surgical techniques (Tsintou et al., 2015) have been developed to reconstruct a long segment of a damaged peripheral nerve (Sedaghati et al., 2014). Advancements in the field of tissue engineering and biomaterial science (Tsintou et al., 2015) have led researchers to develop synthetic nerve conduits as an alternative to nerve autografting. However, to date, the clinical use of the clinically approved nerve conduits is limited to small diameter nerves with short gaps (< 30 mm) as their basic hollow tube designs fail to mimic extracellular matrix (ECM) nanostructure (Moore et al., 2009). So, they become incompetent to support axonal regeneration in defects with longer gaps and larger diameter (Matsumoto et al., 2000).

Extracellular matrix (ECM) is one of the important components that influence neural repair and regeneration. ECM molecules regulate Schwann cells (SCs) morphology, migration and myelination by providing support and anchorage site for these cells (Platt et al., 2003; Armstrong et al., 2007). It is well documented that ECM regulates axonal growth *via* providing binding sites and guides the growing axons during development and regeneration (Baron-Van Evercooren et al., 1982; Chernousov and Carey, 2000). Furthermore, it

has been discovered that the interaction between SCs and ECM molecules is essential for the release of diffusible nerve growth factors from SCs which are crucial for neurite outgrowth (Armstrong et al., 2007).

Influence of Arginine-Glycine-Aspartic Acid (RGD) Peptide on Neural Regeneration

Among the ECM adhesion receptors, integrins play key roles in tissue regeneration. They not only have effect on the cell adhesion but also on transducing growth related signals by influencing intracellular signalling, particularly through the mitogen-activated protein kinase kinase (MEK) pathway (Juliano et al., 2004; Tucker and Mearow, 2008). It has been revealed that SCs up-regulate expression of integrins on contact with dorsal root ganglion neurons (Einheber et al., 1993). Several isolated and purified forms of ECM peptide sequences, like RGD, have been used experimentally to enhance neural regeneration (Rogers et al., 1983; Santiago et al., 2006; Webber and Zochodne, 2010). Many adhesive proteins present in ECM, such as fibronectin, vitronectin, collagen and laminin, contain RGD cell adhesion sequence in their integrin recognition sites, which is cell surface receptor that recognizes the RGD sequence of various proteins. When this sequence is recognised by its integrin, it provides signals for cell attachment, growth, maturation and differentiation (Hersel et al., 2003). **Figure 1** shows schematic diagram of cell surface integrin interaction with RGD coated surface. Extensive research over the last decade has revealed that the ECM derived RGD sequence, can act as an integral cell adhesion recognition sequence which interacts with a

variety of integrins and promote cell attachment, neurite outgrowth and differentiation (Ruoslahti, 1996; Plow et al., 2000; Rashid et al., 2004). As a result of these molecular changes, SCs proliferate and develop a scaffold ahead of the re-growing axon fibers which encourage nerve regeneration (Webber and Zochodne, 2010). Incorporation of a small amount of RGD peptide, for instance 1 fmol/cm², has shown effectively improved cell adhesion to the non-adherent surface (Rafiuddin Ahmed and Jayakumar, 2003). RGD peptide facilitates SC outgrowth at low doses but will disrupt endogenous fibronectin signalling and regeneration at higher doses (Liu et al., 2009). Addition of RGD has shown to affect the physiochemical properties of neural scaffold's surface. Surface topography and chemistry of biomaterial have been shown to be extremely important in determining cell-material interactions by influencing cellular properties such as cell adhesion, proliferation, differentiation, cell-cell reactions and cytoskeleton organisation (Jell et al., 2009).

For instance, a significant reduction in water contact angle of RGD-POSS-PCL (polyhedral oligomeric silsesquioxane modified poly (caprolactone) urea urethane) sample compared to POSS-PCL sample is a good indication of a decrease in the surface hydrophobicity of the RGD-POSS-PCL samples (Sedaghati et al., 2014). This peptide may alter nanocomposite' surface hydrophobicity to a more polar surface by introducing charged functional groups on polymer surface whereby water molecules in the proximity of the polymer surface bind strongly onto it (Jell et al., 2009). Hydrophilicity is known to promote Schwann cell attachment by influencing the adsorption of cell adhesion ligands present in the ECM (Lee et al., 2003; Sun and Downes, 2009; Hong and Kim, 2010). SCs morphology and process outgrowth on RGD-POSS-PCL revealed a significant increase in the SC spreading and process at day 3 of cell culture studied by S100 immuno-staining and haematoxylin. Whilst cells grown on tricalcium phosphate (TCP) sample retained their undifferentiated flat polyhedral morphology with no measurable process and tended to cluster together and rather than spreading. It is therefore speculated that RGD-POSS-PCL surface with less hydrophobicity not only favours better cell attachment but also enhances further morphological differentiation and spreading of neural cells. These findings confirm that recognition of RGD peptide by its integrin on cell membrane may provide signals for neural cell spreading, migration and differentiation (Jifeng et al., 2010; Huang et al., 2013). Similar results were obtained from study performed by Yun et al. (2014) using PC12 cells.

In another study, Qiu et al. (2014) showed that incorporation of RGD and β -TCP in the PDLLA conduit resulted in the microenvironment rich in nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which assist to neutralize the oxidative stress and to improve the cytoskeletal protein expressions *in vivo*. Sciatic nerve regeneration was faster when the RGD peptide was present in a rat model (Xiao et al., 2013). Addition of other bioactive molecules such as NGF and FK506 into conduit containing RGD motifs enhanced functional outcome similar to that of a nerve autograft following neural repair (Yan et al., 2012).

Neural Regeneration Using Nanocomposite and Nano-fibrous Scaffolds

Nerve conduits have emerged as alternatives to the nerve autografting for defects with a gap length of up to 30 mm in order to eliminate its drawbacks such as unavailability of appropriate-sized nerves and donor site morbidities. Several biological and synthetic materials have been tested for development of nerve conduits with different physiochemical properties (Sedaghati et al., 2011).

Nanotechnology and nano-based materials have attracted considerable amount of attention among researchers in last three decades. A nanocomposite is a multi-phase compact material where the dimension of the one of its phases is in the size range of 1–100 nm. These nanoparticles can be used as the link between the molecules of different composite polymers due to their advantageous size and directly affect nanocomposite's thermal, mechanical, electrical, catalytic, optical and chemical properties (Sun and Downes, 2009; Torabinejad et al., 2014; Yazdimamaghani et al., 2014). Furthermore, considerable increase in the surface to volume ratio of nanocomposite in addition to the decrease in the volume distinguishes this material from usual composites (Figure 2). These features allow a higher number of reactions happen on the surface of a nanocomposite. There is a great hope that bio-nanocomposite materials can potentially resemble the characteristic of native ECM (Shekaran and Garcia, 2011). Natural ECM proteins, such as collagen and laminin molecules, exhibit specific nano-structural features (*i.e.*, nano-meter scale (10-9)).

POSS nanoparticles are one of the most promising nanomaterials for medical applications (Kannan et al., 2005; Ghanbari et al., 2011b), consisting of a distinctive nanocage structure comprising an inner inorganic framework of silicon and oxygen atoms and an outer shell of organic functional groups. The chemical composition of this nanoparticle makes it a unique nanoparticle that could potentially be used to improve the physiochemical properties of the copolymers (Ghanbari et al., 2011a). Incorporation of this nanoparticle into poly-caprolactone (PCL) resulted in the synthesis of POSS- incorporated poly (caprolactone) urea/urethane (POSS-PCL) nanocomposite polymer with considerably enhanced physiochemical properties including increased in tensile strength and surface roughness compared to conventional PCL (Chawla et al., 2014). Scaffold made of this nanocomposite are currently under investigation for nerve (Sedaghati et al., 2014) and skin (Yildirimer and Seifalian, 2015) tissue regeneration and implants needed for paediatric cases.

Nano-fibrous scaffolds also offer great potential in the field of neural tissue engineering (Olakowska et al., 2010). They mimic native tissue tubular structures including axons, microtubules and ion channels. Nano-fibrous scaffolds can be produced by different fabrication technique such as electrospinning and self-assembly (Ellis-Behnke et al., 2006; Guo et al., 2007; Guo et al., 2009) using numerous materials, such as synthetic polymers, proteins, lipids, DNA and glass. Processing parameters such as solution-flow rate, applied

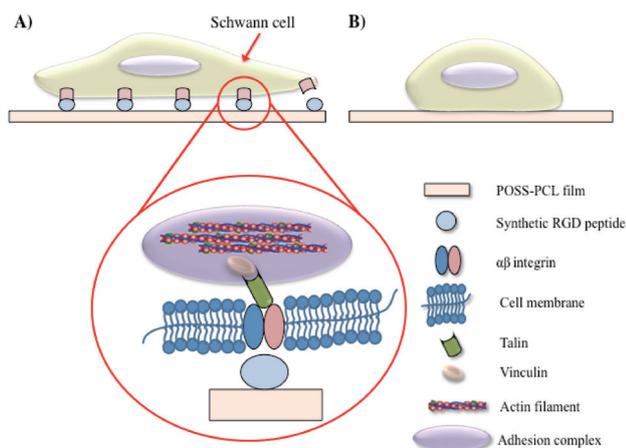


Figure 1 Schematic diagram of cell attachment and spreading on un-coated (A) and Arginine-Glycine-Aspartic acid (RGD)-coated (B) surfaces (adapted from Sedaghati et al., 2014).

The interaction between cellular integrin receptor and RGD sequence on the surface of a biomaterial is important in controlling the intracellular signalling pathway.

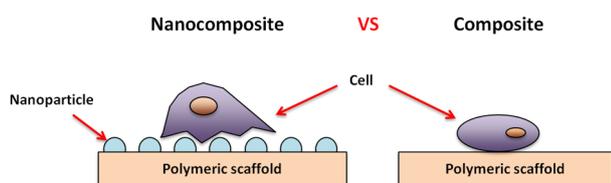


Figure 2 Surface modification of conventional composite biomaterial including topography and chemistry by incorporating nanoparticles can influence cell attachment, growth and differentiation during tissue regeneration.

voltage, polymer concentration and molecular weight and the distance between the needle tip to the ground collection plate can directly or indirectly affect the properties of nano-fibrous scaffolds (Hu et al., 2011; Puppi et al., 2011). Nano-fibers have larger surface area relative to their dimensions (Biazar et al., 2010). This feature can enhance tissue regeneration where it provides the possibility of coating the outside of a nano-fiber with various biochemical substances essential for cell survival, growth and differentiation (Tyseling-Mattiace et al., 2008; Masaeli et al., 2014). PC12 cells grown on fibrous scaffold secreted a higher amount of dopamine compared to the control tissue cultures. Furthermore, neurites of differentiated PC12 cells were highly aligned and longer on parallel PHB fibres than random fibres, thereby indicating the importance of fiber orientation for neural regeneration (Figure 3).

Conclusion

ECM components, especially RGD peptide sequence, have been exhibited to promote neural tissue regeneration by providing a favourable environment in animal models. Potential treatments under research including alteration of the intrinsic ECM and incorporation of nanoparticles into the scaffolds allow the delivery of combination of neurotrophic factors and cells. Nanotubes and nano-fibres also have shown to be

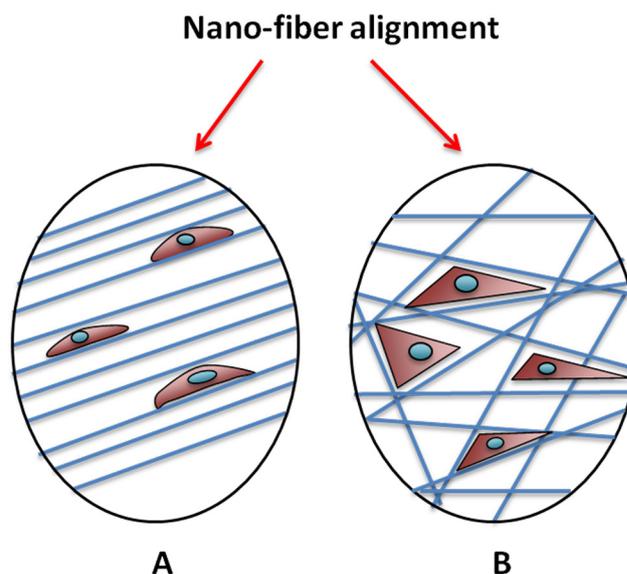


Figure 3 Schematic diagram of parallel (A) and randomly (B) aligned electrospun nano-fibers.

Nano-fiber alignment, orientation and chemistry can control cellular behaviour such as proliferation and differentiation.

promising strategies for neural tissue engineering in which they support and enhance axonal growth based on their nanometre-scale physiochemical properties. Many of these changes have demonstrated noticeable ability to enhance neural regeneration *in vivo*. Whilst there is potential of using such scaffolds and biomolecules for therapeutic improvements in clinical setting, no clinical study is published yet.

Conflicts of interest: None declared.

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