

# Polymer based tissue engineering strategies for neural regeneration

## Abstract

Damage to nervous system causes great trauma to the patients involved and their caregivers. The lack of an effective treatment strategy has led to shifting of focus from traditional methods to alternative treatments such as neural scaffolds containing biological cues. Neural regeneration in central and peripheral nervous systems using various synthetic and naturally occurring polymers have been experimentally successful as evidenced from the numerous publications in this area. This review attempts to enlist the various kinds of polymers that have been used in neural tissue engineering.

**Keywords:** autografts, xenografts, allografts, biomaterial scaffolds, stem cell therapy, biological grafts, polysaccharides, chelation, chitosan, gelatin, dextran

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**Abbreviations:** PEG, poly ethylene glycol; PEO, poly ethylene oxide; EVA, poly ethylene-covinyl acetate; PLA, poly lactic acid; PGA, poly glycolic acid; pHEMA-MMA, 2-hydroxyethyl methacrylate-co-methyl methacrylate; CNS, central nervous system; PNS, peripheral nervous system; ECM, extracellular matrix

## Tissue Engineering

### Introduction

Tissue engineering is a multidisciplinary field that uses the knowledge of medicine, nanotechnology, molecular biology, chemical engineering, bioengineering, physiology, developmental biology, and material science. It is used in the development of functional tissues to repair or replace tissues or organs lost due to age, disease, damage or congenital defects. The shortage of donor organs and the issue of organ transplant rejection led to the development of tissue engineering and regenerative medicine.<sup>1</sup> Tissue engineering aims at designing and producing scaffold with similar mechanical, chemical and physical properties to that of the three dimensional biological systems. The ideal scaffold should favor cell penetration, growth and integration into the host system. It should also undergo degradation either during healing or after healing into non-toxic by-products.

The scaffolds for tissue engineering can be prepared from natural or synthetic materials/ polymers. The predominant techniques which are being used in the design and synthesis of scaffolds include electro spinning, solvent casting, particulate leaching, sheet lamination, adhesion bonding, laser sintering, photo polymerization, self assembly, droplet deposition, lyophilisation, and bioprinting.<sup>2-10</sup> Incorporation of neural cells and various biochemical cues at the time of scaffold preparation have helped in increasing the efficiency of scaffolds manifold.<sup>11-13</sup>

Neural injuries are difficult to treat and the currently available inadequate treatment methods to replace lost neuron increase the relevance of tissue engineering constructs. The main causes of trauma are motor vehicle accidents, sports, and recreational activities, work related accidents and falls at home. At present, there is no universally

accepted treatment for this condition. Pharmacological applications and cell therapy which have been primarily used so far in clinical trials have showed very little reproducibility and promise as reliable therapeutic methods. Physiotherapy is also needed for rehabilitation of the injury-affected individuals. The current trends in neural tissue engineering research appear to be very promising for the millions of people traumatised by neural injuries. This review looks at the various kinds of polymeric materials used for preparing tissue engineered constructs.

## Physiology of the nervous system

The physiology of the nervous system poses several challenges for tissue engineering when compared to other organ systems. The nervous system is classified into the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes mainly the brain and spinal cord and PNS consists of the cranial nerves arising from the brain, the spinal nerves originating from the spinal cord, and sensory nerve cell bodies. A key difference between the PNS and CNS is the capacity for peripheral nerves to regenerate; CNS axons do not regenerate appreciably in their native environment. The physiological response to injury in the CNS is also different from that of PNS.<sup>14</sup> After injury in the CNS, macrophage infiltration to the site of injury is much slower when compared to macrophage infiltration in the PNS. This delays the removal of inhibitory myelin from the CNS (Figure 1).<sup>15</sup>

The scaffolds for neural tissue engineering are usually prepared from synthetic or naturally occurring polymers. These scaffolds are usually aligned vertically in order to facilitate the growth of neuronal cells and regeneration of lost neurons. In case of injury to spinal cord and larger peripheral nerves, tubular and vertically aligned scaffolds are prepared. These scaffolds are filled with hydrogels and various biochemical cues to support the growth of new neurons.<sup>16-18</sup>

## Neural tissue engineering

Neural tissue engineering offers great potential for overcoming the effects of disease, aging, or injury on the nervous system. Neural

tissue engineering broadly spans a collection of approaches (like biomaterial scaffolds, biological grafts and stem cell therapy) for applications ranging from surgical, regenerative, disease treatment to deep brain stimulation.

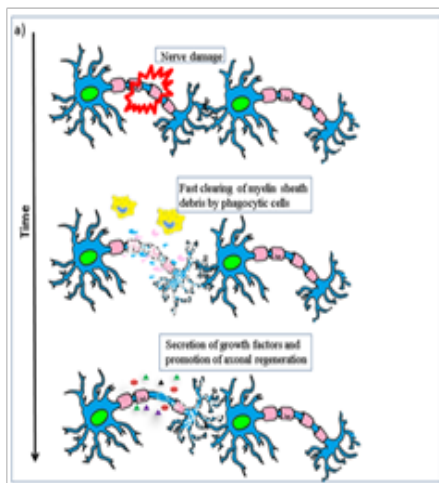


Figure 1A Regeneration in PNS

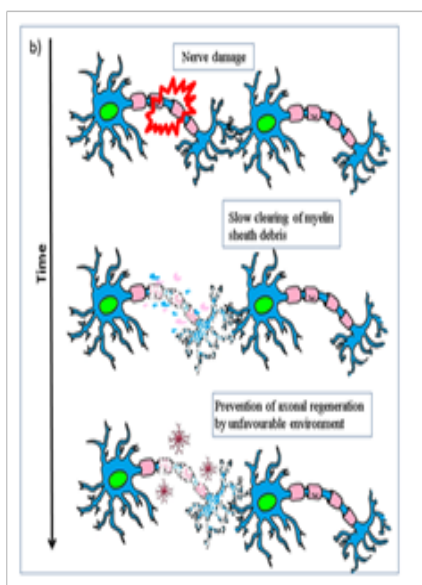


Figure 1B Regeneration in CNS.

In the peripheral nervous system (PNS), direct end-to-end surgical reconnections are commonly used for treatment of nerve transections, if the injury gap is small. Nerve autografts are considered as the ‘gold standard’ for bridging larger nerve gaps. However, the use of autografts are limited by shortage of donor grafts, the potential loss of function at donor sites and the requirement of multiple surgeries.<sup>19–21</sup> Also promises of allografts and xenografts as substitute to autologous nerve grafts are hampered by immunological rejections and the chances of disease transfer. Central nervous system (CNS) injury is even more challenging to manage as the inhibitory environment formed after injury in the CNS restricts nerve regeneration.<sup>22</sup> The mechanisms of regeneration in CNS and PNS are depicted in Figure 1. There are no universally accepted treatments that are effective enough to restore nervous functions in the CNS. Due to this, tissue engineered scaffolds are increasingly exploited for their potential to facilitate neural repair.

The physical and chemical properties of artificial grafts can be tailored based on applications.

Most strategies of neural tissue engineering focus on developing biomaterials that mimic the extracellular matrix by providing trophic support and biochemical cues. Neural tissue engineering technology involves use of engineered tissue constructs to directly replace lost function and/or to facilitate and augment the capacity of host nervous system regeneration. The key attributes of tissue engineered constructs are alignment, three-dimensionality, and scaffold support structure. Typically, tissue engineered constructs are completely or partially created *in vitro*. This has significant applications to replace defective tissue, destroyed tissue, dysfunctional tissue and promoting tissue repair and functional regeneration through polymer matrices by its contact with the healthy neural tissue.<sup>23,24</sup>

One of the first and most important considerations when designing a scaffold for neural tissue engineering is the choice of material. A wide range of materials are available for such applications. These materials must conform to the dimensions of the implantation site and maintain an appropriate shape after implantation. The ability to sterilise the scaffold prior to implantation is also a prerequisite for the synthesis of a scaffold. The materials should not cause any immune response. While selecting a suitable scaffold, its ability to degrade, its degradation rate, ability to provide controlled release and other chemical and physical properties must be considered (Figure 2). Some of the commonly used materials which meet the requirement of an ideal scaffold are as follows:

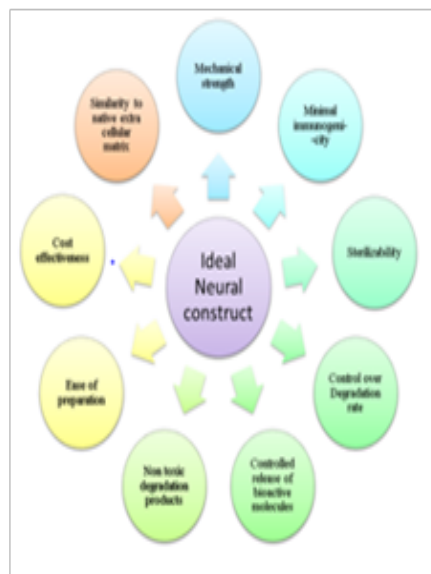


Figure 2 Characteristics of ideal scaffolds for neural regenerations.

### Synthetic materials

Synthetic materials have been extensively used as scaffold material and these can be tailored for a wide range of mechanical properties and degradation rates. They also have known compositions and can be designed to minimize the immune response. Finally, synthetic polymers can be reacted together to combine the properties that are unique to each.

Examples of such materials are:

**Poly (ethylene glycol)/Poly (ethylene oxide):** Poly (ethylene glycol)

(PEG) or poly (ethylene oxide) (PEO) resists protein adsorption and cell adhesion<sup>25</sup> and these minimise the immune response after implantation. This polymer also seals cell membranes after injury, which is highly beneficial to limit cell death. Hydrophilic PEG hydrogels can be synthesised through a variety of cross-linking methods to create scaffolds with varying degradation rates and rates of drug release.<sup>26–30</sup> Further insight into its chemistry can be explored to modify these gels to provide sites for cell adhesion or extracellular matrix (ECM) molecules to allow cells to infiltrate into these scaffolds and thereby enhancing its potential applications.<sup>26,31–35</sup>

**Poly (ethylene-co-vinylacetate):** Poly (ethylene-covinyl acetate) (EVA) is a non-degradable, biocompatible and commercially available polymer. It has been extensively used for drug delivery applications,<sup>36–39</sup> while more recently it has been investigated for neural tissue engineering applications, such as repair of peripheral nerve injury.<sup>40–42</sup>

**Poly (glycolic acid)/Poly (lactic acid)/Poly (lactic-coglycolic acid):** Poly (glycolic acid) (PGA) and poly (lactic acid) (PLA) are biodegradable synthetic polymers, which can be reacted to form the copolymer poly (lactic-co-glycolic acid).<sup>43–45</sup> After implantation, the ester bonds in the backbone of the polymer can be hydrolyzed into by-products. These by-products can be absorbed by the body and may cause pH changes around the implantation site. The degradation rate of the scaffolds can be altered by varying the ratio of PGA to PLA in the scaffold.<sup>46,47</sup>

**Poly (2-hydroxyethyl methacrylate) and poly (2- hydroxyethyl methacrylate-co-methyl methacrylate):** Similar to EVA, poly (2-hydroxyethyl methacrylate) (pHEMA) and poly (2-hydroxyethyl methacrylate-co-methyl methacrylate) (pHEMA-MMA) polymers are not degradable. As a result, scaffolds made from pHEMA and pHEMA-MMA remain stable after implantation.<sup>48–50</sup> Such scaffolds can be molded into a variety of shapes, including being modified to channels that can be filled with therapeutic drugs or ECM proteins.<sup>49</sup> The mechanical properties can also be modulated by adding multiple layers of pHEMA to strengthen the scaffold.<sup>51</sup>

**Polypyrrole:** Polypyrroles (Ppys) are polymers that are made up of connected pyrrole ring structures. In addition to being biocompatible, Ppy also is highly conductive, making it convenient for use in neural prosthetic applications.<sup>52</sup> It can be molded into a variety of shapes and allows cell adhesion to take place.<sup>53</sup> A vast amount of work has been done on engineering Ppy in different ways to improve its functionality as a neural tissue engineering scaffold.<sup>54–56</sup>

## Natural materials

Natural materials possess many properties that make them attractive for neural tissue applications. Many of these materials contain sites conducive for cell adhesion and cell infiltration. These materials also exhibit similar properties to the soft tissues that they are supposed to replace. Since these materials are obtained from natural sources, they must be purified to ensure that foreign body response doesn't occur after implantation. Homogeneity of product between different batches can be an issue with natural materials.

**Agarose/alginate:** Agarose and alginate are linear polysaccharides obtained from seaweed and algae respectively. These materials are easily obtained and can be cross-linked to form three dimensional scaffolds.<sup>57–62</sup> Alginate scaffolds are formed by calcium cross-linking and can be degraded by calcium chelation, while agarose forms a gel

based on its thermodynamic properties above a certain temperature. Both polysaccharides must undergo extensive purification to prevent immune responses after implantation. Previous work has shown a relationship between the stiffness of the agarose gel and neurite extension to have neural tissue applications.<sup>57,58</sup>

**Chitosan/methylcellulose/nitrocellulose:** Chitosan, methylcellulose, and nitrocellulose are all polysaccharides that possess similar properties and have been characterized for use in tissue engineering applications. Cellulose is the most abundant polysaccharide found in nature. Chitin is the second most abundant. Methylcellulose can form thermo responsive scaffolds, allowing it to be delivered in an injectable form.<sup>63,64</sup> Though not as widely used, nitrocellulose binds proteins non-specifically, making it convenient for drug loading.<sup>65</sup> The deacetylated chitin named chitosan can be induced to form gels at neutral pH and injectable versions of chitosan scaffolds have been produced. Chitosan can be functionalized using the appropriate chemistry to further modify these scaffolds.<sup>66,67</sup>

**Collagen:** Collagen is one of the most common ECM proteins. It has been extensively characterized as a potential scaffold for neural tissue engineering. Collagen can be isolated from mammals, including rats, bovines, and humans. By changing the pH of collagen solutions, gel formation can be induced. Denatured collagen, known as gelatin, has also been evaluated for use as a potential scaffold.<sup>68</sup> These scaffolds contain sites for cell adhesion and can be covalently modified.<sup>69–71</sup> Moreover, the scaffold properties can be varied by using different concentrations of collagen. Collagen gels are natural materials but an immune response could arise if cross-species transplantation is used.

**Dextran:** Dextran is a complex polysaccharide derived from bacteria and it consists of glucose subunits. It possesses antithrombotic properties. Scaffolds made from dextran are resistant to protein and cell adhesion and have been investigated for use as coatings for neural implants. Dextran can be chemically modified to add selective cell adhesion sites and growth factors.<sup>72</sup> Recent fabrication techniques have led to creation of macroporous dextran scaffolds that can allow cell infiltration.<sup>73</sup>

**Fibrin:** Fibrin serves as the natural wound healing matrix that form after injury and its precursor, fibrinogen, can be obtained from pooled plasma. Fibrin scaffolds are formed when thrombin cleaves fibrinogen into fibrin monomers that assemble to form a non-covalent scaffold. Similar to collagen, fibrin scaffolds have sites for cell adhesion and the scaffold properties vary depending on the concentration of fibrin used.<sup>74</sup> Fibrin can also be covalently modified to alter its properties.<sup>75–78</sup>

**Fibronectin:** Fibronectin, obtained from bovine or human plasma, is a high molecular weight glycoprotein that can bind collagen, fibrin and heparin. It is found in its soluble form in blood and participates in wound healing process. It can be aggregated to form mats, which can be used as scaffolds for the repair of neural tissue.<sup>79–83</sup> These mats contain pores that all orient in the same direction to allow for guidance of regenerating neurons, provide cell adhesion sites, and can absorb growth factors, and store them as reservoir.

**Hyaluronan/hyaluronic acid:** Hyaluronic acid, is a linear glycosaminoglycan found in the brain ECM, making it an attractive choice for central nervous system (CNS) tissue engineering. Despite being generally non adhesive to cells, it does contain sites that promote cell adhesion and is non-immunogenic. It helps promote wound healing, which is important to promote tissue repair. One disadvantage of using hyaluronan is its water soluble nature which

makes it difficult to develop an injectable version without adding additional components to help cross-link it into a stable scaffold.<sup>84–86</sup>

## Future perspectives

Various approaches have been used for the treatment of neural defects, though these therapies give temporary relief, a permanent and reliable solution is awaited. Stem cell therapy has proved to be useful in to a small extent in some cases and its reproducibility is subject to survival of the cells in the region of injury. Neural scaffolds have been extensively used in animals with great success. Hence the future of neural tissue engineering looks very bright with the fast paced development of various biomaterial scaffolds for bridging the gap between damaged neurons, repairing axonal disruptions and replacing damaged neurons in the CNS and the PNS. The use of a combination of biomaterials, neural cells and advanced technologies for the preparation of tissue engineered construct will lead ultimately to the development of a reliable source of treatment for every neural injury that has been plaguing the biomedical field over the years.

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## Conflict of interest

The author declares no conflict of interest.

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