



Aligned coaxial-electrospun artificial nerve conduits for repair of peripheral nerve injuries: A review

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Received: 20 May 2025 — Revised: 2 Jun 2025 — Accepted: 3 Jun 2025 — Published: 25 Jun 2025

Keywords:

*Peripheral nerve conduit,
Nerve regeneration,
Coaxial electrospinning,
Polymers,
Biomaterials
Tissue engineering*

Abstract — In humans, the nervous system is divided into two: the central nervous system and the peripheral nervous system. Peripheral nerves form an extensive network that connects the brain and spinal cord to all other parts of the body. They are fragile and easily damaged. When these nerves are severed, it significantly affects the patients' quality of life, and even if the peripheral nerves regenerate themselves, regeneration does not occur in cases of major damage. There are treatment options available to support nerve regeneration, depending on the extent of the damage. One of these is the use of autografts, which is still the gold standard in tissue engineering applications. A disadvantage of autografts is that mobility in the region negatively affects the patient's life and there are various difficulties such as finding a donor. In cases where autografts are insufficient in nerve regeneration, peripheral nerve regeneration (PNR) is provided by supporting with biomaterials synthetically fabricated from natural or synthetic polymers. Although various tissue scaffold production techniques are available for the fabrication of biomaterials for nerve regeneration, producing materials that can mimic the extracellular matrix (ECM) by electrospinning is frequently used today as a cheap and easy method. In this review, synthetic and natural polymers used in coaxial electrospinning technique to support nerve regeneration and the properties required for the peripheral nerve canal are discussed.

1. Introduction

The main function of the peripheral nerve system (PNS) is to transmit information between the central nervous system and various parts of the body, controlling muscle movement. The PNS also regulates autonomic nervous functions such as heart rate, blood pressure, digestion and excretion in the body, and responds to environmental stimuli. As a result of its superficial location, peripheral nerves are easily damaged (Figure 1) by trauma, traffic accidents, tumors, etc. [1]. Nerve injuries are becoming an important health problem as well as negatively affecting human life. These injuries lead to paralysis, reduced dexterity, sensitivity to cold, poor sensory and motor function, and severe pain [2]. Surgical treatment is necessary in patients with severe nerve damage. Less than 50% of patients return to normal function after tension-free end-to-end suturing for nerve injury. Therefore, treatment options are few and post-treatment results may not be as expected [3]. After injury,

peripheral nerves can self-repair to some extent, but functional recovery is not complete. Surgery is required for severe peripheral nerve injury (PNI) [4, 5]. After significant peripheral nerve injury, where nerve regeneration is not possible, some form of graft must be placed between the nerve stumps to bridge the gap and promote axonal regrowth. Implantation of an autologous nerve graft, usually taken from another part of the body, is considered the gold standard treatment for repairing the gap created in the peripheral nerve space. However, autologous nerve grafts have disadvantages such as limited donor source, need for a second surgery, donor site morbidity, and incompatibility between the donor nerve and the recipient site, which have led to the discovery of alternatives to autologous nerve grafts [6-9]. In this review, the properties of three-dimensional polymeric conduits fabricated by electrospinning as an alternative to autologous nerve grafts for use in neural tissue engineering for peripheral nerve regeneration will be discussed.

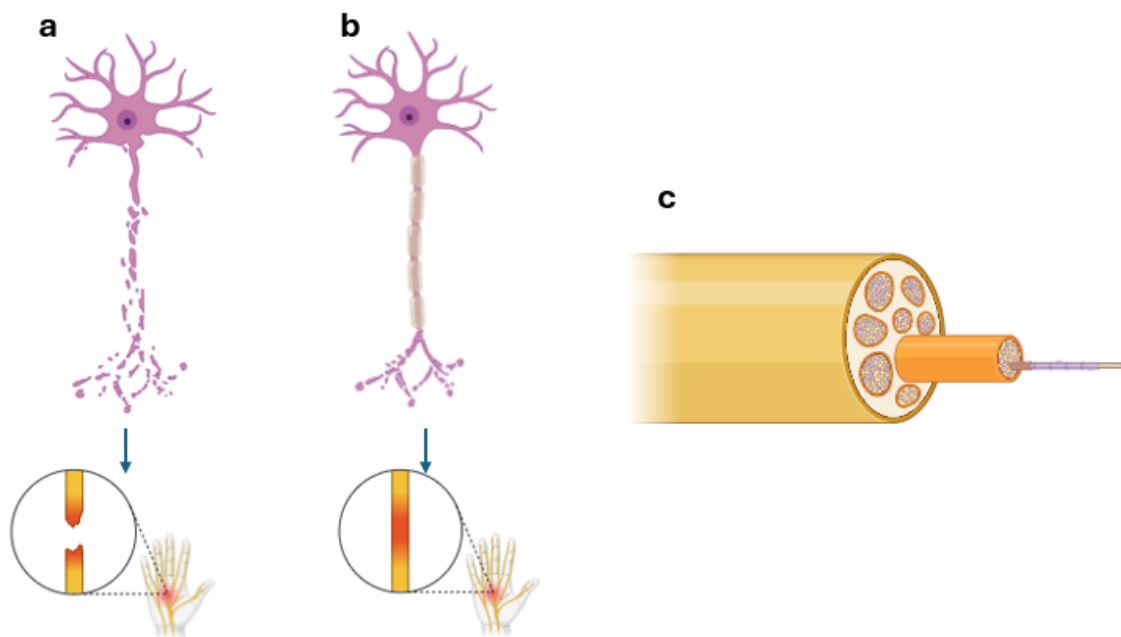


Figure 1. Schematic representation of peripheral nerve injuries, nerve degeneration (a), nerve regeneration (b), structure of nerve conduit (created from www.BioRender.com)

2. Polymeric materials used in peripheral nerve conduits

Peripheral nerve grafts enable axon regeneration in the injured nerve and protect the nerve; they also play a role in the transmission of biochemical and axonal compounds [10-12]. Many synthetic and natural biomaterials have been used in the production of neural conduits. The primary material used in the first manufactured neural conduits was non-biodegradable, biologically inert silicone conduits. Nowadays, different biodegradable synthetic polymers, including aliphatic polyesters, poly (phosphoesters), polyurethanes, piezoelectric polymers and some conductive polymers, have been used as peripheral nerve conduit materials in neural tissue engineering and rapid progress has been realized in the studies. [6, 11, 13].

Biomaterials used in nerve conduits fabrication can be divided into two categories; autologous neural tissues and desellularized neural tissues and (2) natural polymers, including extracellular matrix (ECM) molecules (collagen type I-II, laminin, fibrin, fibronectin and hyaluronic acid) and certain polysaccharide structures (chitosan, alginate, agarose) and protein structures (silk fibroin, keratin) [14, 15]. Various commercially available US/European approved products (Neurotube NeuroGen, NeuroFlex, NeuroMax, NeuroWrap and NeuroMend)

are made from Type I collagen [6]. Any biomaterial used to produce peripheral nerve conduits must have appropriate physicochemical, mechanical and biological properties. The first category includes conduits porosity and permeability properties, while the second involves the balance between flexibility and stiffness. Although many biomaterials are essentially non-toxic, allergenic, mutagenic and non-carcinogenic, they are probably produce a wide range of adverse reactions in the human body [16].

2.1. Natural polymers

Chitosan and collagen-based nerve conduits are natural materials that are biodegradable and used for nerve regeneration. They are used to connect nerve spaces in cases of peripheral nerve damage [2]. Rein et al. reported the use of chitosan nerve conduits for surgical removal of neuromas and nerve grafting, as well as for the treatment of severe nerve injuries. In the study, the shape and structure of the chitosan conduits showed minimal changes, revealing that they were covered by a vascularized fibrous membrane [17]. Lin et al. performed in-vitro characterizations on morphological similarities, mechanical properties, wettability and degradation of the conduits in their study with chitosan and collagen-based nerve conduits. They reported that they produced suitable materials for sciatic nerve applications.

Alginate is a well-known natural polymer with enzymatically degradable, highly biocompatible, tunable chemical properties, negligible immunoreaction and ECM-like structure, and is reported to have promising potential for nerve regeneration but is mechanically unstable and therefore its use in synthetic neural conduit fabrication is limited [19].

Silk is an extraordinary natural biomaterial with a multitude of properties, preferred in the field of tissue engineering due to its high mechanical strength, flexibility and relatively low cost [20]. Silk is widely used as a suture material because it is biodegradable and does not cause local tissue response [21]. Escobar et al. integrated electrically conductive nanoparticles, poly(3,4-ethylenedioxythiophene) (PEDOT), into silk fibroin polymers and reported the production and suturability of three-dimensional nerve conduits that are mechanically durable and have good electrical conductivity [22].

2.2. Synthetic polymers

Polyester is a category of polymers that contain one or two ester bonds in each repeating unit of their main chain. Poly lactic acid (PLA), poly L-lactic acid (PLLA), Poly glycolic acid (PGA), Poly-L-Lactid co-glycolic acid (PLGA), Polycaprolactone (PCL), Polyethylene glycol (PEG) are the most widely used polyesters for the production of neural canals. PLA has been used as a neural canal material in many studies. In studies in rats, successful regeneration from the cavity was observed 8 weeks after the operation. In another study, a PLA nerve conduit was fabricated by immersion deposition to close a 20 mm long gap in a rabbit sciatic nerve transection model [23, 24].

PLLA is a stereoregular and highly crystalline form of PLA and is also widely used in tissue engineering applications [23]. The amorphous regions of PLLA generally degrade faster, leading to an increased crystal concentration of the remaining materials that become resistant to degradation. Therefore, they can remain in the body for a long time, accompanied by inflammatory cells [25]. As an easy-to-obtain and easy-to-process plastic material, a single PLLA nerve conduit was implanted in rats to repair a 12 mm defect in the right sciatic nerve. The long-term (8 months) healing process was evaluated and demonstrated that the PLLA nerve conduit was able to maintain structural integrity and induce vascularization [26].

PGA is a thermoplastic polymer with high crystallinity, high tensile modulus and low solubility in organic solvents. PGA retains both hydrophilic properties in its repeating units, allowing it to be processed and modified into different forms without additives [27]. Due to non-specific ester chain breakage, PGA materials can rapidly loss their strength (2 months) and total mass (6-13 months) in vivo [28, 29]. A randomized clinical

controlled trial of 136 nerve repairs in 98 patients was performed by Weber et al. using PGA nerve canals. Of these, 43% of conventional repairs and 44% of PGA canal repairs had excellent results, 43% of standard repairs and 30% of PGA canal repairs had good results, while 14% of conventional repairs and 26% of PGA canal repairs had poor results. Although the final results demonstrated superior function of conventional repairs, PGA nerve conduit repairs had better static two-point separation and yielded results comparable to autologous nerve grafting in nerve defects smaller than 4 mm [30].

PLGA is one of the most popular biodegradable copolymers composed of two different monomers, polylactic acid and polyglycolic acid, and can be synthesized by ring-opening copolymerization in the presence of catalysts. Several factors can influence the degradation of PLGA copolymers, such as molecular weight, crystallinity and the ratio of lactic acid to glycolic acid. In general, PLGA degrades faster when the fraction of glycolic acid in the main chain increases (<50%) [25]. A research group recently fabricated a porous PLGA conduit composed of aligned electrospun PLGA fibers using capillary force lithography and salt washing. This conduit demonstrated significantly improved regenerative performance over eight weeks in a 10 mm rat sciatic nerve defect [31].

PCL is widely used in nerve repair structures [32]. PCL, a linear synthetic biodegradable aliphatic polyester, is inexpensive and capable of being molded into a variety of shapes, setting it apart from other biomaterials used in scaffolding and is FDA approved. Moreover, the simple processing of PCL as well as its excellent mechanical properties made this polymer attractive for the fabrication of peripheral nerve conduits. On the other hand, the slow degradation of PCL is one of the advantages, which is essential for the regeneration time. However, the disadvantage is that PCL polymer is hydrophobic. This is not suitable for cell-scaffold interaction and this problem can be solved by combining it with synthetic polymers such as chitosan and PEG in the form of composites [33]. In a recent in vivo study by Quan et al., a nerve conduit composed of PCL/chitosan aligned electrospun fibers was used to close a 15 mm gap in the sciatic nerve of rats. Sciatic function index results showed a functional improvement of 40% in the electrospun conduit group and 45% in the autograft group after 12 weeks [34]. These results show that synthetic conduits have a functional recovery close to autografts.

3. Fabrication of peripheral nerve conduits by electrospinning

There are many techniques can be used to fabricate nerve conduits, including leaching, freeze-drying, dip-coating, and electrospinning. Electrospinning produces random or aligned fiber matrices with fiber diameters ranging from nanometers to micrometers (Figure 2). The morphological structure of fibers produced by electrospinning method is affected by different parameters such as polymer solution parameters, process parameters and environmental factors. All of these parameters must be carefully adjusted to obtain uniform and smooth fiber structures. The electrospinning technique enables the production of three-dimensional nano and microfiber structures that can mimic the natural ECM using different synthetic and natural polymers [35]. With electrospinning, parameters such as polymer solution concentration, polymer solution viscosity, applied voltage, and collector rotation speed can be manually varied, thereby controlling properties such as the desired fiber diameter, fiber alignment, and even the integration of certain bioactive agents. This adaptability helps facilitate critical processes such as cellular adhesion, migration and elongation of neurites and is crucial for successful nerve regeneration to occur. The ability of the electrospinning method to produce nanofibers that mimic the natural microenvironment makes it an ideal choice for scaffolds that effectively guide the regenerative process of nerve tissue [36].

3.1. Morphological characteristics of the peripheral nerve conduit

3.1.1. Diameter of nerve conduit

Choosing a wide conduit technically enables implantation into the nerve. However, regardless of the polymeric material used to fabricate the nerve conduit, width leads to a significant collapse of the nerve conduit, which can result in poor regeneration, muscle atrophy, low muscle weight and poor contractile strength. The mechanical properties of the nerve canals and the final collapse of the canal-shaped structure also depend on the scaffold diameter. Adjustment of the tubular canal structure directly affects nerve regeneration, the number and diameter of regenerating axons and myelination, as well as subsequent muscle reinnervation [37,38].

3.1.2. Nerve conduit wall thickness

Increasing the wall thickness of a nerve conduit promotes greater retention of growth factors within the lumen, which increases the survival capacity of neurons. However, such conditions lead to a decrease in the amount of oxygen in the nerve conduit and a decrease in the exchange of essential nutrients such as glucose and lysozyme between the internal and external environment within the lumen [38]. However, thinner nerve conduits can be useful for surgical interventions but mechanical failures can occur leading to nerve conduits collapse, as revealed in in vivo experiments [39]. Therefore, an appropriate nerve canal wall thickness must provide sufficient mechanical resistance with a minimum thickness that allows adequate diffusion and manipulation of nutrients to allow nutrients to reach neurological cells and regenerate nerve tissue while maintaining neurotrophic factors [37-39].

3.1.3. Nerve conduit porosity

The structure of the conduit influences cellular behavior in peripheral nerve regeneration. Therefore, the design and selection of a nerve conduit with high porosity is of great importance to achieve positive results in nerve repair. Nerve conduit porosity plays an important role in the exchange of oxygen, certain nutrients and neurological factors between the internal and external environment that stimulates and promotes cell orientation, infiltration and migration, as well as providing a positive effect for axonal growth after nerve injury. Therefore, porosity is an important parameter that determines both the diffusion of proteins and the permeability of molecules such as glucose through the wall to provide a great environment for peripheral nerve regeneration [37, 39, 40].

3.1.4. Pore diameter

The pore diameter of the peripheral nerve conduit determines which molecule will pass from the surrounding tissue to the regenerating nerve through the graft [41]. Large pores can increase vascularization within peripheral nerve conduits, but when the pores are too large, there are risks of cellular infiltration and blockage of the nerve canal by fibrous tissue, which affects axonal growth and results in low density and number of nerve fibers [42]. However, low pore diameters in nerve conduits reduce nerve regeneration. Therefore, the size of the pores in the different layers of the nerve conduit should not be underestimated in order to achieve the desired results in peripheral nerve regeneration [37].

3.1.5. Fiber diameter

A neural conduit biomimetically must define the optimal architecture to preserve cell organization, survival, proliferation and differentiation, while mimicking the form and function of the ECM. The ECM in peripheral nerves acts as a three-dimensional scaffold composed of polysaccharide fibers and proteins (collagen fibers, elastin) ranging from tens to hundreds of nanometers [34]. Electrospinning is a suitable method to mimic the ECM structure because it can produce micro or nano-scale polymeric fibers and provides a three-dimensional area for growth cells to adhere more. [43]. Moreover, the average diameter of the electrospun fibers in the

scaffolds is an important control variable to observe the release profile of the growth factors. Therefore, the polymeric fiber diameter is of great importance to promote nerve regeneration and this is achieved by varying different electrospinning parameters (Figure 2) such as polymer concentration, flow rate of the polymer, voltage, distance between the needle and collector and collector speed [37].

3.1.6. Alignment of polymer fibers

During nerve regeneration, axon growth and neurite outgrowth occur in response to chemical and physical signals derived from the local microenvironment. Therefore, the topography of fibers within the engineered scaffold plays a crucial role in mimicking the ECM [44, 45]. In nerve regeneration, the superiority of electrospun fibrous nerve conduit architecture that can be appropriately organized to provide better topographical cues for cells has been demonstrated. The native ECM has a specific architecture that is important for tissue function.

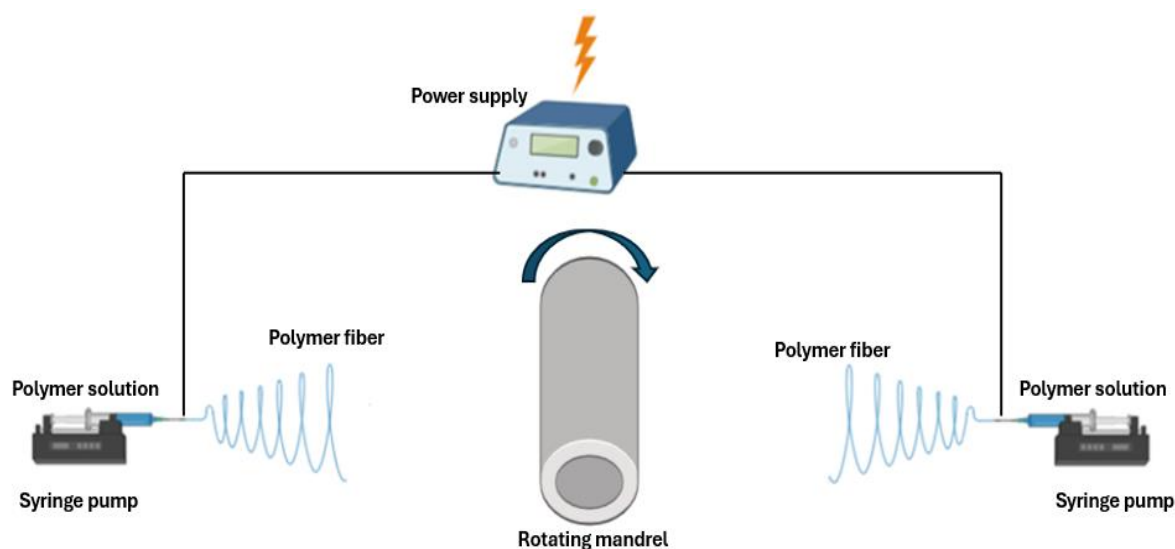


Figure 2. Coaxial electrospinning process (created by www.BioRender.com)

Therefore, once a nerve injury has occurred, a well-defined structure is required to naturally mimic the ECM to correctly guide nerve regeneration. This is why the oriented polymer fibers of the NGC are crucial for promoting nerve regeneration. Furthermore, the distribution of fibers affects the adhesion, proliferation, survival, differentiation, migration and growth of nerve cell types involved in peripheral nerve regeneration. [37-41].

4. Conclusion

In recent years, many polymeric materials have been developed for peripheral nerve injuries. Meeting the tissue requirement is one of the biggest challenges in the production of nerve conduits. This review can be of significant benefit to identify the key morphological characteristics that should be present in materials produced to provide nerve regeneration. In general, an ideal nerve conduit should be biocompatible, biodegradable, mechanically flexible and durable, swell when exposed to water, electrically conductive, hydrophilic and porous. In addition to fine adjusting the electrospinning parameters to avoid cell infiltration, the conduit diameter and wall thickness should be large enough to support nerve regeneration. The lack of optimal levels of these properties negatively affects biodegradability and biomechanical properties. Coaxial electrospinning of polymeric nerve conduits contributes to the production of a hydrophilic composite nerve conduit by spraying a hydrophobic polymer such as PCL onto a collector simultaneously with a hydrophilic polymer such as PEG. Further in vitro and in vivo studies need to be performed in the future for nerve regeneration to become a complete alternative to autografts.

Conflicts of Interest

The authors declare that there is no conflict of interest for this article.

Authors' Contributions

Authors have equal contributions

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