

UTILIZATION OF BIODEGRADABLE POLYMERS FOR PERIPHERAL NERVE RECONSTRUCTION

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Abstract

Peripheral nerve injuries are one of frequent cause of disability. Results of surgical procedures are not still completely satisfactory, though fast development of microsurgical technician improve their efficiency. One of the major problems in surgical treatment of the peripheral nerve injuries is ends coaptation in the case of large loss of nerve tissue. And so finding substitute implants not rejected by the organism for the recipient and providing with the success is necessary of regeneration. Recently, several synthetic nerve guide implants have been introduced and approved for clinical use to replace autologous transplants. This alternative therapy is based on pioneering studies with experimental nerve guides. This paper present a review of published studies involving biodegradable nerve guides.

Key words: *polysaccharides, biodegradable nerve guides, nerve reconstruction.*

1. Introduction

Peripheral nerve injuries are one of frequent cause of disability. Peripheral nerve lesions are caused primarily by traffic accidents, tumour resection or iatrogenic side effects of various types of surgery, including orthopaedic intervention, intravenous aspiration and cosmetic facial surgery [1]. Results of surgical procedures are not still completely satisfactory, though fast development of microsurgical technician improve their efficiency. One of the major problems in surgical treatment of the peripheral nerve injuries is ends coaptation in the case of large loss of nerve tissue. And so finding substitute implants not rejected by the organism for the recipient and providing with the success is necessary of regeneration.

Microsurgical methods of treatment seem to be extremely effective and their results have been encouraging. Despite surgical repair, however, residual disability often persists for the lifetime of many patients [2]. Transplantation of autogenous nerve grafts represents the current “gold standard” for reconstructive surgery, despite obvious side effects such as the deficit created by the graft harvest.

The use of cutaneous nerves as grafts, which until today was considered to be the gold standard, was already suggested 1915 by Foerster [3]. The sural nerve is the most commonly used donor nerve [3]. Other suitable donor sites are the lateral and medial antebrachial cutaneous or posterior interosseus nerves. In contrast to a vessel graft, a nerve graft is not a “plug’n play”-tool. The graft must not only cover the defect between the proximal and distal nerve stump, it must also provide the optimal conditions and environment for axonal regeneration, such as Schwann cells, revascularization and endoneurial morphology.

Nevertheless, success is limited by several factors such as:

- 1) size of the defect,
- 2) quality of the wound bed and surrounding soft tissue,
- 3) quality of vascularization of the donor nerve,
- 4) alteration of the fascicular architecture in the graft and
- 5) limited availability of donor nerves.

Furthermore, nerve grafting implicates two suture lines at the proximal and distal coaptation side, which can lead to ‘neuroma-in-continuity’-formation, due to increased fibrosis and scar tissue formation especially at the distal coaptation side, which ‘close the door’ to the distal nerve stump. Unsolved problems are also apoptosis of motoneurons, especially in nerve lesions close to the neuron and a limited time frame in which muscles can be reinnervated by successful reconnection of the motor end plate to regenerated axons. [4] Although sensory nerves somehow inhibit regeneration of motor nerves [5] - which is a rather irrelevant finding for clinical use, no tubular or other type of conduit has proved to be superior in comparison with the autologous nerve graft, at least not for reconstruction of substantial human motor nerves, such as the ulnar or median nerve trunk. The ideal nerve reconstruction technique should be one that:

- 1) permits immediate nerve reconstruction at time of injury,
- 2) allows a tension free nerve suture,
- 3) does not require sacrificing a donor nerve,

- 4) does not add additional intraoperative time,
- 5) does not place foreign material permanently into the body,
- 6) does not require additional medication (e.g. immunosuppressive therapy in the case of allografts),
- 7) does not create a potential nerve entrapment site and
- 8) that permits concepts of modern neurobiology to enhance nerve regeneration (e.g., specific drug release of growth factors).

The development of an artificial nerve tube as an alternative to autogenous nerve grafting is a current focus of interest in peripheral nerve repair. This review provides a brief overview of various preclinical trials conducted to evaluate the utility of artificial nerve tubes for regenerating peripheral nerves.

2. Basic nerve guide concepts

Due to the specialised conditions required for successful autografting to enable the growth and reinnervation described above, the use of artificial nerve guide tubes has become essential. Three mechanical properties for this type of guide are necessary.

Firstly, the guide tube must provide an adequate scaffold for axon regeneration. It is widely accepted that physical guidance of axons is critical for efficient nerve repair. If a nerve guide tube is too soft, it will not withstand pressure from the surrounding tissues, and conversely, if it is too hard, it will often damage surrounding tissues such as nerve stumps and blood vessels.

Secondly, the guide tube must be semi-permeable. Controlled exchange between the intra- and extrachannel environments is important for the regeneration of severed peripheral nerves through synthetic guidance channels [6]. It is well known that extended regeneration achieved with the use of permeable chambers is due to a combination of

- 1) metabolic exchange across the tube wall (diffusion of nutrients such as glucose, oxygen, etc, and elimination of waste products),
- 2) diffusion into the guide lumen of growth or trophic factors generated in the external environment (wound healing factors) and
- 3) retention of growth or trophic factors secreted by the nerve stumps.

Thirdly, the tube must degrade at an appropriate speed as the nerve regenerates. The most important property related to achieving a synthetic resorbable nerve prosthesis, besides the biocompatibility of the material, is to obtain a resorption rate that is not too rapid and stability of the walls for a sufficiently long period to warrant a space in which the regeneration and reorganisation of the nerve is effective [7]. If the nerve guide breaks down at an early stage, fibrous tissue forms inside the tube and impairs further maturation of the regenerated nerve [8,9]. Moreover, too rapid degradation may cause the regenerated axons to suffer mechanical or biochemical damage. In order to be satisfactory for clinical use, an artificial guide must ensure satisfactory nerve repair, at least to a level equivalent to that obtained with autogenous grafts.

3. Experimental study of artificial nerve guides

A graft made of biodegradable materials is a promising alternative for promoting successful long-term recovery, as has been confirmed both experimentally and clinically, because, after serving as an appropriate scaffold for regeneration, the conduit eventually degrades. A variety of biodegradable materials have been examined for peripheral nerve regeneration.

3.1. Collagen

In the USA, several types of collagen tubes incorporating biological materials have often been used both experimentally and clinically. Collagen is a biological molecule that can be used for constructing nerve guidance channels. Addition of collagen gels to the lumina of nerve conduits speeds the rate of nerve regeneration [10]. A number of collagen-based nerve tubes have been shown to support regeneration over nerve defects in rat, mice [11, 12], rabbits [12] and primates [14 - 17]. However, repair was limited to gaps of less than 30 mm [18].

Since Collin and Donolf. reported nerve regeneration applying a collagen tube [19], other investigators also has observed that collagen is able to promote nerve regeneration [20 - 22]. When preparing bioabsorbable collagen tubes, type I atelo collagen is mostly used. To enhance mechanical strength and control time needed for absorption of the collagen tube, many cross-linking methods can be used, i.e. chemical cross-linkage using formaldehyde, hexamethylene diisocyanate, glutaraldehyde (GA) or polyepoxy compounds, physical cross-linking using irradiation, ultraviolet (UV) irradiation or heat treatments [23].

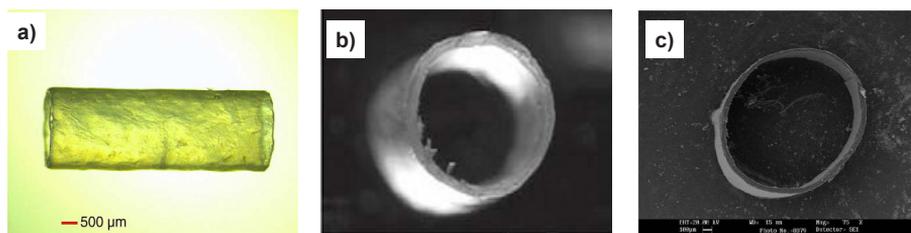


Figure 1. a) Micrograph of a typical C-NC (10× magnification), b) micrographs of cross-sections of NC in the dry (20 × magnification) [24], c) nerve guide uncrosslinked collagen tube (11,500 x magnification) [25].

3.2. Chitosan

Removing calcium phosphate and proteins from crab tendons, crystalline chitosan tubes are obtained [26], having a tubular structure and a suitable size for peripheral nerve reconstruction. Its mechanical strength however is still low perpendicular to its longitudinal axis, resulting in the swelling of the tube surface wall and causing stenosis (due to swelling inside the tube). To improve the mechanical property of a chitosan tube, it is heat-pressed into a triangular shape and surface-modified into apatite using an alternate soaking method – fig [26 - 28]. In study [29], the mechanical property, biocompatibility and efficacy as a nerve conduit of the hydroxyapatite-coated and laminina peptides adsorbed tubes were examined.

In study [27], the chitosan tubes were made to react with the apatite using an alternate soaking method [30 - 32] to enhance the strength of the tube walls.



Figure 2. An apatite-coated chitosan tube with a triangular shape of side 2.1 mm and length 15 mm. [27].

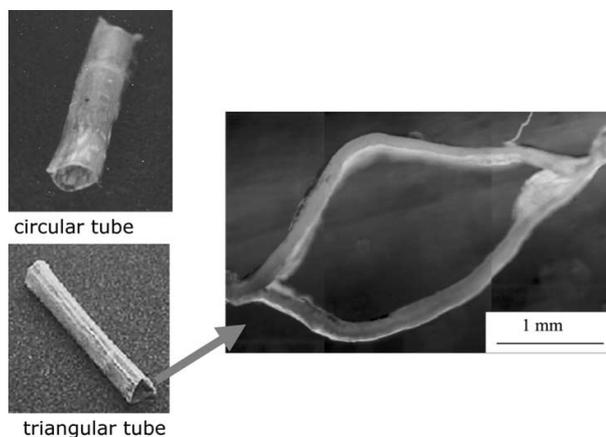


Figure 3. The tendon chitosan tube. The figure of the right side shows an optical microscope image of the cross section perpendicular to the longitudinal axis of the t-chitosan tube. [28].

3.3. Alginate

Alginate, a biodegradable polysaccharide, may be applied to facilitate nerve regeneration.

A novel material for nerve regeneration, alginate, was employed in both tubulation and nontubulation repair of a long peripheral nerve defect injury [33]. Twelve cats underwent severing of the right sciatic nerve to generate a 50-mm gap, which was treated by tubulation repair (n = 6) or nontubulation repair (n = 6). In the tubulation group, a nerve conduit consisting of polyglycolic acid mesh tube filled with alginate sponge was implanted into the gap and the tube was sutured to both nerve stumps. In the nontubulation group, the nerve defect was repaired by a simple interpolation of two pieces of alginate sponge without

any suture. The animals in both groups exhibited similar recovery of locomotor function. Three months postoperatively, successful axonal elongation and reinnervation in both the afferent and efferent systems were detected by electrophysiological examinations. Intracellular electrical activity was also recorded, which is directly indicative of continuity of the regenerated nerve and restoration of the spinal reflex circuit. Eight months after operation, many regenerated myelinated axons with fascicular organization by perineurial cells were observed within the gap, peroneal and tibial branches were found in both groups, while no alginate residue was found within the regenerated nerves. In morphometric analysis of the axon density and diameter, there were no significant differences between the two groups. These results suggest that alginate is a potent material for promoting peripheral nerve regeneration. It can also be concluded that the nontubulation method is a possible repair approach for peripheral nerve defect injury.

Matsuura et al.[34] attempted to regenerate excised cavernous nerves by filling the gap with a biodegradable alginate gel sponge sheet without sutures. Bilateral cavernous nerves of male Wistar rats were excised to make an approximately 2-mm gap. A piece of freeze-dried alginate sheet was then placed over the gap to cover each stump without suturing (alginate group). The results of animal study have demonstrated that by simply filling the nerve gap using an alginate sheet, the cavernous nerve can be regenerated and erectile function may be restored.

Tried to regenerate peripheral nerve by using alginate tubular or non-tubular nerve regeneration devices, and compared their efficacies [35 - 37]. However, there was no significant difference between both groups at electrophysiological and morphological evaluation. Although, nowadays, nerve regeneration materials being marketed mostly have a tubular structure, our results suggest that the tubular structure is not indispensable for peripheral nerve regeneration.

Freeze-dried alginate sponge cross-linked with covalent bonds has been demonstrated to enhance nerve regeneration in peripheral nerves and spinal cords [38]. These findings suggest that alginate contributed to reducing the barrier composed of connective tissues and reactive astrocytic processes, and served as a scaffold for the outgrowth of regenerating axons and elongation of astrocytic processes.

The study [39] analyzed nerve regeneration through alginate gel in the early stages within 2 weeks and the late stages up to 21 months after implantation. Much better nerve regeneration was found in alginate gel, than collagen sponge-, and fibrin glue-implanted distal stump 12 months after surgery. These results indicate that alginate gel has good biocompatibility for regenerating axon outgrowth and Schwann cell migration, and that regenerated fibers can have a diameter as thick as that of normal fibers in the long term. Alginate gel is a promising material for use as an implant for peripheral nerve regeneration.

3.4. Biodegradable synthetic polymers

On the other hand, many experiments have also been conducted using biodegradable synthetic polymers such as PGA, PLLA, PLGA and PLCL. Polyglactin grafts resulted in an inflammatory response and poor nerve regeneration [40]. Better results were obtained

with polyglycolic acid [40-44] and poly(L-lactide-co-caprolactone) [45] conduits. A micro-porous polyglycolic acid braided tube coated with crosslinked collagen to promote tissue proliferation has also successfully supported nerve regeneration [44, 46 - 49].

Study [50] attempted to enhance the efficacy of peripheral nerve regeneration using our previously tested poly(L-lactic acid) (PLLA) conduits by incorporating them with allogenic Schwann cells (SCs).

In study [51] various asymmetric poly(DL-lactic acid-co-glycolic acid) (PLGA) conduits were controlled. The results of the directional permeable films indicated the statistically significant proliferation of Schwann cells, compared with the other films. In the in vivo trial, the PLGA conduits seeded with Schwann cells were implanted into 10 mm right sciatic nerve defects in rats. Histological examination after 6 week verified that directional permeable conduits had markedly more A-type and B-type myelin fibers in the midconduit and distal nerve.

Of these types of materials, nerve guide implants made of collagen, PGA and PLCL are now approved by the US Food and Drug Administration (FDA) for human application and are already in clinical use.

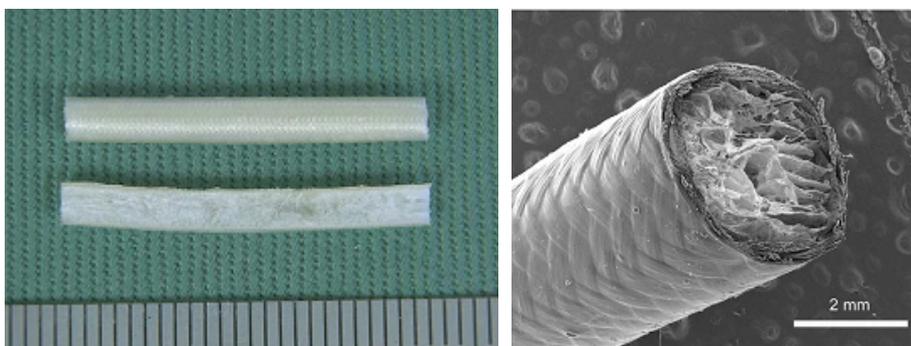


Figure 4. Resorbable nerve guide tube was made from woven PGA fibers. The inner space was filled with collagen sponge to enhance nerve regeneration. (B) Scanning electron microscopic view of PGA-collagen tube [46].

3.5. Growth factors

A more recent study has shown that the ability of an artificial nerve guide to deliver growth factors and Schwann cells is important for enhancing functional recovery [52]. Biodegradable grafts also have a significant advantage in that, as they degrade, they can be made to release growth or trophic factors trapped in, or adsorbed to, the polymer. Entrapment of a promoting factor in the polymer mesh and its release during degradation enables prolonged bioavailability of the factor during the regeneration process. Cross-linking brain-derived neurotrophic factor (BDNF) to collagen nerve tubes enables favourable functional recovery of limb muscles and the largest mean axon diameters proximal and distal to the repair site [53]. An alternative to controlled release of growth factors is to add Schwann cells

directly into the lumen of a biodegradable artificial nerve graft. Schwann cells suspended in gelatin within the lumen of a polyglycolic acid conduit have been shown to support nerve regeneration over a 30-mm gap in the peroneal nerve in rabbits [54].

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