Carbohydrate Polymers 315 (2023) 120934

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Natural polysaccharides and their derivatives as potential medical materials and drug delivery systems for the treatment of peripheral nerve injuries

Sergey O. Solomevich [a,b], Carlo M. Oranges [c], Daniel F. Kalbermatten [c,d], Anna Schwendeman [a,e], Srinivas Madduri [c,d,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI, USA
[b] Research Institute for Physical Chemical Problems of the Belarusian State University, Minsk, Belarus
[c] Plastic, Reconstructive and Aesthetic Surgery Division, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland
[d] Bioengineering and Neuroregeneration Laboratory, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland
[e] Biointerfaces Institute, University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

Keywords:
Nerve regeneration
Nerve repair
Nerve guidance conduits
Hydrogel
Schwann cells
Growth factors

ABSTRACT

Peripheral nerve repair following injury is one of the most serious problems in neurosurgery. Clinical outcomes are often unsatisfactory and associated with a huge socioeconomic burden. Several studies have revealed the great potential of biodegradable polysaccharides for improving nerve regeneration. We review here the promising therapeutic strategies involving different types of polysaccharides and their bio-active composites for promoting nerve regeneration. Within this context, polysaccharide materials widely used for nerve repair in different forms are highlighted, including nerve guidance conduits, hydrogels, nanofibers and films. While nerve guidance conduits and hydrogels were used as main structural scaffolds, the other forms including nanofibers and films were generally used as additional supporting materials. We also discuss the issues of ease of therapeutic implementation, drug release properties and therapeutic outcomes, together with potential future directions of research.

1. Introduction

Nerve injuries are critical and clinically challenging. The rapid urbanization of modern society, technological progress, an increasing number of natural and man-made disasters, local armed conflicts and road traffic accidents, as well as the emergence of new extreme sports, have caused a steady annual increase in patients with peripheral nerve injuries (PNIs). In developing countries, these types of injuries affect from 13 to 23 people per 100,000 per year (Li, Liu, et al., 2014) and are the main cause of life-long disability (Böcker et al., 2022).

Although treatment strategies continue to improve (Deng et al., 2022), the choice of treatment after PNI depends on the extent and type of damage (Samadian et al., 2020) and the completeness and speed of recovery remain difficult to predict (Ruijs et al., 2005). According to different authors, the effectiveness of surgical interventions on peripheral nerves ranges from 36 % to 51.6 %, depending on the nature of the injury and the intervention (Chen et al., 2020; Grinsell & Keating, 2014; Ruijs et al., 2005). In the absence of a gap between nerve stumps, end-to-side or end-to-end suture is usually used for treatment (Bontioti et al., 2005). Postoperative damage recovery should include immobilization for up to six weeks, depending on the severity of the injury (Griffin et al., 2014). The result of nerve repair mainly (up to 50 %) depends on age (Rosén et al., 1994) and treatment timings. This is due to age-related changes in the reactivity of macrophages, Schwann cells (SCs), axons and neurons (Verdú et al., 2000; Wang, Jiang, et al., 2022). The result of nerve repair mainly (up to 50 %) depends on age (Rosén et al., 1994) and treatment timings. This is due to age-related changes in the reactivity of macrophages, Schwann cells (SCs), axons and neurons (Verdú et al., 2000; Wang, Jiang, et al., 2022). In rodents, these changes begin at the age of 12 months and become marked after 20 months (Kovačič et al., 2009; Pestronk et al., 1980; Pola et al., 2004; Santos et al., 2000; Stratton et al., 2020; Verdú et al., 2000; Willows et al., 2023). Human

* Corresponding author at: Plastic, Reconstructive and Aesthetic Surgery Division, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland.
E-mail address: srinivas.madduri@unige.ch (S. Madduri).

https://doi.org/10.1016/j.carbpol.2023.120934
Received 9 February 2023; Received in revised form 7 April 2023; Accepted 17 April 2023
Available online 20 April 2023

tissue measurements reveal such age-related changes after 40 years of age, which become significant between 60 and 70 years old. However, such age-related cellular, molecular and functional changes of peripheral nerves vary significantly among individuals as reported earlier (Verdú et al., 2000). Moreover, chronic nerve injuries and the delayed repair result in nerve fibrosis and senescence of repair SCs (Ghosh et al., 2020). Other factors that affect the rate of healing after nerve injury include the distance from the lesion to nerve stumps and the presence of soft tissue and vascular injury (Gilbert et al., 2006). However, proximal injury involving nerve suturing often results in limited motor recovery due to a limited regeneration capacity and time constraints to reach the target organ (Evans et al., 1991; Raza et al., 2020).

In the case of severe nerve damage where there are large gaps or long scars that prevent effective regeneration and innervation of the distal nerve stump, the standard treatment is autologous nerve grafting (Böcker et al., 2022; Millesi et al., 1972). However, functional recovery after autologous nerve grafting is often limited due to a lack of functional reinnervation (Kline et al., 1998). Nerve transfer, a technique when distal nerve is not available for co-aptation, has become an effective treatment for treating proximal and complex upper limb PNIs (Isaacs & Cochran, 2019; Midha, 2006). Nerve transfer is technically different from nerve graft in that nerve transfer is usually carried out closer to the paralyzed muscle. Therefore, the nerve fibers have a shorter distance to regenerate, while the nerve graft is performed at the site of damage, which may be far away from the target muscle, resulting in a longer regeneration time and thus a slower recovery. Garg et al., showed that double nerve grafting in patients with complete C5-C6 superior brachial plexus injuries resulted in a better recovery of shoulder and elbow functional mobility than conventional autologous nerve grafting (Garg et al., 2011). Nerve transfer is also preferable to nerve grafting in the treatment of ulnar nerve injuries as it provides a better recovery of motor strength and grip functions (Sallam et al., 2017). However, comorbidities associated with autologous nerve grafting and a limited functional recovery after transfer has resulted in the development of nerve guidance conduits (NGCs). Of note, several comprehensive reviews on animal and *in vitro* models used for the development of novel NGCs have already been published (Geuna et al., 2016; Navarro, 2016). Over time, many synthetic NGCs have been created from polymeric materials, such as poly(L-lactide) (Hsu et al., 2011), poly(lactic glycolic acid) (Sasaki et al., 2011), poly(ε-caprolactone) (Jiang et al., 2014), polyamides (Yannas & Hill, 2004), and others (Pertici et al., 2014). Materials for NGCs must meet a number of requirements such as biocompatibility, non-immunogenicity and biodegradability (Garg et al., 2011; Subramanian et al., 2009).

Improved functional recovery after nerve repair can be achieved by promoting the speed and quality of axonal growth (Grinsell & Keating, 2014). Approaches for enhancing axonal regeneration include creating a favorable environment for axon cross-coaptation, delaying or altering Wallerian degeneration, and shortening muscle denervation time (El Soury et al., 2023). Topical pharmaceuticals are also used for the treatment of PNI (Degrugillier et al., 2021). These pharmacological agents are designed to reduce scarring at the repair site and to improve restoration of nerve function. Despite the exceptional importance of immunosuppression in preventing tissue rejection, it is used infrequently and, as a rule, only for allotransplantation of nerves and composite tissues (Grinsell & Keating, 2014).

Implantation of a biomaterial scaffold at the site of nerve injury can provide sustained local delivery of immunosuppressants and growth factors, while simultaneously supporting the guided axonal growth through the lesion cavity (Francis et al., 2017). Within this context, polysaccharides and their derivatives are widely accepted as excellent materials for drug delivery and tissue engineering applications due to their specific properties, such as ease of chemical and structural modification, an ability to absorb and drug release properties, including biocompatibility and biodegradability (Debele et al., 2016; Ko et al., 2014; Nakajima et al., 2017; Ninan et al., 2013; Sampath et al., 2016; Yurkshtovich et al., 2019). It is important to note that these biomaterials do not cause immunostimulation or immunosuppression when used in nerve regeneration (Rosales-Cortés et al., 2003). The limitations of natural polysaccharides and their derivatives for use in the pharmaceutical and medical industry include difficulties in extraction and purification, uncontrolled mechanical properties (*i.e.*, viscosity and hydration rate), microbial contamination, and batch-to-batch variability (Barclay et al., 2019; Mohammed et al., 2021). Other drawbacks to the use of polysaccharides include the poor solubility, variable chemical composition, particularly in the case of multiple sources, and high polydispersity (Wen & Oh, 2014). However, these limitations can be compensated to a large extent by the chemical and structural modification of polysaccharides, as well as the combinational use of polysaccharides with other natural, semi-synthetic and synthetic polymers (Tudu & Samanta, 2023; Wang, Jiang, et al., 2022).

Several recent publications have reviewed current evidence on the use of biomaterials for peripheral nerve regeneration. Notably, two reviews are devoted to natural biomaterials in peripheral nerve repair and describe treatment strategies using hollow NGCs and cell therapy (Carvalho et al., 2019; Powell et al., 2021). Fornasari et al., and Samadian et al., described the use of chitosan, hyaluronic acid and alginate to support peripheral nerve regeneration (Fornasari et al., 2020; Samadian et al., 2020). However, the focus of these reviews on polysaccharide-based biomaterials for the treatment of nerve injuries is limited. To our knowledge, there are no dedicated review articles over the last 5 years highlighting the usefulness of various polysaccharides and their multidisciplinary approaches for the treatment of nerve injuries. Thus, the aim of this review is to summarize the current state of knowledge on the use of polysaccharides and their derivatives to create NGC alone or in combination with bioactives for nerve repair and to discuss future directions.

2. Methods

We performed a literature search according to the PRISMA guidelines. PubMed and Web of Science were searched for articles published in English until 31 January 2023 reporting natural polysaccharides and their derivatives as a potential source of drug delivery systems for the treatment of PNIs. The following search terms were applied: “peripheral nerve repair”, “peripheral nerve regeneration”, “polysaccharide-based materials for nerve regeneration”, “chitosan for treating nerve injuries”, “alginate for treating nerve injuries”, “hyaluronic acid for treating nerve injuries”, “neurotrophic factors delivery for peripheral nerve regeneration” “pharmacological treatment of nerve injuries”, and “drug delivery for peripheral nerve regeneration”. Publications with incomplete data or conclusions and those not directly related to PNIs were excluded, although a limited number of unrelated publications involving polysaccharide-based drug delivery systems were incorporated for repurposing and for extracting further insights for future directions.

3. Polysaccharide-based biomaterials for PNI treatment

Polysaccharides are biocompatible polymers, which can be modified to produce derivatives with physicochemical properties necessary for nerve regeneration (Fig. 1) (Jiang et al., 2023; Nectow et al., 2012). In this section, we discuss various ways of repairing nerve defects using polysaccharide-based materials in the form of NGCs, hydrogels and films.

3.1. Supporting materials for solid NGCs

As a rule, the implantation of any foreign material into the body causes an inflammatory reaction, the intensity of which can vary depending on the material structure and its physicochemical properties (Rosales-Cortés et al., 2003). In this regard, research in recent years has focused on the creation of new implants for the regeneration of

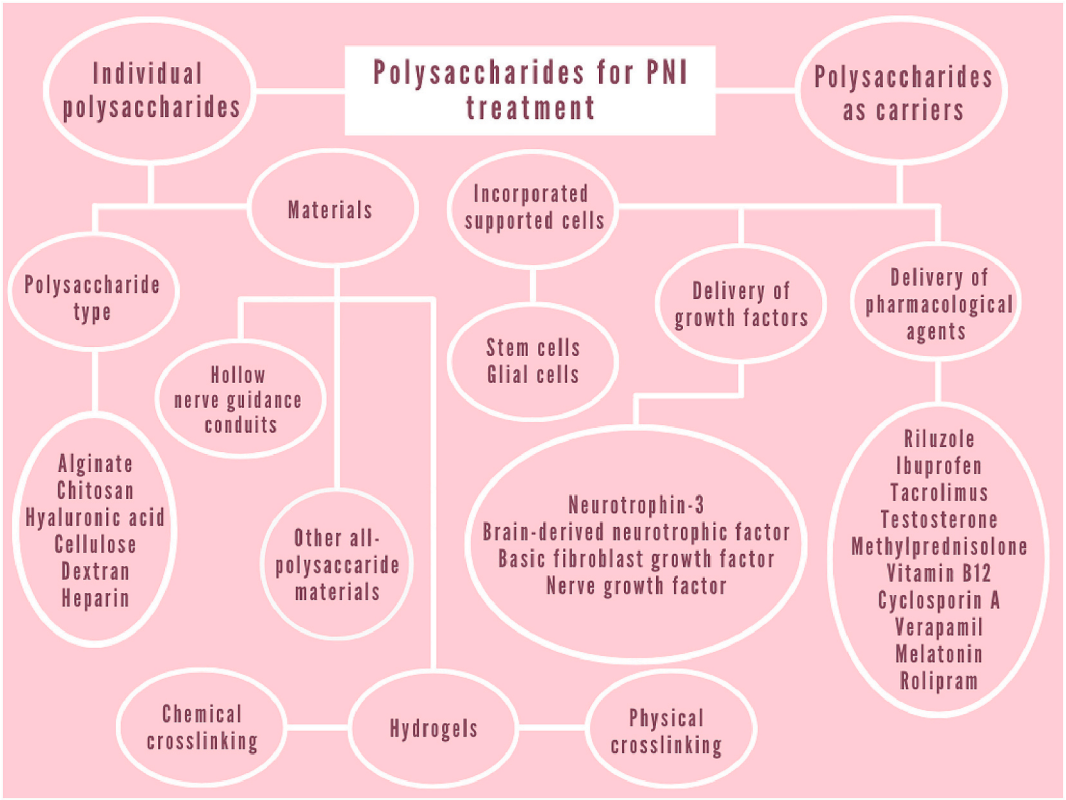

Fig. 1. Various forms of polysaccharides and their composites for peripheral nerve regeneration.

peripheral nerves in the form of NGCs. Prostheses, including NGCs, made from biodegradable materials cause mild tissue inflammation, which is important as inflammation promotes the production of enzymes that degrade the implanted material. However, in the case of an excessive inflammatory reaction, significant tissue destruction occurs at the implantation site, leading to impaired tissue regeneration (Peluso et al., 1994). Much research is currently ongoing on the application of natural biopolymers (especially proteins and polysaccharides) to NGC production (Sarker et al., 2018). These polysaccharides are biocompatible, non-immunogenic, biodegradable, non-toxic, and pro-regenerative.

Chitosan (poly(1,4)-β-D-2-aminoglucose) (50–400 kDa), the deacetylated form of chitin, is a positively-charged polysaccharide with good bioadhesive properties and a beneficial effect on nerve regeneration (Wang et al., 2022; Matica et al., 2019; Siemionow et al., 2010). Chitosan is most commonly extracted from crab tendon by the separation of proteins and calcium phosphates. The polysaccharide is highly biocompatible, microbicidal, non-toxic and biodegradable by the lysozyme (Solomevich, Dmitruk, Bychkovsky, et al., 2021). The main limitation of chitosan polymer for medical purposes is its insolubility in aqueous solutions. The chemical modification of chitosan enables improved solubility and, consequently, it opens up the potential for a wide range of medical applications (Li et al., 2022; Zhao et al., 2018). The use of chitosan NGCs for sciatic nerve repair resulted in a faster recovery compared to control groups, as well as increased axonal growth as evidenced by histological analysis (Boecker et al., 2019; Dietzmeyer, Förthmann, et al., 2020; Patel et al., 2006). De Lima et al. reported a chitosan-based composite NGC for the repair and regeneration of a 5 mm nerve gap injury in the rabbit. Nerve autograft reinforced with composite NGC exhibited an enhanced regeneration compared to individual treatment groups (de Lima et al., 2021). Zhang et al. used the Sprague-Dawley rat model of sciatic nerve defect to investigate the feasibility of creating artificial peripheral nerves using chitosan NGCs (length, 12 mm; wall thickness, 0.1 mm; inner diameter, 1.5 mm) (Zhang et al., 2008). The repair effects of chitosan NGCs with supporting fibers used to bridge a 10 mm gap in the nerve were better than the effects of simple conduits. Another study demonstrated that gamma irradiation-sterilized chitosan/type I collagen (300 kDa)/gelatin (3–6 kDa) composites did not cause any inflammatory reaction in the muscles or demonstrate liver and kidney toxicity, and helped to regenerate sciatic nerve damage in Wistar rats, aged 6–8 weeks (León-López et al., 2019; Wang et al., 2012). In addition, electrofabrication technology enabled the single-step fabrication of chitosan NGC with tunable mechanical properties, which confirmed tunability, scalability and biocompatibility *in vitro* (Nawrotek et al., 2020) and further exhibited beneficial effects on nerve regeneration at the level of autografting (Liu et al., 2021).

To improve chitosan NGC properties, a graft made of chitosan and graphene oxide was developed. SCs cultured on chitosan/graphene oxide films showed elevated Krox20 and Zeb2 responsible for cell proliferation and myelin differentiation. These NGCs further supported nerve regeneration across a 10 mm sciatic nerve defect at the level of autograft as evidenced by the histomorphometric analysis of nerve and muscle tissue, the sciatic function index (SFI) and nerve conduction properties (Zhao et al., 2023). Further studies on a longer nerve defect would enhance the clinical translation of these NGCs. Composite NGC consisting of polyacrylamide/chitosan tailored with mechanical and topographical guidance cues were applied to treat a 15 mm sciatic nerve defect in rabbits (Liu, Xu, et al., 2022). Interestingly, polyacrylamide/chitosan NGC with 8.4 kPa and a surface groove with 30 μm range resulted in improved nerve regeneration comparable to autologous nerve grafting as measured by wet-weight ratio, electrophysiology and nerve histomorphometry. Subsequent molecular analysis revealed the underlying mechanisms to be linked to vinculin, p-FAK, and Rho A proteins, which are all involved in axon growth and elongation features (Liu, Xu, et al., 2022). However, further investigation is needed to elucidate the toxicological effects of the residual acrylamide in greater detail in a long-term study and to establish the safety and biocompatibility. In the context of luminal enrichment within the NGC, a chitosan

NGC filled with carboxymethyl chitosan sponge promoted nerve regeneration and positively influenced the functional recovery comparable to an autologous group in a 10 mm rat sciatic nerve defect model, as evidenced by the SFI, CMAP, conduction speed, muscle wet-weight and nerve histomorphometry (Zhang et al., 2022). In a further improvement, multichannel Chitosan NGC with warp-knitted scaffold and internally aligned fibers showed a comparable performance with autograft treatment in rats over a 10 mm-long sciatic nerve defect. It seems that these multichannel NGC structures protected SCs from apoptosis by blocking Bcl-2/Bax/caspase-3 pathway (Jiang et al., 2023). Electrical properties of the therapeutic materials play a key role in the process of nerve regeneration. For enhancing the conductive properties of NGC, a double-layered chitosan-based NGC was fabricated. The NGC main structural scaffold was prepared by chitosan, whereas the luminal conductive hydrogel was prepared by modified carboxymethyl chitosan and pluronic F-127 (F127-CHO) using the polyaniline and aldehyde functional groups. The resulting NGC with improved conductive properties supported sciatic nerve regeneration in rats after a 10 mm gap injury and compared well with autologous nerve grafting (Deng et al., 2022, p.).

In a step forward using the chitosan NGC, a randomized, prospective clinical study involving 47 patients with varying nerve defects ranging from 9 mm to 23 mm confirmed the safety and non-inferiority to autologous nerve grafting. These conclusions were supported by the outcome measurements covering the two-point discrimination test, Semmes Weinstein monofilament testing, patient satisfaction and pain assessment (Böcker et al., 2022). Thus, these observations highlight the potential of the chitosan NGC for human clinical use. However, further studies involving the critical nerve gap injuries ranging from 40 to 60 mm will enhance their clinical potential.

Sufan et al. synthesized an NGC consisting of polyglycolic acid tubes filled with alginate sponge and used them for the regeneration of 50 mm PNIs in adult American, domestic, short-haired cats (Sufan et al., 2001). Animals were divided into two groups of six. In the first group, alginate-filled tubes were placed in the gaps, while in the second, only alginate sponges were used to fill the nerve gaps. Animals in both groups showed the same restoration of locomotor function. Three months after the operation, reinnervation and elongation of axons were observed and intracellular electrical activity was recorded, thus indicating that the continuity of the nerves was restored and the spinal reflex circuit recovered. These results show that alginate promotes peripheral nerve regeneration and that non-tubulation has a great potential for repairing PNIs.

Hyaluronic acid (HA) (3–4000 kDa) is a naturally occurring, negatively charged, linear, high molecular weight polysaccharide with residues of glucuronic acid and *N*-acetylglucosamine (Agarwal et al., 2020; Lee et al., 2021; Mendichi et al., 2003). HA plays an important role in wound morphogenesis, angiogenesis, tissue remodeling and scar-free healing (López-Cebral et al., 2017). HA nanocomposite NGC consisting of carbon nanotubes and mesoporous silica nanoparticles (NPs) exhibited enhanced modulus and electrical permittivity as evidenced by SC adhesion and survival *in vitro* (Ruiz et al., 2021). Within this context, a hyaluronic acid (HA)-collagen composite in combination with poly (ε-caprolactone) nanofibers better supported SC proliferation and axonal growth and guidance (Entekhabi et al., 2021). However, the impact of the different components such as HA-collagen alone on SCs is unclear. Therefore, further experiments both *in vitro* and *in vivo* are needed to provide an improved understanding of this system. Roca et al. reported the beneficial effects of HA-silk fibroin composite NGC for the reconstruction of mice sciatic nerve transection injury. After 8 weeks of implantation, tissue regeneration was enhanced by the presence of new blood vessels, particularly in the presence of silk fibroin supporting scaffolds, thus indicating the improved bio-mechanical support (Gisbert Roca et al., 2020).

3.2. Hydrogels

As described above, polysaccharides have a number of unique biological and chemical properties and hydrogels based on these materials are widely used, in particular in the food industry, agriculture, medicine and pharmaceuticals (Catoira et al., 2019; de Jong et al., 2001; Rinaudo, 2006; Solomevich, Dmitruk, Aharodnikau, et al., 2021; Tayler & Stowers, 2021). In addition, the low cost of production and rich natural supply make them extremely attractive (Chen et al., 2022; Ullah & Lim, 2022). Crosslinking mechanisms of hydrogels, as well as the density of crosslinking, directly affects the properties of finished polysaccharide hydrogels, *e.g.*, stability, durability, resorption kinetics, and the release rate of bioactive compounds, and consequently the process of tissue regeneration. Therefore, different ways (physical and chemical) of crosslinking the hydrogels hold a significance for nerve repair and regeneration. Furthermore, the choice of crosslinking depends on the type and severity of the nerve defect. The advantages of using chemically crosslinked hydrogels include controlled mechanical strength and cell size, allowing the hydrogels to function as drug delivery systems with tunable release properties, while physical crosslinking is a simple way to obtain safe hydrogels without the use of crosslinkers (Samadian et al., 2020). Hydrogels are described below in two subsections, despite the fact that some of them are crosslinked both chemically and physically and could be presented in either subsection.

3.2.1. Chemically crosslinked hydrogels

Chemically crosslinked hydrogels are usually more stable and have improved mechanical properties than physically crosslinked hydrogels due to the formation of covalent bonds. Various methods are used for chemical crosslinking, including click chemistry, Michael-type addition reaction, Schiff base, enzymatic and photo-crosslinking (Nezhad-Mokhtari et al., 2019).

Navrotek et al. developed a method for synthesizing chitosan-based hydrogel implants for topical application immediately prior to treatment (Nawrotek et al., 2016). By adding hydroxyapatite, the mechanical strength of the hydrogel can be tuned to the size of the gap between the damaged nerve stumps. A chitosan-based, photo-crosslinkable hydrogel has been described as a promising coupling agent for peripheral nerve anastomosis (Rickett et al., 2011). The hydrogel was synthesized by conjugating 4-azidobenzoic acid with low and high molecular weight chitosan polymers. The samples showed no toxicity and were cytocompatible and mechanically suitable for use as bioadhesives in peripheral neurosurgical operations. Wang et al. showed that double-crosslinked composite hydrogels, which consist of HA and alginate, have biocompatibility comparable to pure HA hydrogels (Wang et al., 2013). Ionically and covalently crosslinked porous 3D scaffolds can be prepared by prototyping from composite materials. In another study, carboxymethylchitosan (CMCS) was investigated with respect to degradation and crosslinking processes and eventually converted into a hydrogel usable for supporting peripheral nerve regeneration through radiation-induced crosslinking with an electron-beam dose of 25 kGy (Wach et al., 2020). Furthermore, the resulting CMCS hydrogel was injected into a biodegradable NGC comprised of a mixture of poly(trimethylene carbonate) and poly(lactic acid). The non-toxicity of the gel was shown in *in vitro* experiments with L-929 mouse fibroblasts and *in vivo* experiments involving subcutaneous implantation in male Wistar rats (weighing above 250 g). However, further studies are needed to evaluate the beneficial effects of CMCS NGC in a nerve defect model in a pre-clinical animal model.

HA is often used in the treatment of PNI, both as an individual compound and as a drug delivery system. Several HA-based hydrogels will be described in this section, including those used for the loading of bioactive factors, such as growth factors and small molecular drugs. Wu et al. reported the preparation of cryogel-based NGC by free radical cryopolymerization from methacrylated HA, 4-arm poly(ethylene glycol)acrylate and methacrylated gelatin (Fig. 2) (Wu et al., 2019).

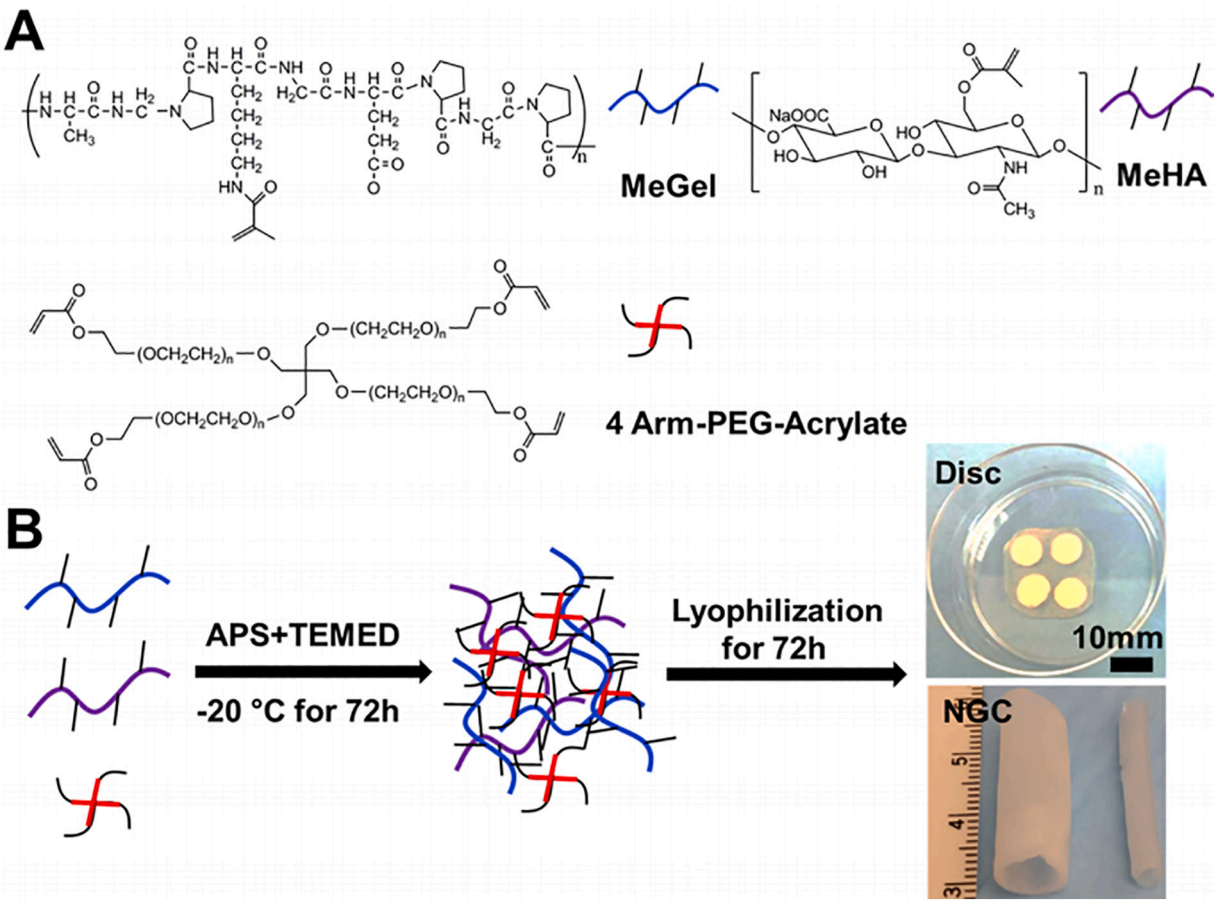

Fig. 2. Preparation of NGC by a free radical cryopolymerization method from methacrylated HA, 4-arm poly(ethylene glycol)acrylate and methacrylated gelatin. (A) Chemical structures of reaction components. (B) Obtaining cryogels in the form of discs and NGC constructs. (Figure reproduced (Wu et al., 2019) with permission from the American Chemical Society.)

Cryogel-based NGC was mechanically strong, had reductive properties, and no obvious limitations in geometric design. For *in vivo* studies of the hybrid cryogel on adult Sprague−Dawley rats, a 10 mm-long incision of the rat sciatic nerve was used. Results showed that the cryogel supported axonal regeneration and remyelination after 16 weeks of recovery, with a comparable myelination diameter and regenerated nerve fiber density in the autograft group.

In order to fabricate the microarchitecture of natural nerve basal lamina, aligned microchannel structures were fabricated under a magnetic field using magnetic alginate microparticle. The resulting HA hydrogels showed the guidance of axonal growth both *in vitro* and *in vivo* (conducted on 10 weeks-old Lewis rats) compared to HA without a microchannel (Lacko et al., 2020). However, functional improvement of the animals needs to be further investigated.

Alginate (32–600 kDa) is a linear polysaccharide copolymer of α-L-guluronic acid and (1–4)-linked β-D-mannuronic acid extracted from brown seaweed (Ahmad Raus et al., 2021; Fu et al., 2011). It is biodegradable, sterilizable without degradation, has good compatibility with cells, and its physical and rheological properties can be easily modified by changing the ratio of monomers and the molecular weight of the polymer chain (Szarek et al., 2013). However, alginate has a number of limitations, which include low mechanical properties, poor stability and thermal sensitivity (Gheorghita Puscaselu et al., 2020). Alginate-based NGC or/and hydrogels showed beneficial effects after nerve regeneration application (Abdelbasset et al., 2022). Poly(ε-caprolactone) (80 kDa) based NGC reinformed with alginate hydrogel on the inner layer of the NGC promoted nerve regeneration, as evidenced by the structural and functional recovery of the completely transected nerve injury in young adult male Sprague−Dawley rats (150–180 g) (Askarzadeh et al., 2020; Noroozi et al., 2012; Vurat & Parmaksiz, 2021).

3.2.2. *Physically crosslinked hydrogels*

Physically crosslinked hydrogels based on polysaccharides as drug carriers represent a growing field of interest (Li et al., 2017; Li, Xiao, et al., 2017; Meka et al., 2017). The main interactions between the two polyelectrolyte polymers include strong, but reversible electrostatic and dipole-dipole associations, as well as hydrogen and hydrophobic bonds. Unlike chemically crosslinked hydrogels, physically crosslinked hydrogels are generally non-toxic, well tolerated, and biocompatible. Covalently crosslinked hydrogels often require additional purification steps as many chemical crosslinkers are toxic (Ahn et al., 2019). For example, glyoxal is known to be mutagenic while glutaraldehyde is neurotoxic (Parhi, 2017). Most synthetic, chemically crosslinked polymers are immunogenic. Therefore, the need to process such systems under harsh conditions can lead to the inactivation or denaturation of encapsulated drugs (Pisal et al., 2010). Physically crosslinked, polysaccharide-based hydrogels are typically prepared by ionic crosslinking with multivalent ions, electrostatic interactions between oppositely charged polyelectrolytes, and through hydrophobic interactions and hydrogen bonds (Dragan & Dinu, 2019). The formation of polyelectrolyte complexes (PECs) depends on the conformation of polyelectrolyte macromolecules, the degree of deacetylation (for chitosan), the degree of polydispersity, the order of their mixing and molecular weight, as well as the dissociation constant of functional groups and their distribution along the polymer chain. At present, the development of chitosan PECs with various polyelectrolytes is very active (Wu et al., 2020). Many synthetic

and natural polyanions have been thoroughly investigated for the formation of PEC with chitosan. Such chitosan PECs have been tested for use in biomedicine, pharmaceuticals, and the food industry, among others. (Gómez-Burgaz et al., 2009; He et al., 2020).

NGCs made from alginate/chitosan PECs are very well suited for nerve regeneration and may be of particular interest due to their stiffness, permeability, hydrophilicity, and relative ease of preparation (Pfister et al., 2007). A solution of chitosan in acetic acid can be used to prepare a gel sponge by neutralization with excess ammonia, followed by freeze drying (Ishikawa et al., 2007). The resulting gel has been used as a material for repairing an 8 mm gap in male Wistar rats aged 4 weeks after removal of a segment of the sciatic nerve. For this purpose, two pieces of chitosan gel sponge were placed around the proximal and distal stumps.

A significant number of publications reported that electrically conductive hydrogels can enhance neurite outgrowth and control nerve cell differentiation even without electrical stimulation (Wang et al., 2017; Guo et al., 2013; Jin & Li, 2014). Poly (3,4-ethylenedioxythiophene) (PEDOT), a polythiophene derivative with excellent chemical stability in aqueous solutions, is one of the most promising conductive polymers due to its controlled electro-optical properties (Balint et al., 2014; Ghasemi-Mobarakeh et al., 2011). Despite the fact that the good biocompatibility of PEDOT and its ability to maintain the proliferation and adhesion of neurons in nerve cells have been observed in numerous studies (Bolin et al., 2009), the application of this material is limited due to its high rigidity and non-biodegradability (Xu et al., 2018). To improve the mechanical properties and biodegradability of PEDOT, researchers are exploring composite hydrogels made by combining PEDOT with a biodegradable flexible material. Abidian et al. described a new method for preparing mechanically-reinforced NGCs from agarose, which are additionally made conductive using a thin layer of PEDOT (Abidian et al., 2012). The resulting hydrogel stimulated the growth of axons in 10 mm neural spaces in 8- to 10-month-old adult males, specific-pathogen-free Fischer-344 rats significantly better than simple agarose conduits. This hybrid material can be used not only to regenerate nerve tissue, but also to obtain a biocompatible interface between the electronic systems of neural prostheses and peripheral nerves. In another study, porous conductive scaffolds for nerve regeneration were prepared by immobilizing conductive HA-doped PEDOT NPs in a gelatin/chitosan matrix (Wang, Lu, et al., 2017). The introduction of HA-doped PEDOT into the framework improved the mechanical and electrical properties, while reducing water absorption, porosity and creating controlled biodegradability. Another report by Xu et al. investigated a biodegradable, conductive, composite hydrogel based on carboxymethyl chitosan and PEDOT (PEDOT/CMCS) (Xu et al., 2018). PEDOT/CMCS hydrogels had high conductivity (up to 4.68×10^{-3} S cm^{-1}), improved mechanical properties and biodegradability. Thus, weight loss during *in vitro* degradation after 10 weeks for PEDOT/CMCS hydrogels was up to 35 % depending on PEDOT content, while for pure CMCS hydrogel it was about 71 %. These hydrogels were not cytotoxic and had the ability to promote the adhesion, viability and proliferation of PC12 cells, even in the absence of electrical stimulation. Thus, these hydrogel NGCs with improved biomechanical properties hold great promise for nerve tissue engineering applications, but further studies in an animal nerve defect model will be required for enhanced knowledge and understanding of their application.

3.3. Other polysaccharide materials

HA and its derivatives can help repair damaged nerves by rearranging the fibrin matrix and thus provide the necessary environment for axon outgrowth (Mekaj et al., 2014). In addition, covering the sciatic nerve with HA prevents intraneural and extraneural scarring after neurolysis (Ikeda et al., 2003). Adanali et al. reported that a membrane based on a mixture of carboxymethyl cellulose and HA (HA-CMC) suppressed perineural scar formation and promoted nerve regeneration (Adanali et al., 2003). The number of myelinated axons was higher in the experimental than in the control group, as evidenced by morphometric analysis. Histological sections showed that intraneural and extraneural fibrosis were significantly lower in the experimental group. Another study showed that a rigid membrane is not suitable for repairing a soft tissue of peripheral nerves. Consequently, the authors studied the effect of a solution of HA-CMC on perineural scar formation after PNI (Park et al., 2011). The sciatic nerve of rats weighing 300–400 g was cut and the epineurium was then immediately restored using Ethilon 10–0 monofilament polyamide-nylon suture. 1 ml of the HA-CMC solution was applied around the nerve in the experimental group, while 1 ml of saline was used in the control group. Application of the HA-CMC solution remarkably reduced the number of inflammatory and fibroblast cells. As early as 4 days after the operation, regenerating axons appeared, while the diameter of myelin fibers increased significantly between two and four months after the operation. Overall, these findings indicate the potential of HA-CMC for scar-free nerve regeneration.

Chen et al. used electrospinning of water-based polyurethane (WPU) and *Bletilla striata* polysaccharide (BSP) for nerve repair (Chen et al., 2020) and for reducing nerve fibrosis. BSP consisting of (1,4)-β-D-glucopyranose and (1,2)-α-D-mannopyranose exhibited anti-inflammatory, antioxidative, antitumor properties and wound healing effects, and further promoted the proliferation of endothelial cells from human cornea and umbilical veins (Jiang et al., 2013; Peng et al., 2014; Wu et al., 2010). BSP/WPU nanofibrous membranes promoted axonal proliferation and improved the proliferation and migration of SCs due to the secondary effect of BSP on nerve regeneration (Chen et al., 2020). Using BSP/WPU in a rabbit model of sciatic nerve recovery significantly improved the isometric muscle contraction strength, compound muscle action potential and nerve conduction velocity, while alleviating muscle atrophy. In another study, electrospinning was used to produce nanofibers of gum tragacanth (GT) and poly (l-lactic acid) (PLLA) at two different ratios (75:25 and 50:50) (Ranjbar-Mohammadi et al., 2016). GT is a biocompatible mixture of water-swellable (bassorin) and water-soluble (tragacanthin) polysaccharides with high protein and arabinose content. The resulting PLLA/GT composite scaffolds were characterized by their hydrophilic nature and suitable mechanical strength. Compared to randomly-oriented PLLA nanofibers, they improved neurite growth, proliferation and cell differentiation.

Anisotropic structures hold promise for axonal growth and guidance. Interestingly, chitosan films with aligned microstructures of an asymmetric nature promoted SC proliferation, growth and elongation in contrast to symmetric microstructures (Scaccini et al., 2021). Although these findings are new and interesting, further studies in animals will provide more insights.

4. Natural polysaccharides as a drug delivery system for promoting nerve regeneration

Various polysaccharide-based vehicles are used for the delivery of pharmacological agents, cells, as well as neurotrophic factors. The delivery systems in use include hydrogels and microspheres, which are selected depending on the desired rates of drug release (Jahromi et al., 2019; Li et al., 2020; Manoukian et al., 2020; Nectow et al., 2012; Tajdaran et al., 2019). In this section, we describe examples of drug delivery by polysaccharides and their derivatives containing functional groups with the capacity to immobilize medicinal substances used in modern clinical practice.

4.1. Delivery of pharmacological agents

The regeneration of repaired nerves can be improved with pharmaceuticals, which are mostly used topically and can prevent scarring. Several pharmaceuticals, including tacrolimus, CsA, nimodipine, and acetyl-L carnitine, have been tested for the treatment of PNI (Manoukian

et al., 2020). It should be noted that the mechanisms of action of these pharmaceuticals differ significantly and remain unclear in some cases (Lee et al., 2020). Acetyl-L-carnitine suppresses the mitochondrial oxidative stress that occurs after nerve damage, thereby promoting the survival and proliferation of SCs and nerve regeneration. In the case of nimodipine, several possible mechanisms were proposed (Curran et al., 2016; Dolmetsch et al., 2001; Tang et al., 2015; Yang et al., 2012). The key mechanisms are the blocking of L-type voltage-gated channels and activation of the p38 MAPK pathway, thus regulating together the SCs proliferation, differentiation and myelin regeneration. In contrast, cyclosporin and tacrolimus bind to CyP40 and FK506-FKBP-52 and eventually result in the activation of the MAPK/ERK pathway,thus resulting in the augmentation of the nerve regeneration process. Furthermore, initiation of cyclosporin-CyP40 and tacrolimus-FKBP-52 complexes results in the inhibition of calcineurin, which in turn promotes GAP43 phosphorylation-dependent nerve plasticity and tissue regeneration (Degrugillier et al., 2021; Dheer et al., 2018; Tung, 2015). The pharmaceuticals mentioned above have been approved by the United States Food and Drug Administration for clinical use through systemic administration or local injection in the treatment of PNIs. However, no effective delivery methods that could improve their efficacy by prolonging their release are currently registered (Manoukian et al., 2020). Other neuroregenerative pharmaceuticals with good pharmacokinetic properties are currently in preclinical development. This section highlights the use of polysaccharides to deliver pharmacological agents used to treat PNI. Although not all of the tailored drug delivery systems described below have not yet been tested for nerve repair applications, these strategies can be repurposed for developing an advanced NGC endowed with a drug delivery system for regulating nerve tissue regeneration.

4.1.1. Chitosan

A number of hydrogels based on chitosan have been developed for the delivery of pharmacological agents for the regeneration of peripheral nerves. Brief descriptions of these formulations are listed in Table 1.

Tacrolimus is an immunosuppressant belonging to the family of immunophilins and is approved for the prevention of allograft rejection. Tacrolimus has also demonstrated neuroregenerative and neuroprotective properties in various models of nerve injury (Dheer et al., 2018). It is believed that the immunosuppressive and neuroregenerative effects of tacrolimus act through different mechanisms (Tung, 2015). Li et al. carried out a comparative analysis of biodegradable chitosan guides with and without tacrolimus (Li et al., 2010). Tubular guide fabrication was accomplished by casting chitosan solution into a tube and impregnating it with 5 % sodium hydroxide solution. Subsequently, the authors showed that the incorporation of tacrolimus into chitosan guides was effective and resulted in a more mature type of myelin fibers eight weeks after surgery. They also observed motor-functional reinnervation with a rate of complex muscle action potentials of 73 % and an amplitude of 60 % relative to the norm. A later report by the same authors suggested a possible mechanism by which tacrolimus-loaded chitosan promotes peripheral nerve recovery (Zhao et al., 2014). Furthermore, the authors performed immunohistochemical staining of a brain-derived neurotrophic factor (BDNF) and receptor tropomyosin kinase B (TrkB) in motor neurons of the lumbar spinal cord. In adult male Wistar rats (6 weeks old, 180–200 g) treated with chitosan alone or chitosan and an injection of tacrolimus, TrkB and BDNF expression increased to relatively high levels within 72 h and then decreased back to baseline in the second week. In contrast, in rats injected with tacrolimus-loaded chitosan channels, the expression of TrkB and BDNF increased to a maximum level within the first week and remained significantly higher than in all other groups, even four weeks later.

A considerable number of publications have reported on the benefits of the local administration of methylprednisolone-loaded chitosan-β-glycerophosphate. For example, Mehrshad et al. showed that chitosan tubes with methylprednisolone-loaded hydrogels improved the functional recovery of transected sciatic nerve in male White Wistar rats weighing approximately 290 g (Mehrshad et al., 2017). In addition, electrophysiological and functional investigations confirmed that methylprednisolone-loaded hydrogel sped up axonal recovery compared to pure hydrogel. Another study showed that the locally sustained release of methylprednisolone from chitosan-β-glycerophosphate hydrogel is beneficial for the regeneration and thermal sensation recovery of injured facial nerve (Chao et al., 2015), making it a prospective injectable drug delivery system.

Another widely used potent immunosuppressant is cyclosporin A (CsA), which has been widely recognized for its neurotrophic and neuritogenic functions, although it is originally known as an

Table 1
Summary of some of the recently developed formulations of chitosan and its derivatives used as drug delivery systems for peripheral nerve regeneration.

Chitosan derivative	Internal architecture	Medicinal substance	PNI type	Main achievements	Reference
Chitosan	Tubular gel	Tacrolimus	Rat model of a 3 mm sciatic nerve injury	Quicker onset of target reinnervation	(Li et al., 2010)
Chitosan	Tubular gel	Tacrolimus	Rat model of a 7 mm sciatic nerve injury	Increase in the density of myelinated nerve fibers	(Zhao et al., 2014)
Chitosan-β-glycerophosphate	Hydrogel	Methylpredni-solone	Rat model of a 10 mm sciatic nerve injury	Fast axonal recovery	(Mehrshad et al., 2017)
Chitosan-β-glycerophosphate	Hydrogel	Methylpredni-solone	Rat model of a facial nerve crush injury	Acceleration of facial function recovery	(Chao et al., 2015)
Chitosan	Prostheses	Progesterone	Rabbit model of a 10 mm facial nerve injury	Long-term progesterone delivery system	(Chávez-Delgado et al., 2003)
Chitosan-glycerol	Tubular	CsA	Rat model of a 10 mm sciatic nerve injury	Improvement of functional recovery	(Mohammadi et al., 2014)
Chitosan - polycrapolactone	Tubular	Verapamil	Rat model of a 10 mm sciatic nerve injury	Fast recovery of regenerated axons	(Alizadeh- Mohajer et al., 2019)
Chitosan-glycerol	Tubular	Vitamin E and pyrroloquino-line quinone	Rat model of a 10 mm sciatic nerve injury	Significant myelin fibers	(Azizi et al., 2014)
Chitosan-glycerol	Tubular	Triiodothyro-nine	Rabbit model of a 10 mm sciatic nerve injury	High number of myelinated fibers with large diameter	(Mohammadi et al., 2013)
Chitosan-hydroxyapatite	Tubular	Laminin-1	Rat model of a 10 mm sciatic nerve injury	Improved nerve function	(Itoh et al., 2003)
Chitosan	Tubular	CYIGSR	Rat model of a 10 mm sciatic nerve injury	Reduced muscle attrophy	(Wang, Jiang, et al., 2022)
Chitosan	Tubular	IKVAV KLT	Rat model of a 10 mm sciatic nerve injury	Enhanced CMAP	(Shen et al., 2022)

PNI – peripheral nerve injury; CsA – cyclosporine A.

immunosuppressive drug. A research group reported the use of chitosan conduit with glycerin added to reduce fragility for the local delivery of CsA and showed that the diameter and number of myelinated fibers in animals treated with CsA-loaded conduits were significantly higher than in the control group (Mohammadi et al., 2014). However, the detailed mechanism of the action of CsA in nerve regeneration remains unclear (Erkutlu et al., 2015), although some studies have evidenced GAP 43 phosphorylation-dependent action as described earlier.

Many other systems for the delivery of immunosuppressants are being developed based on chitosan. Using light microscopy and morphometric analysis, Chávez-Delgado et al. evaluated nerve regeneration 45 days after implantation of progesterone-loaded chitosan prostheses in rabbits with 10 mm facial nerve breaks (Chávez-Delgado et al., 2003). In this study, progesterone-loaded chitosan prostheses provided a long-acting progesterone delivery system and promoted facial nerve regeneration. Another study showed that verapamil-loaded chitosan tubes can improve morphometric parameters and accelerate functional recovery of the sciatic nerve (Alizadeh- Mohajer et al., 2019). Chitosan/hydroxyapatite tubes with adsorbed laminin peptides were developed for peripheral nerve recovery (Itoh et al., 2003). The findings of the histological examination, which included an assessment of the size and number of myelinated axons, as well as the study of mechanical properties, suggested that the triangular shape of the hydroxyapatite-coated tube promoted nerve regeneration. In a novel approach, an active peptide, cysteine-tyrosine-isoleucine-glycine-serine-arginine (CYIGSR), capable of promoting cell adhesion and axonal elongation was incorporated into the chitosan NGC scaffold for slow release in response to endogenous esterase activity. The resulting bioactive NGC supported nerve regeneration over a 10 mm sciatic nerve gap injury and showed a significant increase in target muscle weight and nerve physiological function (Wang et al., 2023). In a similar line of approach, chitosan NGC containing the self-assembling nanofibrous hydrogels containing IKVAV and KLT sequences derived from laminin and VEGF exhibited enhanced nerve regeneration over a 10 mm-long sciatic nerve defect. Outcome measurements including nerve histomorphometry, CMAP, target muscle innervation and the SFI compared well with autologous nerve grafting (Shen et al., 2022).

Zheng et al. synthesized a conductive reduced graphene oxide and asiaticoside liposome-loaded, oxidized hydroxyethyl cellulose/CS hydrogel (OHEC/CS/rGO/asiaticoside liposome hydrogel) for PNI treatment (Zheng et al., 2020). Liposomes obtained by a modified ethanol injection method were loaded into the solution and asiaticoside liposome-loaded OHEC/CS/rGO hydrogel was then prepared after crosslinking of chitosan and OHEC (Fig. 3). Asiaticoside is a triterpenoid that interferes with DNA synthesis in fibroblasts and reduces collagen production, thus inhibiting scar tissue formation (Qi et al., 2008). This highly biocompatible hydrogel design was chosen because its porous 3D structure retards the release of asiaticoside and results in the growth of nerve cells. In addition, conductive rGO can provide a medium for electrical stimulation, which promotes nerve regeneration. A biodegradation study of the hydrogels showed that the rate of mass loss ranged from 76.14 ± 4.45 % to 99.41 ± 5.31 %, depending on the ratio of the polysaccharides. It was shown that 80–90 % of the asiaticoside was released within seven days from the hydrogel with an OHEC/CS weight ratio of 1:2 due to the low crosslinking density. The lowest cumulative release rate (71.43 %) was observed for the 2:2 polysaccharide ratio. It was further shown in NIH/3 T3 and RSC96 cells that the hydrogel was suitable for the proliferation and adhesion of nerve cells and non-toxic.

4.1.2. Alginate

A significant number of publications have reported the application of alginate for the delivery of tacrolimus (Cong et al., 2017; Wang et al., 2020). Due to the low solubility of tacrolimus, various strategies for its incorporation are being investigated to increase drug loading and thus its efficacy (Fig. 4). Wang et al. prepared dibenzocyclooctyne-modified derivatives of immunosuppressants such as mycophenolic acid, rapamycin and tacrolimus and performed targeted click-chemistry conjugation with azido-modified alginate (alginate-N3) hydrogels both *in vitro* and *in vivo* (Fig. 4A) (Wang et al., 2020). Such clickable prodrugs with degradable linkers placed in transplanted tissues provided sustained local drug release. To study the uptake of prodrugs *in vivo*, Balb/c mice were injected subcutaneously with azido-modified alginate hydrogel or control alginate gel before intravenous injection of immunosuppressants 1 h later. Alginate-N3 gel was able to absorb significantly more prodrug compared to the control gels (rapamycin by 130 %, tacrolimus by 105 %, and mycophenolic acid by 80 %), which confirms the effectiveness of using click chemistry *in vivo* for the delivery of functionalized immunosuppressants. In another study, micelles were synthesized from hydrophilic polymannuronic acid and hydrophobic oleyl amine loaded with tacrolimus (FK506-PM-C_{18}; Fig. 4B) (Cong et al., 2017). FK506-PM-C_{18} was characterized by an encapsulation efficiency of over 90 % and a long-lasting release behavior *in vitro*. Although this work showed the

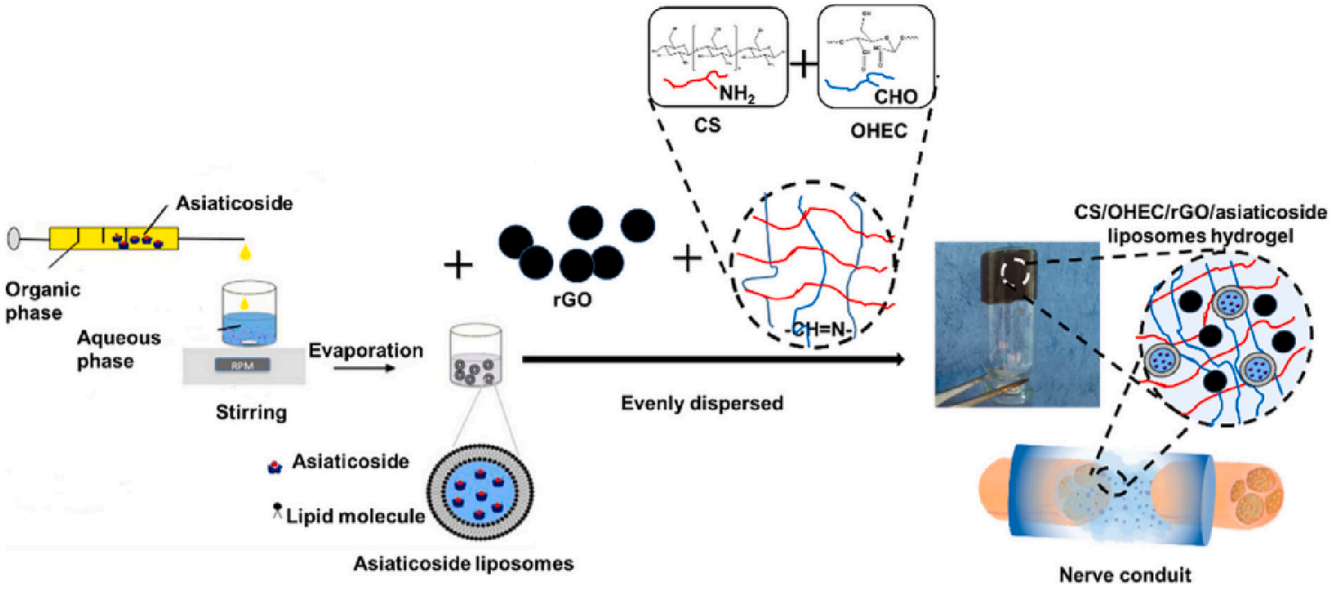

Fig. 3. Sequential preparation of OHEC/CS/rGO/asiaticoside liposome hydrogel. (Figure reproduced (Zheng et al., 2020) with permission from the American Chemical Society.)

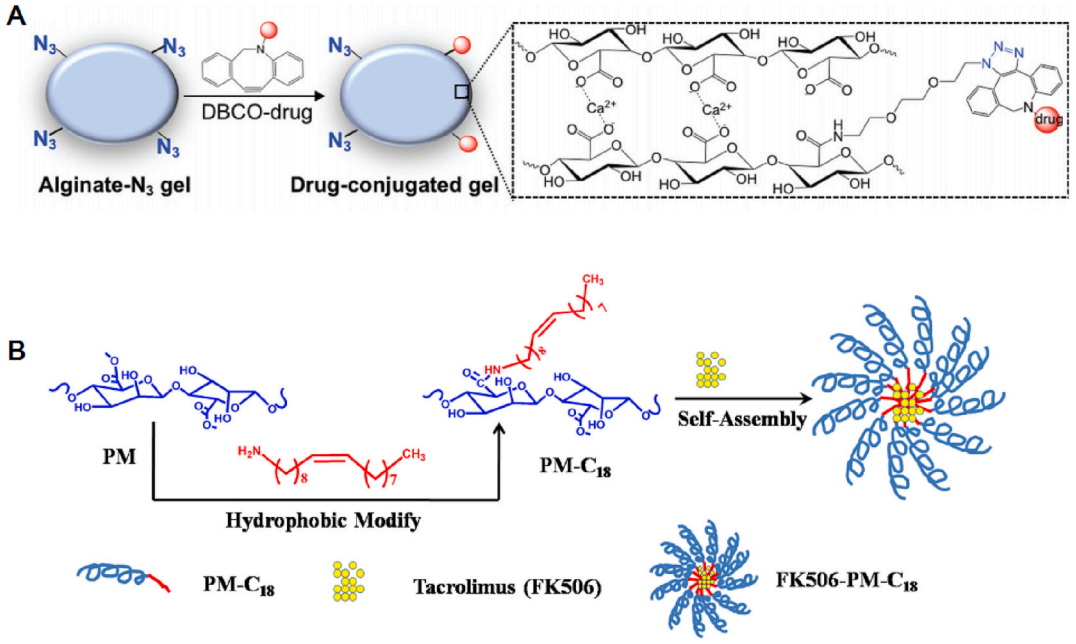

Fig. 4. Strategies for incorporating poorly soluble tacrolimus into alginate. (A) Schematic drug binding to the alginate-N3 gels. (Figure reproduced (Wang et al., 2020) with permission from The Royal Society of Chemistry.) (B) Schematic modification of polymannuronic acid with hydrophobic oleyl amine and subsequent formation of micelles with tacrolimus. (Figure reproduced (Cong et al., 2017) with permission from Elsevier Ltd.)

efficacy of using FK506-PM-C_{18} in the treatment of allergic conjunctivitis, the described tacrolimus delivery systems may be of interest for peripheral nerve regeneration. Parteni et al. prepared a biomaterial consisting of alternating layers of chitosan and sodium alginate with tacrolimus injected in-between (Parteni & Doru Radu, 2016). The biomaterial consisted of 10 layers of polysaccharides and their gradual dissolution led to a sustained release of tacrolimus by preventing the "burst effect". Barberine is known to possess anti-tumor, anti-fibrotic and neuronal protection properties and 1 % barberine-loaded alginate/chitosan composite gels showed a better effect on axonal growth and myeline recovery (Rahmati et al., 2021). However, their effect on completely transected nerve injuries is unknown.

The synthesis of a liposome-in-microsphere (LIMs) oral carrier system for the intestinal delivery of CsA was reported by Park et al. (Park et al., 2006). This system was obtained from CsA-loaded liposomes mixed with alginate solution. Droplets of the resulting dispersion were released into $CaCl_2$ solution using the micro-nozzle of a spray dryer to crosslink the alginate and form LIMs. The study found that the release rate of CsA in simulated gastric fluid was significantly lower than in simulated intestinal fluid, and that the release rates of CsA were lower for compositions containing liposomes compared to compositions without them.

4.1.3. *Hyaluronic acid*

Zhuo et al. proposed an effective way to encapsulate tacrolimus in polymer NPs (Zhuo et al., 2018). Chitosan nanoparticles were obtained using the high pressure homogenization-evaporation method and then loaded with tacrolimus (NPs-CS-TCP) (Pandey et al., 2019). To further improve the pharmacological benefits, the drug-loaded NPs were decorated with HA (NPs-CS-TCP-HA). The *in vitro* release rate of tacrolimus from NPs with and without HA was measured using dialysis membrane and compared. During the first 8 h, approximately 75 % and 25 % of tacrolimus was released from NPs-CS-TCP and NPs-CS-TCP-HA, respectively. This confirms that the coating of NPs with HA provides an additional barrier to drug diffusion and makes NPs-CS-HA excellent candidates for tacrolimus delivery. Preclinical studies of NPs-CS-TCP-HA have shown a pronounced efficacy against atopic dermatitis as they gradually reduced the rate of transepidermal water loss, erythema intensity and the dermatitis index.

Mekaj et al. demonstrated the equally positive effects of HA and tacrolimus for ameliorating the anatomical and functional properties of peroneal nerve injury in a rabbit model after 12 weeks of topical treatment (Mekaj et al., 2015). In a further study, HA prevented perineural scar formation in rabbits after complete nerve transection injury in an end-to-end suture repair model (Mekaj et al., 2017). The resulting data are promising and showed equally positive effects of tacrolimus (FK506) drug treatment. These findings encourage further studies in a longer-gap injury model. Chitosan NGC combined with HA significantly reduced scar adhesions and promoted better nerve regeneration after 12 weeks of sciatic nerve crush injury (Li, Liu, et al., 2018). Taken together, these findings indicate the potential of HA in regulating nerve fibrosis and scar tissue development and holds promise for treating severed nerve injuries.

Another strategy for tacrolimus delivery are colloidal systems based on niosomes. Niosomes are self-assembling vesicles based on non-ionic surfactants and are characterized by high chemical stability, low toxicity, increased permeability conferred by surfactants, and ease of manufacture (Ojeda et al., 2016; Puras et al., 2014). To improve the penetration of tacrolimus into the cornea, Li et al. employed a colloidal system of niosomes derived from proniosomes (Li, Li, et al., 2014). Niosomes effectively increased the penetration of tacrolimus into the cornea and increased the pharmacodynamics of counteracting rejection *in vitro* and *in vivo* (the study was carried out on New Zealand albino rabbits (weighing 2–3 kg), Wistar rats (weighing 200–220 g, 6–8 weeks old) and Sprague–Dawley rats (weighing 200–220 g, 6–8 weeks old)). In another study by the same group, an ocular tacrolimus delivery system based on mucoadhesive HA coating of the niosomes was developed (Zeng et al., 2016). The use of the HA coating led to an increase in the strength and rate of adhesion of niosomes to mucin. HA-coated niosomes prolonged the *in vivo* precorneal retention of tacrolimus (the study was carried out on New Zealand albino rabbits (weighing 2–2.5 kg) and Sprague–Dawley rats (weighing 200–220 g)), improved its pharmacokinetics and ophthalmic bioavailability compared to uncoated niosomes, thus suggesting that the described formulation may be a promising approach for targeted delivery of tacrolimus.

In parallel to the liposome-loaded, alginate-based microspheres

described above (Park et al., 2006), another group proposed to use microspheres of HA for the delivery of CsA (Woo et al., 2007). A spray-drying technique was used to prepare microspheres of sodium lauryl sulfate/sodium hyaluronate/CsA. The resulting microspheres showed improved bioavailability of CsA and a two-fold higher rate of drug dissolution compared to CsA powder and therefore they can be useful for delivery of the poorly water-soluble CsA.

HA-laminin-hydrogel was used as a luminal filler in collagen or chitosan NGC for the repair of a 15 mm sciatic nerve gap injury in rats at an age of 12 weeks. Assessment of anatomical and electrophysiological outcomes showed no improvement over autologous nerve grafting (Dietzmeyer, Huang, et al., 2020). Further studies are needed to optimize the gel composition and modulus, together with guidance structures for enhancing the outcome.

4.1.4. Other polysaccharides

Among various drug delivery systems, polymer micelles represent a promising carrier for poorly water-soluble pharmaceutical substances. Hydrophobically-modified polysaccharides form polymer micelles in water. Their stability and size depend on the architecture of the polymer, its chemical composition and molecular weight (Akiyoshi et al., 2000). Francis et al. reported the preparation of hydrophobically-modified dextran and hydroxypropylcellulose micelles for the delivery of CsA (Svenson, 2006). CsA is characterized by a very low water solubility (23 μg/ml at 20 °C) due to the presence of four intramolecular hydrogen bonds. The degree of CsA immobilization in micelles based on the hydrophobically-modified polysaccharide was significantly higher than for the corresponding non-modified polysaccharides. Polymer micelles showed low cytotoxicity towards Caco-2 cells, as well as increased

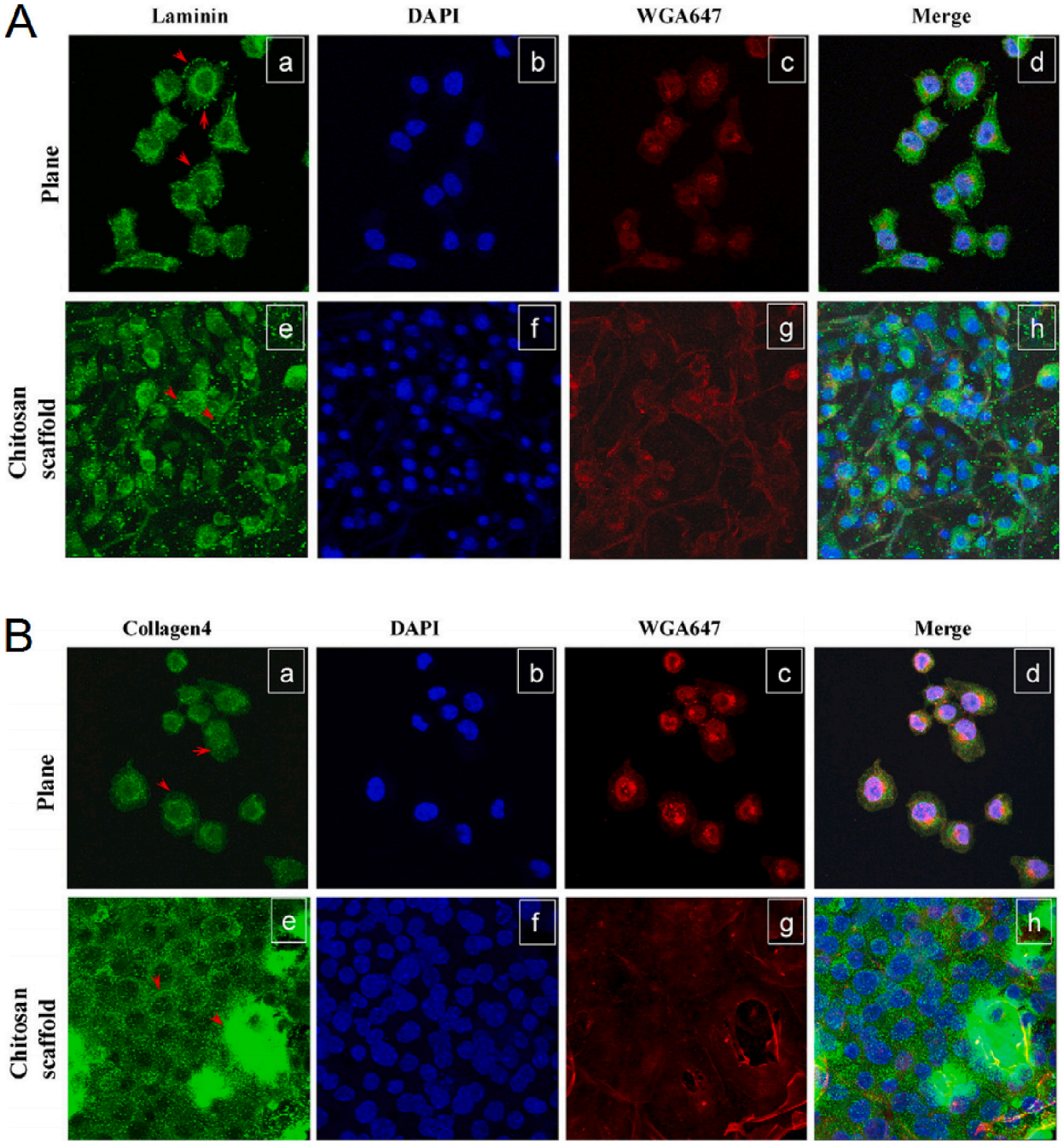

Fig. 5. Immunofluorescence images of laminin β1 (A) and collagen 4 (B) expressed by rat SCs grown in a plane and on the chitosan scaffolds. (Figure reproduced (Lin et al., 2014) with permission from Elsevier Ltd.)

apical and basal drug permeability compared to free CsA.

4.2. Delivery of glial and stem cells

At present, there is a strong focus on the use of polysaccharide-based materials for the delivery of glial and stem cells to the area of the damaged nerve. Several comprehensive reviews have been published on current advances in stem cell therapy with respect to maintaining nerve regeneration and achieving functional recovery after severed nerve damage (Kubiak et al., 2020; Sumarwoto et al., 2021). SCs, the glial cells of the peripheral nervous system, are crucial components of the microenvironment for peripheral nerve regeneration. They can be divided into myelinating SCs, non-myelinating SCs, satellite cells, and perisynaptic SCs (Armati & Mathey, 2014). SCs cells promote cellular reprogramming, which leads to the generation of cells specialized in nerve regeneration and repair (Jessen & Arthur-Farraj, 2019). In this regard, the efforts of many researchers are focused on the development of polymer tubes for obtaining nerve conductors with SCs included in the composition. Chitosan, alginate and HA provide excellent mechanical and biological support for the survival and proliferation of SCs and other supportive cells, thus indicating their potential for therapeutic applications (Ning et al., 2018).

Chitosan fibers and membranes have excellent neuroglial cell affinity and bioartificial nerve grafts consisting of biomaterial pre-seeded with SCs have proven to be an effective substrate for enhancing nerve regeneration (Yuan et al., 2004). The resulting NGC construct with mechanical and biological support is highly interesting and scalable with tunable dimensions, but further studies in animals are needed. Lin et al. evaluated extracellular matrix protein expression using rat SCs grown on porous chitosan scaffolds (Lin et al., 2014). Chitosan scaffolds seeded with SCs secreted more laminin and collagen than SCs grown in a plane (Fig. 5) and showed that a higher expression of extracellular matrix proteins can significantly improve peripheral nerve regeneration. In another study, two cell types - mesenchymal stromal cells and SCs - were seeded in chitosan films to create tissue-engineered nerve grafts that can be used instead of autologous nerve transplantation (Wrobel et al., 2014). Chitosan films with a 5 % degree of deacetylation supported axonal growth, as well as the growth and function of resident SCs in communicating nerve endings, thereby demonstrating the high potential of using such films in tissue nerve grafts.

To bridge the longer nerve-gap injury using the cells, SC cylinders were fabricated using a 3-layered HA-based conduit with an inbuilt gradient pore system that selectively regulates cell mobility across the NGC scaffold (Vilariño-Feltrer et al., 2016). The resulting HA scaffolds encourage further studies in animals and holds promise for cell delivery applications. Another study showed that engineered multi-modular HA-PLA NGC involving several HA mini-conduits loaded with longitudinal PLA (128–152 kDa) microfibers and SC cells promoted a 15 mm sciatic nerve-gap injury in rabbits over 6 months with improved myelin regeneration compared to groups treated with cell-free NGC (Roca et al., 2022; Way et al., 2013).

Hydrogels based on natural and synthetic biodegradable polymers occupy a special place among various forms of scaffolds for the delivery of cells due to their excellent biocompatibility and mechanical properties similar to soft tissues (Atoufi et al., 2017; Ulery et al., 2011). For instance, alginate/chitosan hydrogels carrying olfactory mesenchymal stromal cells promoted nerve regeneration over a 3 mm sciatic nerve injury model and showed improved axonal growth, muscle weight and functional recovery than cell-free constructs (Salehi et al., 2019). However, small gap injuries are relatively not challenging for clinical repair. Therefore, further research is needed to test the functional efficacy of the above strategy for bridging large-gap nerve injuries. Itai et al. reported that the co-axial arrangement of chitosan and collagen hydrogel seeded with SCs in the form of NGC supported axonal growth and elongation *in vitro* (Itai et al., 2020). Alginate/chitosan hydrogels containing barberine supported the regeneration of crush sciatic nerve injury. Raimondo et al. reported on the growth-promoting effects of alginate hydrogel releasing vascular endothelial growth factor (VEGF) and IGF-1 for axonal growth, innervation, and toe spread function both in C57BL/6 J mice (aged 6 or 14 weeks) and viable muscle transfer in adult New Zealand rabbits (4–4.5 kg), thus indicating a promising therapeutic development for nerve and muscle regeneration (Raimondo et al., 2019). HA-laminin hydrogel loaded with FGF2 releasing SCs within the chitosan NGC promoted nerve regeneration (Dietzmeyer, Huang, et al., 2020). Conversely, NGCs loaded with SCs or FGF2-SCs failed to promote nerve regeneration. Although molecular analysis revealed the HA-laminin mediated downregulation of neurotrophic factors from SCs, further research is needed to optimize the gel composition for cell delivery and to improve the outcome measures.

Peptide-polysaccharide hydrogels are also being evaluated as potential cell carriers that promote regeneration after damage to peripheral nerves. Suri and Schmidt developed interpenetrating polymer network hydrogels based on HA and collagen with laminin as scaffolds for SCs (Suri & Schmidt, 2010). The method for preparing 3D interpenetrating polymer network hydrogels with encapsulated SCs is shown in Fig. 6. SCs encapsulated in interpenetrating polymer network hydrogels were viable and secreted BDNFs and nerve growth factors (NGFs). In addition, an increase in the amount of NGFs and BDNFs from the cells was noted when laminin was added to these hydrogels. Another report by McGrath et al. demonstrated a method for obtaining conduits based on cellulose membrane filled with alginate/fibronectin hydrogel containing SCs. The alginate/fibronectin formed hydrogel clusters in the canal, which interfered with the interaction between axons and the transplanted SCs, thereby inhibiting regeneration. The authors found no significant differences in axonal regeneration in conduits with alginate/fibronectin hydrogel alone or alginate/fibronectin supplemented with SCs. However, the use of macroporous alginate hydrogel fibers with immobilized rat dorsal root ganglion cells and gelatin particles, which were used as pore formers and to ensure cell adhesion, led to a noticeable outgrowth of neurites to 150 μm on the 11th day of cell culture. Hence, this formulation provided a favorable environment for the expansion of neurites (Lin et al., 2017). Using confocal laser scanning microscopy, it was shown that gelatin located in the macropores migrated into the alginate fibers, thus providing cell adhesion molecules that are useful for stimulating nerve cell proliferation and axon elongation. Another study demonstrated that 1 % alginate hydrogels loaded with SCs with the genetically-induced release of glial cell-derived neurotrophic factor (GDNF) showed good viability, proliferation and secretion of GDNF (de Guzman et al., 2008).

Despite the great importance of SCs in nerve regeneration, their clinical use is limited due to the lack of a source of the cells and their poor proliferation (Jessen & Mirsky, 2019). For this reason, research is being carried out to identify stem cells that can differentiate into SCs. As an example, it has been shown that some stem cells, such as adipose-derived stem cells and bone marrow mesenchymal stromal cells, can differentiate into a SC-like phenotype (Caddick et al., 2006; di Summa et al., 2010; Keilhoff et al., 2006). Luo et al. reported obtaining NGCs by filling a tube of cellulose and soy protein with gelatin methacryloyl hydrogel containing dental pulp stem cells and basic fibroblast growth factor (FGF-2) (Luo et al., 2021). The resulting material was used to restore a 15 mm gap in the sciatic nerve in the rat and regenerated two types of nerve cells: axonal and SCs. Functional recovery at the physiological level, assessed by SFI studies, was achieved 12 weeks after implantation surgery. Chitosan NGC filled with adipose-derived stem cell (ASC)-loaded fibrin-collagen hydrogel supported early nerve regeneration over a 15 mm long-sciatic nerve defect, although the NGC failed after 15 weeks. During the early regeneration over 2 weeks, upregulation of the pro-regenerative genes NRG1 *vs* ErbB2, VEGF, BDNF, ATF3 and C-Jun was observed in response to the ASC-loaded NGC treatment (El Soury et al., 2023). In another study, Moattari et al. used mesenchymal stem cells associated with chitosan film for the repair of transected sciatic nerve in the rat (Moattari et al., 2018). The study

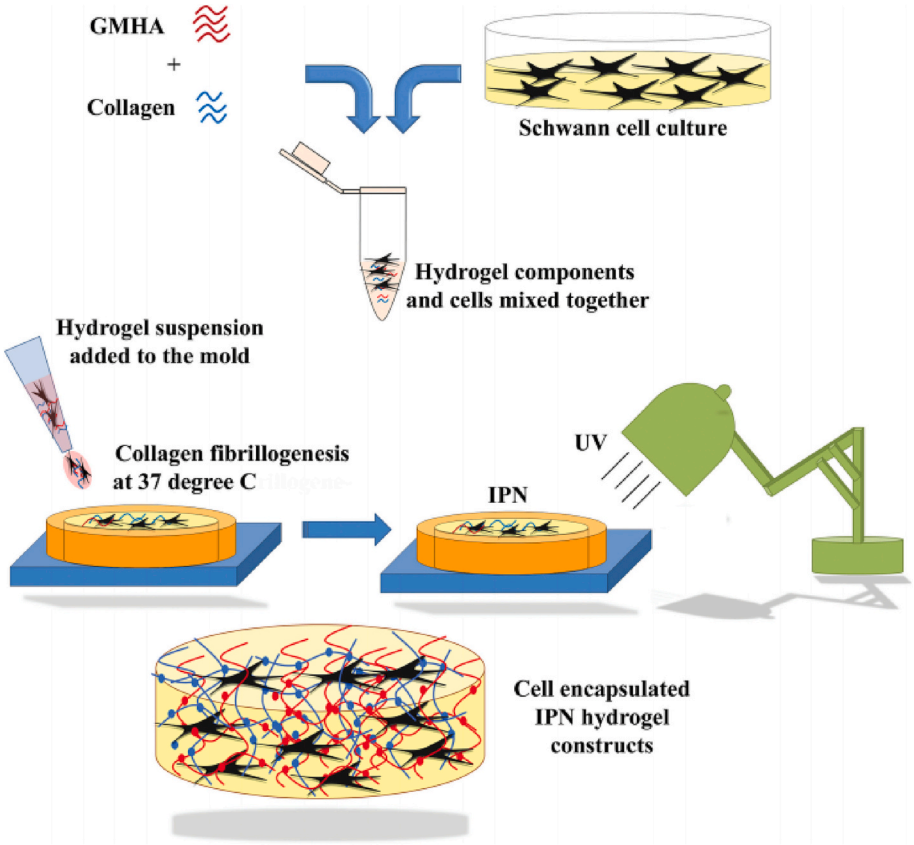

Fig. 6. SC encapsulation in hydrogels with a 3D interpenetrating polymer network. (Figure reproduced (Suri & Schmidt, 2010) with permission from Mary Ann Liebert, Inc.)

found a significant decrease in the SFI and an increase in the amplitude of nerve impulses and the number of nerve fibers of various diameters, which indicates an improvement in the histomorphological and functional properties of the sciatic nerve after PNI when using chitosan films to deliver mesenchymal stem cells.

Stiffness of the HA hydrogels played an important role in the therapeutic efficacy of the mesenchymal stem cell-derived exosomes for supporting the nerve repair. Soft hydrogel (0.4 %) facilitated a quicker release of exosomes and the inhibition of proinflammatory cytokines and consequently macrophages, resulting in an improved sciatic nerve repair after acute nerve compression injury (Liu et al., 2022; Liu, Xu, et al., 2022).

Neural stem cells (NSCs) can also differentiate under different conditions into different types of cells, such as neurons, oligodendrocytes and SCs, and are used both in spinal cord injuries (Li et al., 2021; Qi et al., 2022, p.) and in PNIs (Song et al., 2021; Wang, Lu, et al., 2017). It has been established that this type of cell grows and multiplies well on chitosan films (Wang et al., 2006). Moreover, most of the cells differentiated into neuron-like cells after 4 days of culture. The study also demonstrated that by adapting the production technique, both soft molded and braided conduits can be formed. The former can be used as cell carriers for spinal cord injuries or short peripheral nerve defects, while the latter can be used for peripheral nerve regeneration, especially in the case of long gaps. Wei et al. designed a self-healing injectable hydrogel based on N-carboxyethyl chitosan and oxidized sodium alginate (CEC-l-OSA) to promote the differentiation of NSCs into neurons and treat neurological diseases (Fig. 7) (Wei et al., 2016). Hydrogels with a stiffness similar to brain tissue (100– 1000 Pa) were formed *in situ* through dynamic imine bonds produced by mixing solutions of polysaccharides in a physiological environment. It has been demonstrated that CEC-l-OSA hydrogels are biocompatible and beneficial support materials for NSC proliferation in a 3D microenvironment, as well as for the promotion of the neuronal differentiation of NSCs. 3D cell cultures inside CEC-l-OSA hydrogels expressed much more neuronal marker than the control after 9 days of continuous culture. The authors associated their results not only with the appropriate rigidity of the hydrogels, but also with the presence of dissociated amino groups, which can promote neuronal differentiation. The use of other polysaccharides for cell differentiation is also possible. However, it is necessary to take into account the important data obtained by Ren et al. on the cultivation of NSCs on glass surfaces modified with various chemical groups, including methyl ($–CH_3$), mercapto (–SH), carboxyl (–COOH), amino ($–NH_2$), sulfonic ($–SO_3H$), and hydroxyl (–OH) groups (Ren et al., 2009). The extent of migration of NSCs from the initial aggregates to the test surfaces after 5 days of culture depended on the group with $–OH < –CH_3 < –SO_3H < < –SH = –COOH < –NH_2$.

4.3. Delivery of growth factors

At present, various growth factors such as NGF, BDNF, neurotrophin-3, BDNF and FGF are used for nerve regeneration as they can bind with high affinity to neurotrophic receptors, which play an important role in axo-glial plasticity and regeneration (Dutta et al., 2011; Schultheis et al., 2014). Polysaccharide derivatives widely used for delivery of growth factors are listed in Table 2.

NGFs, which are produced by target organs of the sensory and sympathetic nerves, stimulate the growth of neurites and promote the survival of sensory ganglia, including those that give rise to sensory nerves in the spinal cord and sciatic nerve (Zarrintaj et al., 2020). Similar to other neurotrophic factors used to stimulate nerve regeneration, an NGF has a short half-life, while nerve regeneration requires several weeks. Therefore, the development of sustained-release delivery

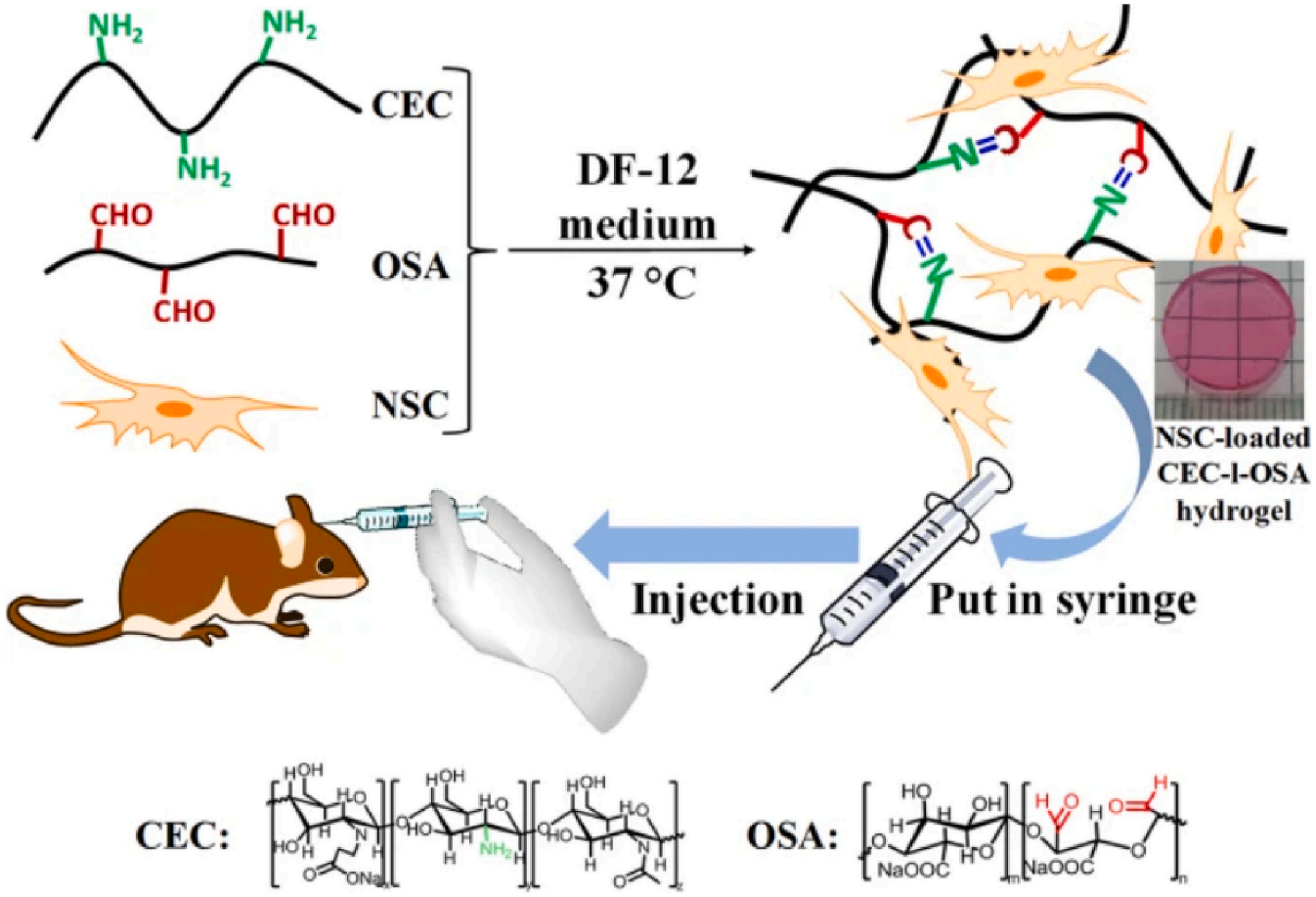

Fig. 7. Schematic implantation of NSC-loaded CEC-l-OSA hydrogels. Figure was duly reproduced (Wei et al., 2016) after receiving permission from Springer Nature.

Table 2
Polysaccharides for delivery of growth factors.

Polysaccharide	Internal architecture	Growth factor	co-factors	Experimental model	Reference
Agarose	Hydrogel	NGF	Laminin-1	Rat model of 20 mm sciatic nerve injury	(Dodla & Bellamkonda, 2008)
Chitosan/HA	Hydrogel	NGF	–	*In vitro* (RSC96 cells)	(Xu et al., 2017)
Chitosan	Hydrogel	NGF	–	Rat model of 5 mm facial nerve injury	(Chao et al., 2016)
Heparin/chitosan	Scaffold	NGF	–	*In vitro* (Schwann Cells)	(Li, Xiao, et al., 2017)
Chitosan	Conduit	Transforming growth factors-β1	SC	Rat model of 10 mm sciatic nerve injury	(Nie et al., 2014)
Chondroitin sulfate/ chitosan	Conduit	NGF	–	Rat model of 10 mm sciatic nerve injury	(Xu et al., 2011)
Alginate/chitosan	Conduit	NGF	–	*In vitro* (rat PC12 cells)	(Pfister et al., 2008)
Heparin	Hydrogel	FGF-2 and NGF	–	Diabetic rat model of sciatic nerve crush injury	(Li, Li, et al., 2018)
Alginate	Capsules	BDNF	–	Rat model of sciatic nerve injury	(Vögelin et al., 2006)
Chitosan	Microspheres	BDNF	–	Rat model of 20 mm sciatic nerve injury	(Zeng et al., 2020)
Heparin	Conduit	NGF and BDNF	–	*In vitro* (chick embryonic explants of dorsal root ganglia)	(Sandoval-Castellanos et al., 2021)
Cellulose	Hydrogel	FGF-2	Dental pulp stem cells	Rat model of 15 mm sciatic nerve injury	(Luo et al., 2021)
Chitosan	Fibrin-collagen	–	ASC	Rat model of 15 mm sciatic nerve injury	(El Soury et al., 2023)
Chitosan	Conduit	NGF	–	Mice embryonic neurons	Nawrotek et al., 2022
Chitosan	Conduit	GDNF	–	Rat model of 3.5 mm facial nerve	Xia et al., 2022

vehicles is extremely important (Dodla & Bellamkonda, 2008). Dodla and Bellamkonda designed and evaluated novel anisotropic agarose scaffolds embedded with gradients of laminin-1 molecules and NGF to stimulate the repair of a 20 mm sciatic nerve gap in the rat (Dodla & Bellamkonda, 2008). Multilayer alginate/chitosan NGC provided a more sustained release of NGF and enabled the studies to elucidate the relationship between release kinetics and nerve regeneration outcome (Pfister et al., 2008). The resulting drug delivery system holds great promise for the further understanding of the growth factor release-dependent nerve tissue growth response and tissue regeneration mechanism. Another study demonstrated the increasing effectiveness of neurotrophic factors involved in NGCs upon the addition of cells known

to enhance nerve regeneration to the composition. In the study by Luo et al. (Luo et al., 2015), a new kind of NGC was constructed using cellulose/soy protein isolate composite membranes as conduits, SC-seeded cells, and pyrroloquinoline quinone as NGF. The effect of NGC on sciatic nerve defect healing in Sprague–Dawley male rats (200–250 g) using an autograft as a control was comparatively assessed by histological and macroscopic analysis, as well as SFI evaluation. The use of composites led to the formation of regenerative nerve fibers accompanied by new blood vessels in the conduits. In another work, chitosan hydrogel-based NGCs were fabricated with a sustained release of NGF over 28 days and the resulting bioactive chitosan conduits showed a confirmed biocompatibility and bioactivity. NGF-loaded microspheres were adsorbed onto the dopamine-coated poly(ε-caprolactone helix, which was further fabricated into chitosan NGC through electrophoretic deposition. Although the resulting chitosan bioactive device showed a sustained NGF release, further testing in animal models is lacking (Nawrotek et al., 2022).

Wei et al. reported that obtaining oxidized bacterial nanocellulose NGC embedded with chitosan NPs releasing NGF promoted nerve regeneration over a 10 mm gap injury in rats by 4 weeks postoperatively. Further amelioration in the structural and functional recovery was observed after 9 weeks (Wei et al., 2021).

Several articles have focused on the creation of materials based on chitosan loaded with NGF for nerve regeneration. It has been shown *in vitro* that chitosan-HA hydrogel is biodegradable (>70 % within 8 weeks), non-toxic, favorable for the adhesion and proliferation of nerve cells, and suitable for releasing NGF (Xu et al., 2017). Another hydrogel based on chitosan-glycerophosphate with immobilized NGF was used to repair damaged facial nerve in the rat (Chao et al., 2016). In this study, nerve regeneration was assessed through the measurement of the *in vitro* release of NGF, vibrissae movement evaluation, histological observation, and electrophysiological assessment. The morphological and functional recovery of the injured facial nerve was better with the hydrogel than with the NGF solution alone. Li et al. showed that NGF-loaded chitosan/heparin scaffolds assembled *via* electrostatic interactions can improve the attachment and proliferation of SCs, as well as effectively promoting the development of their morphology *in vitro* (G. Li, Xiao, et al., 2017). Another promising strategy used a layer-by-layer, electrostatic-assembly technique to obtain a poly (d, L-lactic acid) (PDLLA)/chondroitin sulfate/chitosan conduit with immobilized NGF (Xu et al., 2011). Histological, immunocytochemical and electrophysiological evaluations showed that the functional recovery with PDLLA/chondroitin sulfate/chitosan and PDLLA/chondroitin sulfate/chitosan/NGF nerve conduits was significantly better than with PDLLA alone. Combined with good mechanical properties and *in vivo* degradability conducted on adult Sprague–Dawley rats weighing 200–250 g, this makes them useful materials for nerve damage repair. Another study showed that methacrylated HA-aligned fibers loaded with NGF-releasing microspheres exhibited mechanical, chemical and topographical cues and further enhanced guided axonal outgrowth *in vitro* (Whitehead et al., 2018). The resulting bioactive HA fibers hold promise for promoting axonal regeneration over long distance gap injuries.

Polysaccharide-based vehicles suitable for delivery of other growth factors are also sought after. Li et al. designed a heparin-based hydrogel for the controlled co-delivery of FGF-2 and NGF (Li, Li, et al., 2018, p.). FGF-2 promotes the proliferation of SCs and spinal ganglion neurons and accelerates synaptogenesis, remyelination and angiogenesis (Andrades et al., 2001; Li, Li, et al., 2018, p.). The resulting hydrogels excellently controlled the release of both growth factors, preventing their degradation *in vitro*. When a hydrogel with adsorbed growth factors was administered *in situ* to the sciatic nerve lesion in Wistar rats (weighing 300–350 g), a significant proliferation of SCs was observed. This led to the increased expression of structural proteins of the nerve, increased axon regeneration and remyelination, and a better recovery of motor function than when either the hydrogel or growth factors only were administered.

Together with other neurotrophic factors, BDNF plays an important role in the recovery of function of a damaged sciatic nerve (Frostick et al., 1998; Vögelin et al., 2006). Vögelin et al. synthesized BDNF-loaded calcium alginate capsules and used them on 20 mm gaps in the sciatic nerve of adult syngeneic Sprague–Dawley female rats weighing 352.2 ± 33 g (Vögelin et al., 2006). The calcium alginate spheres provided a biodegradable system for the continuous delivery of BDNF, which stimulated rapid peripheral nerve regeneration and significantly reduced neuropathic pain. In another study, the potential use of ion-crosslinked sodium tripolyphosphate chitosan microspheres for BDNF delivery was assessed (Zeng et al., 2020). The release from the microspheres persisted for up to 7 days, while the mass loss of the microspheres after incubation in phosphate-buffered saline continued for 9 weeks. In a different study, Chitosan NGC releasing GDNF was used for bridging the facial nerve defect over a 3.5 mm-long segment. Electrodiagnostic recordings after 9 weeks showed an outcome similar to uncut healthy nerve, thus indicating the potential of composite bioactive NGC for treating the facial nerve defects. However, the number of regenerated fibers were not higher in the GDNF-treated group, but aberrant fiber growth was significantly reduced (Xia et al., 2022).

5. Summary of clinical trials of polymers in peripheral nerve regeneration

Despite the large number of studies on the use of polysaccharides and their derivatives in nerve regeneration, very few substances reach commercial production. In Table 3, we summarize the state of clinical research on polysaccharides and other polymers (clinicaltrials.gov and other public databases). Only the first three of 18 clinical studies involved polysaccharides, none of which has so far reached commercial production. The first trial (NCT02459015) was terminated due to slow patient recruitment and insufficient patient compliance; the status of the second trial (NCT02372669) is unknown, while the third trial (NCT03359330) currently recruits patients. Among the 15 trials of non-polysaccharide materials, four reported positive outcomes, two have unknown status, and nine are still recruiting patients. All four trials with positive outcomes involved simple conduits based on biodegradable polymers and no delivery systems for pharmacological agents or cells. Considering the numerous *in vitro* and *in vivo* animal studies, only a limited number of products are available on the market. Thus, the development of drug-releasing nerve conduits for nerve regeneration should be a main focus both in academia and industry. A significant increase in the number of clinical trials, data, and an enhanced understanding of human nerve regeneration can be expected in the coming years.

6. Conclusions and future perspectives

Functional repair of nerve injuries is one of the most challenging tasks in the clinical setting. When the gap between the distal and proximal stumps of damaged nerves is large, guidance and protection is needed for regenerating axons. The gold standard treatment of autologous nerve grafting is associated with donor site morbidity. Consequently, the development of artificial NGC has emerged with great promise in the field. Within this context, polysaccharides are the most promising source of materials given their biocompatibility, stability, high sorption capacity, fabrication versatility (conduits, nanofibers, think films, porous scaffolds and hydrogels) and delivery capacity of bioactive substances, *i.e.*, pharmaceuticals, cells, and growth factors). Thus, there is a growing interest for polysaccharides in the field of peripheral nerve repair.

A large number of studies have shown the beneficial effects of polysaccharide-based NGC for nerve repair in various animal models, such as rodents and rabbits, and in humans. However, most trials have focused on small gap injuries, including clinical studies. Therefore, further studies involving the critical nerve gap defects will elucidate the

Table 3
Summary of clinical trials of polymer-based products for peripheral nerve regeneration.

No.	Proprietary name	Composition	Developer	Conditions	NCT number or ref.	Status PI-III	Outcome
1	Reaxon	Chitosan-based nerve guide	Medovent GmbH	• PNI	NCT02459015	Terminated	Slow patient recruitment and insufficient patient compliance
2	–	Chitosan nerve tube	BG Unfallklinik	• Traumatic lesion of sensory nerves of the hand	NCT02372669	Unknown	–
3	–	De-acetyl chitin conduit	Peking University People's Hospital	PN	NCT03359330	Not yet recruiting	–
4	Neurotube	Polyglycolic acid	Synovis Life Technologies, Inc.	Digital nerve reconstruction	(Mackinnon & Dellon, 1990; Weber et al., 2000)	Completed	Positive
5	–	PGA-collagen conduit	Kyoto Prefectural University of Medicine	PNI	(Hagiwara et al., 2002)	Completed	Positive
6	NeuraGen	Collagen tube Conduit	Integra LifeSciences	PNI	(Wangensteen & Kalliainen, 2010)	Completed	Positive
7	NeuroLac	Poly(dl-lactide-ε-caprolactone)	Polyganics B.V.	PNI	(Costa Serrão de Araújo et al., 2017)	Completed	Positive
8	TISSIUM™ Nerve coaptation Device	Poly (Glycerol-*Co*-Sebacate)	Tissium	Digital nerve injury	NCT04327154	Not yet recruiting	–
9	Autologous human Schwann cells	SCs wrapped in a collagen matrix	W. Dalton Dietrich	PNI	NCT03999424	Recruiting	–
10	AxoGuard Nerve cuff	Collagen	Xijing Hospital	PNI	NCT03780855	Unknown	–
11	AxoGuard Nerve Cuff	Collagen	University of Mississippi Medical Center	Spinal accessory nerve injury; cervical lymphadenopathy	NCT03941327	Unknown	–
12	AxoGuard Nerve Cap	Collagen	Axogen Corporation	Symptomatic neuroma; Morton's neuroma; chronic nerve pain	NCT03940963	Recruiting	–
13	AxoGuard Nerve Cap	Collagen	Axogen Corporation	Symptomatic neuroma; amputation; chronic nerve pain	NCT04865679	Not yet recruiting	–
14	–	Polyethylene glycol	Vanderbilt University	PNIs;	NCT02359825	Recruiting	–
15	–	Polyethylene glycol	WellSpan Health	Injury of other nerves at wrist and hand level of unspecified arm	NCT03236064	Recruiting	–
16	–	Polyethylene glycol	Nova Scotia Health Authority	PNI	NCT04270019	Not yet recruiting	–
17	Polynerve	*Co*-polymer of poly-ε-caprolactone and poly-l-lactic acid	University of Manchester	Injury of nerves at wrist and hand level	NCT02970864	Active, not recruiting	–
18	SilkBridge	Silk fibroin-based scaffold	Silk Biomaterials s. r.l.	PNI; digital nerve injury	NCT03673449	Active, not recruiting	–

PNI: peripheral nerve injury; SC: Schwann cell; NCT: National Clinical Trial.

clinical potential of these materials. Notably, there is a paucity of studies involving advanced therapeutic NGCs or combinational products (*i.e.*, inclusion of pharmaceutical, cell and growth factors). This can be attributed due to the lack of suitable delivery strategies for the already FDA-approved drug substances. Within this context, future NGC strategies can leverage on some of the advanced drug release strategies that are not yet tested for nerve regeneration, but discussed in this review. Future studies should also include the crucial experimental models such as delayed nerve repair, chronic nerve compression and diabetic neuropathies, in order to enhance the therapeutic potential of currently available NGCs to a large spectrum of nerve disorders.

The rapid development of 3D printing technology also holds great promise for PNI recovery as it paves the way for more accurate and cost-effective, patient-tailored ways to control nerves. In brief, the use of polysaccharides and their bioactive composites is a promising approach for PNI treatment and 3D printing opens a notable window of possibilities for the customized production of NGCs with cellular, molecular, neurotrophic and topographical guidance features. Thus, future studies may include the multifactorial NGCs to be fabricated using a single and automated bio-fabrication process.

CRediT authorship contribution statement

SM conceived the idea for the review. SM, AS and SS developed the concept. SS performed a review of the literature, and wrote the manuscript. SM, AS, CO, and DK provided the feedback and edits on all aspects of the manuscript. All authors contributed to the article and approved the submitted version. SM supervised the project.

Funding

This work was supported by the Department of Surgery at Geneva University Hospitals and the University of Geneva, and the Geneva University Hospitals (HUG) Private Foundation for Research (RC08-21), Geneva, Switzerland.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to thank the team of the Department of Plastic, Reconstructive and Aesthetic Sergery, Geneva University Hospitals, for their scientific and technical support. Special thanks to Karin Uelfeti and Patricia Berset-Neuhaus for the administrative support. Further special thanks to Rosemary Sudan for language editing support. The authors would like to thank the copyright owners for enabling the use of their images in this manuscript.

References

Abdelbasset, W. K., Jasim, S. A., Sharma, S. K., Margiana, R., Bokov, D. O., Obaid, M. A., Hussein, B. A., Lafta, H. A., Jasim, S. F., & Mustafa, Y. F. (2022). Alginate-based hydrogels and tubes, as biological macromolecule-based platforms for peripheral nerve tissue engineering: A review. *Annals of Biomedical Engineering, 50*(6), 628–653. https://doi.org/10.1007/s10439-022-02955-8

Abidian, M. R., Daneshvar, E. D., Egeland, B. M., Kipke, D. R., Cederna, P. S., & Urbanchek, M. G. (2012). Hybrid conducting polymer-hydrogel conduits for axonal growth and neural tissue engineering. *Advanced Healthcare Materials, 1*(6), 762–767. https://doi.org/10.1002/adhm.201200182

Adanali, G., Verdi, M., Tuncel, A., Erdogan, B., & Kargi, E. (2003). Effects of hyaluronic acid-carboxymethylcellulose membrane on extraneural adhesion formation and peripheral nerve regeneration. *Journal of Reconstructive Microsurgery, 19*(1), 029–036. https://doi.org/10.1055/s-2003-37188

Agarwal, G., Agiwal, S., & Srivastava, A. (2020). Hyaluronic acid containing scaffolds ameliorate stem cell function for tissue repair and regeneration. *International Journal of Biological Macromolecules, 165*, 388–401. https://doi.org/10.1016/j.ijbiomac.2020.09.107

Ahmad Raus, R., Wan Nawawi, W. M. F., & Nasaruddin, R. R. (2021). Alginate and alginate composites for biomedical applications. *Asian Journal of Pharmaceutical Sciences, 16*(3), 280–306. https://doi.org/10.1016/j.ajps.2020.10.001

Ahn, J., Ryu, J., Song, G., Whang, M., & Kim, J. (2019). Network structure and enzymatic degradation of chitosan hydrogels determined by crosslinking methods. *Carbohydrate Polymers, 217*, 160–167. https://doi.org/10.1016/j.carbpol.2019.04.055

Akiyoshi, K., Kang, E.-C., Kurumada, S., Sunamoto, J., Principi, T., & Winnik, F. M. (2000). Controlled Association of Amphiphilic Polymers in water: Thermosensitive nanoparticles formed by self-assembly of hydrophobically modified pullulans and Poly(N -isopropylacrylamides). *Macromolecules, 33*(9), 3244–3249. https://doi.org/10.1021/ma991798d

Alizadeh- Mohajer, M., Raisi, A., Farjanikish, G., & Mohammadi, R. (2019). Effect of local Administration of Verapamil Combined with chitosan based hybrid nanofiber conduit on transected sciatic nerve in rat. *Bulletin of Emergency and Trauma, 7*(1), 28–34. https://doi.org/10.29252/beat-070104

Andrades, J. A., Wu, L. T., Hall, F. L., Nimni, M. E., & Becerra, J. (2001). Engineering, expression, and renaturation of a collagen-targeted human bFGF fusion protein. *Growth Factors, 18*(4), 261–275. https://doi.org/10.3109/08977190109029115

Armati, P. J., & Mathey, E. K. (2014). Clinical implications of schwann cell biology. *Journal of the Peripheral Nervous System, 19*(1), 14–23. https://doi.org/10.1111/jns5.12057

Askarzadeh, N., Nazarpak, M. H., Mansoori, K., Farokhi, M., Gholami, M., Mohammadi, J., & Mottaghitalab, F. (2020). Bilayer cylindrical conduit consisting of electrospun polycaprolactone nanofibers and DSC cross-linked sodium alginate hydrogel to bridge peripheral nerve gaps. *Macromolecular Bioscience, 20*(9), Article 2000149. https://doi.org/10.1002/mabi.202000149

Atoufi, Z., Zarrintaj, P., Motlagh, G. H., Amiri, A., Bagher, Z., & Kamrava, S. K. (2017). A novel bio electro active alginate-aniline tetramer/ agarose scaffold for tissue engineering: Synthesis, characterization, drug release and cell culture study. *Journal of Biomaterials Science, Polymer Edition, 28*(15), 1617–1638. https://doi.org/10.1080/09205063.2017.1340044

Azizi, A., Azizi, S., Heshmatian, B., & Amini, K. (2014). Improvement of functional recovery of transected peripheral nerve by means of chitosan grafts filled with vitamin E, pyrroloquinoline quinone and their combination. *International Journal of Surgery, 12*(1), 76–82. https://doi.org/10.1016/j.ijsu.2013.10.002

Balint, R., Cassidy, N. J., & Cartmell, S. H. (2014). Conductive polymers: Towards a smart biomaterial for tissue engineering. *Acta Biomaterialia, 10*(6), 2341–2353. https://doi.org/10.1016/j.actbio.2014.02.015

Barclay, T. G., Day, C. M., Petrovsky, N., & Garg, S. (2019). Review of polysaccharide particle-based functional drug delivery. *Carbohydrate Polymers, 221*, 94–112. https://doi.org/10.1016/j.carbpol.2019.05.067

Böcker, A., Aman, M., Kneser, U., Harhaus, L., Siemers, F., & Stang, F. (2022). Closing the gap: Bridging peripheral sensory nerve defects with a chitosan-based conduit a randomized prospective clinical trial. *Journal of Personalized Medicine, 12*(6), 900. https://doi.org/10.3390/jpm12060900

Boecker, A., Daeschler, S. C., Kneser, U., & Harhaus, L. (2019). Relevance and recent developments of chitosan in peripheral nerve surgery. *Frontiers in Cellular Neuroscience, 13*, 104. https://doi.org/10.3389/fncel.2019.00104

Bolin, M. H., Svennersten, K., Wang, X., Chronakis, I. S., Richter-Dahlfors, A., Jager, E. W. H., & Berggren, M. (2009). Nano-fiber scaffold electrodes based on PEDOT for cell stimulation. *Sensors and Actuators B: Chemical, 142*(2), 451–456. https://doi.org/10.1016/j.snb.2009.04.062

Bontioti, E., Kanje, M., Lundborg, G., & Dahlin, L. B. (2005). End-to-side nerve repair in the upper extremity of rat. *Journal of the Peripheral Nervous System, 10*(1), 58–68. https://doi.org/10.1111/j.1085-9489.2005.10109.x

Caddick, J., Kingham, P. J., Gardiner, N. J., Wiberg, M., & Terenghi, G. (2006). Phenotypic and functional characteristics of mesenchymal stem cells differentiated along a schwann cell lineage. *Glia, 54*(8), 840–849. https://doi.org/10.1002/glia.20421

Carvalho, C. R., Oliveira, J. M., & Reis, R. L. (2019). Modern trends for peripheral nerve repair and regeneration: Beyond the hollow nerve guidance conduit. *Frontiers in Bioengineering and Biotechnology, 7*, 337. https://doi.org/10.3389/fbioe.2019.00337

Catoira, M. C., Fusaro, L., Di Francesco, D., Ramella, M., & Boccafoschi, F. (2019). Overview of natural hydrogels for regenerative medicine applications. *Journal of Materials Science: Materials in Medicine, 30*(10), 115. https://doi.org/10.1007/s10856-019-6318-7

Chao, X., Fan, Z., Han, Y., Wang, Y., Li, J., Chai, R., Xu, L., & Wang, H. (2015). Effects of local application of methylprednisolone delivered by the C/GP-hydrogel on the recovery of facial nerves. *Acta Oto-Laryngologica, 135*(11), 1178–1184.

Chao, X., Xu, L., Li, J., Han, Y., Li, X., Mao, Y., Shang, H., Fan, Z., & Wang, H. (2016). Facilitation of facial nerve regeneration using chitosan- β -glycerophosphate-nerve growth factor hydrogel. *Acta Oto-Laryngologica, 136*(6), 585–591. https://doi.org/10.3109/00016489.2015.1136432

Chávez-Delgado, M. E., Mora-Galindo, J., Gómez-Pinedo, U., Feria-Velasco, A., Castro-Castañeda, S., López-Dellamary Toral, F. A., Luquin-De Anda, S., García-Segura, L. M., & García-Estrada, J. (2003). Facial nerve regeneration through progesterone-loaded chitosan prosthesis. A preliminary report: Facial nerve regeneration with chitosan prosthesis. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 67B*(2), 702–711. https://doi.org/10.1002/jbm.b.10059

Chen, S.-H., Chou, P.-Y., Chen, Z.-Y., Chuang, D. C.-C., Hsieh, S.-T., & Lin, F.-H. (2020). An electrospun nerve wrap comprising Bletilla striata polysaccharide with dual function for nerve regeneration and scar prevention. *Carbohydrate Polymers, 250*, Article 116981. https://doi.org/10.1016/j.carbpol.2020.116981

Chen, Y., Hao, Y., Mensah, A., Lv, P., & Wei, Q. (2022). Bio-inspired hydrogels with fibrous structure: A review on design and biomedical applications. *Biomaterials Advances.* , Article 212799. https://doi.org/10.1016/j.bioadv.2022.212799

Cong, M., Wu, Z., Xu, X., Yin, J., Li, H., Zhuang, B., Wang, C., & Zhao, X. (2017). Novel oleyl amine-modified polymannuronic acid micelle loading tacrolimus for therapy of allergic conjunctivitis. *International Journal of Biological Macromolecules, 104*, 862–873. https://doi.org/10.1016/j.ijbiomac.2017.06.064

Costa Serrão de Araújo, G., Couto Neto, B., Harley Santos Botelho, R., & Carpi Malta, M. (2017). Clinical evaluation after peripheral nerve repair with caprolactone neurotube. *Hand, 12*(2), 168–174. https://doi.org/10.1177/1558944716643277

Curran, M. W. T., Olson, J., Morhart, M., Sample, D., & Chan, K. M. (2016). Acetyl-L-carnitine (ALCAR) to enhance nerve regeneration in carpal tunnel syndrome: Study protocol for a randomized, placebo-controlled trial. *Trials, 17*(1), 200. https://doi.org/10.1186/s13063-016-1324-2

de Guzman, R. C., Ereifej, E. S., Broadrick, K. M., Rogers, R. A., & VandeVord, P. J. (2008). Alginate-matrigel microencapsulated schwann cells for inducible secretion of glial cell line derived neurotrophic factor. *Journal of Microencapsulation, 25*(7), 487–498. https://doi.org/10.1080/02652040802054745

de Jong, S. J., van Eerdenbrugh, B., van Nostrum, C. F., Kettenes-van den Bosch, J. J., & Hennink, W. E. (2001). Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: Degradation and protein release behavior. *Journal of Controlled Release, 71*(3), 261–275. https://doi.org/10.1016/S0168-3659(01)00228-0

de Lima, G. G., Júnior, E. L.d. S., Aggio, B. B., Shee, B. S., Fournet, M. B., Devine, D. M., … Segundo, F. A.d. S. (2021). Nanocellulose for peripheral nerve regeneration in rabbits using citric acid as crosslinker with chitosan and freeze/thawed PVA. *Biomedical Materials, 16*(5), Article 055011. https://doi.org/10.1088/1748-605X/ac199b

Debele, T. A., Mekuria, S. L., & Tsai, H.-C. (2016). Polysaccharide based nanogels in the drug delivery system: Application as the carrier of pharmaceutical agents. *Materials Science and Engineering: C, 68*, 964–981. https://doi.org/10.1016/j.msec.2016.05.121

Degrugillier, L., Prautsch, K. M., Schaefer, D. J., Guzman, R., Kalbermatten, D. F., Schären, S., & Madduri, S. (2021). Systematic investigation and comparison of US FDA-approved immunosuppressive drugs FK506, cyclosporine and rapamycin for neuromuscular regeneration following chronic nerve compression injury. *Regenerative Medicine, 16*(11), 989–1003. https://doi.org/10.2217/rme-2020-0130

Deng, P., Chen, F., Zhang, H., Chen, Y., & Zhou, J. (2022). Multifunctional double-layer composite hydrogel conduit based on chitosan for peripheral nerve repairing. *Advanced Healthcare Materials, 11*(13), Article 2200115. https://doi.org/10.1002/adhm.202200115

Dheer, D., Jyoti, Gupta, P. N., & Shankar, R. (2018). Tacrolimus: An updated review on delivering strategies for multifarious diseases. *European Journal of Pharmaceutical Sciences, 114*, 217–227. https://doi.org/10.1016/j.ejps.2017.12.017

di Summa, P. G., Kingham, P. J., Raffoul, W., Wiberg, M., Terenghi, G., & Kalbermatten, D. F. (2010). Adipose-derived stem cells enhance peripheral nerve regeneration. *Journal of Plastic, Reconstructive & Aesthetic Surgery, 63*(9), 1544–1552. https://doi.org/10.1016/j.bjps.2009.09.012

Dietzmeyer, N., Förthmann, M., Grothe, C., & Haastert-Talini, K. (2020). Modification of tubular chitosan-based peripheral nerve implants: Applications for simple or more complex approaches. *Neural Regeneration Research, 15*(8), 1421. https://doi.org/10.4103/1673-5374.271668

Dietzmeyer, N., Huang, Z., Schüning, T., Rochkind, S., Almog, M., Nevo, Z., Lieke, T., Kankowski, S., & Haastert-Talini, K. (2020). In vivo and in vitro evaluation of a novel hyaluronic acid-laminin hydrogel as luminal filler and carrier system for genetically engineered schwann cells in critical gap length tubular peripheral nerve graft in rats. *Cell Transplantation, 29*. https://doi.org/10.1177/0963689720910095, 096368972091009.

Dodla, M. C., & Bellamkonda, R. V. (2008). Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. *Biomaterials, 29*(1), 33–46. https://doi.org/10.1016/j.biomaterials.2007.08.045

Dolmetsch, R. E., Pajvani, U., Fife, K., Spotts, J. M., & Greenberg, M. E. (2001). Signaling to the nucleus by an L-type Calcium Channel-calmodulin complex through the MAP kinase pathway. *Science, 294*(5541), 333–339. https://doi.org/10.1126/science.1063395

Dragan, E. S., & Dinu, M. V. (2019). Polysaccharides constructed hydrogels as vehicles for proteins and peptides. A review. *Carbohydrate Polymers, 225*, Article 115210. https://doi.org/10.1016/j.carbpol.2019.115210

Dutta, P., Koch, A., Breyer, B., Schneider, H., Dittrich-Breiholz, O., Kracht, M., & Tamura, T. (2011). Identification of novel target genes of nerve growth factor (NGF) in human mastocytoma cell line (HMC-1 (V560G c-Kit)) by transcriptome analysis. *BMC Genomics, 12*(1), 196. https://doi.org/10.1186/1471-2164-12-196

El Soury, M., García-García, Ó., Tarulli, I., Chato-Astrain, J., Perroteau, I., Geuna, S., Raimondo, S., Gambarotta, G., & Carriel, V. (2023). Chitosan conduits enriched with fibrin-collagen hydrogel with or without adipose-derived mesenchymal stem cells for the repair of 15-mm-long sciatic nerve defect. *Neural Regeneration Research, 18*(6), 1378. https://doi.org/10.4103/1673-5374.358605

Entekhabi, E., Haghbin Nazarpak, M., Shafieian, M., Mohammadi, H., Firouzi, M., & Hassannejad, Z. (2021). Fabrication and in vitro evaluation of 3D composite scaffold based on collagen/hyaluronic acid sponge and electrospun polycaprolactone nanofibers for peripheral nerve regeneration. *Journal of Biomedical Materials Research Part A, 109*(3), 300–312. https://doi.org/10.1002/jbm.a.37023

Erkutlu, I., Alptekin, M., Geyik, S., Geyik, A., Gezgin, I., & Gök, A. (2015). Early cyclosporin a treatment retards axonal degeneration in an experimental peripheral nerve injection injury model. *Neural Regeneration Research, 10*(2), 266. https://doi.org/10.4103/1673-5374.152381

Evans, P. J., Bain, J. R., Mackinnon, S. E., Makino, A. P., & Hunter, D. A. (1991). Selective reinnervation: A comparison of recovery following microsuture and conduit nerve repair. *Brain Research, 559*(2), 315–321. https://doi.org/10.1016/0006-8993(91)90018-Q

Fornasari, B. E., Carta, G., Gambarotta, G., & Raimondo, S. (2020). Natural-based biomaterials for peripheral nerve injury repair. *Frontiers in Bioengineering and Biotechnology, 8*, Article 554257. https://doi.org/10.3389/fbioe.2020.554257

Francis, N. L., Hunger, P. M., Donius, A. E., Wegst, U. G. K., & Wheatley, M. A. (2017). Strategies for neurotrophin-3 and chondroitinase ABC release from freeze-cast chitosan–alginate nerve-guidance scaffolds. *Journal of Tissue Engineering and Regenerative Medicine, 11*(1), 285–294. https://doi.org/10.1002/term.1912

Frostick, S. P., Yin, Q., & Kemp, G. J. (1998). Schwann cells, neurotrophic factors, and peripheral nerve regeneration. *Microsurgery, 18*(7), 397–405. https://doi.org/10.1002/(SICI)1098-2752(1998)18:7<397::AID-MICR2>3.0.CO;2-F

Fu, S., Thacker, A., Sperger, D. M., Boni, R. L., Buckner, I. S., Velankar, S., Munson, E. J., & Block, L. H. (2011). Relevance of rheological properties of sodium alginate in solution to calcium alginate gel properties. *AAPS PharmSciTech, 12*(2), 453–460. https://doi.org/10.1208/s12249-011-9587-0

Garg, R., Merrell, G. A., Hillstrom, H. J., & Wolfe, S. W. (2011). Comparison of nerve transfers and nerve grafting for traumatic upper plexus palsy: A systematic review and analysis. *Journal of Bone and Joint Surgery, 93*(9), 819–829. https://doi.org/10.2106/JBJS.I.01602

Geuna, S., Raimondo, S., Fregnan, F., Haastert-Talini, K., & Grothe, C. (2016). In vitro models for peripheral nerve regeneration. *European Journal of Neuroscience, 43*(3), 287–296. https://doi.org/10.1111/ejn.13054

Ghasemi-Mobarakeh, L., Prabhakaran, M. P., Morshed, M., Nasr-Esfahani, M. H., Baharvand, H., Kiani, S., Al-Deyab, S. S., & Ramakrishna, S. (2011). Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine, 5*(4), e17–e35. https://doi.org/10.1002/term.383

Gheorghita Puscaselu, R., Lobiuc, A., Dimian, M., & Covasa, M. (2020). Alginate: From food industry to biomedical applications and management of metabolic disorders. *Polymers, 12*(10), 2417. https://doi.org/10.3390/polym12102417

Ghosh, N., Kalbermatten, D., Madduri, S., & Guzman, R. (2020). Fibrosis and regulation of nerve regeneration in the peripheral and central nervous systems. *CNS & Neurological Disorders - Drug Targets, 19*(8), 560–571. https://doi.org/10.2174/1871527319666200726222558

Gilbert, A., Pivato, G., & Kheiralla, T. (2006). Long-term results of primary repair of brachial plexus lesions in children. *Microsurgery, 26*(4), 334–342. https://doi.org/10.1002/micr.20248

Gisbert Roca, F., Lozano Picazo, P., Pérez-Rigueiro, J., Guinea Tortuero, G. V., Monleón Pradas, M., & Martínez-Ramos, C. (2020). Conduits based on the combination of hyaluronic acid and silk fibroin: Characterization, in vitro studies and in vivo biocompatibility. *International Journal of Biological Macromolecules, 148*, 378–390. https://doi.org/10.1016/j.ijbiomac.2020.01.149

Gómez-Burgaz, M., Torrado, G., & Torrado, S. (2009). Characterization and superficial transformations on mini-matrices made of interpolymer complexes of chitosan and carboxymethylcellulose during in vitro clarithromycin release. *European Journal of Pharmaceutics and Biopharmaceutics, 73*(1), 130–139. https://doi.org/10.1016/j.ejpb.2009.04.004

Griffin, M. F., Malahias, M., Hindocha, S., & Wasim, S. K. (2014). Peripheral nerve injury: Principles for repair and regeneration. *The Open Orthopaedics Journal, 8*(1), 199–203. https://doi.org/10.2174/1874325001408010199

Grinsell, D., & Keating, C. P. (2014). Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. *BioMed Research International, 2014*, 1–13. https://doi.org/10.1155/2014/698256

Guo, B., Glavas, L., & Albertsson, A.-C. (2013). Biodegradable and electrically conducting polymers for biomedical applications. *Progress in Polymer Science, 38*(9), 1263–1286. https://doi.org/10.1016/j.progpolymsci.2013.06.003

Hagiwara, A., Nakashima, S., Itoh, T., Sakakura, C., Otsuji, E., Yamagishi, H., Okajima, S., Kusuzaki, K., Hase, H., Kubo, S., Soh, J., Miki, T., Toba, T., Nakamura, T., & Shimizu, Y. (2002). Clinical application of PGA-tube for regeneration of intrapelvic nerves during extended surgery for intrapelvic recurrent rectal cancer. *Gan to Kagaku Ryoho. Cancer & Chemotherapy, 29*(12), 2202–2204.

He, M., Shi, L., Wang, G., Cheng, Z., Han, L., Zhang, X., Wang, C., Wang, J., Zhou, P., & Wang, G. (2020). Biocompatible and biodegradable chitosan/sodium polyacrylate polyelectrolyte complex hydrogels with smart responsiveness. *International Journal of Biological Macromolecules, 155*, 1245–1251. https://doi.org/10.1016/j.ijbiomac.2019.11.092

Hsu, S., Chan, S.-H., Chiang, C.-M., Chi-Chang Chen, C., & Jiang, C.-F. (2011). Peripheral nerve regeneration using a microporous polylactic acid asymmetric conduit in a rabbit long-gap sciatic nerve transection model. *Biomaterials, 32*(15), 3764–3775. https://doi.org/10.1016/j.biomaterials.2011.01.065

Ikeda, K., Yamauchi, D., Osamura, N., Hagiwara, N., & Tomita, K. (2003). Hyaluronic acid prevents peripheral nerve adhesion. *British Journal of Plastic Surgery, 56*(4), 342–347. https://doi.org/10.1016/S0007-1226(03)00197-8

Isaacs, J., & Cochran, A. R. (2019). Nerve transfers for peripheral nerve injury in the upper limb: A case-based review. *The Bone & Joint Journal, 101-B*(2), 124–131. https://doi.org/10.1302/0301-620X.101B2.BJJ-2018-0839.R1

Ishikawa, N., Suzuki, Y., Ohta, M., Cho, H., Suzuki, S., Dezawa, M., & Ide, C. (2007). Peripheral nerve regeneration through the space formed by a chitosan gel sponge. *Journal of Biomedical Materials Research Part A, 83A*(1), 33–40. https://doi.org/10.1002/jbm.a.31126

Itai, S., Suzuki, K., Kurashina, Y., Kimura, H., Amemiya, T., Sato, K., Nakamura, M., & Onoe, H. (2020). Cell-encapsulated chitosan-collagen hydrogel hybrid nerve guidance conduit for peripheral nerve regeneration. *Biomedical Microdevices, 22*(4), 81. https://doi.org/10.1007/s10544-020-00536-x

Itoh, S., Yamaguchi, I., Suzuki, M., Ichinose, S., Takakuda, K., Kobayashi, H., Shinomiya, K., & Tanaka, J. (2003). Hydroxyapatite-coated tendon chitosan tubes with adsorbed laminin peptides facilitate nerve regeneration in vivo. *Brain Research, 993*(1–2), 111–123. https://doi.org/10.1016/j.brainres.2003.09.003

Jahromi, M., Razavi, S., & Bakhtiari, A. (2019). The advances in nerve tissue engineering: From fabrication of nerve conduit to in vivo nerve regeneration assays. *Journal of Tissue Engineering and Regenerative Medicine, 13*(11), 2077–2100. https://doi.org/10.1002/term.2945

Jessen, K. R., & Arthur-Farraj, P. (2019). Repair schwann cell update: Adaptive reprogramming, EMT, and stemness in regenerating nerves. *Glia, 67*(3), 421–437. https://doi.org/10.1002/glia.23532

Jessen, K. R., & Mirsky, R. (2019). The success and failure of the schwann cell response to nerve injury. *Frontiers in Cellular Neuroscience, 13*, 33. https://doi.org/10.3389/fncel.2019.00033

Jiang, F., Li, W., Huang, Y., Chen, Y., Jin, B., Chen, N., Ding, Z., & Ding, X. (2013). Antioxidant, antityrosinase and antitumor activity comparison: The potential utilization of fibrous root part of Bletilla striata (Thunb.) Reichb.f. *PLoS ONE, 8*(2), Article e58004. https://doi.org/10.1371/journal.pone.0058004

Jiang, X., Mi, R., Hoke, A., & Chew, S. Y. (2014). Nanofibrous nerve conduit-enhanced peripheral nerve regeneration: Nanofibrous nerve conduit-enhanced peripheral nerve regeneration. *Journal of Tissue Engineering and Regenerative Medicine, 8*(5), 377–385. https://doi.org/10.1002/term.1531

Jiang, Z., Zhang, Y., Wang, Y., Wang, S., Chang, J., Liu, W., & Han, B. (2023). Multichannel nerve conduit based on chitosan derivates for peripheral nerve regeneration and schwann cell survival. *Carbohydrate Polymers, 301*, Article 120327. https://doi.org/10.1016/j.carbpol.2022.120327

Jin, G., & Li, K. (2014). The electrically conductive scaffold as the skeleton of stem cell niche in regenerative medicine. *Materials Science and Engineering: C, 45*, 671–681. https://doi.org/10.1016/j.msec.2014.06.004

Keilhoff, G., Goihl, A., Langnäse, K., Fansa, H., & Wolf, G. (2006). Transdifferentiation of mesenchymal stem cells into schwann cell-like myelinating cells. *European Journal of Cell Biology, 85*(1), 11–24. https://doi.org/10.1016/j.ejcb.2005.09.021

Kline, D. G., Kim, D., Midha, R., Harsh, C., & Tiel, R. (1998). Management and results of sciatic nerve injuries: A 24-year experience. *Journal of Neurosurgery, 89*(1), 13–23. https://doi.org/10.3171/jns.1998.89.1.0013

Ko, E., Jeong, D., Kim, J., Park, S., Khang, G., & Lee, D. (2014). Antioxidant polymeric prodrug microparticles as a therapeutic system for acute liver failure. *Biomaterials, 35*(12), 3895–3902. https://doi.org/10.1016/j.biomaterials.2014.01.048

Kovačič, U., Sketelj, J., & Bajrović, F. F. (2009). Chapter 26 age-related differences in the reinnervation after peripheral nerve injury. In , *Vol. 87. International review of neurobiology* (pp. 465–482). Elsevier. https://doi.org/10.1016/S0074-7742(09)87026-8.

Kubiak, C. A., Grochmal, J., Kung, T. A., Cederna, P. S., Midha, R., & Kemp, S. W. P. (2020). Stem-cell–based therapies to enhance peripheral nerve regeneration. *Muscle & Nerve, 61*(4), 449–459. https://doi.org/10.1002/mus.26760

Lacko, C. S., Singh, I., Wall, M. A., Garcia, A. R., Porvasnik, S. L., Rinaldi, C., & Schmidt, C. E. (2020). Magnetic particle templating of hydrogels: Engineering naturally derived hydrogel scaffolds with 3D aligned microarchitecture for nerve repair. *Journal of Neural Engineering, 17*(1), Article 016057. https://doi.org/10.1088/1741-2552/ab4a22

Lee, B. M., Park, S. J., Noh, I., & Kim, C.-H. (2021). The effects of the molecular weights of hyaluronic acid on the immune responses. *Biomaterials Research, 25*(1), 27. https://doi.org/10.1186/s40824-021-00228-4

Lee, J. I., Hur, J. M., You, J., & Lee, D. H. (2020). Functional recovery with histomorphometric analysis of nerves and muscles after combination treatment with erythropoietin and dexamethasone in acute peripheral nerve injury. *PLOS ONE, 15*(9), Article e0238208. https://doi.org/10.1371/journal.pone.0238208

León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., & Aguirre-Álvarez, G. (2019). Hydrolyzed collagen—Sources and applications. *Molecules, 24*(22), 4031. https://doi.org/10.3390/molecules24224031

Li, G., Xiao, Q., Zhang, L., Zhao, Y., & Yang, Y. (2017). Nerve growth factor loaded heparin/chitosan scaffolds for accelerating peripheral nerve regeneration. *Carbohydrate Polymers, 171*, 39–49. https://doi.org/10.1016/j.carbpol.2017.05.006

Li, G., Zhang, B., Sun, J., Shi, L., Huang, M., Huang, L., Lin, Z., Lin, Q., Lai, B., Ma, Y., Jiang, B., Ding, Y., Zhang, H., Li, M., Zhu, P., Wang, Y., Zeng, X., & Zeng, Y. (2021). An NT-3-releasing bioscaffold supports the formation of TrkC-modified neural stem cell-derived neural network tissue with efficacy in repairing spinal cord injury. *Bioactive Materials, 6*(11), 3766–3781. https://doi.org/10.1016/j.bioactmat.2021.03.036

Li, P., Ruan, L., Jiang, G., Sun, Y., Wang, R., Gao, X., Yunusov, K. E., Aharodnikau, U. E., & Solomevich, S. O. (2022). Design of 3D polycaprolactone/ε-polylysine-modified chitosan fibrous scaffolds with incorporation of bioactive factors for accelerating wound healing. *Acta Biomaterialia, 152*, 197–209. https://doi.org/10.1016/j.actbio.2022.08.075

Li, P., Zhao, J., Chen, Y., Cheng, B., Yu, Z., Zhao, Y., Yan, X., Tong, Z., & Jin, S. (2017). Preparation and characterization of chitosan physical hydrogels with enhanced mechanical and antibacterial properties. *Carbohydrate Polymers, 157*, 1383–1392. https://doi.org/10.1016/j.carbpol.2016.11.016

Li, Q., Li, Z., Zeng, W., Ge, S., Lu, H., Wu, C., Ge, L., Liang, D., & Xu, Y. (2014). Proniosome-derived niosomes for tacrolimus topical ocular delivery: In vitro cornea permeation, ocular irritation, and in vivo anti-allograft rejection. *European Journal of Pharmaceutical Sciences, 62*, 115–123. https://doi.org/10.1016/j.ejps.2014.05.020

Li, R., Li, D., Zhang, H., Wang, J., Li, X., & Xiao, J. (2020). Growth factors-based therapeutic strategies and their underlying signaling mechanisms for peripheral nerve regeneration. *Acta Pharmacologica Sinica, 41*(10), 1289–1300. https://doi.org/10.1038/s41401-019-0338-1

Li, R., Li, Y., Wu, Y., Zhao, Y., Chen, H., Yuan, Y., Xu, K., Zhang, H., Lu, Y., Wang, J., Li, X., Jia, X., & Xiao, J. (2018). Heparin-poloxamer thermosensitive hydrogel loaded with bFGF and NGF enhances peripheral nerve regeneration in diabetic rats. *Biomaterials, 168*, 24–37. https://doi.org/10.1016/j.biomaterials.2018.03.044

Li, R., Liu, H., Huang, H., Bi, W., Yan, R., Tan, X., Wen, W., Wang, C., Song, W., Zhang, Y., Zhang, F., & Hu, M. (2018). Chitosan conduit combined with hyaluronic acid prevent sciatic nerve scar in a rat model of peripheral nerve crush injury. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2018.8388

Li, R., Liu, Z., Pan, Y., Chen, L., Zhang, Z., & Lu, L. (2014). Peripheral nerve injuries treatment: A systematic review. *Cell Biochemistry and Biophysics, 68*(3), 449–454. https://doi.org/10.1007/s12013-013-9742-1

Li, X., Wang, W., Wei, G., Wang, G., Zhang, W., & Ma, X. (2010). Immunophilin FK506 loaded in chitosan guide promotes peripheral nerve regeneration. *Biotechnology Letters, 32*(9), 1333–1337. https://doi.org/10.1007/s10529-010-0287-8

Lin, C.-Y., Li, L.-T., & Su, W.-T. (2014). Three dimensional chitosan scaffolds influence the extra cellular matrix expression in schwann cells. *Materials Science and Engineering: C, 42*, 474–478. https://doi.org/10.1016/j.msec.2014.05.063

Lin, S. C.-Y., Wang, Y., Wertheim, D. F., & Coombes, A. G. A. (2017). Production and in vitro evaluation of macroporous, cell-encapsulating alginate fibres for nerve repair. *Materials Science and Engineering: C, 73*, 653–664. https://doi.org/10.1016/j.msec.2016.12.016

Liu, F., Xu, J., Liu, A., Wu, L., Wang, D., Han, Q., Zheng, T., Wang, F., Kong, Y., Li, G., Li, P., Gu, S., & Yang, Y. (2022). Development of a polyacrylamide/chitosan composite hydrogel conduit containing synergistic cues of elasticity and topographies for promoting peripheral nerve regeneration. *Biomaterials Science, 10*(17), 4915–4932. https://doi.org/10.1039/D2BM00327A

Liu, H., Zhao, Y., Tong, J., Shi, X., Chen, Y., & Du, Y. (2021). Electrofabrication of flexible and mechanically strong tubular chitosan implants for peripheral nerve regeneration. *Journal of Materials Chemistry B, 9*(27), 5537–5546. https://doi.org/10.1039/D1TB00247C

Liu, Z., Tong, H., Li, J., Wang, L., Fan, X., Song, H., Yang, M., Wang, H., Jiang, X., Zhou, X., Yuan, H., & Wang, Y. (2022). Low-stiffness hydrogels promote peripheral nerve regeneration through the rapid release of exosomes. *Frontiers in Bioengineering and Biotechnology, 10*, Article 922570. https://doi.org/10.3389/fbioe.2022.922570

López-Cebral, R., Silva-Correia, J., Reis, R. L., Silva, T. H., & Oliveira, J. M. (2017). Peripheral nerve injury: Current challenges, conventional treatment approaches, and new trends in biomaterials-based regenerative strategies. *ACS Biomaterials Science & Engineering, 3*(12), 3098–3122. https://doi.org/10.1021/acsbiomaterials.7b00655

Luo, L., Gan, L., Liu, Y., Tian, W., Tong, Z., Wang, X., Huselstein, C., & Chen, Y. (2015). Construction of nerve guide conduits from cellulose/soy protein composite membranes combined with schwann cells and pyrroloquinoline quinone for the repair of peripheral nerve defect. *Biochemical and Biophysical Research Communications, 457*(4), 507–513. https://doi.org/10.1016/j.bbrc.2014.12.121

Luo, L., He, Y., Jin, L., Zhang, Y., Guastaldi, F. P., Albashari, A. A., Hu, F., Wang, X., Wang, L., Xiao, J., Li, L., Wang, J., Higuchi, A., & Ye, Q. (2021). Application of bioactive hydrogels combined with dental pulp stem cells for the repair of large gap peripheral nerve injuries. *Bioactive Materials, 6*(3), 638–654. https://doi.org/10.1016/j.bioactmat.2020.08.028

Mackinnon, S. E., & Dellon, A. L. (1990). Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. *Plastic and Reconstructive Surgery, 85*(3), 419–424. https://doi.org/10.1097/00006534-199003000-00015

Manoukian, O. S., Baker, J. T., Rudraiah, S., Arul, M. R., Vella, A. T., Domb, A. J., & Kumbar, S. G. (2020). Functional polymeric nerve guidance conduits and drug delivery strategies for peripheral nerve repair and regeneration. *Journal of Controlled Release, 317*, 78–95. https://doi.org/10.1016/j.jconrel.2019.11.021

Matica, Aachmann, Tøndervik, Sletta, & Ostafe. (2019). Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *International Journal of Molecular Sciences, 20*(23), 5889. https://doi.org/10.3390/ijms20235889

Mehrshad, A., Shahid Bahonar University of Kerman, K., Iran, Shahraki, M., Urmia University, U., Iran, & Ehteshamfar, S. Department Clinical Sciences, Faculty of Veterinary Medicine, Department of Internal Medicine and Clinical Pathology, Faculty of Veterinary Medicine. (2017). Local administration of methylprednisolone laden hydrogel enhances functional recovery of transected sciatic nerve in rat. *Bulletin of Emergency and Trauma, 5*(4), 231–239. https://doi.org/10.18869/acadpub.beat.5.4.509

Meka, V. S., Sing, M. K. G., Pichika, M. R., Nali, S. R., Kolapalli, V. R. M., & Kesharwani, P. (2017). A comprehensive review on polyelectrolyte complexes. *Drug Discovery Today, 22*(11), 1697–1706. https://doi.org/10.1016/j.drudis.2017.06.008

Mekaj, A., Morina, A., Manxhuka-Kerliu, S., Neziri, B., Duci, S., Kukaj, V., & Miftari, I. (2015). Electrophysiological and functional evaluation of peroneal nerve regeneration in rabbit following topical hyaluronic acid or tacrolimus application after nerve repair. *Nigerian Postgraduate Medical Journal, 22*(3), 179. https://doi.org/10.4103/1117-1936.170738

Mekaj, A. Y., Manxhuka-Kerliu, S., Morina, A. A., Duci, S. B., Shahini, L., & Mekaj, Y. H. (2017). Effects of hyaluronic acid and tacrolimus on the prevention of perineural scar formation and on nerve regeneration after sciatic nerve repair in a rabbit model. *European Journal of Trauma and Emergency Surgery, 43*(4), 497–504. https://doi.org/10.1007/s00068-016-0683-4

Mekaj, A. Y., Morina, A. A., Bytyqi, C. I., Mekaj, Y. H., & Duci, S. B. (2014). Application of topical pharmacological agents at the site of peripheral nerve injury and methods used for evaluating the success of the regenerative process. *Journal of Orthopaedic Surgery and Research, 9*(1), 94. https://doi.org/10.1186/s13018-014-0094-3

Mendichi, R., Šoltés, L., & Giacometti Schieroni, A. (2003). Evaluation of radius of gyration and intrinsic viscosity molar mass dependence and stiffness of hyaluronan. *Biomacromolecules, 4*(6), 1805–1810. https://doi.org/10.1021/bm0342178

Midha, R. (2006). Emerging techniques for nerve repair: Nerve transfers and nerve guidance tubes. *Clinical Neurosurgery, 53*, 185–190.

Millesi, H., Meissl, G., & Berger, A. (1972). The interfascicular nerve-grafting of the median and ulnar nerves. *The Journal of Bone and Joint Surgery. American Volume, 54*, 727–750.

Moattari, M., Kouchesfehani, H. M., Kaka, G., Sadraie, S. H., Naghdi, M., & Mansouri, K. (2018). Chitosan-film associated with mesenchymal stem cells enhanced regeneration of peripheral nerves: A rat sciatic nerve model. *Journal of Chemical Neuroanatomy, 88*, 46–54. https://doi.org/10.1016/j.jchemneu.2017.10.003

Mohammadi, R., Amini, K., Yousefi, A., Abdollahi-Pirbazari, M., Belbasi, A., & Abedi, F. (2013). Functional effects of local administration of thyroid hormone combined with chitosan conduit after sciatic nerve transection in rats. *Journal of Oral and Maxillofacial Surgery, 71*(10), 1763–1776. https://doi.org/10.1016/j.joms.2013.03.010

Mohammadi, R., Heydarian, H., & Amini, K. (2014). Effect of local administration of cyclosporine a on peripheral nerve regeneration in a rat sciatic nerve transection model. *Chinese Journal of Traumatology = Zhonghua Chuang Shang Za Zhi, 17*(1), 12–18.

Mohammed, A. S. A., Naveed, M., & Jost, N. (2021). Polysaccharides; classification, chemical properties, and future perspective applications in fields of pharmacology and biological medicine (A review of current applications and upcoming Potentialities). *Journal of Polymers and the Environment, 29*(8), 2359–2371. https://doi.org/10.1007/s10924-021-02052-2

Nakajima, H., Dijkstra, P., & Loos, K. (2017). The recent developments in biobased polymers toward general and engineering applications: Polymers that are upgraded from biodegradable polymers, analogous to petroleum-derived polymers, and newly developed. *Polymers, 9*(12), 523. https://doi.org/10.3390/polym9100523

Navarro, X. (2016). Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: A critical overview. *European Journal of Neuroscience, 43*(3), 271–286. https://doi.org/10.1111/ejn.13033

Nawrotek, K., Kubicka, M., Gatkowska, J., Wieczorek, M., Michlewska, S., Bekier, A., Wach, R., & Rudnicka, K. (2022). Controlling the spatiotemporal release of nerve growth factor by chitosan/polycaprolactone conduits for use in peripheral nerve regeneration. *International Journal of Molecular Sciences, 23*(5), 2852. https://doi.org/10.3390/ijms23052852

Nawrotek, K., Tylman, M., Adamus-Włodarczyk, A., Rudnicka, K., Gatkowska, J., Wieczorek, M., & Wach, R. (2020). Influence of chitosan average molecular weight on degradation and stability of electrodeposited conduits. *Carbohydrate Polymers, 244*, Article 116484. https://doi.org/10.1016/j.carbpol.2020.116484

Nawrotek, K., Tylman, M., Rudnicka, K., Balcerzak, J., & Kamiński, K. (2016). Chitosan-based hydrogel implants enriched with calcium ions intended for peripheral nervous tissue regeneration. *Carbohydrate Polymers, 136*, 764–771. https://doi.org/10.1016/j.carbpol.2015.09.105

Nectow, A. R., Marra, K. G., & Kaplan, D. L. (2012). Biomaterials for the development of peripheral nerve guidance conduits. *Tissue Engineering Part B: Reviews, 18*(1), 40–50. https://doi.org/10.1089/ten.teb.2011.0240

Nezhad-Mokhtari, P., Ghorbani, M., Roshangar, L., & Soleimani Rad, J. (2019). Chemical gelling of hydrogels-based biological macromolecules for tissue engineering: Photo- and enzymatic-crosslinking methods. *International Journal of Biological Macromolecules, 139*, 760–772. https://doi.org/10.1016/j.ijbiomac.2019.08.047

Nie, X., Deng, M., Yang, M., Liu, L., Zhang, Y., & Wen, X. (2014). Axonal regeneration and remyelination evaluation of chitosan/gelatin-based nerve guide combined with transforming growth factor-β1 and Schwann cells. *Cell Biochemistry and Biophysics, 68*(1), 163–172. https://doi.org/10.1007/s12013-013-9683-8

Ninan, N., Muthiah, M., Park, I.-K., Elain, A., Thomas, S., & Grohens, Y. (2013). Pectin/carboxymethyl cellulose/microfibrillated cellulose composite scaffolds for tissue engineering. *Carbohydrate Polymers, 98*(1), 877–885. https://doi.org/10.1016/j.carbpol.2013.06.067

Ning, L., Sun, H., Lelong, T., Guilloteau, R., Zhu, N., Schreyer, D. J., & Chen, X. (2018). 3D bioprinting of scaffolds with living schwann cells for potential nerve tissue engineering applications. *Biofabrication, 10*(3), Article 035014. https://doi.org/10.1088/1758-5090/aacd30

Noroozi, N., Thomson, J. A., Noroozi, N., Schafer, L. L., & Hatzikiriakos, S. G. (2012). Viscoelastic behaviour and flow instabilities of biodegradable poly (ε-caprolactone) polyesters. *Rheologica Acta, 51*(2), 179–192. https://doi.org/10.1007/s00397-011-0586-6

Ojeda, E., Puras, G., Agirre, M., Zarate, J., Grijalvo, S., Eritja, R., Martinez-Navarrete, G., Soto-Sánchez, C., Diaz-Tahoces, A., Aviles-Trigueros, M., Fernández, E., & Pedraz, J. L. (2016). The influence of the polar head-group of synthetic cationic lipids on the transfection efficiency mediated by niosomes in rat retina and brain. *Biomaterials, 77*, 267–279. https://doi.org/10.1016/j.biomaterials.2015.11.017

Pandey, M., Choudhury, H., Gunasegaran, T. A. P., Nathan, S. S., Md, S., Gorain, B., Tripathy, M., & Hussain, Z. (2019). Hyaluronic acid-modified betamethasone encapsulated polymeric nanoparticles: Fabrication, characterisation, in vitro release kinetics, and dermal targeting. *Drug Delivery and Translational Research, 9*(2), 520–533. https://doi.org/10.1007/s13346-018-0480-1

Parhi, R. (2017). Cross-linked hydrogel for pharmaceutical applications: A review. *Advanced Pharmaceutical Bulletin, 7*(4), 515–530. https://doi.org/10.15171/apb.2017.064

Park, H.-J., Lee, C.-M., Lee, Y.-B., & Lee, K.-Y. (2006). Controlled release of cyclosporin a from liposomes-in-microspheres as an oral delivery system. *Biotechnology and Bioprocess Engineering, 11*(6), 526–529. https://doi.org/10.1007/BF02932078

Park, J. S., Lee, J. H., Han, C. S., Chung, D. W., & Kim, G. Y. (2011). Effect of hyaluronic acid-carboxymethylcellulose solution on perineural scar formation after sciatic nerve repair in rats. *Clinics in Orthopedic Surgery, 3*(4), 315. https://doi.org/10.4055/cios.2011.3.4.315

Parteni, O., & Doru Radu, C. (2016). The release of tacrolimus from a cotton biomaterial to dermis. *Journal of Pharmaceutics & Drug Delivery Research, 05*(01). https://doi.org/10.4172/2325-9604.1000142

Patel, M., Vandevord, P. J., Matthew, H., Wu, B., Desilva, S., & Wooley, P. H. (2006). Video-gait analysis of functional recovery of nerve repaired with chitosan nerve guides. *Tissue Engineering, 12*(11), 3189–3199. https://doi.org/10.1089/ten.2006.12.3189

Peluso, G., Petillo, O., Ranieri, M., Santin, M., Ambrosic, L., Calabró, D., Avallone, B., & Balsamo, G. (1994). Chitosan-mediated stimulation of macrophage function. *Biomaterials, 15*(15), 1215–1220. https://doi.org/10.1016/0142-9612(94)90272-0

Peng, Q., Li, M., Xue, F., & Liu, H. (2014). Structure and immunobiological activity of a new polysaccharide from Bletilla striata. *Carbohydrate Polymers, 107*, 119–123. https://doi.org/10.1016/j.carbpol.2014.02.042

Pertici, V., Laurin, J., Féron, F., Marqueste, T., & Decherchi, P. (2014). Functional recovery after repair of peroneal nerve gap using different collagen conduits. *Acta Neurochirurgica, 156*(5), 1029–1040. https://doi.org/10.1007/s00701-014-2009-9

Pestronk, A., Drachman, D. B., & Griffin, J. W. (1980). Effects of aging on nerve sprouting and regeneration. *Experimental Neurology, 70*(1), 65–82. https://doi.org/10.1016/0014-4886(80)90006-0

Pfister, L. A., Alther, E., Papaloïzos, M., Merkle, Gander, B., & Hans, P. (2008). Controlled nerve growth factor release from multi-ply alginate/chitosan-based nerve conduits. *European Journal of Pharmaceutics and Biopharmaceutics, 69*(2), 563–572. https://doi.org/10.1016/j.ejpb.2008.01.014

Pfister, L. A., Papaloïzos, M., Merkle, H. P., & Gander, B. (2007). Hydrogel nerve conduits produced from alginate/chitosan complexes. *Journal of Biomedical Materials Research Part A, 80A*(4), 932–937. https://doi.org/10.1002/jbm.a.31052

Pisal, D. S., Kosloski, M. P., & Balu-Iyer, S. V. (2010). Delivery of therapeutic proteins. *Journal of Pharmaceutical Sciences, 99*(6), 2557–2575. https://doi.org/10.1002/jps.22054

Pola, R., Aprahamian, T. R., Bosch-Marcé, M., Curry, C., Gaetani, E., Flex, A., Smith, R. C., Isner, J. M., & Losordo, D. W. (2004). Age-dependent VEGF expression and intraneural neovascularization during regeneration of peripheral nerves. *Neurobiology of Aging, 25*(10), 1361–1368. https://doi.org/10.1016/j.neurobiolaging.2004.02.028

Powell, R., Eleftheriadou, D., Kellaway, S., & Phillips, J. B. (2021). Natural biomaterials as instructive engineered microenvironments that direct cellular function in peripheral nerve tissue engineering. *Frontiers in Bioengineering and Biotechnology, 9*, Article 674473. https://doi.org/10.3389/fbioe.2021.674473

Puras, G., Mashal, M., Zárate, J., Agirre, M., Ojeda, E., Grijalvo, S., Eritja, R., Diaz-Tahoces, A., Martínez Navarrete, G., Avilés-Trigueros, M., Fernández, E., & Pedraz, J. L. (2014). A novel cationic niosome formulation for gene delivery to the retina. *Journal of Controlled Release, 174*, 27–36. https://doi.org/10.1016/j.jconrel.2013.11.004

Qi, S. H., Xie, J.-L., Pan, S., Xu, Y.-B., Li, T.-Z., Tang, J.-M., Liu, X.-S., Shu, B., & Liu, P. (2008). Effects of asiaticoside on the expression of smad protein by normal skin fibroblasts and hypertrophic scar fibroblasts. *Clinical and Experimental Dermatology, 33*(2), 171–175. https://doi.org/10.1111/j.1365-2230.2007.02636.x

Qi, Z., Zhang, T., Kong, W., Fu, C., Chang, Y., Li, H., Yang, X., & Pan, S. (2022). A dual-drug enhanced injectable hydrogel incorporated with neural stem cells for combination therapy in spinal cord injury. *Chemical Engineering Journal, 427*, Article 130906. https://doi.org/10.1016/j.cej.2021.130906

Rahmati, M., Ehterami, A., Saberani, R., Abbaszadeh-Goudarzi, G., Rezaei Kolarijani, N., Khastar, H., Garmabi, B., & Salehi, M. (2021). Improving sciatic nerve regeneration by using alginate/chitosan hydrogel containing berberine. *Drug Delivery and Translational Research, 11*(5), 1983–1993. https://doi.org/10.1007/s13346-020-00860-y

Raimondo, T. M., Li, H., Kwee, B. J., Kinsley, S., Budina, E., Anderson, E. M., Doherty, E. J., Talbot, S. G., & Mooney, D. J. (2019). Combined delivery of VEGF and IGF-1 promotes functional innervation in mice and improves muscle transplantation in rabbits. *Biomaterials, 216*, Article 119246. https://doi.org/10.1016/j.biomaterials.2019.119246

Ranjbar-Mohammadi, M., Prabhakaran, M. P., Bahrami, S. H., & Ramakrishna, S. (2016). Gum tragacanth/poly(l -lactic acid) nanofibrous scaffolds for application in regeneration of peripheral nerve damage. *Carbohydrate Polymers, 140*, 104–112. https://doi.org/10.1016/j.carbpol.2015.12.012

Raza, C., Riaz, H. A., Anjum, R., & Shakeel, N.u. A. (2020). Repair strategies for injured peripheral nerve: Review. *Life Sciences, 243*, Article 117308. https://doi.org/10.1016/j.lfs.2020.117308

Ren, Y.-J., Zhang, H., Huang, H., Wang, X.-M., Zhou, Z.-Y., Cui, F.-Z., & An, Y.-H. (2009). In vitro behavior of neural stem cells in response to different chemical functional groups. *Biomaterials, 30*(6), 1036–1044. https://doi.org/10.1016/j.biomaterials.2008.10.028

Rickett, T. A., Amoozgar, Z., Tuchek, C. A., Park, J., Yeo, Y., & Shi, R. (2011). Rapidly photo-cross-linkable chitosan hydrogel for peripheral neurosurgeries. *Biomacromolecules, 12*(1), 57–65. https://doi.org/10.1021/bm101004r

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*(7), 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001

Roca, F. G., Santos, L. G., Roig, M. M., Medina, L. M., Martínez-Ramos, C., & Pradas, M. M. (2022). Novel tissue-engineered multimodular hyaluronic acid-polylactic acid conduits for the regeneration of sciatic nerve defect. *Biomedicines, 10*(5), 963. https://doi.org/10.3390/biomedicines10050963

Rosales-Cortés, M., Peregrina-Sandoval, J., Bañuelos-Pineda, J., Sarabia-Estrada, R., Gómez-Rodiles, C. C., Albarrán-Rodríguez, E., Zaitseva, G. P., & Pita-López, M. L. (2003). Immunological study of a chitosan prosthesis in the sciatic nerve regeneration of the axotomized dog. *Journal of Biomaterials Applications, 18*(1), 15–23. https://doi.org/10.1177/0885328203018001002

Rosén, B., Lundborg, G., Dahlin, L. B., Holmberg, J., & Karlson, B. (1994). Nerve repair: Correlation of restitution of functional sensibility with specific cognitive capacities. *Journal of Hand Surgery, 19*(4), 452–458. https://doi.org/10.1016/0266-7681(94)90209-7

Ruijs, A. C. J., Jaquet, J.-B., Kalmijn, S., Giele, H., & Hovius, S. E. R. (2005). Median and ulnar nerve injuries: A meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair. *Plastic and Reconstructive Surgery, 116*(2), 484–494. https://doi.org/10.1097/01.prs.0000172896.86594.07

Ruiz, I. M., Vilariño-Feltrer, G., Mnatsakanyan, H., Vallés-Lluch, A., & Monleón Pradas, M. (2021). Development and evaluation of hyaluronan nanocomposite conduits for neural tissue regeneration. *Journal of Biomaterials Science, Polymer Edition, 32*(17), 2227–2245. https://doi.org/10.1080/09205063.2021.1963930

Salehi, M., Bagher, Z., Kamrava, S. K., Ehterami, A., Alizadeh, R., Farhadi, M., Falah, M., & Komeili, A. (2019). Alginate/chitosan hydrogel containing olfactory ectomesenchymal stem cells for sciatic nerve tissue engineering. *Journal of Cellular Physiology, 234*(9), 15357–15368. https://doi.org/10.1002/jcp.28183

Sallam, A. A., El-Deeb, M. S., & Imam, M. A. (2017). Nerve transfer versus nerve graft for reconstruction of high ulnar nerve injuries. *The Journal of Hand Surgery, 42*(4), 265–273. https://doi.org/10.1016/j.jhsa.2017.01.027

Samadian, H., Maleki, H., Fathollahi, A., Salehi, M., Gholizadeh, S., Derakhshankhah, H., Allahyari, Z., & Jaymand, M. (2020). Naturally occurring biological macromolecules-based hydrogels: Potential biomaterials for peripheral nerve regeneration. *International Journal of Biological Macromolecules, 154*, 795–817. https://doi.org/10.1016/j.ijbiomac.2020.03.155

Sampath, U., Ching, Y., Chuah, C., Sabariah, J., & Lin, P.-C. (2016). Fabrication of porous materials from natural/synthetic biopolymers and their composites. *Materials, 9*(12), 991. https://doi.org/10.3390/ma9120991

Sandoval-Castellanos, A. M., Claeyssens, F., & Haycock, J. W. (2021). Bioactive 3D scaffolds for the delivery of NGF and BDNF to improve nerve regeneration. *Frontiers in Materials, 8*, Article 734683. https://doi.org/10.3389/fmats.2021.734683

Santos, A. R., Wada, M. L. F., Langone, F., & Oliveira, A. L. R. (2000). Differential Schwann cell migration in adult and old mice: An in vitro study. *Brain Research, 881*(1), 73–76. https://doi.org/10.1016/S0006-8993(00)02815-8

Sarker, M. D., Naghieh, S., McInnes, A. D., Schreyer, D. J., & Chen, X. (2018). Regeneration of peripheral nerves by nerve guidance conduits: Influence of design, biopolymers, cells, growth factors, and physical stimuli. *Progress in Neurobiology, 171*, 125–150. https://doi.org/10.1016/j.pneurobio.2018.07.002

Sasaki, R., Aoki, S., Yamato, M., Uchiyama, H., Wada, K., Ogiuchi, H., Okano, T., & Ando, T. (2011). PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. *Journal of Tissue Engineering and Regenerative Medicine, 5*(10), 823–830. https://doi.org/10.1002/term.387

Scaccini, L., Mezzena, R., De Masi, A., Gagliardi, M., Gambarotta, G., Cecchini, M., & Tonazzini, I. (2021). Chitosan micro-grooved membranes with increased asymmetry for the improvement of the schwann cell response in nerve regeneration. *International Journal of Molecular Sciences, 22*(15), 7901. https://doi.org/10.3390/ijms22157901

Schultheis, A. M., Bos, M., Schmitz, K., Wilsberg, L., Binot, E., Wolf, J., Büttner, R., & Schildhaus, H.-U. (2014). Fibroblast growth factor receptor 1 (FGFR1) amplification is a potential therapeutic target in small-cell lung cancer. *Modern Pathology, 27*(2), 214–221. https://doi.org/10.1038/modpathol.2013.141

Shen, X., Qu, F., Pei, Y., Lei, S., Xia, S., Liang, J., Li, S., Sun, X., & Liu, L. (2022). Repairing sciatic nerve injury with self-assembling peptide nanofiber scaffold-containing chitosan conduit. *Frontiers in Neurology, 13*, Article 867711. https://doi.org/10.3389/fneur.2022.867711

Siemionow, M., Bozkurt, M., & Zor, F. (2010). Regeneration and repair of peripheral nerves with different biomaterials: Review. *Microsurgery, 30*(7), 574–588. https://doi.org/10.1002/micr.20799

Solomevich, S. O., Dmitruk, E. I., Aharodnikau, U. E., Salamevich, D. A., Bychkovsky, P. M., Golub, N. V., & Yurkshtovich, T. L. (2021). Characterization of H3PO4/HNO3–NANO2 oxidized bacterial cellulose and its usage as a carrier for the controlled release of cephalexin. *Cellulose, 28*(14), 9425–9439. https://doi.org/10.1007/s10570-021-04130-z

Solomevich, S. O., Dmitruk, E. I., Bychkovsky, P. M., Salamevich, D. A., Kuchuk, S. V., & Yurkshtovich, T. L. (2021). Biodegradable polyelectrolyte complexes of chitosan and partially crosslinked dextran phosphate with potential for biomedical applications. *International Journal of Biological Macromolecules, 169*, 500–512. https://doi.org/10.1016/j.ijbiomac.2020.12.200

Song, S., McConnell, K. W., Amores, D., Levinson, A., Vogel, H., Quarta, M., Rando, T. A., & George, P. M. (2021). Electrical stimulation of human neural stem cells via conductive polymer nerve guides enhances peripheral nerve recovery. *Biomaterials, 275*, Article 120982. https://doi.org/10.1016/j.biomaterials.2021.120982

Stratton, J. A., Eaton, S., Rosin, N. L., Jawad, S., Holmes, A., Yoon, G., Midha, R., & Biernaskie, J. (2020). Macrophages and associated ligands in the aged injured nerve: A defective dynamic that contributes to reduced axonal regrowth. *Frontiers in Aging Neuroscience, 12*, 174. https://doi.org/10.3389/fnagi.2020.00174

Subramanian, A., Krishnan, U. M., & Sethuraman, S. (2009). Development of biomaterial scaffold for nerve tissue engineering: Biomaterial mediated neural regeneration. *Journal of Biomedical Science, 16*(1), 108. https://doi.org/10.1186/1423-0127-16-108

Sufan, W., Suzuki, Y., Tanihara, M., Ohnishi, K., Suzuki, K., Endo, K., & Nishimura, Y. (2001). Sciatic nerve regeneration through alginate with tubulation or nontubulation repair in cat. *Journal of Neurotrauma, 18*(3), 329–338. https://doi.org/10.1089/08977150151070991

Sumarwoto, T., Suroto, H., Mahyudin, F., Utomo, D. N., Romaniyanto, Tinduh, D., Notobroto, H. B., Sigit Prakoeswa, C. R., Rantam, F. A., & Rhatomy, S. (2021). Role of adipose mesenchymal stem cells and secretome in peripheral nerve regeneration. *Annals of Medicine and Surgery, 67*, Article 102482. https://doi.org/10.1016/j.amsu.2021.102482

Suri, S., & Schmidt, C. E. (2010). Cell-laden hydrogel constructs of hyaluronic acid, collagen, and laminin for neural tissue engineering. *Tissue Engineering Part A, 16*(5), 1703–1716. https://doi.org/10.1089/ten.tea.2009.0381

Svenson, S. (2006). *Polymeric drug delivery I: Particulate drug carriers* (Vol. 923). American Chemical Society. https://doi.org/10.1021/bk-2006-0923

Szarek, D., Marycz, K., Bednarz, P., Tabakow, P., Jarmundowicz, W., & Laska, J. (2013). Influence of calcium alginate on peripheral nerve regeneration: In vivo study: Influence of CaAlg on peripheral nerve regeneration. *Biotechnology and Applied Biochemistry, 60*(6), 547–556. https://doi.org/10.1002/bab.1096

Tajdaran, K., Chan, K., Gordon, T., & Borschel, G. H. (2019). Matrices, scaffolds, and carriers for protein and molecule delivery in peripheral nerve regeneration. *Experimental Neurology, 319*, Article 112817. https://doi.org/10.1016/j.expneurol.2018.08.014

Tang, Y., Zheng, X., Ying, T., Yuan, Y., & Li, S. (2015). Nimodipine-mediated re-myelination after facial nerve crush injury in rats. *Journal of Clinical Neuroscience, 22*(10), 1661–1668. https://doi.org/10.1016/j.jocn.2015.03.048

Tayler, I. M., & Stowers, R. S. (2021). Engineering hydrogels for personalized disease modeling and regenerative medicine. *Acta Biomaterialia, 132*, 4–22. https://doi.org/10.1016/j.actbio.2021.04.020

Tudu, M., & Samanta, A. (2023). Natural polysaccharides: Chemical properties and application in pharmaceutical formulations. *European Polymer Journal, 184*, Article 111801. https://doi.org/10.1016/j.eurpolymj.2022.111801

Tung, T. (2015). Clinical strategies to enhance nerve regeneration. *Neural Regeneration Research, 10*(1), 22. https://doi.org/10.4103/1673-5374.150641

Ulery, B. D., Nair, L. S., & Laurencin, C. T. (2011). Biomedical applications of biodegradable polymers. *Journal of Polymer Science Part B: Polymer Physics, 49*(12), 832–864. https://doi.org/10.1002/polb.22259

Ullah, A., & Lim, S. I. (2022). Bioinspired tunable hydrogels: An update on methods of preparation, classification, and biomedical and therapeutic applications. *International Journal of Pharmaceutics, 612*, Article 121368. https://doi.org/10.1016/j.ijpharm.2021.121368

Verdú, E., Ceballos, D., Vilches, J. J., & Navarro, X. (2000). Influence of aging on peripheral nerve function and regeneration. *Journal of the Peripheral Nervous System, 5*(4), 191–208. https://doi.org/10.1111/j.1529-8027.2000.00026.x

Vilariño-Feltrer, G., Martínez-Ramos, C., Monleón-de-la-Fuente, A., Vallés-Lluch, A., Moratal, D., Barcia Albacar, J. A., & Monleón Pradas, M. (2016). Schwann-cell cylinders grown inside hyaluronic-acid tubular scaffolds with gradient porosity. *Acta Biomaterialia, 30*, 199–211. https://doi.org/10.1016/j.actbio.2015.10.040

Vögelin, E., Baker, J. M., Gates, J., Dixit, V., Constantinescu, M. A., & Jones, N. F. (2006). Effects of local continuous release of brain derived neurotrophic factor (BDNF) on peripheral nerve regeneration in a rat model. *Experimental Neurology, 199*(2), 348–353. https://doi.org/10.1016/j.expneurol.2005.12.029

Vurat, M., & Parmaksiz, M. (2021). Mechanical evaluation of 3D printed Poly (Caprolactone) scaffolds: Effect of molecular weight. *International Journal of 3D Printing Technologies and Digital Industry.*. https://doi.org/10.46519/ij3dptdi.966777

Wach, R. A., Adamus-Wlodarczyk, A., Olejnik, A. K., Matusiak, M., Tranquilan-Aranilla, C., & Ulanski, P. (2020). Carboxymethylchitosan hydrogel manufactured by radiation-induced crosslinking as potential nerve regeneration guide scaffold. *Reactive and Functional Polymers, 152*, Article 104588. https://doi.org/10.1016/j.reactfunctpolym.2020.104588

Wang, A., Ao, Q., He, Q., Gong, X., Gong, K., Gong, Y., Zhao, N., & Zhang, X. (2006). Neural stem cell affinity of chitosan and feasibility of chitosan-based porous conduits as scaffolds for nerve tissue engineering*. *Tsinghua Science & Technology, 11*(4), 415–420. https://doi.org/10.1016/S1007-0214(06)70210-3

Wang, C., Lu, C., Peng, J., Hu, C., & Wang, Y. (2017). Roles of neural stem cells in the repair of peripheral nerve injury. *Neural Regeneration Research, 12*(12), 2106. https://doi.org/10.4103/1673-5374.221171

Wang, H., Sobral, M. C., Snyder, T., Brudno, Y., Gorantla, V. S., & Mooney, D. J. (2020). Clickable, acid labile immunosuppressive prodrugs for in vivo targeting. *Biomaterials Science, 8*(1), 266–277. https://doi.org/10.1039/C9BM01487J

Wang, M.-D., Zhai, P., Schreyer, D. J., Zheng, R.-S., Sun, X.-D., Cui, F.-Z., & Chen, X.-B. (2013). Novel crosslinked alginate/hyaluronic acid hydrogels for nerve tissue engineering. *Frontiers of Materials Science, 7*(3), 269–284. https://doi.org/10.1007/s11706-013-0211-y

Wang, Q., Yang, X., Ren, M., Hu, Y., Chen, Q., Xing, L., Meng, C., & Liu, T. (2012). Effect of chitosan/type I collagen/gelatin composites in biocompatibility and nerve repair. *Neural Regeneration Research, 7*, 1179–1184. https://doi.org/10.3969/j.issn.1673-5374.2012.15.009

Wang, R., Jiang, G., Aharodnikau, U. E., Yunusov, K., Sun, Y., Liu, T., & Solomevich, S. O. (2022). Recent advances in polymer microneedles for drug transdermal delivery: Design strategies and applications. *Macromolecular Rapid Communications, 43*(8), Article 2200037. https://doi.org/10.1002/marc.202200037

Wang, S., Guan, S., Zhu, Z., Li, W., Liu, T., & Ma, X. (2017). Hyaluronic acid doped-poly (3,4-ethylenedioxythiophene)/chitosan/gelatin (PEDOT-HA/Cs/Gel) porous conductive scaffold for nerve regeneration. *Materials Science and Engineering: C, 71*, 308–316. https://doi.org/10.1016/j.msec.2016.10.029

Wang, Y., Li, Y., Huang, Z., Yang, B., Mu, N., Yang, Z., Deng, M., Liao, X., Yin, G., Nie, Y., Chen, T., & Ma, H. (2022). Gene delivery of chitosan-graft-polyethyleneimine vectors loaded on scaffolds for nerve regeneration. *Carbohydrate Polymers, 290*, Article 119499. https://doi.org/10.1016/j.carbpol.2022.119499

Wang, Y., Zhu, L., Wei, L., Zhou, Y., Yang, Y., & Zhang, L. (2023). A bio-orthogonally functionalized chitosan scaffold with esterase-activatable release for nerve regeneration. *International Journal of Biological Macromolecules, 229*, 146–157. https://doi.org/10.1016/j.ijbiomac.2022.12.113

Wangensteen, K. J., & Kalliainen, L. K. (2010). Collagen tube conduits in peripheral nerve repair: A retrospective analysis. *The Hand, 5*(3), 273–277. https://doi.org/10.1007/s11552-009-9245-0

Way, C., Wu, D. Y., Cram, D., Dean, K., & Palombo, E. (2013). Processing stability and biodegradation of polylactic acid (PLA) composites reinforced with cotton linters or maple hardwood fibres. *Journal of Polymers and the Environment, 21*(1), 54–70. https://doi.org/10.1007/s10924-012-0462-1

Weber, R. A., Breidenbach, W. C., Brown, R. E., Jabaley, M. E., & Mass, D. P. (2000). A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plastic and Reconstructive Surgery, 106*(5), 1036–1045. https://doi.org/10.1097/00006534-200010000-00013

Wei, Z., Hong, F. F., Cao, Z., Zhao, S.-Y., & Chen, L. (2021). In situ fabrication of nerve growth factor encapsulated chitosan nanoparticles in oxidized bacterial nanocellulose for rat sciatic nerve regeneration. *Biomacromolecules, 22*(12), 4988–4999. https://doi.org/10.1021/acs.biomac.1c00947

Wei, Z., Zhao, J., Chen, Y. M., Zhang, P., & Zhang, Q. (2016). Self-healing polysaccharide-based hydrogels as injectable carriers for neural stem cells. *Scientific Reports, 6*(1), 37841. https://doi.org/10.1038/srep37841

Wen, Y., & Oh, J. K. (2014). Recent strategies to develop polysaccharide-based nanomaterials for biomedical applications. *Macromolecular Rapid Communications*. https://doi.org/10.1002/marc.201400406. n/a-n/a.

Whitehead, T. J., Avila, C. O. C., & Sundararaghavan, H. G. (2018). Combining growth factor releasing microspheres within aligned nanofibers enhances neurite outgrowth. *Journal of Biomedical Materials Research Part A, 106*(1), 17–25. https://doi.org/10.1002/jbm.a.36204

Willows, J. W., Gunsch, G., Paradie, E., Blaszkiewicz, M., Tonniges, J. R., Pino, M. F., Smith, S. R., Sparks, L. M., & Townsend, K. L. (2023). Schwann cells contribute to demyelinating diabetic neuropathy and nerve terminal structures in white adipose tissue. *IScience, 26*(3), Article 106189. https://doi.org/10.1016/j.isci.2023.106189

Woo, J., Piao, M., Li, D., Ryu, D., Choi, J., Kim, J., Kim, J., Jin, S., Kim, D., & Lyoo, W. (2007). Development of cyclosporin A-loaded hyaluronic microsphere with enhanced oral bioavailability. *International Journal of Pharmaceutics, 345*(1–2), 134–141. https://doi.org/10.1016/j.ijpharm.2007.08.050

Wrobel, S., Serra, S. C., Ribeiro-Samy, S., Sousa, N., Heimann, C., Barwig, C., Grothe, C., Salgado, A. J., & Haastert-Talini, K. (2014). In vitro evaluation of cell-seeded

chitosan films for peripheral nerve tissue engineering. *Tissue Engineering Part A, 20* (17–18), 2339–2349. https://doi.org/10.1089/ten.tea.2013.0621

Wu, D., Zhu, L., Li, Y., Zhang, X., Xu, S., Yang, G., & Delair, T. (2020). Chitosan-based colloidal polyelectrolyte complexes for drug delivery: A review. *Carbohydrate Polymers, 238*, Article 116126. https://doi.org/10.1016/j.carbpol.2020.116126

Wu, S., Kuss, M., Qi, D., Hong, J., Wang, H.-J., Zhang, W., Chen, S., Ni, S., & Duan, B. (2019). Development of cryogel-based guidance conduit for peripheral nerve regeneration. *ACS Applied Bio Materials, 2*(11), 4864–4871. https://doi.org/10.1021/acsabm.9b00626

Wu, X., Xin, M., Chen, H., Yang, L., & Jiang, H. (2010). Novel mucoadhesive polysaccharide isolated from Bletilla striata improves the intraocular penetration and efficacy of levofloxacin in the topical treatment of experimental bacterial keratitis. *Journal of Pharmacy and Pharmacology, 62*(9), 1152–1157. https://doi.org/10.1111/j.2042-7158.2010.01137.x

Xia, S., Zhang, M., Li, M., Song, X., Chen, D., Zhu, M., Zheng, H., & Chen, S. (2022). The bridging effect of controlled-release glial cell-derived neurotrophic factor microcapsules within nerve conduits on rat facial nerve regeneration. *Disease Markers, 2022*, 1–7. https://doi.org/10.1155/2022/8942985

Xu, C., Guan, S., Wang, S., Gong, W., Liu, T., Ma, X., & Sun, C. (2018). Biodegradable and electroconductive poly(3,4-ethylenedioxythiophene)/carboxymethyl chitosan hydrogels for neural tissue engineering. *Materials Science and Engineering: C, 84*, 32–43. https://doi.org/10.1016/j.msec.2017.11.032

Xu, H., Yan, Y., & Li, S. (2011). PDLLA/chondroitin sulfate/chitosan/NGF conduits for peripheral nerve regeneration. *Biomaterials, 32*(20), 4506–4516. https://doi.org/10.1016/j.biomaterials.2011.02.023

Xu, H., Zhang, L., Bao, Y., Yan, X., Yin, Y., Li, Y., Wang, X., Huang, Z., & Xu, P. (2017). Preparation and characterization of injectable chitosan–hyaluronic acid hydrogels for nerve growth factor sustained release. *Journal of Bioactive and Compatible Polymers, 32*(2), 146–162. https://doi.org/10.1177/0883911516662068

Yang, D. P., Kim, J., Syed, N., Tung, Y.-J., Bhaskaran, A., Mindos, T., Mirsky, R., Jessen, K. R., Maurel, P., Parkinson, D. B., & Kim, H. A. (2012). P38 MAPK activation promotes denervated schwann cell phenotype and functions as a negative regulator of Schwann cell differentiation and myelination. *Journal of Neuroscience, 32*(21), 7158–7168. https://doi.org/10.1523/JNEUROSCI.5812-11.2012

Yannas, I. V., & Hill, B. J. (2004). Selection of biomaterials for peripheral nerve regeneration using data from the nerve chamber model. *Biomaterials, 25*(9), 1593–1600. https://doi.org/10.1016/S0142-9612(03)00505-2

Yuan, Y., Zhang, P., Yang, Y., Wang, X., & Gu, X. (2004). The interaction of schwann cells with chitosan membranes and fibers in vitro. *Biomaterials, 25*(18), 4273–4278. https://doi.org/10.1016/j.biomaterials.2003.11.029

Yurkshtovich, T. L., Golub, N. V., Solomevich, S. O., Kosterova, R. I., Yurkshtovich, N. K., Alinovskaya, V. A., & Bychkovsky, P. M. (2019). Acid-Base properties and biological activity of starch phosphate microgels. *Colloid Journal, 81*(3), 329–336. https://doi.org/10.1134/S1061933X19030165

Zarrintaj, P., Zangene, E., Manouchehri, S., Amirabad, L. M., Baheiraei, N., Hadjighasem, M. R., Farokhi, M., Ganjali, M. R., Walker, B. W., Saeb, M. R., Mozafari, M., Thomas, S., & Annabi, N. (2020). Conductive biomaterials as nerve conduits: Recent advances and future challenges. *Applied Materials Today, 20*, Article 100784. https://doi.org/10.1016/j.apmt.2020.100784

Zeng, W., Chang, Z., Liu, Z., Zhu, L., Wang, M., Hao, D., & He, B. (2020). Controlled delivery of bioactive BDNF for potential treatment of peripheral nerve injury. *Polymer Degradation and Stability, 181*, Article 109296. https://doi.org/10.1016/j.polymdegradstab. 2020.109296

Zeng, W., Li, Q., Wan, T., Liu, C., Pan, W., Wu, Z., Zhang, G., Pan, J., Qin, M., Lin, Y., Wu, C., & Xu, Y. (2016). Hyaluronic acid-coated niosomes facilitate tacrolimus ocular delivery: Mucoadhesion, precorneal retention, aqueous humor pharmacokinetics, and transcorneal permeability. *Colloids and Surfaces B: Biointerfaces, 141*, 28–35. https://doi.org/10.1016/j.colsurfb.2016.01.014

Zhang, P., Xue, F., Kou, Y., Fu, Z., Zhang, D., Zhang, H., & Jiang, B. (2008). The experimental study of absorbable chitin conduit for bridging peripheral nerve defect with nerve fasciculu in rats. *Artificial Cells, Blood Substitutes, and Biotechnology, 36*(4), 360–371. https://doi.org/10.1080/10731190802239040

Zhang, Y., Jiang, Z., Wang, Y., Xia, L., Yu, S., Li, H., Zhang, W., Liu, W., Shao, K., & Han, B. (2022). Nerve regeneration effect of a composite bioactive carboxymethyl chitosan-based nerve conduit with a radial texture. *Molecules, 27*(24), 9039. https://doi.org/10.3390/molecules27249039

Zhao, D., Yu, S., Sun, B., Gao, S., Guo, S., & Zhao, K. (2018). Biomedical applications of chitosan and its derivative nanoparticles. *Polymers, 10*(4), 462. https://doi.org/10.3390/polym10040462

Zhao, J., Zheng, X., Fu, C., Qu, W., Wei, G., & Zhang, W. (2014). FK506-loaded chitosan conduit promotes the regeneration of injured sciatic nerves in the rat through the upregulation of brain-derived neurotrophic factor and TrkB. *Journal of the Neurological Sciences, 344*(1–2), 20–26. https://doi.org/10.1016/j.jns.2014.06.005

Zhao, Y.-N., Wu, P., Zhao, Z.-Y., Chen, F.-X., Xiao, A., Yue, Z.-Y., Han, X.-W., Zheng, Y., & Chen, Y. (2023). Electrodeposition of chitosan/graphene oxide conduit to enhance peripheral nerve regeneration. *Neural Regeneration Research, 18*(1), 207. https://doi.org/10.4103/1673-5374.344836

Zheng, F., Li, R., He, Q., Koral, K., Tao, J., Fan, L., Xiang, R., Ma, J., Wang, N., Yin, Y., Huang, Z., Xu, P., & Xu, H. (2020). The electrostimulation and scar inhibition effect of chitosan/oxidized hydroxyethyl cellulose/reduced graphene oxide/asiaticoside liposome based hydrogel on peripheral nerve regeneration in vitro. *Materials Science and Engineering: C, 109*, Article 110560. https://doi.org/10.1016/j.msec.2019.110560

Zhuo, F., Abourehab, M. A. S., & Hussain, Z. (2018). Hyaluronic acid decorated tacrolimus-loaded nanoparticles: Efficient approach to maximize dermal targeting and anti-dermatitis efficacy. *Carbohydrate Polymers, 197*, 478–489. https://doi.org/10.1016/j.carbpol.2018.06.023