The use of chitosan-based scaffolds to enhance regeneration in the nervous system.

The Use of Chitosan-Based ScaffoldstoEnhanceRegeneration in the Nervous System

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Abstract Various biomaterials have been proposed to build up scaffolds for promoting neural repair. Among them, chitosan, a derivative of chitin, has been raising more and more interestamongbasicandclinicalscientists.Anumberofstudieswithneuronalandglial cellcultures haveshownthat thisbiomaterial has biomimetic properties,which make it a good candidate for developing innovative devices for neural repair. Yet, in vivo experimental studies have shown that chitosan can be successfully used to create scaffolds that promote regeneration both in the central and in the peripheral nervous system. In this review, the relevant literature on the use of chitosan in the nervous tissue, either alone or in combination with other components, is overviewed. Altogether, the promising in vitro and in vivo experimental results make it possible to foresee that time for clinicaltrialswithchitosan-basednerveregeneration-promotingdevicesisapproaching quickly.

1. INTRODUCTION

Chitin and its main derivative, chitosan, are becoming increasingly relevant among the novel families of biomacromolecules because of their wide potential application in biomedicine and tissue engineering (Domard & Domard, 2002; Khor & Lim, 2003; Kumar, 2002; Singh & Ray, 2000; Suh & Matthew, 2000). Chitin and chitosan represent a family of linear polysaccharides made up of b(1–4)-linked N-acetyl D-glucosamine and D-glucosamine units (Domard &Domard, 2002; Muzzarelli, 1977).Dependingon the processing method used to derive the biopolymer, glucosamine units may be randomly or block distributed throughout the biopolymer chain. Chitin is primarily obtained from the exoskeleton of arthropods, shellfish such as crabs and shrimps, cuticles of insects, and cell wall of fungi (Muzzarelli, 1977). Based on the chain organization in sheets or stacks, chitin can be classified into three crystalline isoforms: a, b, and g. The structure of a-chitin has been investigated more extensively than that of either the b or the g form, because it is the most common polymorphic form. Commercial chitins are usually isolated from marine crustaceans, because of large amount of waste derived from food processing. In this case, a-chitin is produced while squid pens are used to produce b-chitin (Aranaz et al., 2009). Crustacean shells consist of proteins, calcium carbonate, chitin, and contain pigments such as carotenoids. Chitin is extracted by acid treatment to dissolve the calcium carbonate followed by alkaline extraction to dissolve the proteins and by a depigmentation step to obtain a colorless product (Aranaz et al., 2009; Muzzarelli, 1977). Chitosan, on the other hand, although occurring in some fungi

(Mucoraceae), is produced industrially by cleavage of the N-acetyl groups of the chitin N-acetyl D-glucosamine residues (Muzzarelli, 1977).

Chitosan is prepared by alkaline hydrolysis of acetamide groups of chitin. High temperature (100 °C) combined with strong aqueous alkali treatments are used to deacetylate chitin (N-acetylation degree lower than 30%), in order to obtain chitosan. Two different methods of preparing chitosan from chitin with varying degrees of acetylation are known: heterogeneous deacetylation of solid chitin and homogeneous deacetylation of preswollen chitin under vacuum in an aqueous medium (Aranaz et al., 2009; Muzzarelli, 1977). The main limitations in the use of chitosan in several applications are its high viscosity and low solubility at neutral pH. Different experimental variables should be taken into account when working with chitosan solutions such as the nature of the salt counterion, length of polymer chain, molecular weight (Mw), pH, ionic strength, the addition of a nonaqueous solvent, and the degree of N-acetylation (Aranaz et al., 2009; Muzzarelli, 1977). Their different solubilities in dilute acids are commonly used to distinguish between chitin and chitosan. Chitosan, the soluble form, can have a degree of acetylation between 0% and about 60%, the upper limit depending on parameters such as processing conditions, molar mass, and solvent characteristics (Aiba, 1992). Thanks to the protonation of free amine groups present along the chitosan chain; this macromolecule can be dissolved in diluted aqueous acidic solvents, rendering the corresponding chitosan salt in solution. Degradation rate can be tuned based on its degree of deacetylation (DD), whereas fully deacetylated (DD¼100%) chitosan is nondegradable (Freier, Koh, Kazazian, & Shoichet, 2005; Tomihata & Ikada, 1997) and partially deacetylated (DD¼70%) chitosan is fully degradable (Tomihata & Ikada, 1997). Chitin and chitosan are interesting candidates for use in the medical and pharmaceutical applications because they have positive properties such as biocompatibility, biodegradability, and nontoxicity that make them suitable in biomedical field (Khor & Lim, 2003). Moreover, other properties such as analgesic effect, antitumor activity, hemostatic, anticholesterolemic, antimicrobial, permeation enhancing effect, and antioxidant properties have also been reported (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Several chitosan products have been approved by the Food and Drug Administration. Furthermore, chitosan has been used to generate laseractivated film surgical adhesive (SurgiLux) that can be very useful as an alternative to microsurgery for peripheral nerve reconstruction (Foster & Karsten, 2012). SurgiLux has been tested in vitro and in vivo on different tissues including nerve, intestine, dura mater, and cornea, demonstrating a good biocompatibility (Foster & Karsten, 2012).

The American Society of Testing Materials (ASTM F04 division IV) is making efforts to establish standard guidelines for tissue engineered medical products (ASTM, 2001). The F2103 guide covers the evaluation of chitosan salts suitable for use in medical applications. Moreover, chitosan hydrochloride (a derivative of chitosan) has been included in the European Pharmacopeia in 2002 (Pharmacopeia, 2002). In order to be approved as a biomedical material, sterility is an important issue to resolve. Chitosan products intended for parenteral administration andthosein contactwithserousfluids, forexample,wounds,haveto besterilized before use. Common methods for the sterilization include exposure to dry heat, saturated steam, ethylene oxide, or g radiation. Before using any of these methods for chitosan product sterilization, their effects on polymer

properties and end performance have to be tested, as they can cause irreversible damage to the morphological, physical, mechanical, and biological characteristics. Dry heat sterilization method resulted in lower aqueous solubility for chitosan and in insolubility in acidic aqueous media (Lim, Khor, & Ling, 1999). Saturated steam and g irradiation caused an acceleration in the rate and extent of chitosan chain scission events, respectively. The use of 70% ethanol, as a sterilizing agent, is a suitable method as it did not alter chitosan-membrane characteristic; however, it is limited to small-scale applications. Ethylene oxide is a simple technique to be used for sterilization of industrially produced chitosan membrane, preserving chitosan-membrane morphology, percentage of strain at break, and in vitro cytotoxicity to Vero cells. Moreover, this method can be used for industrial sterilization of chitosan membrane (Marreco, da Luz Moreira, Genari, & Moraes, 2004). The long-term storage may have effects and implications on the integrity of chitin and chitosan materials (Kam, Khor, & Lim, 1999), and further investigation is needed to optimize sterilization and storage method conditions. It has been shown that chitosan is capable of forming large phospholipid aggregates by inducing the fusion of small dipalmitoyl phosphatidylcholine bilayers, which are a major component of the plasma membrane (Pavinatto et al., 2007; Zuo et al., 2006). Thus, the use of chitosan as a "fusogen" might be more advantageous as a potential clinical tool relative to nonionic polymers (e.g., PEG or P188). Due to its high biodegradability and biocompatibility,togetherwithitsspecific interactions with components of the extracellular matrix (ECM) and growth factors, chitosan employment is growing in a variety of applications, including implantable and injectable orthopedic and periodontal lsystems,drug delivery systems, wound-healing agents, lung surfactant additives, and tissue engineering scaffolds for tissue regeneration of skin, bone, and cartilage (Drury & Mooney, 2003; Gan & Wang, 2007; Janes, Fresneau, Marazuela, Fabra, & Alonso, 2001; Madihally & Matthew, 1999; Roy, Mao, Huang, & Leong, 1999; Suh & Matthew, 2000; Ueno et al., 1999; Yuan, Zhang, Yang,Wang,&Gu,2004;Zuoetal.,2006).Yet,chitosanisaversatilematerial currentlyusedinclinicalwounddressings,primarilyforitshemostaticproperty (Gustafson, Fulkerson, Bildfell, Aguilera, & Hazzard, 2007). Another important application of chitosan is the development of drug delivery systems such as nanoparticles, hydrogels, microspheres, films, and tablets. The abundance of primary amine groups enables chitosan to be ionically or covalently coupled to various biomolecules because the amine moieties become predominantly protonated and positively charged below pH 6.5, whereas they are increasingly deprotonated at pH 6.5 and above. As a result of its cationic character, chitosan is able to react with polyanion giving rise to polyelectrolyte complexes (Acosta, Aranaz, Peniche, & Heras, 2003; Peniche, Arguelles-Monal, Peniche, & Acosta, 2003). Moreover, due to its positive charge, chitosan can interact with negative molecules such as DNA. This property has been used to prepare a nonviral vector gene delivery system (Mumper, Wang, Claspell, & Rolland, 1995). Among the different tissue organs, many studies have investigated the use of chitosan for repair, not only because of its biocompatibility, biodegradability, low toxicity, and cost but also because of its excellent potential for supporting three-dimensional organization of regenerating tissues (Evans et al., 1999; Ho et al., 2005; Ma et al., 2003; Madihally & Matthew, 1999; Novikova, Novikov, & Kellerth, 2003; Vasconcelos & Gay-Escoda, 2000). Here, we review the main chitosan-based bioengineering strategies for peripheral nerve and spinal cord injury (SCI) repair. This review has been divided into the following sections:

in the first part, we report in vitro studies on the evaluation of chitosan properties, the second and the third parts cover in vivo studies for spinal cord and peripheral nerve repairs, respectively.

2. IN VITRO EVIDENCE: CHITOSAN PROPERTIES, BIOCOMPATIBILITY, AND SURFACE MODIFICATION

2.1. Chitosan physical properties

2.1.1 Mechanical strength

Chitosan matrices have been shown to have low mechanical strength under physiological conditions (Itoh et al., 2003; Madihally & Matthew, 1999) and to be unable to maintain a predefined shape for transplantation, which has initially limited their use as nerve guidance conduits (NGC; Freier, Montenegro, Koh, & Shoichet, 2005; Itoh, Suzuki, et al., 2003; Itoh et al., 2003; Yamaguchi, Itoh, Suzuki, Osaka, & Tanaka, 2003). To improvechitosan mechanicalproperties,Ao et al.(2006) useda novel mold and thermally induced phase-separation technique with a unidirectional temperature gradient to produce chitosan conduits containing longitudinally aligned microfibers. This preparation method may allow the incorporation of therapeutic agents into the matrix for sustained release, as no toxic substances have been used (Ao et al., 2006). In vitro characterization using Neuro-2a-cells verified that the mold-based multimicrotubule chitosan conduit had suitable mechanical strength, microtubule diameter distribution, porosity, swelling, biodegradability, and nerve cell affinity, for applications in nerve tissue engineering (Ao et al., 2006). A novel method to create poroustubular chitosan scaffolds with desirable mechanical proprieties and controllable inner structure has been developed by Wang et al. (2006). Inner matrix, with multiple axially oriented macrochannels and radially interconnected micropores, was produced using acupuncture needles as mandrel during the molding process (Wang et al., 2006). In vitro characterization demonstrated that the scaffolds possessed suitable mechanical (porosity, swelling, and biodegradability) and biological (differentiated Neuro-2a cells grew along the oriented macrochannels) properties for applications in nerve tissue engineering (Wang et al., 2006). However, these scaffolds have a low mechanical strength under physiological conditions, thus limiting their applicability. In order to increase mechanical strength, chitosan conduits have been reinforced with additives (Yang et al., 2004) or cross-linked with chemical substances such as formaldehyde (Wang et al., 2005). Recently, a mold-casting/lyophilization method was used to fabricate porous chitosan nerve conduits; however, these conduits still have a low mechanical strength under physiological conditions. The porous structure of the chitosan conduit was reinforced by introducing braided chitosan fibers, leading to an increase in tensile strength (Wang et al., 2007). These conduits were permeable to molecules ranging in molecular size from 180 to 66,200 Da (Wang et al., 2007). Moreover, in vitro direct contact cytotoxicity test, using Neuro-2a cells, showed that the conduits were not cytotoxic (Wang et al., 2007). While chitosan has low mechanical strength under physiological conditions (Madihally & Matthew, 1999; Yamaguchi et al., 2003; Yang et al., 2002), chitin gels prepared by selective N-acetylation of chitosan amine groups (Hirano, Ohe, & Ono, 1976) are known to be mechanically strong (Vachoud & Domard, 2001), suggesting that they may be able to overcome the insufficient strength described for chitosan-based NGCs. Chitosan hydrogel tubes have been

fabricated from chitosan solution using acylation chemistry and mold-casting techniques followed by alkaline hydrolysis that results in chitosan tube formation, with the extent of hydrolysis controlling the resulting amine content (Freier, Montenegro, et al., 2005). Chitosan tubes resulted to be mechanically stronger to support adhesion and differentiation of primary chick dorsal root ganglion neurons and to significantly enhance neurite outgrowth (Freier, Montenegro, et al., 2005). Also, the DD affects chitosan mechanical properties, and it has been shown that the swelling index of chitosan films decreases and the elastic modulus and tensile strength increase with the increase in DD (Wenling et al., 2005). Finally, Wang et al. showed that a bilayered chitosan mesh tube, with an inner layer of oriented nanofibers and an outer layer of random nanofibers, increased the resistance to the compression force compared with the random fiber mesh tubes (Wang, Itoh, Matsuda, Ichinose, 2008; Wang et al., 2008).

2.1.2 Porosity

Porosity of a regenerative scaffold is an important factor in tissue engineering. Huang et al. described an easy method for the production of longitudinally oriented channels made of chitosan by using a lyophilizing and wire-heating process. Ni-Cu wires were used as a mandrel because of their high level of resistance (Huang, Huang, Huang, & Liu, 2005). In comparison with poly-lactic-co-glycolic acid (PLGA), the permeability and porous structure of chitosan improved its effectiveness for the nerve tissue engineering (Huang, Onyeri, Siewe, Moshfeghian, & Madihally, 2005). The employ of a weak base, to neutralize chitosan, can be used to influence the porous structure, making it more uniform (Huang, Onyeri, et al., 2005). Porosity geometry can also be controlled by a production method based on inverted colloid crystals (Kuo & Lin, 2013).

2.1.3 Chitosan biodegradability

Concerning biodegradability, chitin and chitosan are degraded in vivo by proteasespresentin allmammaltissues,suchaslysozyme, papain,andpepsin, leading to the release of nontoxic oligosaccharides of variable length which can be incorporated into glycosaminoglycans and glycoproteins (Pangburn, Trescony, & Heller, 1982). The length of the chains also affects the degradation rate (Huang, Khor, & Lim, 2004; Tomihata & Ikada, 1997; Zhang & Neau, 2001). Controlling degradation rate of chitin- and chitosan-based biopolymers is essential in drug delivery and tissue regeneration applications. The degradation rate also affects the biocompatibility as fast degradation rate results in amino sugars accumulation that may lead to inflammatory response. Chitosan samples with low DD may induce an inflammatory response, whereas chitosan samples with high DD do not because of the low degradation rate (Hirano, Tsuchida, & Nagao, 1989; Kurita, Kaji, Mori, & Nishiyama, 2000; Sashiwa, Saimoto, Shigemata, Ogawa, & Tokura, 1991). The degradation rate of chitosan can be influenced by physical parameters such as porosity, fiber diameter, blending with other polymers, or the use of cross-linking agents. Chitosan scaffolds with high porosity degrade faster than scaffolds with smaller pore diameter. Within the same range of porosity, scaffolds with smaller pore diameter degrade faster (Cunha-Reis et al., 2007). Adjusting the pH of the solution of a nerve conduit has been reported to influence degradation properties. In particular, increasing the layer numbers and overcoming the acidity-caused autoacceleration of poly-D, L-lactic acid/ chondroitin sulfate/chitosan (PDLLA/CS/CHS) nerve conduit decrease its

biodegradability rate retaining its integrity up to 3 months (Xu, Yan, Wan, & Li, 2009). The degradation kinetics is inversely related to the crystallinity degree which can be controlled by acting on the DD and on the distribution of acetyl groups. The absence of acetyl groups or their homogeneous/random distribution results in low enzymatic degradation rates (Aiba, 1992; Suh & Matthew, 2000). Chitosan nano-/microfiber meshes with a deacetylation of 78% have a faster biodegradation rate than meshes with a deacetylation of 93% and collapse over the time, causing occlusion of the tube made from these meshes (Wang, Itoh, Matsuda, Ichinose, et al., 2008). Degradation of chitosan films with very low (about 0.5%) or high (about 99.2%) acetylation is minimal over 4-week period, whereas progressive mass loss to greater than 50% has been reported for chitosan film with 30–70% acetylation (Freier, Koh, et al., 2005). Blending of chitin with other biomaterials like gelatin results in a faster degradation rate and significant loss of material compared with chitosan alone (Huang, Onyeri, et al., 2005).

Finally, the use of cross-linking agents such as hexamethylene diisocyanate (HDI), epichlorohydrin (ECH), and glutaraldehyde (GA) may also result in significant decrease in degradation rate compared with noncross-linked chitosan (Cao et al., 2005).

2.2. Chitosan biocompatibility

Biocompatibility properties of chitin and chitosan depend on the sample characteristics such as natural source, preparation method, Mw, and DD. In particular, residual proteins, in chitin and chitosan, derived from production methods could cause allergic reactions such as hypersensitivity. Biocompatibility is an important issue in the choice of a biomaterial for peripheral nerve regeneration or SCI repair. In vitro studies have shown that chitosan exerts biomimetic in the peripheral nervous system, allowing neuronal adhesion, differentiation, and growth (Cheng, Cao, et al., 2003; Yang et al., 2004). Different blends of chitosan and gelatin cross-linked with genipin allow cell adhesion and proliferation of NIH3T3 mouse fibroblasts and S5Y5 neuroblastoma cells. Cross-linked samples were found to be biocompatible in particular blends containing 8% gelatin supporting very well neuroblastoma cell adhesion and proliferation (Chiono et al., 2008). Among different blends of chitosan with polyacrylamide, ethyl acrylate, and hydroxyethyl acrylate, chitosan, poly(methyl acrylate), and 50% (w/w) blends of ethyl acrylate and hydroxyethyl acrylate were the most suitable polymers to promote in vitro cell adhesion and differentiation of neural explants from the medial ganglionic eminence and the cortical ventricular zone of embryonic rat brains (Soria et al., 2006). Polymeric biomaterial composite of chitin, chitosan, and gelatin, with a pore geometry of inverted colloidal crystals, induced pluripotent stem (iPS) cell adhesion and proliferation and has the potential to guide and accelerate differentiation of iPS cells toward a neuron phenotype (Kuo & Lin, 2013). It has been also shown that rat pheochromocytoma cell line (PC12), grown on chitosan–gelatin–fibronectin-composed films, differentiates more rapidly and extends longer neurite than on pure chitosan films (Cheng, Deng, et al., 2003). Another study reported that the blending of chitosan with polycaprolactone (PCL) increased cell viability and redistribution of actin cytoskeletal fibers of mouse embryonic fibroblasts cultured in vitro (Sarasam & Madihally, 2005).

In addition, biodegradable chitosan microgrooved polymers were successfully used to align Schwann cells (SCs) and cells of the glial cell line C6. SCs display high degree of alignment and express neurotrophic factors, like glial-derived neurotrophic factor (GDNF) and nerve growth factor (NGF; Hsu, Lu, Ni, & Su, 2007). Yuan et al. also reported that chitosan membranes and fibers have excellent neuroglial cell affinity and good biological compatibility. SCs grown on chitosan membranes displayed a spherical shape, whereas on chitosan fibers, they had an elongated morphology (Yuan et al., 2004). Yet, Wang et al. (2009) reported that a chitosan nonwoven nanofiber mesh tube with an inner layer of oriented nanofibers, produced by electrospinning method, induced alignment of immortalized adult mouse SCs (IMS32). Cells of the rat SCs line RT4-D6P2T cultured on PCL/chitosan blend nanofibrous scaffold showed higher cell proliferation in comparison with cells grown on PCL scaffolds alone and maintained their characteristic cell morphology, with spreading bipolar elongation (Prabhakaran et al., 2008). Interestingly, culturing adult rat SCs on chitosan films with low acetylation resulted in better cell spreading and proliferation (Wenling et al., 2005). Cross-linking agents may also influence the adhesion and proliferation of cultured SCs. Cao et al. (2005) showed that HDI cross-linked chitosan films enhanced the spread and the proliferation of SCs, whereas ECH and GA cross-linked films delayed cell proliferation. Electrical stimulation, through conductive polymers, can be used to enhance neurite outgrowth and peripheral nerve regeneration. Conductive polypyrrole chitosan membranes have been shown to support SC adhesion, spreading with and without electrical stimulation (Huang, Hu, et al., 2010). Interestingly, this study provides confirmation of cell biocompatibility on chitosan conductive polymers. Concerning employability of chitosan for neural repair, in vitro studies using neural stem cells (NSCs), human adipose-derived stem cells (hADSCs),neuroepithelial stemcells(NEPs),andiPS cellshave beencarried out since these cells have a great potential as a cell replacement therapy for SCI. Chitosan/collagen membrane showed low cytotoxicity supporting rat NSC (at the neurosphere level) survival, proliferation, and differentiation. In particular, cells migrate out from the neurospheres and differentiate into neurons (Yang, Mo, Duan, & Li, 2010). NSCs have been also cultured on laminin-coated chitosan channels (Guo et al., 2012).

Moreover, chitosan carrier loaded with neurotrophin 3 (NT-3) provided an ideal environment for adhesion, proliferation, and differentiation of NSCs (Yang, Duan, Mo, Qiao, & Li, 2010). NSCs seeded in fibrin scaffolds within a chitosan channel, containing PLGA microspheres releasing dibutyryl cyclic-AMP, differentiate in vitro tob-III-tubulin positive neurons providing further confirmation of chitosan scaffold biocompatibility (Kim, Zahir, Tator, & Shoichet, 2011). Yet, scaffolds made of chitin, chitosan, and gelatinwith pore geometry of inverted colloidal crystals have been successfully used to guide the differentiation of iPS cells into neurons (Kuo & Lin, 2013). Finally, hADSCs have been successfully transdifferentiated from mesenchymal into the neural lineage onto a chitosan-coated surface (Hsueh, Chiang, Wu, & Lin, 2012). hADSCs are a subset of multipotent mesenchymal stem cells with less ethical conflict and minimal invasive surgical procedure to obtain cells and can thus be tentatively proposed as agents for promoting nerve regeneration in patients. NEPs are NSCs with multipotentiality for neuronal and glial differentiation. NEPs have been reported to adhere and grow on chitosan fibers. Moreover, they

could differentiate into neurons and glia (Fang et al., 2010). This study demonstrated that chitosan fibers have good biocompatibility with NEPs.

2.3. Chitosan surface modification

Improving nerve cell affinity for chitosan is a key issue for improving its effectiveness for neural regeneration (Dhiman, Ray, & Panda, 2004; Haipeng et al., 2000; Zhu, Gao, He, Liu, & Shen, 2003; Zielinski & Aebischer, 1994). Combining chitosan with poly-L-lysine, laminin, laminin peptide, or collagen may increase cell adhesion, growth, and viability. Blending chitosan with poly-L-lysine improved PC12 cell affinity in comparison with chitosan and chitosan–collagen films as demonstrated by increasing attachment, growth, and differentiation into nerve cells. The increased cell affinity might be due to both the increased surface charge and hydrophilicity of composite materials (Mingyu et al., 2004). Another study also reported that poly-L-lysine-, collagen-, or albuminblended chitosan exhibit better nerve cell affinity, neurite outgrowth, and proliferation of PC12 and fetal mouse celebral cortex cells than original chitosan (Cheng, Cao, et al., 2003).

Thermoresponsive chitosan/glycerophosphate salt hydrogel coated with poly-D-lysine immobilized via azidoaniline photocoupling improves cell adhesion and morphology and neurite outgrowth compared with uncoated chitosan/glycerophosphatesalt hydrogel(Cromptonetal., 2007).Increasing poly-D-lysine concentration did not alter cell survival but significantly inhibited neurite outgrowth (Crompton et al., 2007). Lamininisan180-KDaglycoproteinthatplaysanimportantroleinneuronal cell adhesion, differentiation, and neurite outgrowth (Madison, da Silva, Dikkes, Sidman, & Chiu, 1987; Manthorpe et al., 1983). Two peptides of the lamin-1 molecule, namely, YIGSR (Tyr-Ile-Gly-Ser-Arg) and IKVAV (Ile-Lys-Val-Ala-Val) sequences, mediate receptor-specific neural cell adhesion and are known to promote cell adhesion and neurite outgrowth, respectively (Graf et al., 1987; Kleinman et al., 1988; Pierschbacher & Ruoslahti, 1984; Sephel, Burrous, & Kleinman, 1989; Tashiro et al., 1989). Moreover, these domains enhance SC migration. Surface modification of a biomaterial may improve its biocompatibility. Matsuda et al. developed a new biomaterial consisting of molecularly aligned chitosan with IKVAV and YIGSRpeptidesbondedcovalently.Briefly,chitosanwasthiolatedbyreacting 4-thiobutyrolactonewiththechitosanaminogroupandthiolgroupofcysteine locatedattheendofthesyntheticlamininpeptidesthatwerereactedchemically with thiolated chitosan to form chitosan-S-S-laminin peptide (Matsuda, Kobayashi, Itoh, Kataoka, & Tanaka, 2005). A novel chitosan gel has been synthesized by reaction of chitosan amine group with methacrylic anhydride, resulting in methacrylamide chitosan (Yu, Kazazian, & Shoichet, 2007). Maleimide-terminated cell adhesive peptides, mi-GDPGYIGSR and mi-GQASSIKVAV, have been coupled to a thiolated form of methacrylamide chitosan, resulting in increased neuronal adhesion and neurite outgrowth (Yu et al., 2007). Synthetic surface modification methods often lead to alterations of the original material's physical proprieties. The plasma surface modification processhasbeenshowntobeabletomodifythesurfacepropertiesofabiomaterial without affecting its bulk physical properties (Yeh, Iriyama, Matsuzawa, Hanson, & Yasuda, 1988). Compared with the

conventional chemical method, the percentage of laminin incorporated on chitosan films by plasma treatment is significantly higher (Huang, Huang, Huang, & Chen, 2007). Moreover, laminin-modified chitosan membrane significantly increases SC adhesion (Huang et al., 2007). Carbon nanotube/chitosan fibers coated with laminin, via an oxygen plasma technique, allowed PC12 cell adhesion, growth, and guided oriented neurite outgrowth (Huang et al., 2011).

Gliosarcoma cells (9 L) and primary neurons have been cultured on chitosan, GA-cross-linked chitosan, GA-cross-linked chitosan–gelatin conjugate, a chitosan–gelatin mixture, chitosan coated with poly-L-lysine, chitosan coated with laminin, and chitosan coated with serum revealed that coated chitosan, especially chitosan coated with laminin, has excellent nerve cell affinity, promoted better cell adhesion, spread, and growth in comparison with cross-linked-chitosan or chitosan alone (Haipeng et al., 2000). In conclusion, it was shown that chitosan precoated with ECM molecules, in particular laminin, improves nerve cell affinity. The ECM molecules adsorbed on the materials, and the physicochemical properties of the material improve the adhesion and spread of nerve cells on the biomaterials.

2.4. Chitosan as a tool for neurotrophic factor delivery

To enhance axonal regeneration, spatial and temporal delivery of therapeutic molecules combined with biomaterials may be helpful. Current methods for therapeutic agent delivery, such as oral and intravenous administration, are inadequate for local delivery as they have limitation of dose control, premature drug degradation, not specific action, and may lead to undesirable side-effects and/or system effects. In order to provide a system for local and sustained growth factor release, poly-lactide-co-glycide microspheres have been incorporated into chitosan guidance channels by spin-coating the interior of a chitosan channel with a chitosan solution containing microspheres minimizing the exposure of PLGA microspheres to acidic solution (Kim, Tator, & Shoichet, 2008). Alkaline phosphatase, used as a model protein to test the release and bioactivity, showed high encapsulation efficiency and bioactivity profile over a 90-day period in vitro (Kim et al., 2008). Poly-lactide-co-glycide microspheres have been physically entrapped in between two concentric tubes consisting of a chitosan inner tube and a chitin outer tube (Goraltchouk, Scanga, Morshead, & Shoichet, 2006). Bovine serum albumin (BSA), used as a model drug, was released up to 84 days after encapsulation in the microspheres (Goraltchouk et al., 2006). Epidermal growth factor (EGF), coencapsulated with BSA, was released for 56 days with a similar profile to that of BSA and was found to be active up to 14 days (Goraltchouk et al., 2006). Moreover, microstructured polymer filaments used as a nerve implant have been successfully loaded with chitosan/siRNA nanoparticles to promote nerve regeneration and ensure local delivery of nanotherapeutics. The nanoparticles were internalized by the cells resulting in target mRNA reduction and enhanced neurite outgrowth (Mittnacht et al., 2010). Pfister et al. reported that NGF release kinetics could be regulated by embedding NGF at different radial locations within a nerve conduit. In particular, polyelectrolyte alginate/chitosan conduit was coated with poly(lactide-co-glycolide) to control the release of the embedded NGF (Pfister, Alther, Papaloizos, Merkle, & Gander, 2008). A sustained release of NGF in low nanogram concentration per day was obtained for up to 15 days in vitro (Pfister et al., 2008). Hydrogels are cross-linked polymers

characterized by high water content. They can have a possible application as growth factor-releasing systems. Biodegradable hydrogels made up of oppositely charged polysaccharide alginate and chitosan showed high water uptake (84% (w/w)) and permitted good permeation of fluorescent-labeled dextran in a molecular-weightdependent manner (Pfister, Papaloizos, Merkle, & Gander, 2007). Spatially defined patterns of NGF can be created using photochemical immobilization technique made possible by UV confocal laser pattering. NGF has been chemically immobilized on chitosan films in distinct areas or as concentration gradients remaining bioactive as demonstrated by in vitro culturing of dissociated primary neuron from rat superior cervical ganglia. When neurons are plated on a chitosan film characterized by distinct immobilized NGF-patterned areas surrounded by unmodified chitosan, remained as single spread cells in the NGF-patterned region and formed clusters resulting in lower cell survival in the unmodified chitosan areas. Moreover, the immobilized NGF induces axonal sprouting compared with the unmodified chitosan (Yu, Wosnick, & Shoichet, 2008). Finally, a recent study showed that photo-cross-linkable streptavidinmodified methacrylamide chitosan 3D hydrogel, along with the recombinant biotin–interferon-g promotes neuronal differentiation of neuronal stem/progenitor cells (NSPCs, Leipzig, Wylie, Kim, & Shoichet, 2011).

3. CHITOSAN FOR CENTRAL NERVOUS SYSTEM REPAIR

So far, the potential use of chitosan for central nervous system (CNS) nerve repair has been focused on SCI, a common outcome of traffic accidents like motor vehicle crashes, sports injuries, and trauma that may lead to life-long paralysis, for which, unfortunately, there is no effective cure. In fact, axons of adult mammals regenerate poorly and in a disorganized manner or fail to regenerate spontaneously after SCI. When nervous tissue loss occurs, different methods have been used to bridge the spinal cord gap. For example, transplantation of peripheral nerves (Bray, Villegas-Perez, Vidal-Sanz, & Aguayo, 1987; Cheng, Cao, & Olson, 1996), SCs (Bunge, 2002; Novikova, Pettersson, Brohlin, Wiberg, & Novikov, 2008; Xu, Zhang, Li, Aebischer, & Bunge, 1999), olfactory ensheathing cells (Li, Field, & Raisman, 1997; Ramon-Cueto, Plant, Avila, & Bunge, 1998), and NSCs has been used (Teng et al., 2002; Xue et al., 2012; Zheng & Cui, 2012). These studies have shown that CNS axons can regenerate in an appropriate microenvironment and injured axons can recover part of their function. However, the above-mentioned methods have limitations for clinical applications, such as damage to the donors of peripheral nerves and immunological rejection. Biomaterials are becoming increasingly popular as a potential tool for the treatment of SCI as a mean to restore the ECM at the site of injury. Various materials, both of natural and synthetic origin, have been investigated for potential applications in the spinal cord (Nomura, Tator, & Shoichet, 2006; Novikova et al., 2003; Samadikuchaksaraei, 2007; Straley, Foo, & Heilshorn, 2010). These materials can support endogenous tissue regeneration (Tysseling-Mattiace et al., 2008; Woerly, Pinet, de Robertis, Van Diep, & Bousmina, 2001), promote directed axonal regrowth (Li and Hoffman-Kim, 2008; Yoshii, Ito, Shima, Taniguchi, & Akagi, 2009), enhance cell transplant survival and integration (Itosaka et al., 2009; Teng et al., 2002), deliver drugs (Johnson, Parker, & Sakiyama-Elbert, 2009; Kang, Poon, Tator, & Shoichet, 2009; Willerth & Sakiyama-Elbert, 2007), and seal damaged dura mater (Gazzeri et al., 2009). Biomaterials designed for spinal cord repair should provoke minimal chronic inflammation and immune responses when implanted into

the body (Anderson, Rodriguez, & Chang, 2008; Williams, 2008). These responses depend not only on the intrinsic properties of the material itself but also on the form in which the material is presented, for example, implant shape (Di Vita et al., 2008), size (Kohane et al., 2006), and porosity (Ghanaati et al., 2010). In particular, it is important to monitor over time degradation kinetics and secondary product formation of biomaterials because degradation products can elicit inflammatory responses that may be different than those elicited by the implanted material. Regarding degradation kinetics, chitosan is an attractive material because of its degradation rate that can be regulated by acting on its DD. Fully deacetylated (DD¼100%) chitosan is nondegradable (Freier, Koh, et al., 2005; Tomihata & Ikada, 1997), whereas partially deacetylated (DD¼70%) is fully degradable (Kofuji, Ito, Murata, & Kawashima, 2001; Tomihata & Ikada, 1997). In recent years, chitosan, either alone or in combination with other biomaterials (Table 1.1), adhesion peptides (Table 1.2), supportive cells (Table 1.3), or growth factors (Table 1.4), has been widely used for spinal cord repair. Ex vivo and in vivo SCI models demonstrate that chitosan is able to restore compromised membrane integrity following spinal cord trauma, reduces injury-mediated production of reactive oxygen species (ROS), and restricts continuing lipid peroxidation, displaying a potent neuroprotective role even though it did not show any ROS, or acrolein, scavenging ability (Cho et al., 2010). Yet, the use of chitosan has therapeutic potential through site-specific delivery following traumatic spinal cord and head injury (Cho et al., 2010). To increase the potential for axonal regeneration and functional recovery, implantation of autograft combined with biomaterials appears to be a promisingstrategytoo.Nomura,Baladie,etal.(2008) haveshownthatintracavitary implantation of chitosan guidance channels containing peripheral nerve grafts after subacute SCI resulted in a thicker bridge containing a larger number of myelinated axons compared with chitosan channels alone. Peripheral nervefilled chitosan conduits showed an excellent biocompatibility with the adjacent neural tissue with no signs of degradation and minimal tissue reaction at 14 weeks after implantation (Nomura, Baladie, et al., 2008).

3.1. Surface modification of chitosan conduits for CNS repair

A promising strategy for facilitating nerve regeneration is the combination of biomaterials with adhesion molecules (Table 1.2), such as laminin (Cheng et al., 2007; Lemmon, Burden, Payne, Elmslie, & Hlavin, 1992), L1 (Lemmon et al., 1992), N-cadherin (Lemmon et al., 1992), and collagen (Li et al., 2009). These molecules may be positioned in the inner portion of the tube device in order to guide neurite growth. Chitosan conduits enriched with adhesion molecules have been already used in vivo with the goal of better directing the repair of damaged axons following SCI. Biodegradable porous chitosan nerve conduits, filled with semifluid type I collagen, have been developed using lyophilizing and wire-heating process (Li et al., 2009) and implanted into the injured spinal cord of a rat model. Results showed that collagen serves as a directional guide to facilitate correctly aligned axon regrowth and enhances nerve regeneration across a gap. Yet, the chitosan tube blocked the invasion of glial scar tissue into the lesion site (Li et al., 2009).

Table 1.1 Types of chitosan conduits used in spinal cord repair

Intrinsic framework

Nerve tube	Framework	Internal filler	Animal	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan	–	–	Female Sprague–Dawley rats	Laminectomy T8 vertebral level	2	Histological evaluation	Degradable polyglycolide and nondegradable expanded polytetrafluoroethylene tubes	12 months	Chitosan, in comparison with the other tested materials, does not elict immune response. At 12 months, postimplantation chitosan is not degraded	Kim et al. (2011)
Chitosan membrane	–	–	Guinea pigs	Complete transection or compression injury in the midthoracic region	–	Somatosensory evoked potential (SSEP)	Subcutaneous injection of Ringer's solution	2 weeks	Topical application of chitosan after complete transection or compression restored the conduction of nerve impulses through the length of spinal cord	Cho, Shi, and Borgens (2010)
Chitosan tube	Rat intercostal nerve from T7 to T11	Peripheral nerve graft	Female Sprague–Dawley rats	Laminectomy at T7–T9 and spinal cord compression at T8 with a 50-g clip for 1 min	8 mm length and 1.8 mm diameter	Basso–Beattie–Bresnahan (BBB) test, anterograde axonal tracing with biotin dextran amine, and histological evaluation	Empty chitosan tube	14 weeks	Chitosan tube, containing peripheral nerve graft, contains a higher number of myelinating axons compared with chitosan tube alone. SCs from the peripheral nerve graft have high myelination capacity. Chitosan tube shows excellent biocompatibility	Nomura et al. (2008)

Table 1.2 Modifications to chitosan conduits surface used in central nervous system repair
Surface modification

Nerve tube	Surface modification	Internal filler	Animal	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan tube	Collagen type-I	Semifluid collagen type-I	Wistar rats	Laminectomy from T7 to T10 vertebrae + lateral incision at T9 to excise a segment of spinal cord of 4 mm length and 2 mm width	About two-third spinal cord	Basso–Beattie–Bresnahan (BBB) test, anterograde axonal tracing with biotin dextran amine (BDA), retrograde axonal tracing with FluoroGold (FG), and histological evaluation	Empty chitosan tube and lesion without tube implantation	12 months	Axons from the proximal spinal cord regenerate, cross the lesion area inside the tube, and lead to functional restoration of paralyzed hind limbs	Li, Yang, Zhang, Wang, and Chen (2009)
Porous chitosan tube	Laminin coating of the inner surface by oxygen plasma treatment	–	Female Sprague–Dawley rats	Complete transection at T8 and removal of 5 mm piece of spinal cord tissue	5	Basso–Beattie–Bresnahan (BBB), CCombined Behavior Score (CBS) test, and histological evaluation	Empty chitosan tube	2 months	Laminin-coated chitosan tube improves functional recovery by guiding damaged axon regrowth, through the lesioned area, without inducing inflammation or apoptosis	Cheng, Huang, Chang, and Huang (2007)
Polyglycolic acid–chitosan tube	Coating with recombinant L1-Fc	–	Rats	Optic nerve transection	–	Anterograde and retrograde tracing and histological evaluation	Polyglycolic acid–chitosan tube	–	The polyglycolic acid–chitosan conduit coated with L1-Fc is more effective to promote axonal regeneration and remyelination	Xu et al. (2004)

Table 1.3 Types of supportive cells used to enhance chitosan tubes in spinal cord repair
Combination with cells

Nerve tube	Cell type	Internal filler	Animal	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Laminin-coated chitosan tube	Neural stem/progenitor cells (NSPCs)	NSPCs preseeded on laminin-coated chitosan tube in combination with nogo-66 receptor protein, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth fator (PDGF)	Female Sprague–Dawley rats	Complete spinal cord transection T7–T9 laminae were removed, the facets at the same levels were removed, and the dura mater was longitudinally incised in the midline and then excised at T8	–	Basso–Beattie–Bresnahan (BBB) test and histological evaluation	Chitosan tube without growth factors or nogo-66 receptor protein	12 weeks	The combination of nogo-66 receptor protein, growth factors, and NSPCs increases the survival of transplanted NSPCs and enhances axonal regeneration	Guo et al. (2012)
Chitosan tube	Bone marrow stromal cells (BMSCs)	–	Rats	Complete spinal cord transection	–	Basso–Beattie–Bresnahan (BBB) test, retrograde tracing, and histological evaluation	Chitosan tube alone	12 weeks	Enhanced axonal regrowth, remyelination, and functional recovery	Chen et al. (2011)
Chitosan tube	Neural stem/progenitor cells (NSPCs)	Poly-lactic-*co*-glycolic acid (PLGA) microspheres containing dibutyryl cyclic-	Female Sprague–Dawley rats	Complete spinal cord transection laminectomy was performed on T7–T9 exposing the spinal cord. The	–	Functional and histological evaluation	Chitosan tube containing untreated NSPCs	6 weeks	dcb-AMP treatment results in greatest number of NSPCs differentiated into neurons.	Kim et al. (2011)

Continued

Table 1.3 Types of supportive cells used to enhance chitosan tubes in spinal cord repair—cont'd

Combination with cells

Nerve tube	Cell type	Internal filler	Animal	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
		AMP (dbcAMP) and NSPCs		facets of the vertebrae at T7–T9 were also removed					Combination of NSPCs with chitosan tube results in extensive axonal regeneration into the injury site and improvement of functional recovery	
Chitosan tube	Neural stem cells (NSCs)	–	Female Sprague–Dawley rats	Laminectomy at T7–T9 and spinal cord compression at T8 with a 35-g clip for 1 min	5	Basso–Beattie–Bresnahan (BBB) test and histological evaluation	NSCs transplanted without chitosan channel	6 weeks	Chitosan channels enhance the survival of transplanted NSCs. There is no difference in functional recovery between treatment and control group	Bozkurt et al. (2010)
Chitosan tube	Radial glial cells	–	Female Sprague–Dawley rats	Complete spinal cord transection at T8 level	3	Histological evaluation	–	14 weeks	After 14 weeks, radial glial cells are organized in longitudinal cord. Axons regenerate across the chitosan tube	Nomura et al. (2010)
Chitosan–alginate tube	Bone marrow stromal cells (BMSCs)	Chitosan–alginate freeze-dried sponge scaffold	Female Sprague–Dawley rats	Hemitransection at the T9 level	–	Basso–Beattie–Bresnahan (BBB) test, germ agglutinin–horseradish peroxidase retrograde tracing and histological evaluation	Direct suture of spinal dura mater after the lesion	6 weeks	Chitosan–alginate scaffolds combination with BMSCs result in better functional recovery and axonal regrowth in comparison with control group	Wang et al. (2010) and Wang, Wen, Lan, and Li (2010)
Chitosan tube	Neural stem cells (NSCs)	Multicellular sheets	Rats	Complete transection model of spinal cord injury	–	Histological evaluation	NSCs transplanted without chitosan channel	5 weeks	*In vivo* survival of NSCs and differentiation into astrocytes and oligodendrocytes. Host neurons were identified in the tissue bridge formed within the chitosan tubes	Zahir et al. (2008)

Table 1.4 Types of neurotrophic and neuroprotective factors used to enhance chitosan tubes in spinal cord repair

Combination growth factors/neuroprotective molecules

Nerve tube	Growth factor(s)/ neuroprotective molecules	Internal filler	Animal	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Laminin-coated chitosan tube	Nogo-66 receptor protein, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth fator (PDGF)	NSPCs preseeded on laminin-coated chitosan tube in combination with nogo-66 receptor protein, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth fator (PDGF)	Female Sprague–Dawley rats	Complete spinal cord transection T7–T9 laminae were removed, the facets at the same levels were removed and the dura mater was longitudinally incised in the midline and then excised at T8	–	Basso–Beattie–Bresnahan (BBB) test and histological evaluation	Chitosan tube without growth factors or nogo-66 receptor protein	12 weeks	The combination of nogo-66 receptor protein, growth factors, and NSPCs increases survival of transplanted NSPCs and enhances axonal regeneration	Guo et al. (2012)
Chitosan tube	Dibutyryl cyclic-AMP	Poly-lactic-*co*-glycolic acid (PLGA) microspheres containing dibutyryl cyclic-AMP (dbcAMP) in combination with NSPCs	Female Sprague–Dawley rats	Complete spinal cord transection laminectomy was performed on T7–T9 exposing the spinal cord. The facets of the vertebrae at T7–T9 were also removed	–	Functional and histological evaluation	Chitosan tube containing untreated NSPCs	6 weeks	dcb-AMP treatment results in greatest number of NSPCs differentiated into neurons. Combination of NSPCs with chitosan tube results in extensive axonal regeneration into the injury site and improvement of functional recovery	Kim et al. (2011)
Chitosan microspheres	Atorvastatin calcium	–	Sprague–Dawley rats	Laminectomy at T7–T9 level	–	Functional evaluation using inclined plane technique of Rivlin and Tator and a modified version of the Tarlov Grading Scale and histological evaluation	Implantation of empty chitosan microspheres	5 days	Chitosan microspheres containing atorvastatin improve functional recovery, attenuate the expression of TNF-α, IL-1 beta, and IL-6, decrease lipid peroxidation levels and preserve cellular uniformity	Eroglu et al. (2010)

In another study, a laminin-coated conduit was shown to enable axons to cross the lesioned area of the spinal cord and to reduce glial scar formation (Cheng et al., 2007). Behavioral analyses evaluating the Basso–Beattie– Breshnahan motor behavior score, the sensorimotor combined behavior score, open-field walking scores, and treadmill analyses demonstrated that following the implantation of the laminin-coated nerve conduit the rats showed a tendency toward behavior improvement and functional recovery (Cheng et al., 2007). Histological and immunocytochemical analyses indicated that the implanted nerve conduit groups were capable of leading the damaged axons through the lesioned area without triggering inflammation or apoptosis (Cheng et al., 2007).

Other cell adhesion molecules, such as L1, have been shown to enhance CNS regeneration, and Xuet al., by using the optic nerve transection animal model, showed that polyglycolic acid (PGA)–chitosan conduits coated with recombinant L1–Fc have a potential role in promoting nerve regeneration by guiding axonal regrowth and remyelination (Xu et al., 2004).

3.2. Chitosan conduits combined with cells for CNS repair

NSPCs, bone marrow mesnchymal stem cells (BMSCs), and radial glial cells have been used in combination with chitosan for SCI repair (Table 1.3). Results showed that, compared to direct NSPCs injection, chitosan channels improved their survival after implantation (Bozkurt et al., 2010; Guo et al., 2012; Kim et al., 2011). In other studies, NSPCs isolated from the subependyma of lateral ventricles of adult green fluorescent protein (GFP) transgenic rat forebrains (Zahir et al., 2008) or derived from brain or spinal cordoftransgenicGFPrats (Nomura, Zahir, et al.,2008)incombinationwith chitosan channels were implanted into the spinal cord after transection injury. These in vivo studies showed excellent survival of NSPCs as well as differentiation into astrocytes and oligodendrocytes (Nomura, Zahir, et al., 2008; Zahiretal.,2008).Moreover,hostneuronswereidentifiedinthetissuebridge that formed within the chitosan tubes and bridged the transected cord stumps (Zahir et al., 2008). The excellent in vivo survival of the NSPCs coupled with their differentiationand maintenance of host neuronsinthe regeneratedtissue bridge demonstrates that the use of three-dimensional chitosan scaffolds combined with adult spinal cord-derived NSPCs is a promising therapeutic strategy for stem cell delivery and enhances regenerative potential restoring spinal cord function after SCI although functional outcome recovery remains poor (Bozkurt et al., 2010; Nomura, Zahir, et al., 2008; Zahir et al., 2008).

BMSCs (Chen et al., 2011; Wang, Wen, Lan, & Li, 2010) and radial glial cells(Nomuraetal.,2010),incombination withchitosanscaffold, havebeen successfully used to promote SCI repair.

3.3. Chitosan conduits combined with neurotrophic factors or neuroprotective molecules for CNS repair

Chitosan conduits combined with neurotrophic factors or neuroprotective molecules have been used for SCI repair as summarized in Table 1.4. Recently,theneuroprotectiveeffectsofAtorvastatin,a drugusedasa cholesterol lowering agent in patients, are becoming the focus of many research studies. Interestingly, chitosan microspheres containing Atorvastatin calcium have been successfully used to improve the functional outcome in an experimental SCI model (Eroglu et al., 2010). Moreover, nogo-66 receptor protein, basic fibroblast growth factor (bFGF), EGFs, and platelet-derived growth factor have been successfully used in combination with NSPCs for SCI repair (Guo et al., 2012).

4. CHITOSAN FOR PERIPHERAL NERVOUS SYSTEM REPAIR

The clinical treatment of large peripheral nerve defects requires bridging the defect that is usually accomplished by means of an autologous nerve graft. However, nerve autografting has various

drawbacks such as sacrificing of a healthy functioning nerve resulting in donor site morbidity, size and quality mismatch, and possible neuroma formation at the donor site. Allografts using nerves from other individuals or animals require an additional immunosuppressant treatment. Various artificial materials have been used as scaffolds for nerve regeneration including chitosan, either alone or in combination with other materials. In this context, chitosan is an attractive material because of its mechanical strength, porosity, biodegradability, and biocompatibility, and thus, it has been recently used for repairing nerve injury, either alone or in combination with other biomaterials (Table 1.5), adhesion molecules (Table 1.6), cells (Table 1.7), or growth factors (Table 1.8). A number of in vivo studies suggested that chitosan conduits are promising candidates as supporting material for tissue engineering application in peripheralnervereconstruction(Huang, Lu, et al.,2010;Ishikawaet al.,2007;Lauto etal.,2007,2008;Marcoletal.,2011;Matsumotoetal.,2010;Pateletal.,2006; Rickett et al., 2011; Rosales-Cortes, Peregrina-Sandoval, Banuelos-Pineda, Sarabia-Estrada, et al., 2003; Simoes et al., 2011; Wang, Itoh, Matsuda, Ichinose, et al., 2008; Wang, Itoh, et al., 2010; Wang et al., 2009; Yamaguchi et al., 2003; Zhang et al., 2005). Anexperimentalstudyreconstructing10-mmgapsintheratsciaticnerve showed that chitosan tubes induce nerve regeneration and are gradually degraded and absorbed in vivo (Yamaguchi et al., 2003). Patel et al. (2006) reported that chitosan nerve guides improve functional nerve recovery, by increasing axonal growth, reduce muscle atrophy, and restore functional strength. It has also been shown that the regeneration of the axotomized dog sciatic nerve can be improved through tubulization with chitosan without affecting the immune response (Rosales-Cortes, Peregrina-Sandoval, Banuelos-Pineda, Sarabia-Estrada, et al., 2003). As an internal conduit framework is concerned, a freeze-dried chitosan gel sponge has been used to bridge a 8-mm gap lesion in the rat sciatic nerve; 14 daysafterthe surgery, the regeneratednerve fibers areextended insidethe conduit along a cell layer provided by infiltrating cells, and 2 months postsurgery, the regenerated nerve appeared well remyelinated, indicating that the chitosan gel sponge material might be a promising graft for peripheral nerve regeneration (Ishikawa et al., 2007). Chitosan nano-/microfiber mesh tubes have been safely used also to successfully regenerate damaged thoracic nerves in beagle dogs, specifically sympathetic and phrenic nerve, resulting in restoration of the respiratory function (Matsumoto et al., 2010). Chitosan nanofibers mesh tubes with or without orientation and bilayered chitosan mesh tubes with an inner layer of oriented nanofibers and an outer layer of randomized nanofibers have been used to bridge sciatic nerve defects in rats. Sprouting of axons and axonal maturation followed by functional recovery occurred in the oriented conduits as well as in the bilayered conduits matching the outcome of the nerve autografts (Wang et al., 2009). Nano-/microfiber mesh tubes with a DD of 78% or 93% investigated in the 10-mm rat sciatic nerve gap repair resulted in better sensory recovery for mesh tubes with a DD of 93%. These tubes have adequate mechanical properties to preserve the tubes internal lumen, resulting in better cell migration and adhesion as well as humoral permeation enhancing nerve regeneration (Wang, Itoh, Matsuda, Ichinose, et al., 2008). Interestingly, a chitosan-based-laser-activated adhesive has been successfully applied to perform sutureless coaptation of the rat tibial nerve without altering axon number and morphology (Lauto et al., 2007, 2008). A photocrosslinkable hydrogel based on chitosan has been in vitro successfully characterized and proposed as a new adhesive for peripheral nerve anastomosis (Rickett et al.,

2011). Chitosan has further been used in combination with other biomaterials for bridging peripheral nerve gaps (Fan et al., 2008; Gu et al., 2012; Jiao et al., 2009; Lin et al., 2008; Liu et al., 2011; Simoes et al., 2010; Wang et al., 2005; Xie et al., 2005; Xie et al., 2008; Xu et al., 2009). Chitosan– polylactid acid (PLA) composite nerve conduits showed good biocompatibility and permeability, good mechanical strength, intensity, and elasticity, facilitating microsuture manipulation, and they provide enough mechanical strength to support nerve regeneration. Chitosan–PLA conduits promoted axonal regeneration of rat sciatic nerves across a defect of 10 mm, with comparable success to nerve autografts, resulting in muscle reinnervation 12 weeks postsurgery (Xie et al., 2008). Yet, tubular grafts made out of chitosan membranes have been successfully used to improve peripheral nerve functional recovery after neurotmesis of the rat sciatic nerve, and they induced better nerve regeneration and functional recovery when compared with poly-lactic-polyglycolic acid (PLGA) control tubes (Simoes et al., 2010). A dual component artificial nerve conduit consisting of an outer chitosan microporous conduit and an internal oriented PGA filament matrix has been used to bridge a 10-mm defect in rats after long-term delay (3 or 6 months), resulting in reinnervation of the atrophic denervated muscle by regenerating neurites through new muscle–nerve connections (Jiao et al., 2009). The same conduit has been used to regenerate 30-mm beagle dog sciatic nerve defects, resulting in reconstruction and restoration of nerve continuity and functional recovery as indicated by improved locomotion activities of the operated limb after target muscle reinnervation (Wang et al., 2005). Finally, a couple of clinical studies in which chitosan scaffolds have been used to repair peripheral nerve have been reported. Chitosan/PGA artificial conduits have been successfully used in repairing a 35-mm-long median nerve defect of a human patient. During the 3-year follow-up period, an ongoing motor and sensory functional recovery postimplantation was detected (Fan et al., 2008). Chitosan/PGA conduits have further been used to repair a 30-mm long median nerve defect in the right distal forearm of a 55-year-old male patient. Thirty-six months after the surgery, reproducible compound muscle action potentials have been recorded on the right abductor policies, the palm adduction of the thumb, and the thumb-index digital opposition recovered and facilitated the accomplishment of fine activities (Gu et al., 2012). These results are very promising and suggest that the chitosan/PGA artificial nerve graft could be used for clinical reconstruction of major peripheral nerves' defects in the forearm.

Table 1.5 Chitosan conduits and luminal framework modifications used in peripheral nerve repair

Intrinsic framework

Nerve tube	Framework	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan/ polyglycolic acid tube	–	–	55–year-old male human patient	Median nerve	Nerve discontinuity	30	Compound muscle action potential recording and ninhydrin test	–	3 years	Recovery of palm abduction of the thumb and thumb–index digital opposition; reproducible compound muscle action potential of the right abductor pollicis	Gu et al. (2012)
–	Microcrystallic chitosan gel	–	Male Wistar rats	Sciatic nerve	Nerve transection	10	Autotomy behavior and histological evaluation	Nerve transection without chitosan gel implantation	20 weeks	In chitosan group neuroma formation, extraneural fibrosis are reduced in comparison to control group. There is no difference in autotomy behavior between groups	Marcol et al. (2011)
Chitosan membranes	Porous structure	–	Female Wistar rats	–	Subcutaneous implantation	–	Histological evaluation	–	8 weeks	Chitosan membranes owing their porous structure and chemical modifications and display high affinity to cellular systems	Simoes et al. (2011)
Chitosan/ polyvinyl alcohol (PVA)	–	–	Macaques	Radial nerve	Nerve transection	20	Electrophysiological and histological evaluation	Autograft (positive control) nongrafted (negative control)	8 months	Axonal regrowth and remyelination. Recovery of compound muscle axon potential	Liu, Hou, Lin, and Wei (2011)
Chitosan tube	Tube wall with longitudinally or randomly oriented pores	–	Rats	Sciatic nerve	Nerve transection	15	Electrophysiology, retrograde labeling, and histological evaluation	Autograft	4 weeks	Axonal regeneration and motor functional recovery are improved by electrical stimulation in animals that received longitudinal pore tube compared to control group	Huang, Hu, et al. (2010) and Huang, Lu, et al. (2010)
Chitosan nano-/ microfiber mesh tube	Hollow tube	–	Beagle dogs	Thoracic sympathetic nerve and phrenic nerve	Nerve transection	10	Skin temperature measurement, X-ray imaging	–	12 months	Improvement of nerve regeneration; restoration of diaphragm mobility	Matsumoto, Kaneko, Oda, and Watanabe (2010)
Freeze-dried chitosan type-III membrane and freeze-dried chitosan type-III tube	End-to-end neurorrhaphy enwrapped by chitosan membrane, 10-mm autograft enwrapped by chitosan membrane and 10-mm autograft enwrapped by chitosan tube guides	–	Sprague–Dawley rats	Sciatic nerve	End-to-end neurorrhaphy and nerve transection	10	Extensor postural thrust (EPT), withdrawal reflex latency (WRL), and ankle kinematics	10-mm nerve autograft, 10-mm PLGA tube and end-to-end surgery alone	20 weeks	Better nerve regeneration in chitosan type III tubulization group than PLGA tubulization control group	Simoes et al. (2010)

Continued

Table 1.5 Chitosan conduits and luminal framework modifications used in peripheral nerve repair—cont'd

Intrinsic framework

Nerve tube	Framework	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Microporous–chitosan tube	Filaments	Polyglycolicacid (PGA) oriented filaments	Female Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Electrophysiological and histological evaluation	10-mm autograft (positive control) and nongrafted (negative control)	6 months	Better axonal regeneration, SC myelination, and reinnervation of atrophic denervated muscle in the chitosan/PGA graft group	Jiao et al. (2009)
Bilayered chitosan nonwoven nanofibers mesh tube	Filaments	Inner layer of orientated nanofibers and outer layer of random nanofibers	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Von Frey hair test, Static toe spread factor (STSF), electrophysiological and histological evaluation	Autograft (positive control) and random nanofibers chitosan mesh tube (negative control)	30 weeks	Functional recovery in the bilayered chitosan nonwoven nanofibers mesh tube group matches autograft group. Sprouting of myelinated axons and axonal maturation occurs in both groups at the same level	Wang et al. (2009)
Poly-D-L-lactic acid/chondroitin sulfate/chitosan tube (PDLLA/CS/CHS)	–	–	Rats	–	–	–	–	PDLLA tube	3 months	The PDLLA/CS/CHS tube allows nerve regeneration without acidity-caused irritation and acidity-induced autoaccelerating degradation behavior typical of PDLLA alone	Xu et al. (2009)
Microporous–chitosan tube	Filaments	Polyglycolic acid (PGA) oriented filaments	37-years-old male human patient	Median nerve	Nerve discontinuity	35	JAMAR Hand evaluation Kit (5030KIT), touch test sensory evaluators, disk-criminator, electrophysiological evaluation, blood and urinary test, and serum biochemical examination	–	3 years	Motor and sensory function recovery	Fan et al. (2008)
Laser-activated chitosan adhesive	–	–	Rats	Tibial and sciatic nerve	Sutureless anastomosis	–	Histological evaluation	–	3 days	Successful nerve anastomosis; myelinated axons display normal number and morphology	Lauto et al. (2008)
Bilayered chitosan film tube	Nano-/microfibers	Nano-/microfibers mesh tube with a DAc of 78% or 93% and film tube with a DAc of 93%	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	15	Von Frey hair test, Static toe spread factor (STSF), electrophysiological and histological evaluation	15-mm autograft	10 weeks	Functional recovery of motor activity delays in each group compared to autograft group; good sensory and functional recovery in autograft group followed by nano-/microfibers mesh tubes with a DAc of 93% group	Wang et al. (2008) and Wang, Itoh, Matsuda, Ichinose, et al. (2008)

Continued

Table 1.5 Chitosan conduits and luminal framework modifications used in peripheral nerve repair—cont'd

Intrinsic framework

Nerve tube	Framework	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Polylactic acid (PLA)/ chitosan tube	Hollow tube	PLGA filler	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Electrophysiological and histological evaluation	10-mm autograft and 10-mm silicone conduit group	12 weeks	Axonal regeneration, sciatic nerve functional recovery, and muscle reinnervation of chitosan–PLA group are close to control group	Xie, Li, Gu, Liu, and Shen (2008)
Chitosan	Microgrooved polymer	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	12	Histological evaluation	12-mm silicone group and smooth chitosan conduits	6 weeks	Microgrooved conduits enhance peripheral nerve regeneration in comparison with the smooth conduits	Hsu et al. (2007)
Chitosan tube	Sponge	Freeze-dried chitosan gel sponge	Male Wistar rats	Sciatic nerve	Nerve transection	8	Histological evaluation	8-mm gap without chitosan tube implantation	4 months	Axonal regeneration and remyelination	Ishikawa et al. (2007)
Chitosan tube	Hollow tube	–	Rats	Sciatic nerve	Nerve transection	10	Walking track analysis and histological evaluation	10-mm autograft	12 weeks	Decrease in muscle atrophy, increase in axonal growth and functional recovery	Patel et al. (2006)
Microporous chitosan tube	Filaments	Polyglycolic acid (PGA)-oriented filaments	Beagle dogs	Sciatic nerve	Nerve transection	30	Electrophysiological, histological evaluation and FluoroGold retrograde tracing	30-mm autograft group (positive control) and nongrafted group (negative control)	6 months	Restoration of nerve continuity, functional recovery, and target muscle reinnervation in the chitosan PGA graft group	Wang et al. (2005)
Chitosan/ PLA tube	–	–	Rats	Sciatic nerve	Nerve transection	5	Electrophysiological and histological evaluation	Silicon conduits and autograft group	12 weeks	Chitosan/PLA tube results in nerve regeneration, axon's quality, and quantity close to autograft's group	Xie, Li, and Zhao (2005)
Chitosan tube	Hollow tube	–	Female Beagle dogs	Sciatic nerve	Axotomy	–	IgG and IgM serum analysis	Intact and axotomized control groups	60 days	Chitosan implants do not affect the immune response	Rosales-Cortes, Peregrina-Sandoval, Bannelos-Pineda, Castellanos-Martinez, et al. (2003) and Rosales-Cortes, Peregrina-Sandoval, Bannelos-Pineda, Sarabia-Estrada, et al. (2003)
Apatite/ chitosan tube	Hollow tube	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Histological evaluation	10-mm chitosan tube	12 weeks	Apatite-treated chitosan hollow tube keeps its shape *in vivo* and induces nerve regeneration	Yamaguchi et al. (2003)

Table 1.6 Modifications to chitosan conduits surface used in peripheral nerve repair

Surface modification

Nerve tube	Surface modification	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan nanofibers mesh tube	Coating with polarized and nonpolarize-β-tricalcium phosphate particles	–	Male Wistar rats	Sciatic nerve	Nerve transection	10	Static toe spread factor (STFS), von Fray hair test, electrophysiological and histological evaluation	Autograft	12 weeks	Motor and sensory nerve function and electrophysiological recovery progress with time in each group. Immunofluorescence reveals more rapid nerve regeneration in the polarized tube group compared with the nonpolarized tube group. The axon density and axon area in the polarized tube group are significantly greater than those in the chitosan mesh tube and nonpolarized group, and it shows no significant differences from the control group	Wang, Itoh, et al. (2010) and Wang, Wen, Lan, and Li (2010)
Chitosan–collagen tube	Blending with collagen	Longitudinally oriented microchannels	Rats	Sciatic nerve	Nerve transection	15	Functional and histological evaluation	Autograft	–	Chitosan–collagen scaffolds achieve nerve regeneration and functional recovery equivalent to autograft.	Hu et al. (2009)
Chitosan–collagen tube	Blending with collagen	–	Rats	Sciatic nerve	Nerve transection	–	Gait analysis and behavioral test	Unblended chitosan nerve guide	12 weeks	Collagen-blended chitosan nerve guide enhances motor and sensory recovery compared with unblended nerve guides	Patel et al. (2008a, 2008b)
Chitosan film	Conjugation with C(G) YIGSR peptide from laminin-1	Chitosan nonwoven nano-/microfibers mesh	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Static toe spread factor (STSF) and histological evaluation	Autograft	10 weeks	Nerve regeneration in the chitosan/CYIGSR group is similar to the autograft	Wang, Itoh, Matsuda, Aizawa, et al. (2008) and Wang, Itoh, Matsuda, Ichinose, et al. (2008)
Thiolated and nonthiolated hydroxyapatite-coated chitosan tube	Adsorbed YIGSR peptide from laminin-1	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	15	Electrophysiological and histological evaluation	Autograft	12 weeks	YIGSR peptide enhances nerve regeneration and axonal sprouting from the proximal stump to the distal one	Itoh et al. (2005)
Circular and triangular cross-section chitosan tube	Absorption of laminin and YIGSR and IKVAV laminin–peptide	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	15	Electrophysiological and histological evaluation	Autograft	12 weeks	Triangular tubes have higher mechanical strength and inner volume than circular ones. SC migration and axonal outgrowth is enhanced. Nerve tissue regeneration occurs in both laminin and laminin–peptide groups	Itoh, Suzuki, et al. (2003)

Continued

Table 1.6 Modifications to chitosan conduits surface used in peripheral nerve repair—cont'd
Surface modification

Nerve tube	Surface modification	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Circular and triangular cross-section chitosan tube coated with hydroxyapatite	Absorption of laminin and YIGSR and IKVAV laminin–peptide	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	15	Electrophysiological and histological evaluation	Autograft	8 weeks	Triangular tubes coated with hydroxyapatite have higher mechanical strength and inner volume than circular ones. Nerve tissue regeneration occurs in both laminin and laminin–peptide groups matching isograft group. Functional recovery is delayed	Itoh, Yamaguchi, et al. (2003)
Triangular cross-section chitosan tube	Covalent binding of laminin and YIGSR and IKVAV laminin–peptide	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	15	Electrophysiological and histological evaluation	Autograft	8 weeks	YIGSR, followed by IKVAV, laminin–peptide matches the effectiveness of intact laminin in enhancing nerve regeneration	Suzuki et al. (2003)
Chitosan–collagen film	Blending with collagen	–	Rats	Sciatic nerve	Nerve transection	5 or 10	Electrophysiological and histological evaluation	Autograft	12 weeks	In 5-mm defects, nerve regeneration is similar to control group. In 10-mm defects, nerve regeneration is inferior to control group. Chitosan–collagen film conduits are degraded at 12 weeks postsurgery	Wei et al. (2003)

Table 1.7 Types of supportive cells used to enhance chitosan tubes in peripheral nerve repair
Combination with cells

Nerve tube	Cell type	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Silicon tube	Autologous bone marrow mesenchymal stem cells (BMSCs)	Laminin-modified chitosan film with BMSCs	Female Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Histological evaluation and FluoroGold retrograde tracing	Empty silicon tube	16 weeks	BMSCs containing scaffolds improve nerve regrowth, muscle mass maintenance, and functional recovery.	Hsu et al. (2013)
Chitosan tube	Autologous bone marrow mesenchymal stem cells (BMSCs)	Longitudinally aligned poly lactic-*co*-glycolic acid (PLGA) fibers	Rhesus monkeys	Median nerve	Nerve transection	50	Electrophysiological and histological evaluation, FluoroGold retrograde tracing	Chitosan–PLGA fibers alone	12 months	Twelve months after grafting, nerve function recovery, and morphological reconstruction of BMSCs containing PLGA/chitosan scaffold is superior to that of chitosan/PLGA scaffold alone	Hu et al. (2013)
Chitosan tube	Autologous bone marrow mesenchymal stem cells (BMSCs)	Poly lactic-*co*-glycolic-acid (PLGA) oriented fibers	Male Beagle dogs	Sciatic nerve	Nerve transection	60	Electrophysiological, retrograde fluorogold tracing, and histological evaluation	Autograft and chitosan/PLGA fibers alone	12 months	MSCs in combination with chitosan/PLGA fibers tube improve nerve repair in comparison to chitosan/PLGA fibers tube alone	Xue et al. (2012)
Chitosan tube	Bone marrow stromal cells (BMSCs)-derived Schwann cells or sciatic nerve-derived Schwann cells (SCs)	–	Rats	Sciatic nerve	Nerve transection	8	Sciatic nerve function index (SFI) and histological evaluation	Chitosan tube alone	6 weeks	Six weeks postsurgery, the SFI, average regenerated fiber density, and fiber diameter in nerve bridged with BMSCs are similar to autograft	Zheng and Cui (2012)

Continued

Table 1.7 Types of supportive cells used to enhance chitosan tubes in peripheral nerve repair—cont'd
Combination with cells

Nerve tube	Cell type	Internal filler	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan tube	Bone marrow stromal cells (BMSCs)-derived Schwann cells or sciatic nerve-derived Schwann cells (SCs)	Matrigel	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	12	Footprint analysis, compound muscle action potential (CMAP) measurements, histological evaluation	Autograft and PBS-filled conduits	3 months	Nerve conduction velocity, average regenerated myelin area, and myelinated axon count in nerve bridged with BMSCs-derived SCs are similar to those treated with sciatic nerve-derived SCs and higher than those bridged with PBS-filled conduits	Ao et al. (2011)
Chitosan/polyglycolic acid tube	Autologous bone marrow mesenchymal stem cells (BMSCs)	–	Beagle dogs	Sciatic nerve	Nerve transection	50	Electrophysiological and histological evaluation, FluoroGold retrograde tracing	Autograft	6 months	Introduction of BMSCs in the conduits promotes nerve regeneration and functional recovery	Ding et al. (2010)
Chitosan-3-glycidoxypropyltrimethoxysilane (GPTMS) cross-linked membranes	Predifferentiated N1E-115 cells	Chitosan membrane covered with a cell monolayer	Female Wistar rats	Sciatic nerve	Crush injury	10	Kinematic analysis and histological evaluation	Unoperated animals	8 weeks	Local enwrapping with chitosan membrane without N1E-115 cells improves axonal regrowth and functional recovery	Amado et al. (2008)
Chitosan–Au–nanocomposites	Neural stem cell (NSC)	–	Male Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Histological evaluation	Chitosan–Aunanocomposites tube without NSC	6 weeks	In comparison to control group, the number of regenerated axons, the regenerated area, and the number of blood vessels are significantly higher in the NSCs preseeded tube group	Lin, Jen, Hsu, and Chiu (2008)

Table 1.8 Types of neurotrophic and neuroprotective factors used to enhance chitosan tubes for peripheral nerve repair
Neurotrophic factors

Nerve tube	Growth factor(s)	Carrier/delivery system	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan tube	Nerve growth factor (NGF)	NGF immobilization via genipin cross-linking	Rats	Sciatic nerve	Nerve transection	10	Electrophysiological and histological evaluation	Autograft	24 weeks	Chitosan/NGF tube promotes nerve regeneration close to the autograft group	Wang et al. (2012)
Poly-D, L-lactic-acid/chondroitin sulfate/chitosan tube	NGF	Immobilization on the tube surface	Sprague–Dawley rats	Sciatic nerve	Nerve transection	10	Electrophysiological and histological evaluation	Autograft	6 months	No connective tissue in growth. Rapid functional recovery and nontoxicity of degradation products. NGF promotes nerve regeneration close to the autograft group	Xu, Yan, and Li (2011)
Chitosan tube	Immunophilin ligand FK506	Drug loading into the semipermeable wall of chitosan tube	Rats	Sciatic nerve	Nerve transection	–	Electrophysiological and histological evaluation	Autograft	8 weeks	FK506 treatment results in more mature appearance of myelinated fibers. The amplitude and velocity of compound muscle action potential of treated group are close to the autograft group	Li et al. (2010)

Continued

Table 1.8 Types of neurotrophic and neuroprotective factors used to enhance chitosan tubes for peripheral nerve repair—cont'd

Neurotrophic factors

Nerve tube	Growth factor(s)	Carrier/delivery system	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan tube	Basis fibroblast growth factor (bFGF)	Heparin–incorporated fibrin–fibronectin matrix	Rats	Sciatic nerve	Nerve transection	10	Conduction velocity recovery index (CVRI), muscle restoration rate (MRR), and histological evaluation	Autograft	3 months	CVRI and MRR in animals of bFGF group are similar to those of autograft group	Han, Ao, Chen, Wang, and Zuo (2010)
Poly lactic–co–glycolic acid (PLGA)/ chitosan tube	CNTF	CNTF blending with chitosan	Cross–bred dogs	Tibial nerve	Nerve transection	25	Electrophysiological and histological evaluation	PLGA/ chitosan tube without CNTF	3 months	PLGA/ chitosan–CNTF tube promotes nerve regeneration close to the autograft group	Shen et al. (2010)
–	Chitooligosaccharides (COSs)	Intravenous injections of 1.5 or 3 mg/kg body weight of COSs over 6-week period	New Zealand rabbits	Common peroneal nerve	Nerve crush injury	20	Electrophysiological and histological evaluation	Saline injections	6 weeks	Compound muscle action potentials, number of regenerated myelinated nerve fibers, thickness of regenerated myelin sheaths and the cross-sectional area of tibialis posterior muscle fibers are significantly improved in animals treated with COSs in a dose-dependent manner. COSs display a neuroprotective effect	Gong, Gong, Gu, and Ding (2009)
–	Chitooligosaccharides (COSs)	Intraperitoneally injections of 3 or 6 mg/kg body weight of COSs over 3-week period	Sprague–Dawley rats	Sciatic nerve	Nerve crush injury	–	Electrophysiological, measurement of withdrawal reflex latency (WRL), walking track analysis, and histological evaluation	Saline injections	3 weeks	COSs promote peripheral nerve regeneration and functional recovery of injured nerves. COSs display a neuroprotective effect	Jiang, Zhuge, Yang, Gu, and Ding (2009)
Chitosan tube	GDNF	GDNF blending with chitosan	Lewis rats	Sciatic nerve	Nerve transection	10	Histological evaluation	Autograft	12 weeks	Chitosan–GDNF tube enhances nerve regeneration process during the initial stages of nerve repair	Patel, Mao, Wu, and VandeVord (2009)
Chitosan tube	GDNF	GDNF blending with chitosan	Lewis rats	Sciatic nerve	Nerve transection	10	Functional gait analysis and sensitivity test	Autograft	12 weeks	Chitosan–GDNF tube increases functional recovery compared to unblended chitosan groups	Patel, Mao, Wu, and Vandevord (2007)

Continued

Table 1.8 Types of neurotrophic and neuroprotective factors used to enhance chitosan tubes for peripheral nerve repair—cont'd
Neurotrophic factors

Nerve tube	Growth factor(s)	Carrier/delivery system	Animal	Nerve	Injury	Gap size (mm)	Methods	Controls	Follow-up	Outcome	Authors and year
Chitosan tube	Progesterone (PROG) and pregnenolone (PREG)	GDNF blending with chitosan	New Zealand rabbits	Facial nerve	Nerve transection	10	Histological evaluation	Chitosan tube without PROG/PREG	45 days	Chitosan–PROG/PREG tube promotes axonal regeneration and myelination	Chavez-Delgado et al. (2005)
Chitin tube	Nerve growth factor (NGF)	–	Sprague–Dawley rats	Sciatic nerve	Nerve transection	5	Electrophysiological and histological evaluation	Epineurium direct suture	8 weeks	The repair effects of chitin conduit bridging peripheral nerve with 5-mm gap are better than epineurium suture directly, and possess the potential to substitute the epineurium suture	Zhang et al. (2005)
Chitosan tube	Progesterone (PROG) and pregnenolone (PREG)	GDNF blending with chitosan	New Zealand rabbits	Facial nerve	Nerve transection	10	Histological evaluation	Chitosan tube without PROG/PREG	45 days	Chitosan–PROG/PREG tube promotes myelination	Chavez-Delgado et al. (2003)
Chitosan tube	Progesterone (PROG)	–	Female dogs	Sciatic nerve	Nerve transection	15	Histological evaluation	Chitosan tube without PROG	–	Chitosan/PROG tube promotes axonal regeneration and myelination	Rosales-Cortes, Peregrina-Sandoval, Banuelos-Pineda, Castellanos-Martinez, et al. (2003) and Rosales-Cortes, Peregrina-Sandoval, Banuelos-Pineda, Sarabia-Estrada, et al. (2003)

4.1. Surface modification of chitosan conduits for PNS repair

Various studies have been dedicated to develop scaffolds with an inner structure mimicking the nerve-guiding basal lamina present in nerve autografts. Designing biomaterial surfaces in order to mediate cellular interactions through coupling of specific cell membrane receptors may allow to control cell adhesion, cell migration, and tissue organization and consequently improve SC migration and organization and axonal outgrowth. To enhance nerve regeneration, a number of

studies, in which laminin has been used to coat the inner tube surface, have been reported (Madison, da Silva, Dikkes, Chiu, & Sidman, 1985; Yoshii, Yamamuro, Ito, & Hayashi, 1987). Although these studies proved the effectiveness of scaffold enrichment with laminin, this molecule is not easy to synthesize and it cannot be applied in human patients because it is a tumor-inducing material (Timpl et al., 1979). To avoid these limitations, laminin peptides (YIGSR and IKVAV) have been used for functionalizing the inner surface of chitosan-based conduits too, with the goal of improving nerve regeneration. YIGSR-treated chitosan–hydroxyapatite (HAp) tubes enhanced SC migration and longdistance growth of regenerated axons in a 15-mm gap rat sciatic nerve injury modelincomparisonwithfull laminin-1-coatedchitosantubes.Histological regeneration, as well as mechanical properties, of the YIGSR-treated chitosan–HAp tubes matched with those of nerve autografts, although functional recovery was delayed (Itoh, Yamaguchi, et al., 2003). Yet, circular and triangular cross-section chitosan tubes combined with YIGSR and IKVAV have been used to bridge a 15-mm gap in the sciatic nerve of rats. The mechanical strength of triangular tubes was higher than that of circular tubes and their inner volume tended to be larger. Again, YIGSR and IKVAV matched the effectiveness of full laminin to enhance nerve regeneration (Itoh, Suzuki, et al., 2003; Suzuki et al., 2003). In another study, thiolated and nonthiolated HAp-coated crab tendon triangular cross-section chitosan tubes, both alone and conjugated with the YIGSR peptide, have been utilized to bridge a 15-mm rat sciatic nerve gap. Histological and functional recovery analyses showed that while thiolation might have delayed nerve tissue regeneration, YIGSR peptides enhanced nerve regeneration by promoting sprouting from the proximal nerve stump and long-distance growth of regenerated axons throughout the tube (Itoh et al., 2005). Moreover, a bilayered chitosan tube that comprised of an outer layer of a chitosan film and an inner layer of a nonwoven chitosan nano-/microfiber mesh coated with the YIGSR peptide (in which a glycin spacer has been introduced) has been successfully used to bridge the 15-mm rat sciatic nerve gap (Wang, Itoh, Matsuda, Aizawa, et al., 2008). Chitosan has also been used in combination with collagen for nerve repair. Collagen-blended chitosan nerve guides have been successfully tested in vivo in rats and enhanced both motor and sensory recoveries in comparison with unblended nerve guides (Patel et al., 2008a; Wei, Lao, & Gu, 2003).Moreover,collagen–chitosannerve guidespromoteandsupportaxonal sprouting, increase axon diameters and the area occupied by regenerated axons, and further improve axonal maturation (Patel et al., 2008b). Collagen–chitosan conduits, characterized by longitudinally orientated microchannels, have also been shown to allow good nerve regeneration and functional recovery across 15-mm-long rat sciatic nerve defects (Hu et al., 2009). A summary of the different studies of chitosan combined with adhesion molecules is reported in Table 1.6.

4.2. Chitosan conduits combined with cells for PNS repair

Directing SC migration by biomaterial substrates is receiving much attention in peripheral nerve tissue engineering (Heath & Rutkowski, 1998). SCs secrete neurotrophic factors and express cell adhesion molecules that enhance peripheral nerve regeneration (Gravvanis et al., 2004; Heath & Rutkowski, 1998; Ide, 1996). They form an endoneurial sheath which acts as a guide for axonal growth from the proximal to the distal nerve stumps. They also play a role in clearing debris and creating an appropriate milieu for nerve regrowth. Due to their role in nerve regeneration, the

behavior of SCs on a biomaterial used for nerve conduit fabrication is clearly a key issue (Guenard, Kleitman, Morrissey, Bunge, & Aebischer, 1992; Heath & Rutkowski, 1998) and it has been shown that SCs can be aligned by culturing them on biomaterial surface groves which provide micrometric dimensions (Hsu et al., 2007; Wang et al., 2009).

Another strategy to support SC colonization inside nerve guides is preenrichment of the conduit with SCs or their precursors. BMSC-derived SCs have been used in combination with chitosan conduits to repair 12-mm rat sciatic nerve gap, resulting in nerve conduction velocities, average regenerated myelin area, and number of myelinated axons similar to those conduits treated with sciatic nerve-derived SCs (Ao et al., 2011). Chitosan-based conduits combined with autologous BMSCs have been successfully utilized to bridge 8-mm-long sciatic nerve defects in adult rats (Zheng & Cui, 2012). Chitosan/PLGA-based neuronal scaffolds, in which autologous BMSCs have been incorporated, promoted dog sciatic nerve regeneration and functional recovery across 50- to 60-mm-long gaps. The outcome was close to that of nerve autografts and better than that of chitosan/PLGA-based scaffolds alone (Ding et al., 2010; Xue et al., 2012). Very recently, chitosan/PLGA nerve grafts combined with autologous BMSCs have been utilized to bridge 50-mm-long medial nerve defects in rhesus monkeys. Functional recovery was more efficient when chitosan/ PLGA nerve grafts were combined with BMSCs instead of the used cell-free chitosan/PLGA grafts. Moreover, blood tests and histopathological examinations demonstrated that BMSCs could be safely used in primates (Hu et al., 2013). Recently, combination ofpeptide and cellsto enhance nerve regeneration has been used to bridge 10-mm gaplesionin rat sciatic nerve.Laminin-coated chitosan conduit in combination with BMSCs results in enhancement of nerve regrowth, muscle mass maintaining, and functional recovery. BMSCs inhibitedneuronalcelldeathandoverturntheinflammatoryresponseinduced by long-term chitosan implantation promoting nerve regeneration (Hsu et al., 2013). Chitosan 3-glycidoxypropyl-methyldiethoxysilane-cross-linked membranes have been used for peripheral nerve reconstruction in the rat sciatic nerve model, either alone or in combination with N1E-115 NSCs. Chitosan membranes showed good biocompatibility and were suitable for N1E-115 cells growth. However, the study of in vivo nerve regeneration after nerve crush injury showed that freeze-dried chitosan membrane without cell enrichment improved axonal regrowth and functional recovery, suggesting that nerve enwrapping with chitosan membrane alone may be an effective method for improving peripheral nerve repair, while enrichment with N1E-115 neural cells is not (Amado et al., 2008; Simoes et al., 2011).

4.3. Chitosan conduits combined with neurotrophic factors or neuroprotective molecules for PNS repair

After an injury, one of the causes contributing to apoptosis and poor functional recovery is the neurotrophic factor deprivation (Ramer, Priestley, & McMahon, 2000). Nerve regeneration has been found to be enhanced by utilizing guidance channels filled with neurotrophic factors, such as GDNF, ciliary nerotrophic factor (CNTF), FGF-2, and NT-3 (Grothe, Haastert, & Jungnickel, 2006; Madduri, Feldman, Tervoort, Papaloizos, & Gander, 2010; Madduri, Papaloizos, & Gander, 2010; Oh et al., 2008; Pfister et al., 2008; Yang et al., 2007). Various types of nerve guides have been

developed by blending chitosan with different growth factors (Table 1.8). Adding growth factors can support nerve regeneration by improving the biological properties of a nerve guide. GDNF has been mixed to chitosan–lamininconduitswhichhavebeenusedtobridge10-mmratsciatic nerve gaps. GDNF–laminin-blended chitosan conduits increased functional recovery and decreased muscle atrophy compared with unblended chitosan conduits (Patel et al., 2007). Histologically, the GDNF–laminin-blended chitosan conduits demonstrated at 6 weeks postsurgery regenerated axons with higher axonal area and myelination in comparison with control conditions. At 9–12 weeks postsurgery, however, control groups matched the GDNF–laminin-blended chitosan group indicating that these kinds of conduits exert their positive effectsduring the initial stages of nerve regeneration only (Patel et al., 2009). In another study, CNTF-coated PLGA chitosan nerve conduit has been utilized to repair 25-mm-long segments in the canine tibial nerve. Histological results demonstrated that the PLGA/chitosan–CNTF conduits were capable of guiding the damaged axons through the lesioned area, resulting in good functional recovery close to the outcome in the nerve autograft group (Shen et al., 2010). Yet, also NGF has been immobilized onto biodegradable PDLLA/CS/ CHS nontoxic nerve conduits, resulting in good functional recovery after bridging 10-mm defects in the rat sciatic nerve (Xu et al., 2011). Moreover, chitosan conduit on which NGF was immobilized via genipin cross-linking resulted in nerve reconstruction and muscle reinnervation in a 10-mm-long sciatic nerve gap in rat (Wang et al., 2012). Chitosan nerve conduits filled with heparin-incorporated fibrin– fibronectin matrix for bFGF delivery have been successfully used to repair sciatic nerve defects of 10-mm in adult rats (Han et al., 2010).

Interestingly, a couple of studies showed that chitooligosaccharides (COSs), the biodegradation products of chitosan, promote peripheral nerve regeneration and functional recovery in the rat sciatic nerve crush injury model and rabbit common peroneal nerve crush injury model, suggesting their potential application in peripheral nerve repair as neuroprotective agents (Gong et al., 2009; Jiang et al., 2009). Moreover, immunophilin FK506 combined with biodegradable chitosan guide provides neurotrophic and neuroprotective actions, promoting nerve regeneration in a rat sciatic nerve defect model (Li et al., 2010). Also neurosteroids, such as progesterone (PROG) and pregnenolone (PREG), have been used for potentiating conduit nerve repair as these hormones are synthesized by SCs (Baulieu & Schumacher, 2000) and induce myelination binding on intracellular receptors which activate the synthesis of myelin protein P0 and PMP22 (Desarnaud et al., 1998; Jung-Testas, Schumacher, Robel, & Baulieu, 1996). Furthermore, neurite outgrowth may be stimulated by a PROG metabolite (5-a-tetrahydroprogesterone) through GABA(A) receptors (Guennoun et al., 2001; Koenig, Gong, & Pelissier, 2000). Chitosan conduits have been used to deliver PROG into a 10-mm rabbit facial nerve gap model. The released PROG promoted nerve regenerationtoa highdegreesothatat45 dayspostsurgerymyelinated fibers were observed both in the proximal and distal nerve stumps (ChavezDelgado et al., 2003). Similar results have been reported using axotomized dog sciatic nerve injured model (Rosales-Cortes, Peregrina-Sandoval, Banuelos-Pineda, Castellanos-Martinez, et al., 2003). Chitosan tubes delivering a combination of PROG and PREG have also been tested. Fifteen days postsurgery, the regenerating tissue contained SCs holding nonmyelinated fibers, whereas at 45 days postsurgery, the regenerating tissue displayed myelinated fibers of different shape, size, and myelin sheath thickness (Chavez-

Delgado et al., 2005). These findings indicate that chitosan conduits allows regeneration of nerve fibers and that long-time release of neurosteroid from the conduits induces faster regeneration.

5. CONCLUSION

Among the many different types of biomaterials that have been proposed as optimal candidates for neural repair scaffolds, chitosan has been receiving growing interests among basic and clinical scientists. In this review, we have overviewed the more relevant articles demonstrating that this biomimetic material exerts a positive effect on cell cultures of both neurons and glial cells. Yet, experimental results obtained from several animal models in vivo and first data from the first few clinical studies which were done so far have shown that chitosan-based scaffolds are good candidates for developing innovative devices for neural repair of both the CNS and PNS.

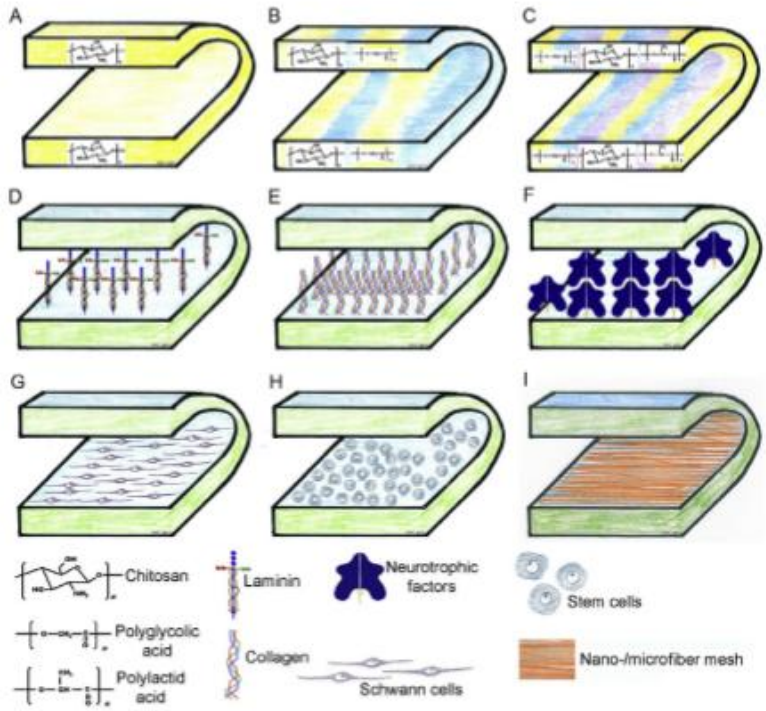

Figure 1.1 Summary of the most common chitosan-blend materialsusedfor the fabrication ofnervegraft devices.In general, basic materials are composed of (A)chitosan in differentdegreesofacetylationtotailortheirrateofbiodegradabilityorchitosan-blendedwith, forexample,polyglycolicacid(PGA)(B),polylactidacid(PLA),orothercommonlyusedpolymers.Also,blendsofchitosanwithmorethanonepolymerhavebeendescribed,forexample, PGA and PLA (C). Chitosan-based materials can further be modified at their surfaces which will be in contact to the regenerating neural tissue: extracellular matrix glycoproteins-like laminin (D) or laminin-derived peptides can be added as an collagen (E) or diverse neurotrophic factors (F), like NGF, GDNF, CNTF, or NT-3. The inner surfaces oftheguidancechannelscanbemanufacturedtoallowseedingofregeneration-supporting celltypes,likealignedSchwanncells(G)ordifferenttypesofstemcells(H).Finally,theinnovativeelectrospinning technique canbe utilized to secondary structure the surface of the chitosan-based materials byadding oriented nano- or microfibers (I).

Chitosan is biocompatible, biodegradable, and its chemicophysical properties can be easily manipulated with the goal to create scaffolds with different structural features (i.e.,

biodegradation time or surface properties). Figure 1.1 summarizes the most cited chitosan and chitosan-blend nerve conduits (Fig. 1.1A–C) as well as the most frequently investigated surface modifications of chitosan-based nerve conduits (Fig. 1.1D–I). After reviewing the literature regarding the use of chitosan in neural repair approaches, it can be foreseen that the time for clinical trials utilizing chitosan-based nerve regeneration-promoting devices is approaching quickly.

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