Electrodeposition of chitosan/graphene oxide conduit to enhance peripheral nerve regeneration

Yan-Nan Zhao1,2,3, Ping Wu1,4, Zi-Yuan Zhao3, Fei-Xiang Chen3, Ao Xiao4, Zhi-Yi Yue4, Xin-Wei Han4, Yong Zheng1, *, Yun Chen1, *

Abstract
Currently available commercial nerve guidance conduits have been applied in the repair of peripheral nerve defects. However, a conduit exhibiting good biocompatibility remains to be developed. In this work, a series of chitosan/graphene oxide (GO) films with concentrations of GO varying from 0–1 wt% (collectively referred to as CHGF-n) were prepared by an electrodeposition technique. The effects of CHGF-n on proliferation and adhesion abilities of Schwann cells, neuronal survival, and Wallerian degeneration were evaluated. The results showed that Schwann cells exhibited elongated spindle shapes and upregulated expression of nerve regeneration-related factors such as Krox20 (a key myelination factor), Zeb2 (essential for Schwann cell differentiation, myelination, and nerve repair), and transforming growth factor β (a cytokine with regenerative functions). In addition, a nerve guidance conduit with a GO content of 0.25% (CHGF-0.25) was implanted to repair a 10-mm sciatic nerve defect in rats. The results indicated improvements in sciatic functional index, electrophysiology, and sciatic nerve and gastrocnemius muscle histology compared with the CHGFC-0 group, and similar outcomes to the autograft group. In conclusion, we provide a candidate method for the repair of peripheral nerve defects using free-standing chitosan/GO nerve conduits produced by electrodeposition.

Key Words: chitosan; electrodeposition; free-standing; graphene oxide; nerve conduit; nerve factors; Schwann cells; tissue engineering

Introduction
Peripheral nerve injury (PNI) caused by trauma, burn, or surgical intervention is a global clinical problem that leads to complete or partial loss of motor, sensory, and autonomic functions, therefore drastically affecting the quality of life of patients (Mobini et al., 2019; Hussain et al., 2020; Chen et al., 2021; Zaming et al., 2021). Regeneration following PNI is accompanied by a multiplex biological process in which Schwann cells (SCs) play an important role (Zaming et al., 2020a; Li et al., 2021). Mechanically, SCs provide a suitable microenvironment for the inventory and regeneration of nerve fibers, furnish vital nutrition, support cell adhesion, and guide directional nerve cell migration (Wang et al., 2021a, c; Cai et al., 2022). SCs inherently participate in Wallerian degeneration, whereby they contribute to phagocytosis of myelin and axon debris, axon regeneration, deposition of a supportive extracellular matrix, neurotrophin release, and macrophage recruitment to clear debris after PNI (Navarro et al., 2007; Novikova et al., 2008; Deumens et al., 2010; Carriel et al., 2017). Thus, implantation of a nerve guidance conduit (NGC) at the site of injury needs to support SC growth to benefit functional recovery of the injured nerve.

Artificial NGCs developed by tissue engineering from materials including poly lactide-caprolactone, polyactic acid, chitosan (CH), collagen, graphene oxide (GO), and cellulose have been widely used for PNI repair (Siemionow et al., 2010; Wu et al., 2020). Among these components, CH is a cationic polysaccharide broadly applied in the field of peripheral nerve regeneration for its biocompatibility, biodegradability, and antimicrobial activity (Lau et al., 2018; Wu et al., 2021; Liu et al., 2022). The oxidized form of graphene, GO, has abundant oxygen-containing groups, such as hydroxyl (-OH) and carboxyl (-C=O) groups, a high specific surface area, good mechanical properties, flexibility, and excellent electrical conductivity (Reina et al., 2014; Gardin et al., 2016). In addition, GO is biocompatible at low concentrations (Nishida et al., 2014; Nagarajan et al., 2016) and acts as an alternative for reinforcing material (Han et al., 2011; Ouyang et al., 2015; Sayyar et al., 2015). Importantly, GO has also been shown to promote the proliferation of SCs, hippocampal cells, PC12 cells, and stem cells (Li et al., 2011b, 2016; Gardin et al., 2016; Liu et al., 2017).

CH-based NGCs can be prepared by chemical crosslinking or mold-forming techniques. The basic function of the NGC is to support the adhesion, proliferation, and migration of nerve and glial cells (Vijayakumarakaran, 2020). To improve the in vivo performance of CH-based NGCs, a series of material modification strategies were developed. Rao et al. (2020) designed an aligned-Ch hydrogel NGC grafted with bioactive peptides, which yielded great application effects for repair of long-distance sciatic nerve defects. Chen et al. (2021) showed that depletion of interleukin 17F in the nerve regeneration microenvironment could improve the repair effect of CH-based NGCs. In addition, numerous physical and biological parameters were shown to be beneficial for nerve regeneration (Grinsell and Keating, 2014; Li et al., 2020). In this work, we hypothesized that incorporation of GO into CH-based NGCs could enhance their biocompatibility. No previous report has described a CH/GO composite NGC, likely because of a lack of an appropriate material processing technology.
Electrodeposition is an attractive material processing technique characterized by programmable assembly in response to device-imposed stimuli (Xi et al., 2009; Sun et al., 2015). Because of its self-assembling and pH-responsive properties, CH can be used to build a film on a conducting electrode using electrodeposition methods (Wang et al., 2003). Compared with conventional techniques, electrodeposition is a more facile and precise method to form stable CH films with controllable thickness, stiffness, and other properties on a variety of substrates (Liu et al., 2011). Specifically, the presence of functional groups of GO greatly enhances its surface ability to bind other materials (Shen et al., 2012; Li et al., 2016). Thus, GO could be homogeneously dispersed into CH solution and subsequently co-deposited along with CH.

This work aimed to fabricate a series of CH/GO composite NGCs using a novel electrodeposition technique, and subsequently evaluate their chemical composition, surface morphology, and in vitro biocompatibility. Our findings suggest that the obtained CH/GO composite NGCs have relatively good performance in vivo for PNI repair.

Methods

Materials

Chitosan powder (200-kDa molecular weight and 80% deacetylation degree) was purchased from TCI Biotechnology (Shanghai, China). GO nanosheets were obtained from Hengqiu Technology (Suzhou, China). A spontaneously immortalized Schwann cell line RSC 96 (CSTR: 19375.09.3101RATGNR6) was supplied by the National Collection of Authenticated Cell Cultures (Shanghai, China). Fetal bovine serum, alpha minimum essential medium (α-MEM), trypsin/EDTA solution, and penicillin-streptomycin solution were supplied by Sigma (St. Louis, MO, USA). HCl, dimethyl sulfoxide (DMSO), and NaOH were obtained from Sinopharm Chemical Reagent (Beijing, China). Other chemicals were used without further purification.

Electrodeposition of chitosan/graphene oxide composites

CH solution (CS, 1 wt%) was prepared by liquefying CH in a 0.25 M HCl solution under vigorous stirring, and then adjusting the pH value to 5.5 using 0.25 M NaOH solution. GO (0.2 wt%) was dispersed into deionized water under continuous stirring. The GO suspension was added to the CS solution and sonicated for 5 minutes to obtain a homogeneous solution. Next, the prepared CS/GO solution was transferred into a beaker for electrodeposition. A titanium plate (0.1 × 40 × 20 mm²) served as the cathode and a platinum wire served as the anode. Electrodeposition was performed by immersing the titanium plate and 1.5 cm of the platinum wire into the CS/GO solution. A programmable DC power supply (Keithley, Shanghai, China) was employed to apply 5–6 V to these two electrodes for 1 hour, resulting in films with a thickness of ~3 mm. Following washing of films with distilled water to clear any unbound GO particles, they were dried for further studies. The resultant films were named CHGF-0.25/0.5/1 or collectively CHGF-n, where CH stands for chitosan, G for graphene oxide, F for film, and n for the concentration of GO (wt%) in the mixed solution. Table 1 shows the sample names and compositions of various CHGF-n preparations. To further prepare CH/GO NGCs containing GO contents of 0 and 0.25 wt%, we replaced the titanium plate of the CHGF-0 with a titanium plate of CHGF-0.25.

| Sample code | CS (mL) | GO (mL) | Deionized water (mL) | Total volume (mL) | Sample type |
|-------------|--------|--------|----------------------|---------------|-------------|
| CHGF-0      | 50     | 0      | 50                   | 100           | Film        |
| CHGF-0.25   | 50     | 12.5   | 37.5                 | 100           | Film        |
| CHGF-0.5    | 50     | 25     | 25                   | 100           | Film        |
| CHGF-1      | 50     | 50     | 0                    | 100           | Film        |
| CHGF-C      | 0      | 0      | 50                   | 100           | Conduit     |
| CHGF-C0.25  | 50     | 12.5   | 37.5                 | 100           | Conduit     |

CHGF: Chitosan/graphene oxide film; CHGF-C: CHGF-based conduit; CS: chitosan; GO: graphene oxide.

Characterization of CHGF

Morphological analysis of CHGF was evaluated with a digital camera (Coolpix P6000, Nikon, Tokyo, Japan) and scanning electron microscope (SEM; TESCAN, Brno, Czech Republic). The morphology, thickness, and wettability of the CHGF films were examined by SEM. CHGF films were coated with Au-Pd (10 nm) and then observed with an accelerating voltage of 20 kV. The surface morphology and roughness of CHGF films were evaluated by atomic force microscopy (AFM; Bruker, Waltham, MA, USA). The water contact angle (WCA) was measured using the sessile drop method.

Table 1  Sample codes and compositions of films and conduits

| Sample code | CS (mL) | GO (mL) | Deionized water (mL) | Total volume (mL) | Sample type |
|-------------|--------|--------|----------------------|---------------|-------------|
| CHGF-0      | 50     | 0      | 50                   | 100           | Film        |
| CHGF-0.25   | 50     | 12.5   | 37.5                 | 100           | Film        |
| CHGF-0.5    | 50     | 25     | 25                   | 100           | Film        |
| CHGF-1      | 50     | 50     | 0                    | 100           | Film        |
| CHGF-C      | 0      | 0      | 50                   | 100           | Conduit     |
| CHGF-C0.25  | 50     | 12.5   | 37.5                 | 100           | Conduit     |

In which A₀, Aᵣ, and Aₕ denote the absorption values of the sample, negative control, and blank control, respectively.

Cell contact experiments

To explore the potential utilization of CHGF as an NGC, SCs were directly cultured on the samples to observe their behaviors. Prior to the seeding stage, CHGF was sterilized and washed three times with distilled water. For cell morphology observation, SCs were seeded at a density of 5 × 10⁴ cells per well on the surface of CHGF and incubated for 48 hours at 37°C. Tissue culture plastic coated with laminin was used as a control. After incubation, cells on the films were fixed with 3.7% parafomaldehyde solution overnight at room temperature. The following day, fixed cells on the surfaces of CHGF samples were dehydrated in a gradient series of ethanol solutions (Zhao et al., 2018b). Finally, samples were coated with gold under vacuum for observation by SEM. SCs on the surface of the CHGF film were further identified by calcine acetoxyethyl ester (calcine AM) staining (50 µg; Calcine AM Assay Kit, ab141420, Abcam, Cambridge, UK). Briefly, SCs on samples were stained with AM dye for 30 minutes at 37°C and observed by fluorescence microscopy (Olympus, Tokyo, Japan).

Quantitative reverse transcription-polymerase chain reaction

mRNA expression of Krox20 (a key myelination factor), transforming growth factor β (TGF-β, a cytokine with regenerative functions), and Zeb2 (essential for Schwann cell differentiation, myelination, and nerve repair) was evaluated in SCs cultured with CHGF-n extracts. After SCs were plated onto tissue culture plastic or CHGF for 24 hours, mRNA expression levels of Krox20, Zeb2, and TGF-β in SCs were determined with quantitative reverse transcription-polymerase chain reaction (qRT-PCR). First, total cellular RNA was extracted from SCs with TRIzol according to the manufacturer’s instructions. Next, cDNA was transcribed from total cellular RNA with a cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Finally, SYBR Green or ROX qPCR Master Mix was employed to perform qRT-PCR. The temperature gradient of amplification was as follows: 95°C for 15 minutes; and 40 cycles of 15 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C. Relative expression levels were calculated using the comparative 2⁻ΔΔCt method. Primers used in this study are listed in Table 2.

Table 2  Primer sequences for quantitative reverse transcription-polymerase chain reaction

| Gene     | Primer sequence (5’–3’) | Product size (bp) |
|----------|-------------------------|-------------------|
| Krox20   | F: GGA GGA GCA AAT GAT GAC CG | 89 |
|          | R: ATC ATG CCA TCT GCA ACC AT | |
| Zeb2     | F: AAA GCA GTC CCC TTC TGC GA | 102 |
|          | R: AGG AGC CAG AGT GTG AAA AG | |
| TGF-β    | F: CTT CTC ACC CCC ACT GAT AC | 97 |
|          | R: AGC CCT GTA TGC CTC CT | |
| GAPDH    | F: AGT GCC AGC CTC TCA TA | 122 |
|          | R: GGT AAC CAG GGC TCC GAT AC | |

F: Forward; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; R: reverse; TGF-β: transforming growth factor β.
Animals and surgical procedures
All animal experiments were performed in accordance with Guiding Opinions on the Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People’s Republic of China, and approved by the Animal Care and Welfare Committee of the Wuhan University School of Medicine (2018116) on October 22, 2018. Forty-two female Sprague-Dawley rats (200–220 g) were provided by Wuhan University Laboratory Animal Center (Wuhan, Hubei, China), and then randomly divided into three groups: autograft, CHGF-C0, and CHGF-C0.25. For surgical procedures, animals were anesthetized by an intraperitoneal administration of 1% pentobarbital sodium (150 mg/kg) (Sigma). Next, the sciatic nerve in the right hind limb was exposed from nerve stump to distal nerve stump, and treated and covered with autograft or a CHGF conduit. For the autograft group, the excised autograft was rotated by 180° and sutured to the proximal and distal nerve stumps. In the CHGF-C0 and CHGF-C0.25 groups, conduits with GO contents of 0% and 0.25% were sutured to the proximal and distal nerve stumps of the injured nerve. After transplantation, the muscle cut and skin were sutured. All procedures were performed under aseptic conditions and penicillin (3 mg/kg) was administered subcutaneously 3 times a day for 3 days. After 90 days, rats were euthanized and evaluated for nerve regeneration.

Sciatic functional index
To explore functional recovery after surgery, sciatic functional index (SFI) was evaluated at 90 days. Black ink was applied to the hind paws of rats, which were subsequently placed in a 15 × 100 cm corridor covered with a sheet of white paper. Three parameters including print length (PL: longest distance from toe to heel), intermediate toe spread (IT: distance from the second to fourth toes), and toe spread (TS: distance from first to fifth toes) were obtained from the paw prints. Data for normal (N) and experimental (E) hind legs were normalized. The formula $SFI = (L_{IE} - L_{NE}) / L_{NE}$ (where $L_{IE}$ is the print length of the injured leg and $L_{NE}$ is the print length of the normal leg) was employed to calculate the SFI. SFI values range between −100 and 0, with −100 denoting complete dysfunction of nerves and 0 denoting good function.

Electrophysiological test
Electrophysiological studies were also used to evaluate functional recovery of the regenerated nerve. At 90 days after implantation, rats were anesthetized and the sciatic nerve on the operative side was re-exposed. A stimulation electrode was applied to the proximal nerve trunk of the rat, and compound muscle action potentials (CMAPs) of the gastrocnemius muscle on the operative side were recorded. The stimulating mode was set to pulse mode with a 1-Hz frequency, 10-mV stimulus intensity, and 1-ms duration.

Histological detection of nerve regeneration
At 90 days after implantation, the distal nerve ends of each group were obtained and fixed in 2.5% glutaraldehyde overnight. After washing with PBS, samples were post-fixed in 1% osmium tetroxide (Sigma) for 1 hour, cleaned, dehydrated, and embedded in Epon 812 epoxy resin (Sigma). Next, the epoxy resin was cut into ultrathin sections of 0.5 mm thickness and stained with lead citrate and uranyl acetate. Finally, stained sections were examined by transmission electron microscopy (FEI, Thermo Fisher Scientific). Image-Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD, USA) was employed to obtain the area of myelinated axons, thickness of myelin sheaths, and number of myelin sheaths.

Gastrocnemius muscle measurement and histological assessment
Morphology and function of the gastrocnemius muscle are reliable parameters to examine nerve regeneration (Xue et al., 2017). At 90 days after implantation, the bilateral gastrocnemius muscles were dissected and weighed. The muscle recovery ratio was calculated as the right-side weight divided by the left-side weight. Next, gastrocnemius muscles were stored in 4% paraformaldehyde until staining with a Masson’s Trichrome Staining Kit (Beyotime) to visualize collagen contents. Images of five randomly selected fields in each sample were acquired with a light microscope (Olympus). Image-Pro Plus software was employed to determine the cross-sectional areas of muscle and collagen fibers. The percentage area of collagen fibers was calculated as the collagen fiber area divided by the sum of collagen and muscle fiber areas.

Statistical analysis
No blinded method was applied in the collection or analysis of the results. Data are expressed as the mean ± standard deviation. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by the least significant difference test using SPSS 20.0 (IBM, Armonk, NY, USA). P < 0.05 was considered statistically significant.

Results
Fabrication of CHGF
The increased pH gradient generated close to the electrode causes CH electrodeposition on the cathode (Zhao et al., 2014). GO sheets are chemically resistant to aqueous solutions, leading to irreversible deposition (Stankovich et al., 2007). Thus, it is reasonable to speculate that the GO sheets could directly attach to the electrode surface (Chen et al., 2011). When a voltage is applied, protons around the electrode surface are consumed, leading to the formation of water. At the same time, positively-charged protons and negatively-charged GO wrap around to the cathode and are co-deposited from bulk solution together. Conclusively, the electrodeposition method is a simple and alternative strategy to fabricate CHGF on conductive substrates.

Characterization of CHGF
Optical images of CHGF-n are shown in Figure 1A. It was observed that CHGF-0 was transparent, while CHGF-0.25, CHGF-0.5, and CHGF-1 appeared light brown, dark brown, and black, respectively. These results confirmed that GO was successively electrodeposited together with CH. Next, the microstructure of CHGF-n was observed by SEM. As shown in Figure 1B, CHGF-0 exhibited a rough surface morphology. Compared with CHGF-0, CHGF-0.25 exhibited the typical crumpled and wrinkled texture associated with the presence of flexible and ultrathin graphene flakes (Liu et al., 2013). These flakes were evenly distributed among the film surface, providing physical cues for cell adhesion growth. In addition, several studies documented that GO could be used as chemical cue to enhance nerve cell growth and neurite extension (Liu et al., 2017). However, the surface changed as the GO content increased, with CHGF-0.5 exhibiting flake-like structures and CHGF-1 exhibiting a relatively smooth surface, potentially caused by GO accumulation. Figure 1C displays SEM images of CHGF-n cross-sections. The cross-section structure of CHGF-0 was observed to be smooth. When the addition of GO, uniform flake-like structures appeared on the cross-section of CHGF-0.25. This flake-like structure was replace by a more obvious multilayered structure in CHGF-0.5. However, the multilayered structure appeared slightly deteriorated in CHGF-1, likely owing to the excessive GO added to CS during the electrodeposition process. Conclusively, GO could be evenly dispersed within the CH film and the structure of deposited CHGF-0.5 was influenced by the proportion of GO.
Effects of CHGF on SC growth and behaviors in vitro

As shown in Figure 3A, the activity of SCs on CHGF-0.25 was highest at 24 hours after incubation on CHGF-0.25 and CHGF-1, consistent with the notion that GO has dose-cytotoxicity. At 48 hours, the cell activity of each group was higher compared with that at 24 hours, and cell activity on CHGF-0.25 remained the highest. At 72 hours, cell activities on CHGF-0, CHGF-0.5, and CHGF-1 began to decrease, and CHGF-25 retained a significant increase of cell viability compared with the control group. These results clearly suggest that cell viability depended on GO content and cell culture duration, and the excellent cytocompatibility of CHGF-0.25 makes it a promising material for constructing CHGF for peripheral tissue engineering applications.

Figure 3C shows SEM images of SCs on CHGF-n. Cells cultured on the CHGF-0, CHGF-0.5, and CHGF-1 were round and clustered together, which may be caused by the hydrophobic surface, microstructure, and chemical composition of these three samples. Cells cultured on CHGF-0.25 were well spread out and exhibited long spindle shapes, possibly due to its hydrophilic characteristics. SCs were further characterized by immunofluorescence staining. As shown in Figure 3B, in contrast to cells on other samples, cells cultured on CHGF-0.25 had spindle shapes. Therefore, samples with a GO content of 0.25% were identified as the favorable cell carrier to promote the spread and growth of SCs.

As shown in Figure 3D–F, mRNA expression levels of Krox20, TGF-β, and Zeb2 in all CHGF-n groups were remarkably higher compared with the control group. Additionally, mRNA expression levels were relatively increased in the CHGF-0.25 group. Conclusively, CHGF-n, especially CHGF-0.25, promoted expression of nerve-related factors such as Krox20, Zeb2, and TGF-β, thus ensuring the regeneration of damaged nerves. Collectively, the results described above show that CHGF-0.25 had the most suitable surface structure, mechanical properties, and cell compatibility for SC growth, and the best performance for nerve-related factor secretion. Thus, the conduit referred to as CHGF-0.25 (based on CHGF-0.25) was selected for animal experiments to evaluate nerve repair effects. CHGF-0 and autograft groups were used as controls.

Animal experiments for CHGF-C

Motor function of rats

There was a remarkable improvement in motor function of the CHGF-C-0.25 group compared with the CHGF-C-0 group (Figure 4A). SFI values of the CHGF-C-0.25 group were similar to those of the autograft group, but SFI values of the CHGF-C-0 group were significantly lower compared with autograft and CHGF-C-0.25 group (P < 0.05 and P < 0.01, respectively; Figure 4B). CMAP amplitudes on the surgical side of the CHGF-C-0 and CHGF-C-0.25 groups were significantly lower compared with the autograft group (P < 0.01 and P < 0.05, respectively; Figure 4D).

Morphology of regenerated nerves

As shown in Figure 5A, regenerated myelinated fibers were scattered in clusters (with the exception of an occasional unmyelinated fiber) and myelinated axons were covered with a clear myelin sheath in the autograft group. Structures of myelinated fibers in the CHGF-C-0.25 group were similar to those of the autograft group. In contrast, myelinated fibers were seldom observed in the CHGF-C-0 group. Areas of myelinated axons, thicknesses of myelin sheaths, and numbers of myelin sheath layers were significantly decreased in the CHGF-C-0 group compared with the autograft group (P < 0.05 or P < 0.01; Figure 5B–D), while thicknesses of myelin sheaths and numbers of myelin sheath layers were significantly increased in the CHGF-C-0 group compared with the CHGF-C-0.25 group (P < 0.01 and P < 0.05). Regenerated nerves in the CHGF-C-0.25 group showed better recovery compared with the CHGF-C-0 group but remained inferior to the autograft group.
been widely used for surface modification of metal devices. Our group and cooperative teams successfully introduced electrodeposition technology into the field of biomaterials, and successively developed a series of chitin/CH biomaterials with good economic benefits (Zhao et al., 2018a; Liu et al., 2021).

Preliminary progress has been made in the development of electrodeposition CH-based NGCs. Notably, this work revealed for the first time that GO could effectively improve the biocompatibility and bioactivity of CH-based NGCs for glial cells, and thus improve the application effect in vivo. GO is an inorganic non-metallic material with desirable conductivity and biocompatibility. Owing to its high performance, publications describing GO in the field of biomedical materials have consistently increased annually (Shende and Pathan, 2021). Our results showed that the introduction of GO into CH significantly changed the chemical composition, micro-structure and mechanical strength, and biocompatibility of the obtained composite NGC. Thus, inclusion of GO might be a key reason for the promotive effects observed for NGCs. In recent years, the biological mechanism of GO has been revealed. We assumed that GO could be swallowed by glial cells during degradation in vivo, thereby it participates in intracellular signal transduction to synergistically promote nerve regeneration. In conclusion, a GO-modified CH-based NGC prepared by electrodeposition technique promoted the treatment effect for PNI, which was attributed to synergistic effects between the NGC and glial cells. Above all, the application of GO can be refined into a general strategy to upgrade existing NGC materials.

Limitations

Even animals with permanently sectioned sciatic nerves tend to develop locomotor strategies that partially compensate for the loss in function induced by nerve damage. Thus, an additional experimental group with impeded reinnervation (a negative control group) is necessary to evaluate the magnitude of treatment responses.

Conclusions

In this study, we constructed a series of CHGFs and evaluated their physicochemical and biological properties. In vitro experiments showed that GO was successfully integrated in CHGF to enhance its mechanical properties, thus promoting CHGF to peel off from the mold to form free-standing films. CHGFC-0.25 significantly enhanced SC growth, extension, and secretion of nerve-related factors such as Krox20, 2eb2, and TGF-B. In vivo results showed that CHGFC-0.25 could guide the regeneration of injured nerves, thus representing a candidate strategy for peripheral nerve repair. CHGF prepared by electrodeposition is green, simple, accurate, nontoxic, and harmless, and provides a new direction for preparation of NGCs.

Discussion

CH is an ideal biomedical polymer that can be processed by mold-forming and other techniques (Hu et al., 2019a). In our previous works, we successfully developed a variety of CH-based NGCs. On this basis, the in vivo application effect of these CH-based NGCs was further improved by physicochemical and biological methods. For example, multi-channel NGCs were prepared to promote the proliferation and directional migration of nerves cells, effectively shortening the time required for nerve recanalization (Zhao et al., 2018a). Electrodeposition, a simple and efficient material processing technology, has been widely used for surface modification of metal devices. Our group and cooperative teams successfully introduced electrodeposition technology into the field of biomaterials, and successively developed a series of chitin/CH biomaterials with good economic benefits (Zhao et al., 2018a; Liu et al., 2021).

Preliminary progress has been made in the development of electrodeposition CH-based NGCs. Notably, this work revealed for the first time that GO could effectively improve the biocompatibility and bioactivity of CH-based NGCs for glial cells, and thus improve the application effect in vivo. GO is an inorganic non-metallic material with desirable conductivity and biocompatibility. Owing to its high performance, publications describing GO in the field of biomedical materials have consistently increased annually (Shende and Pathan, 2021). Our results showed that the introduction of GO into CH significantly changed the chemical composition, micro-structure and mechanical strength, and biocompatibility of the obtained composite NGC. Thus, inclusion of GO might be a key reason for the promotive effects observed for NGCs. In recent years, the biological mechanism of GO has been revealed. We assumed that GO could be swallowed by glial cells during degradation in vivo, thereby it participates in intracellular signal transduction to synergistically promote nerve regeneration. In conclusion, a GO-modified CH-based NGC prepared by electrodeposition technique promoted the treatment effect for PNI, which was attributed to synergistic effects between the NGC and glial cells. Above all, the application of GO can be refined into a general strategy to upgrade existing NGC materials.

Limitations

Even animals with permanently sectioned sciatic nerves tend to develop locomotor strategies that partially compensate for the loss in function induced by nerve damage. Thus, an additional experimental group with impeded reinnervation (a negative control group) is necessary to evaluate the magnitude of treatment responses.

Conclusions

In this study, we constructed a series of CHGFs and evaluated their physicochemical and biological properties. In vitro experiments showed that GO was successfully integrated in CHGF to enhance its mechanical properties, thus promoting CHGF to peel off from the mold to form free-standing films. CHGFC-0.25 significantly enhanced SC growth, extension, and secretion of nerve-related factors such as Krox20, 2eb2, and TGF-B. In vivo results showed that CHGFC-0.25 could guide the regeneration of injured nerves, thus representing a candidate strategy for peripheral nerve repair. CHGF prepared by electrodeposition is green, simple, accurate, nontoxic, and harmless, and provides a new direction for preparation of NGCs.

Acknowledgments: The authors are grateful to the Experimental Teaching Center of Basic Medical Sciences, Wuhan University for technical support. Author contributions: YNZ, PW and YC designed the study and drafted the manuscript. YZ, FXC and AX conducted surgery and behavioral tests. YZ and YC carried out histology and image analysis. YWH and YZ helped to draft the manuscript. YC performed manuscript editing and review. All authors read and approved the final manuscript for publication.

Conflicts of interest: The authors declare no competing financial interest.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

References

Cai M, Shao J, Yung B, Wang Y, Gao NN, Xu X, Zhang HH, Feng YM, Yao DB (2022) Baculoviral inhibitor of apoptosis protein repeat-containing protein 3 delays early Wallerian degeneration after sciatic nerve injury. Neural Regen Res 17:845-853.

Carrie V, Sarzoni I, Campos A, Cornelissen M, Alamrons M (2017) Differential expression of GAP-43 and neurofilament during peripheral nerve regeneration through bioartificial conduits. J Tissue Eng Regen Med 11:153-563.

Chen F, Liu W, Zhang Q, Wu P, Xiao A, Zhao Y, Zhou Y, Wang Q, Chen Y, Tong Z (2021) IL-17F depletion accelerates chitosan conduit guided peripheral nerve regeneration. Acta Neuropathol Commun 9:125.

Chen L, Tang Y, Wang K, Liu C, Luo S (2011) Direct electrodeposition of reduced graphene oxide on glassy carbon electrode and its electrochemical application. Electrochem Commun 13:133-137.

Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, Brook GA (2010) Repairing injured peripheral nerves: Bridging the gap. Prog Neurobiol 92:245-276.

Ferrari A (2007) Raman spectroscopy of graphene and graphite: Disorder, electronphonon coupling, doping and nonadiabatic effects. Solid State Commun 143:47-57.

Gardin C, Piattelli A, Zavan B (2016) Graphene in regenerative medicine: focus on stem cells and neuronal differentiation. Trends Biotechnol 34:435-437.

Grissell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. Biomed Res Int 2014:698256.
Research Article

Rao F, Wang Y, Zhang D, Lu C, Cao Z, Sui J, Wu M, Zhang Y, Pi W, Wang B, Kou Y, Wang X, Ouyang A, Wang C, Wu S, Shi E, Zhao W, Cao A, Wu D (2015) Highly porous core-shell...

Novikova LN, Pettersson J, Brohlin M, Wiberg M, Novikov LN (2008) Biodegradable poly...

Nishida E, Miyaji H, Takita H, Kanayama I, Tsuji M, Akasaka T, Sugaya T, Sakagami R, Nagarajan S, Pochat-Bohatier C, Teyssier C, Balme S, Miele P, Kalkura N, Cavaillès V, Liu Y, Yang S, Niu W (2013) Simple, rapid and green one-step strategy to synthesis of...

Liu H, Zhao Y, Tong J, Shi X, Chen Y, Du Y (2021) Electrofabrication of flexible and mechanically strong tubular chitosan implants for peripheral nerve regeneration. J Mater Chem B 9:5537-5546.

Li L, Xu Y, Wang X, Liu J, Hu X, Tan D, Li Q, Gao J (2021) Acetic acid accelerates Wallerian degeneration after peripheral nerve injury. Neural Regen Res 16:1078-1085.

Li N, Zhang X, Song Q, Su R, Zhang Q, Kong T, Liu L, Jin G, Tang M, Cheng G (2011) The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by graphene substrates. Biomaterials 32:9374-9382.

Li R, Li DH, Zhang HY, Wang J, Li X, Xiao J (2020) Growth factors-based therapeutic strategies and their underlying signaling mechanisms for peripheral nerve regeneration. Acta Pharmacol Sin 41:1289-1300.

Liu FD, Duan HM, Hao F, Zhao W, Gao YO, Hao P, Yang ZY, Li XG (2022) Biomimetic chitosan scaffolds with long-term controlled release of nerve growth factor repairs 20-mm-long sciatic nerve defects in rats. Neural Regen Res 17:1146-1155.

Liu H, Zhao Y, Tong J, Shi X, Chen Y, Du Y (2021) Electrofabrication of flexible and mechanically strong tubular chitosan implants for peripheral nerve regeneration. J Mater Chem B 9:5537-5546.

Li J, Liu Y, Wang X, Wang T, Li D, Xi F, Wang J, Wang E (2014) Functionalization of monolithic and porous three-dimensional graphene by one-step chitosan electrodeposition for enzymatic biosensor. ACS Appl Mater Interfaces 6:19997-20002.

Liu X, Miller AL, 2nd, Park S, Waletzki BE, Zhou Z, Tericz A, Lu L (2017) Functionalized carbon nanotube and graphene oxide embedded electrically conductive hydrogel synergistically stimulates nerve cell differentiation. ACS Appl Mater Interfaces 9:14677-14690.

Li Y, Yang S, Ni WU (2013) Simple, rapid and green one-step strategy to synthesis of graphene/carbon nanotubes/chitosan hybrid as solid-phase extraction for square-wave voltammetric detection of methyl parathion. Colloids Surf B Biointerfaces 108:266-270.

Mobini S, Song YH, McCray MW, Schmidt CE (2019) Advances in ex vivo models and lab-on-a-chip devices for neural tissue engineering. Biomaterials 198:146-166.

Nagarajan S, Pochat-Bohatier C, Teysier C, Balme S, Miele P, Kalkura N, Cavaillès V, Bechelany M (2016) Design of graphene oxide/gelatin electrospun nanocomposite on-a-chip devices for neural tissue engineering. Biomaterials 198:146-166.

Nagarajan S, Pochat-Bohatier C, Teysier C, Balme S, Miele P, Kalkura N, Cavaillès V, Bechelany M (2016) Design of graphene oxide/gelatin electrospun nanocomposite on-a-chip devices for neural tissue engineering. Biomaterials 198:146-166.

Nagarajan S, Pochat-Bohatier C, Teysier C, Balme S, Miele P, Kalkura N, Cavaillès V, Bechelany M (2016) Design of graphene oxide/gelatin electrospun nanocomposite on-a-chip devices for neural tissue engineering. Biomaterials 198:146-166.

Nagarajan S, Pochat-Bohatier C, Teysier C, Balme S, Miele P, Kalkura N, Cavaillès V, Bechelany M (2016) Design of graphene oxide/gelatin electrospun nanocomposite on-a-chip devices for neural tissue engineering. Biomaterials 198:146-166.