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Preparation and Characterization of Poly Lactic Acid/ Graphene Oxide/Nerve Growth Factor Scaffold with Electrical Stimulation for Peripheral Nerve Regeneration *in vitro*

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Abstract: A novel conductive drug-loading system was prepared by using an improved emulsion electrostatic spinning method which contained polylactic acid (PLA), graphene oxide (GO), and nerve growth factor (NGF) coated with bovine serum albumin (BSA) nanoparticles. Firstly, the structure, mechanical properties, morphology and electrical conductivity of PLA/GO electro spun fiber membranes with different GO ratios were characterized. PLA/GO scaffolds can exhibit superior porosity, hydrophilic and biomechanical properties when the GO incorporation rate is 0.5%. The addition of GO in the PLA/GO electro spun fiber membranes can also create appropriate pH environment for the repair of injured nerve when the GO incorporation rate is above 0.5%. Secondly, PLA/GO/BSA/Genipin/NGF particles (with a ratio of BSA/NGF = 3:1) prepared by modified emulsion electro spinning method will release more NGF than PLA/GO/NGF particles. In addition, PLA/0.5%GO/NGF scaffold can maintain its structure stability for at least 8 weeks observed by scanning electron microscope (SEM). Moreover, the degradation of PLA/0.5%GO/NGF scaffold is consistent with its weight loss. Finally, in vitro assay confirmes that PLA/GO composite scaffold exhibits low cytotoxicity to RSC96 cells. Cellular results have demonstrated that PLA/0.5%GO/NGF sustained-release drug sustained-release system with appropriate electrical stimulation (ES) can promote PC12 cell proliferation, and it can maintain its differentiation capability for at least 3 weeks. In conclusion, PLA/0.5%GO/NGF sustained-release drug sustained-release system can maintain its biological activity for at least 3 weeks and promote cell proliferation with appropriate ES.

Key words: poly lactic acid; graphene oxide; nerve growth factor; electrical stimulation; peripheral nerve regeneration

1 Introduction

Although surgical interventions for peripheral nerve injury can achieve favorable outcomes, the regeneration and repair of the peripheral nerve long-gap nerve defects remain challenging in clinical practice. Autologous nerve graft is the gold standard therapy for bridging the peripheral nerve defects[1]. Nevertheless, the rate of donor site morbidity limits further applications of this traditional technique. In addition, the axonal regeneration rate is about 2 to 5 mm/day in autologous nerve graft treatment group[2], which is a major barrier to the functional recovery for the long-gap nerve defects. It is necessary for us to develop a new artificial engineering material for bridging the long-gap nerve defects of the peripheral nerve. Recently, biodegradable nerve conduit has shown promising potential for the treatment of the long-gap nerve defects[3].

Poly lactic acid (PLA) is a biocompatible and biodegradable polymer with good mechanical properties, which shows great promise as the nerve conduit[4]. The PLA tube was efficient as a nerve guide to bridge the nerve defects[5]. The PLA is mainly used for the preparation of the nerve conduit by weaving, molding or

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electro spinning. Particularly, the electro spun polymers have attracted significant attention since they resemble to the native extracellular matrix (ECM)[6,7]. It is convenient to form nanofibers fabricated by electro spinning with a broad range of materials including both natural and synthetic polymers, which can provide a suitable environment for cell adhesion, proliferation, and differentiation. Studies have found that highly oriented nanostructure fibers can greatly improve the growth rate of cells[8]. Moreover, neural scaffold prepared by electrospinning nanofibers can promote the migration of Schwann cells along the orientation direction[9]. However, the neural scaffold with single composition is difficult to meet the requirements for tissue engineering application, hence conductive substances, nerve growth factor (NGF) and other active substances are added to improve the biological performance of composite scaffold[10].

The conductive conduit can not only provide three-dimensional (3D) nanofibers architecture for cell adhesion, but also regulate the cell differentiation by an extra electrical stimulus. Therefore, conductive materials are often employed to provide the surface delivery of an electrical signal for cell differentiation[11]. However, the electrically conductive materials for tissue engineering applications such as poly pyrrole, polyaniline, carbon nanotubes and poly(3,4-ethylenedioxythiophene) often failed owing to these unfavorable characteristics such as fragility, weak electrical conductivity and undesired biodegradation rate, *etc*[12]. Graphene has been regarded as one of the most promising candidates for conductive materials by merit of its superior physicochemical and biocompatible properties[13]. Graphene and its chemical derivatives have great potential for biomedical applications[14]. Graphene oxide (GO) is considered to be an attractive alternative since it exerts superior mechanical strength and electrical conductivity as compared to graphene[15]. In addition, studies have demonstrated that cells on GO substrates have exhibited better adhesion and proliferation capability than those on the graphene substrates[16].

The scaffold with a single composition might not mimic the physiological environment of native neural tissues because it is difficult for this scaffold to display different physical, chemical and electrical properties simultaneously. Therefore composite engineering scaffold with multiple materials needs to be constructed to fulfill a wide range of functional requirements. In this study, a PLA/GO/NGF composite nerve conduit fabricated by electro spinning was constructed for the regeneration and repair of the peripheral nerve, and its physicochemical properties were characterized. It is expected to find an ideal scaffold for neural tissue engineering applications.

2 Experimental

2.1 Materials

PLA (M_w=100 kDa), dichloromethane (DCM), dimethylformamide (DMF) and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, AR) were purchased from Aladdin (Shanghai, China). Sodium bis (2-ethylhexylhexyl) sulfosuccinate(AOT, purity≥99.0%) was purchased from Sigma-Aldrich (St. Louis, USA). Bovine serum albumin (BSA) were purchased from J&K Scientific Ltd., and NGF were purchased from Zhuhai Yisheng Biological Pharmaceutical Co., Ltd. RPMI 1640 medium were purchased from Wuhan Angus Biology Co., Ltd. RSC96 cells and PC12 cells were purchased from China Center for Type Culture Collection in Wuhan (China). All chemicals and solvents were used as received.

2.2 Preparation of PLA/GO/NGF nanofibers

GO was prepared by the improved Hummers method[17]. Briefly, sulfuric acid, sodium nitrate, graphite powder and potassium permanganate were mixed at 0 ℃ for 30 min, and stirred at 35 ℃ for 2 h. Mixed solution was kept in a container at 98 ℃ for 30 mins, and then the supernatant was took out and neutralized (pH=7.4) by ultra-pure water. The GO was dried for 12 h. GO (50.0 mg) and AOT (2 mg) were added into 10 mL of DMF with ultrasonication for 2 h to obtain a homogeneous GO/DMF dispersion of 0.5% concentration (*w/v*). The GO/DMF dispersions were introduced into the PLA solution to obtain a series of the PLA/GO solution. NGF was encapsulated in BSA particles by cross-linking. The PLA/GO mixture solution was ultrasonicated for 10 min. The BSA/NGF particles were then stirred and introduced into the PLA/GO mixture solution to obtain the PLA/GO/NGF mixture solution.

Nanofibers were then fabricated by electro spinning method using an electro spinning machine (SS-2353H, Beijing Ucalery Sci-tech Co., Ltd, China) at applied voltage of 20-22 kV, dispensing rate 0.2 mL/h, and collecting distance 10-15 cm, in ambient conditions of 40%-50% humidity and 23-27 ℃. Scaffolds for cell culture were deposited directly on 14 mm cover slips in layers of 0.15 mm thick. All samples were then dried in a vacuum oven for 1 week.

2.3 Characterization of prepared PLA/GO/NGF nanofibers

2.3.1 Porosity

The porosity of the scaffolds was measured through a specific gravity method[18]. The freeze-dried scaffolds with a regular shape (V_0) were immersed into a sealed container filled with ethanol and allowed to stand until no bubbles emerged from the samples. The weight difference between the initial state (W_0) and the equilibrium state (W_t) was the ethanol weight in the pores of the samples. According to the density of ethanol (ρ), the pore volume (V_p) was calculated using the following formula.

$$V_p = (W_t - W_0)/\rho$$

The formula of porosity (%) is as follows:

$$\text{Porosity (\%)} = V_p/V_0 \times 100\%$$

2.3.2 Hydrophobic property

Based on the droplet method, the contact angles of the material were determined by a contact angle analyzer (FACECA-XP150, Japan). For each drop, the measuring time must be controlled within 10 s. For each specimen, its contact angle was measured randomly from five points to calculate its mean value.

2.3.3 Mechanical properties

The mechanical properties of the scaffolds were measured using an electronic universal testing machine (SANS CMT6503, China), all columned scaffold samples were sectioned into dumb-bell shapes with length (30 mm) × width (10 mm) × thickness (0.09 mm). All the samples used a 6 N load cell and were compressed at a uniform strain rate of 1 mm/min at room temperature.

The tensile strength (MPa) is calculated according to the following formula:

$$\delta = \frac{F}{b \times d}$$

where, δ is the tensile strength (MPa); F is the maximum breaking stress (N); b is the sample width; d is the sample thickness.

Elongation at break (%) is calculated according to the following formula:

$$\varepsilon = \frac{L - L_0}{L_0}$$

where, ε is the Elongation at break (%); L_0 is the distance between the original fixtures of the sample; L is the distance between fixtures when the sample breaks.

2.3.4 Structural groups

Fourier transform infrared (FTIR) spectroscopy spectra of the samples were obtained to map chemical structure of the composite by using a spectrometer (Nexus, Thermo Nicolet, America). Powder of the samples was mixed with spectroscopic grade potassium bromide (KBr) in 1:1 proportion and then pressed to obtain pellets according to the size specialized by Bruker vector 33.

The Raman spectra of the electrospun fiber membranes of GO, PLA/0.1% GO, PLA/1.0% GO, and pure PLA groups were obtained totest the GO component in the fiber membrane by using a spectrometer (RAman Station 400F). The excitation wavelength of He-Ne laser was 633 nm and the output power was 10 mW.

2.3.5 Microstructure

The surface morphologies of the nanofibers were studied by a JSM-5610LV scanning electron microscope (SEM, JEOL, Japan) with an accelerating voltage of 10 kV. Prior to observation, the specimens were sputter-coated with gold for 60 s by a high-vacuum sputter coater ion sputter (Hitachi E-1010, Japan). The mean fiber diameters were calculated from SEM micrographs by measuring 100 fibers using image-analysis software (Image Pro Plus). Transmission electron microscopy (TEM, JEOL 1010, Tokyo, Japan) micrographs were obtained at an accelerating voltage of 100 kV to visualize the internal structure and GO distribution of nanocomposite fibers. The functional groups present in the GO, pure PLA, and PLA/GO composite nanofibers were analyzed using a fully automated laser raman microscope (Lab RAM Aramis, Horiba Job in Yvon, France) with a 633-nm excitation wavelength and a 50× magnification objective at room temperature.

2.4 Degradation *in vitro*

NGF was encapsulated in BSA particles by cross-linking. The PLA/GO mixture solution was ultrasonicated for 10 mins. The BSA/NGF particles were then stirred and introduced into the PLA/GO mixture solution to obtain the PLA/GO/NGF mixture solution. The scaffolds were cut into squares (25 mm×25 mm) for degradation testing in vitro. After being weighed, all specimens were placed in vials containing phosphate-buffer solution (PBS) (0.1 mol/L, pH 7.4±0.01). All vials were kept at 37±0.2 ℃ (50 strokes per min) for 12 weeks. The buffered solution was replaced every 2 weeks. Samples of different periods are dried with absorbent paper at room temperature and then dried in vacuum until a constant weight is obtained. Weight loss percentages were calculated from the dried weight ob-

tained before and after degradation using gravimetrical method. The weight loss ratio (W_L (%)) was calculated using the following equation:

$$W_L(\%)=\frac{W_0-W_r}{W_0}\times 100$$

where, W_0 is the initial weight and W_r is the residual weight of the dried materials. The pH change of the suspension liquor was monitored once a week using a pH meter (PHS-3C, Leici Company, China). Each value was averaged from five specimens.

The pH change of the suspension liquor was monitored once a week using a pH meter (PHS-3C, Leici Company, China).

2.5 *In vitro* drug release

2.5.1 Content determination of NGF

NGF standard curve was drawn by an enzyme linked immunosorbent assay (ELISA). The concentration of the drug in the sample was measured by UV-Vis. 200 mg fiber membrane was added into 3 mL DCM and supernatant was took. The NGF loading amount (m) in the fiber membrane can be calculated by the following formula:

M (%) = M_0 (total amount of drug in supernatant)/ M (original drug loading)

2.5.2 *In vitro* release of NGF

In vitro, the following experiments were carried out: 200 mg of fiber membrane and 15 mL PBS were added into 50 mL centrifuge tube, which was rotated at a speed of 100 r/min at 37 ℃. 3 mL of the release solution was taken out according to the predetermined time points and stored at −80 ℃, and replenish 3 mL of fresh release solution. During the test, the release solution of each time period was taken out at the same time, and the sample diluted by a certain multiple of the release solution that was tested. The concentration of the drug in the sample is measured by UV-Vis. The content of NGF in the release solution is multiplied by the dilution ratio. Calculate the cumulative release percentage of NGF according to formula and draw the release curve.

$$Q(\%)=\frac{V_0\times C_t+V\times\sum_{n=1}^{t-1}C}{M}$$

where, V_0 is the total volume of diluent (ML); C_t is the concentration of NGF in the release solution measured at each time point (ng/mL); V is the volume of each sampling solution (ML); M is the total mass of NGF input (NG).

2.6 Cell evaluation *in vitro*

2.6.1 Cell cytotoxicity assay by CCK-8

RSC96 cells (Chinese Academy of Sciences Cell Resource Center, China) were applied for the measurement of cell proliferation[19]. The OD values of the control, the PLA, PLA/0.1%GO, PLA/0.5%GO, PLA/1.0%GO, and PLA/1.5%GO/NGF films were measured at 450 nm by a multifunctional microplate reader (Thermo 3001, Thermo Fischer Scientific, USA) using a CCK-8 kit.

2.6.2 Electrical stimulation cell proliferation

Rectangular sections of the PLA, PLA/0.5%GO, and PLA/0.5%GO/NGF films were cut and placed in a device as described in previous study[29]. Briefly, each cut film was placed on a thin section of polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, USA) with a glass slide. Two wires were placed on either side of the film and sealed with a PDMS well. External electric stimulation device was used during cell culture. The growth of PC12 cells in films with or without electrical stimulation was recorded and assayed.

2.6.3 Fluorescent staining of cells on PLA/GO/NGF fiber membrane

RSC96 cells were cultured with fiber membrane in 96-well plate for 3 days, and supernatant of each sample was taken out, then washed in PBS three times for 5 min each time and fixed in 4% paraformaldehyde for 30 min, washed in PBS three times containing 0.1% Tritonx-100 for 5 mins each time. 10 μM DiL was diluted into 100 mL PBS. The DiL dilution was added to each well and kept in the shadow for 20 mins at room temperature, then washed in PBS three times for 5 mins each time, and 200 μL DAPI was then added to each well and kept in the shadow for 30 mins at room temperature, washed in PBS three times for 5 mins each time. The fluorescent staining was observed under inverted phase contrast microscope (CK40, OL YMPUS, Japan). Fluorescent stained cells on the PLA/GO/NGF membrane with or without electrical stimulation (ES) were recorded under the same condition. The OD value of each sample was measured at 570 nm by the multifunctional microplate reader (Thermo 3001, Thermo Fischer Scientific, USA) on 2, 4, and 8 hours.

2.6.4 Effect of PLA/GO/ NGF fiber membrane on PC12 differentiation

The samples were put into a 24-well plate, 3 mL of serum-free RPMI 1640 medium was added, and then incubated in a cell incubator at 37 ℃ and 5% CO_2. 2 mL supernatant of each sample was taken out on the 1st, 7th, 14th, and 21st days, respectively. PC12 cells

were cultured on 24-well plate at a density of 1×10^5/mL. In the experiment group, 500 μL of PLA/GO/NGF fiber membrane slow-release solution and serum-free RPMI 1640 culture solution were added to each well. In the control group, 1 mL of PLA/GO fiber membrane slow-release solution, 10 μL of 20 mg/L NGF and 990 μL serum-free RPMI 1 640 culture medium were added to each well. After 3 days of co-culture, the differentiated cells were observed under inverted phase contrast microscope (S-4800, HITACHI, Japan).

In this study, data were processed using SPSS 17.0 software (SPSS Inc, Chicago, USA) and the results were expressed as mean ± standard deviation of three measurements.

3 Results and discussion

Nerve conduit provided a primary treatment for repairing nerve injury. However, hollow nerve conduit is not an ideal candidate to supply enough extracellular matrix components during early nerve repair. Therefore, it is necessary to use different fillers such as micro-nanofibers, protein microspheres, microgel particles, functional hydrogels and growth factors in combination with nerve conduits. PLA has been widely used in nerve tissue engineering because of its excellent biocompatibility, biodegradability and absorbability. Graphene oxide has good conductivity and biocompatibility. It can improve local damage tissue microcirculation through the blood barrier to clear oxygen free radicals, playing an important role in regulating neural stem cells differentiate into neurons. Therefore, graphene oxide is expected to improve the mechanical properties and biological activity of nerve tissue engineering scaffold after the nerve damage. Numerous studies have shown that NGF plays a significant role in the regeneration of peripheral nerves. However, due to its short biological half-life, it is unable to maintain the effective concentration due to environmental changes. In order to improve NGF stability and keep its activity, NGF was encapsulated in BSA particles by cross-linking firstly, and then dispersed in PLA/GO solution. Finally, the PLA/GO/NGF nanofibers were prepared by the improved emulsion electro-spinning (Fig.1).

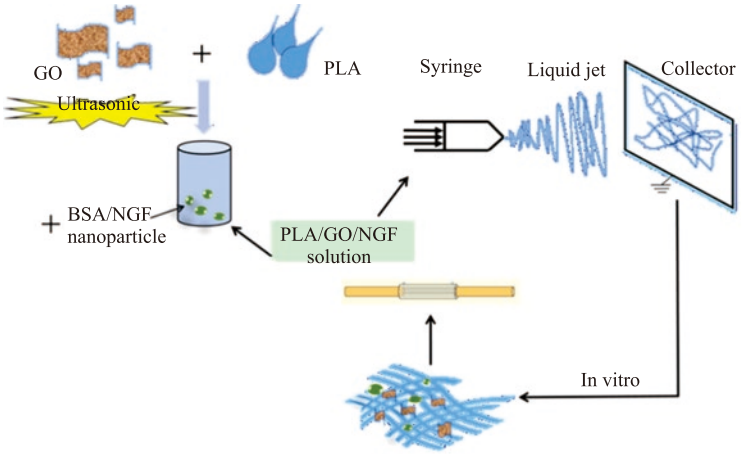


Fig.1 Schematic diagram of prepared PLA/GO/NGF electro spinning sustained-release drug delivery system (PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor)

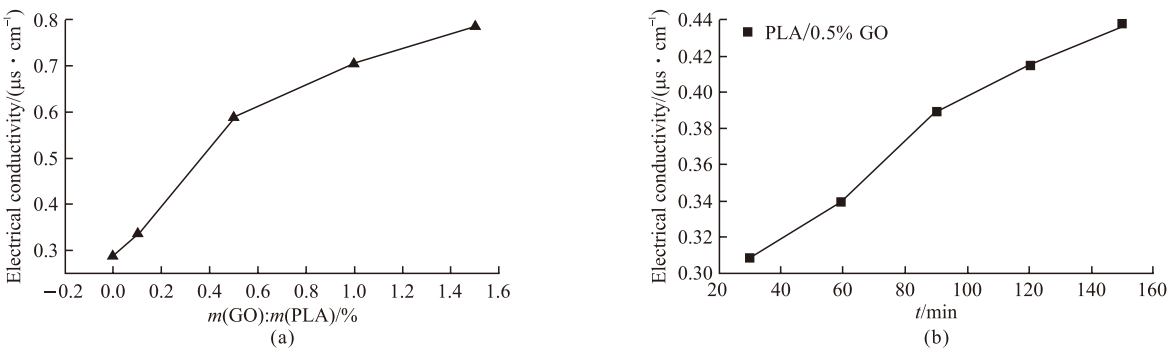


Fig.2 (a) Conductivity of spinning solution with different amount of GO; (b) Conductivity of PLA / 0.5% GO spinning solution at different ultrasonic time (GO: Graphene oxide)

The nanofibers are anticipated to take the advantages of PLA, GO and NGF, providing an ideal nutrient and electrical stimulation microenvironment for nerve regeneration.

3.1 The composition of PLA/GO spinning nanofibers

As shown in Fig.2(a), the conductivity of spinning nanofibers is positively correlated with the GO content. The rising trend is steeper when the GO content exceeds 0.5% as compared with that when the GO content is below 0.5%. Similar to previous study[20], the data suggests that electrical conductivity of GO is increased with the increased incorporation of GO, moreover, 0.5% GO might be the appropriate proportion that contributes the conductivity of spinning nanofibers. Therefore, 0.5% GO was chosen to investigate the relationship between the conductivity of spin nanofibers and the ultrasonic time. As shown in Fig.2(b), the conductivity of spinning nanofibers (PLA/0.5%GO) is positively correlated with the ultrasonic time. When the ultrasonic time exceeds 90 min, the conductivity tends to rise steadily. It indicates that GO is fully dispersed stripped dispersion when the ultrasonic time reaches 90 min, leading to a corresponding conductivity increase of spinning nanofibers. 90-min ultrasound time is then used for the composition of PLA/GO spinning nanofibers.

3.2 Structural groups

Fig.3(a) has shown that GO with less oxide layers are synthesized in the experiment. The edge of the oxide layer is almost transparent with obvious folds. Fig.3(b) is the TEM diagram of single filament containing GO. The surface of fibers is interspersed with layers of GO with different thicknesses. The darker part in the diagram is a result of multi-layer GO accumulation. The Raman spectra of PLA, PLA/1.0%GO, PLA/0.1%GO, and GO are present in the Fig.3(c), respectively. Two characteristic peaks, d-peak (−1 340 cm^{-1}) and g-peak (−1 581 cm^{-1}) are observed. Similar to previous study, characteristic peaks increase with

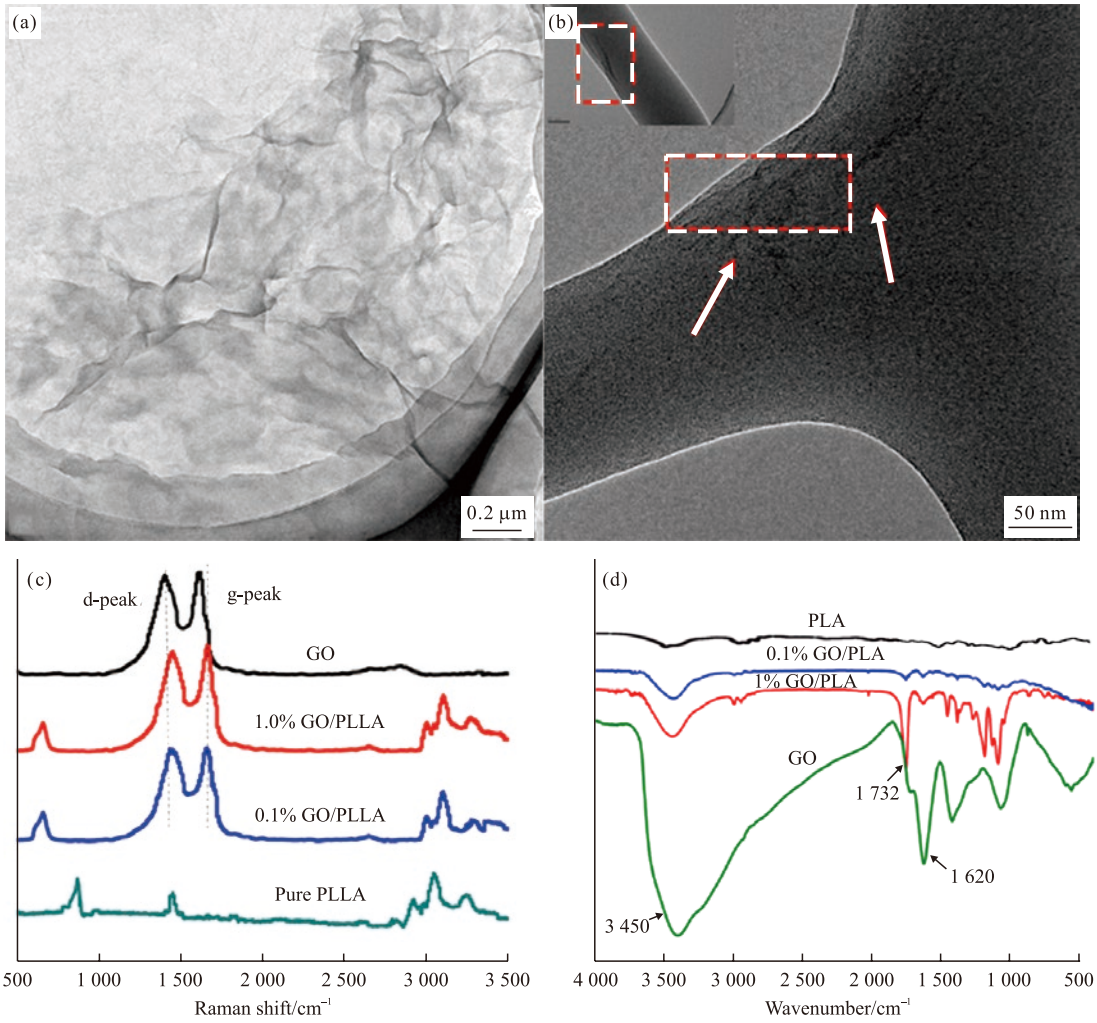


Fig.3 (a) The TEM image of GO lamellae; (b) The TEM image of single PLA/GO filament; (c) Raman spectra of PLA, PLA/1.0% GO, PLA/0.1% GO, and GO; (d) Infrared spectra of PLA, PLA/0.1% GO, PLA/1.0% GO, and GO (TEM: Transmission electron microscopy; PLA: Poly lactic acid; GO: Graphene oxide)

Table 1 Porosity, hydrophobic property, and mechanical properties of PLA/GO/NGF nanofibers with different concentrations

Conductivity of GO catheter and PLA / GO catheter (0%, 5%, 10%, and 15%)				
Mass ratio of GO/PLA	Porosity/%	Contact angle/(°)	Tensile strength/MPa	Elongation at break/%
Pure PLA	54.68+4.6	123.63+0.26	2.36+0.76	5.98 ±3.93
PLA/0.1%GO	62.45±6.35	115.64±0.14	6.86 ±1.47	74.30+2.75
PLA/0.5%GO	68.13+2.36	107.34±0.66	9.20+1.06	107.26+1.47
PLA/1.0%GO	75.35+5.6	98.45 ±0.48	5.90+2.07	125.40±1.46
PLA/1.5%GO	79.34+7.16	93.24 ±0.67	3.65±0.89	103.70±3.36

the increase of GO loading [31]. The FTIR spectra of PLA/0.1% GO, PLA/0.5% GO, PLA/1% GO and GO appear in Fig.3 (D), respectively. The characteristic peaks of GO appear at 3 450 cm^{-1}, 1 732cm^{-1}, and 1 620 cm^{-1}, respectively, which are OH tensile vibration peaks, C=O tensile vibration peaks and C-OH bending vibration peaks on the GO carboxyl group. With the increase of GO content, the intensity of the absorption peak increases at 3 450 cm^{-1}, indicating that GO is successfully incorporated on the PLA/GO fiber membrane. Almost the same absorption peaks are observed on the FTIR spectra of PLA/GO and PLA fiber membranes, and no new absorption peaks appear except for the characteristic peaks of GO, indicating that no new substances were formed by the incorporation of GO and the chemical structure of PLA remains stable. Taken together, these data indicate that GO has been successfully incorporated into the prepared PLA/GO fiber membranes.

3.3 Porosity, hydrophobic property, and mechanical properties

The porosity of PLA/GO/NGF nanofibers with different concentrations of GO is calculated according to the method demonstrated by Abdullah and Khairurrijal[21,22]. The porosity of pure PLA scaffold is 54.68±4.6%, which can not meet the demand for the 3D structure of natural nerve tissue[23]. Studies have shown that the pore on the pure PLA scaffold with low porosity is too small for the cell to adhere and stretch[24]. In our study, the incorporation of GO not only increases the conductivity of the spinning solution, but also increases the porosity of the pure PLA scaffold. Therefore, the PLA/GO electro spun scaffold exhibits significantly higher porosity as compared with pure PLA scaffold. These results suggest that the incorporation of GO can increase the porosity of the electro spun scaffold.

The higher hydrophilicity of the neural tissue engineering scaffold is beneficial for the cell adhesion and proliferation[25]. The hydrophilicity is closely related to the contact angle[26]. When the contact angle is smaller than 90°, the membrane of the scaffold is hydrophilic[27]. Moreover, the contact angle value is negatively correlated with the membrane hydrophilicity[28]. PLA is a hydrophobic polymer material and the contact angle value is 123°. The incorporation of proportional GO in the PLA/GO electrospinning fibers were 0.1%, 0.5%, 1.0%, and 1.5%, respectively. The data presented here indicate that the contact angle value tend to decrease with the increase of GO doping amount. Therefore, the incorporation of GO also improves the membrane hydrophilicity of the PLA/GO electro spun scaffold. We attribute the improved hydrophilicity to the large number of highly polar hydroxyl groups on the GO surface[29].

The tensile strength and elongation at break of pure PLA scaffold are 2.36 ± 0.36 MPa and 5.98 ± 3.93 MPa, respectively. The tensile strength reaches the maximum value when the incorporation ratio of GO is 0.5%, and the value of elongation at break is the highest (125.4±1.46 MPa) when 1.0% GO was added to the PLA scaffold[30]. We speculate that GO is easily dispersed in the spinning solution and can be uniformly distributed throughout the scaffold when the GO incorporation ratio is less than 1%. On the contrary, excess GO might accumulate in the scaffold, and destroy the stress transfer and increase the stress concentration point, which leads to defects in the fiber when the GO incorporation ratio is more than 1%. Therefore, the appropriate concentration range of GO is from 0.5% to 1% (Table 1).

3.4 The pH changes of PLA/GO fiber membrane

The pH value changes of PLA/GO and PLA fiber membrane are presented in Fig.4. The result regarding PLA degradation is consistent with previous study[31]. The structure of PLA/GO electro spun fiber membrane contains lipid bond since the main composite material of PLA/GO electro spun fiber membrane is PLA. The ester bond is easy to form -COOH in natural hydrolysis, resulting in lower pH value in simulated body fluid environment (Fig.4). However, oligo layers of GO

are uniformly dispersed on the surface of fiber membrane, and they can connect the internal space of the fiber membrane as connection points. This structure is capable of slowing down the degeneration of PLA fiber membrane by reducing -COOH accumulation. Therefore, the pH value of PLA/GO fiber membrane is higher than that of pure PLA fiber membrane, and pH value of PLA/1.5%GO fiber membrane is highest among 5 groups. The critical period of injured nerve regeneration is between 7th week and 8th week[32]. The pH of the PLA fiber membrane is acidic (below 6.8) during this period, however, the pH of PLA/GO fiber membrane is stable at 6.95 to 7.13, which can create an appropriate pH environment similar to the nature microenvironment. The data suggest that the addition of GO basically maintains the stability of the pH value, and provides a favorable microenvironment for the repair of injured nerve.

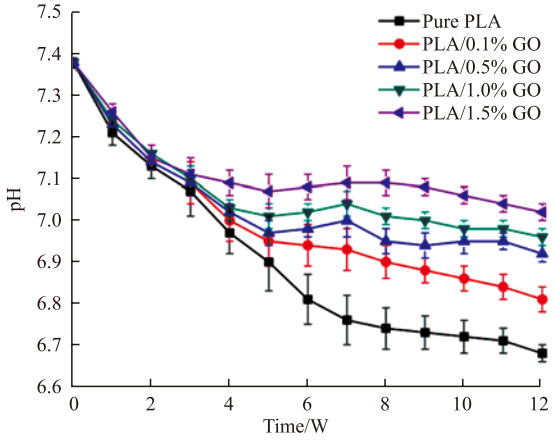


Fig.4 The pH changes of PLA/GO electro spun fiber membrane at different time points (PLA: Poly lactic acid; GO: Graphene oxide)

3.5 Mix of NGF and *in vitro* drug release

NGF is encapsulated in BSA particles by cross-linking. The PLA/GO mixture solution was ultrasonicated for 10 min. The BSA/NGF particles were then stirred and introduced into the PLA/GO mixture solution to obtain the PLA/GO/NGF mixture solution. The PLA/GO electro spun scaffold can achieve superior bio-mechanical properties when the incorporation ratio of GO is 0.5%. There, the incorporation ratio of 0.5%GO was chosen to investigate the degeneration of PLA/GO/NGF scaffold.

As shown in Fig.5(a), there is a good linear relationship between OD value and NGF concentration, when the NGF concentration is in the range of 0-2000 pg/mL at the pH 7.4 value. It suggests that ELISA is sensitive to determine the NGF concentration. The regression equation is obtained by linear fitting: y=0.0007x+0.1512, where x is the concentration, y is the absorption value, and R^2=0.9992. The NGF concentration can be calculated according to the above equation. Fig.5(b) has shown the effect of different constitute BSA/NGF gel particles on the drug release of fiber membrane in vitro when the NGF content maintains constant value of 0.05 mg and the ratio of BSA/NGF

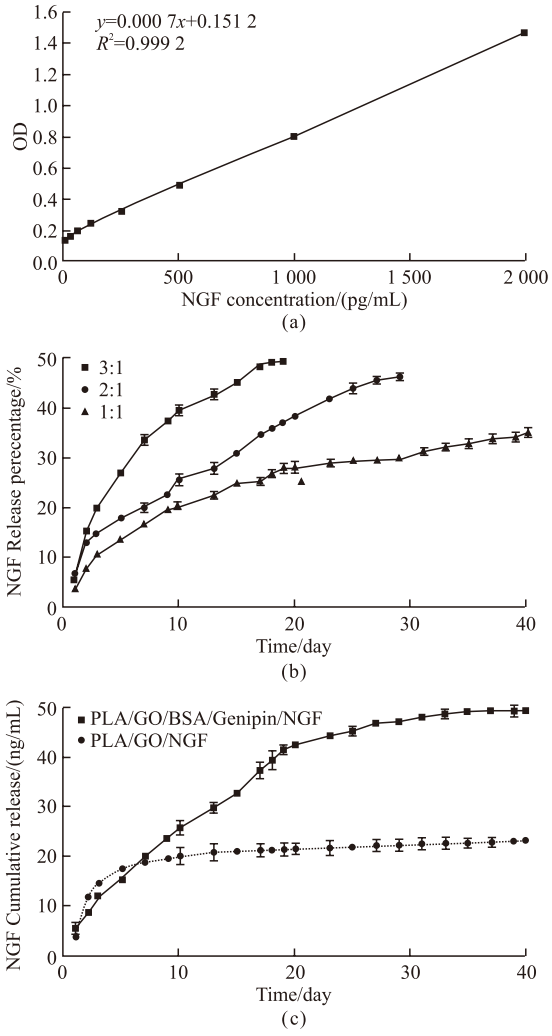


Fig.5 (a) Standard curve of NGF in PBS (pH = 7.4); (b) Cumulative release curves of PLA/GO/NGF electro spun fiber membrane with different BSA/NGF ratios; (c) Cumulative release curves of NGF with or without encapsulated in BSA particles (PBS: phosphate-buffer solution; BSA: bovine serum albumin; PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor)

Table 2 The constitute of PLA/GO/BSA/NGF electro spun fiber membrane

Relative ratio of BSA/NGF	Content of NGF relative to BSA/%	10%BSA:2%Genlpln solution content/mg
1:1	0.05	0.05
1:2	0.05	0.10
1:3	0.05	0.15

is 3:1, 2:1, and 1:1, respectively (Table 2). The cumulative release rate is the highest among three groups when the ratio of BSA/NGF is 3:1 in the electro spun fiber membrane. The gel particles with a higher BSA/NGF ratio exhibit higher drug release capacity, and the ratio of 3:1 was chosen for the following experiments.

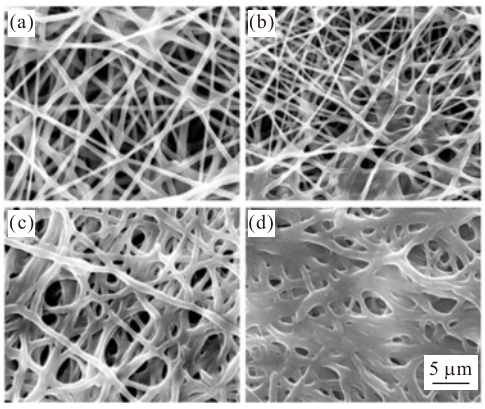
In order to determine the effect of different drug loading methods on NGF, two slow release drug delivery systems, the control group (PLA/GO/NGF) and the experimental group (PLA/GO/BSA/Genipin/NGF), prepared by modified emulsion electro spinning method[33], are detected by ELISA, respectively. The cumulative release concentration of the control group and the experimental group within 40th days (the total volume of solution is 4 mL; the release amount is calculated according to the release concentration) are measured. The release curve is plotted, as shown in Fig.5(c). The drug in the control group is rapidly released from 1st to 5th day, the peak appears at (18.48 ± 2.32) ng, which tends to be stable until the 13th day, and then the basial release is completed from 35th to 40th day. However, the cumulative release of NGF in the experimental group increase rapidly from 1st to 20th day, and maintain at high level from 21st to 40th day. The cumulative release of the experiment group reaches (49.08 ± 1.12) ng before the 40th day, and the cumulative release rate is 85.2%, which is significantly higher than that in the control group (47.82%).

Studies have shown that organic solvent can reduce the release of NGF in PBS solution. PLA/GO/NGF particles release less drug in PBS solution since NGF has lower affinity and permeability to organic solvents, however, PLA/NGF/BSA particles release more drug in PBS solution since BSA reduce the effect of organic solvents on NGF in electro spun fibers. Moreover, the increased hydrophilicity brought by GO doping also contribute to the increased cumulative release of experimental group, since the NGF in the membrane can gradually penetrate through the hydrophilic channel. Taken together, we conclude that PLA/GO/NGF electro spinning sustained release drug delivery system modified by emulsion spinning can release NGF slowly and meet the requirements of nerve regeneration.

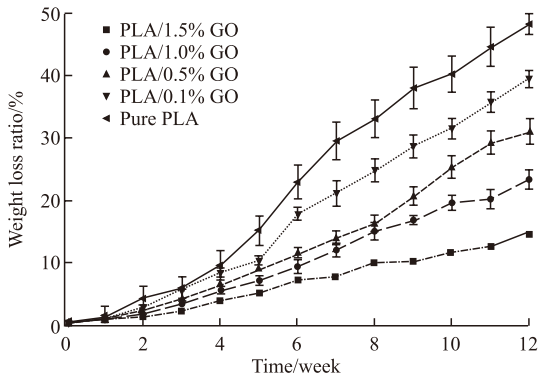
3.6 The degeneration of PLA/GO/NGF scaffold

The Fig.6 (A) has shown the SEM of the degradation of PLA/0.5%GO/NGF scaffold. The fibrous film is connected by fibers with GO layers dispersed on them, which can form a conductive network structure as a bridge between connecting points. There is no obvious difference of the scaffold between 1st-day degeneration group (Fig.6(A-a)) and 4th-week degeneration group (Fig.6(A-b)). However, we find that the scaffold exhibits a little swelling, and some fibers begin to combine or adhere in the 4th-week degeneration group. In the 8th-week degeneration group (Fig.6(A-c)), the scaffold exhibits distinct swelling and some fibers bond with each other in it. Their quality decrease obviously, and the diameter of the pore formed by the fiber become smaller as compared with that in the 4th-week degeneration group. In the 12th-week degeneration group (Fig.6 (A-d)), the scaffold begins to fracture and some fibers collapse due to the decreased mechanical strength caused by the material loss. Finally, their quality further decreases, and the diameter of the pore formed by the fiber become further smaller as compared with that in the 8th-week degeneration group.

In the 12th-week degeneration group (Fig.6(B)),



A



B

Fig.6 (A) SEM images of PLA/0.5%GO/NGF scaffold degradation: (a) 1st-day degeneration group; (b) 4th-week degeneration group; (c) 8th-week degeneration group; (d) 12th-week degeneration group; (B) The weight loss of PLA/GO fiber membrane at different time points (SEM: Scanning electron microscopy; PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor)

the weight loss of PLA/GO fiber membrane is relatively lower as compared with that of the pure PLA fiber membrane. In all degeneration groups, the weight loss remains stable at the beginning 4th weeks degeneration, and it tends to increase steadily during 6th and 10th weeks degeneration. However, the weight loss of PLA/0.1%GO and PLA fiber membrane accelerate in the late stage (the 11st and 12nd weeks) until the degradation is complete. Nevertheless，the weight loss of PLA/GO (GO incorporation ratio more than 0.5%) fiber membrane still increase slowly as compared with that of PLA/0.1%GO and PLA fiber membrane. We attribute the reason to that PLA fibers interspersed with more layers of GO (GO incorporation ratio more than 0.5%) can maintain the mechanical structure more stable as compared with that interspersed with less layers of GO (GO incorporation ratio less than 0.5%) in the late stage of degeneration. Therefore, PLA/GO fiber membrane with GO incorporation ratio more than 0.5% can slow down the degeneration process and reduce weight loss rate. The weight loss of PLA/GO fiber membrane is consistent with the SEM results.

3.7 Cell evaluation *in vitro*

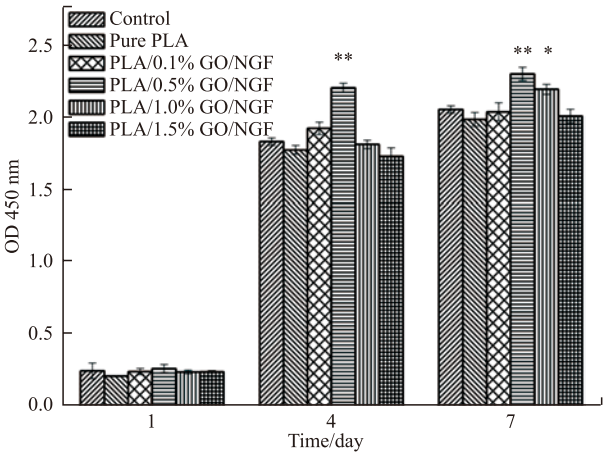


Fig.7 CCK-8 assay of pure PLA and PLA/GO/NGF cultured with RSC96 cells at 1, 4, and 7 days. Control: saline; PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor. $^{**}P$<0.05 represents PLA/0.5%GO/NGF group vs. the other groups; $^{*}P$<0.05 represents PLA/1.0%GO/NGF group vs. (the control, pure PLA, PLA/0.1%GO/NGF and PLA/1.5%GO/NGF group)

CCK-8 assay[34] is applied to investigate the cytotoxicity of RSC96 cells cultured within saline (control group), PLA and PLA/GO composite scaffolds with different GO concentrations (Fig.7). The proliferation rate tends to increase from day 1st to day 7th in all groups. There is no significant difference between the control group and pure PLA scaffolds with regard to proliferation rate pof RSC96 on day 1st (P> 0.05). however, difference can still be observed among groups at day 4 and day 7. There is no significant difference between GO-added PLA/GO scaffolds (0.1%, 1.0%, and 1.5%) and pure PLA scaffolds with regard to proliferation rate of RSC96 on day 4th, however, the proliferation rate of RSC96 in the PLA/0.5%GO scaffold is the highest among 6 groups on day 4th (P<0.05). Similarly, there is no significant difference between GO-added PLA/GO scaffolds (0.1% and 1.5%) and pure PLA scaffolds with regard to proliferation rate of RSC96 on day 7th, however, the proliferation rate of RSC96 in the PLA/0.5%GO scaffold and PLA/1.0%GO scaffold are higher than those in the other scaffolds (P<0.05). The data suggest that the microenvironment provided by PLA/GO composite scaffold is suitable for cell proliferation, and appropriate GO incorporation (0.5% GO) can promote cell proliferation.

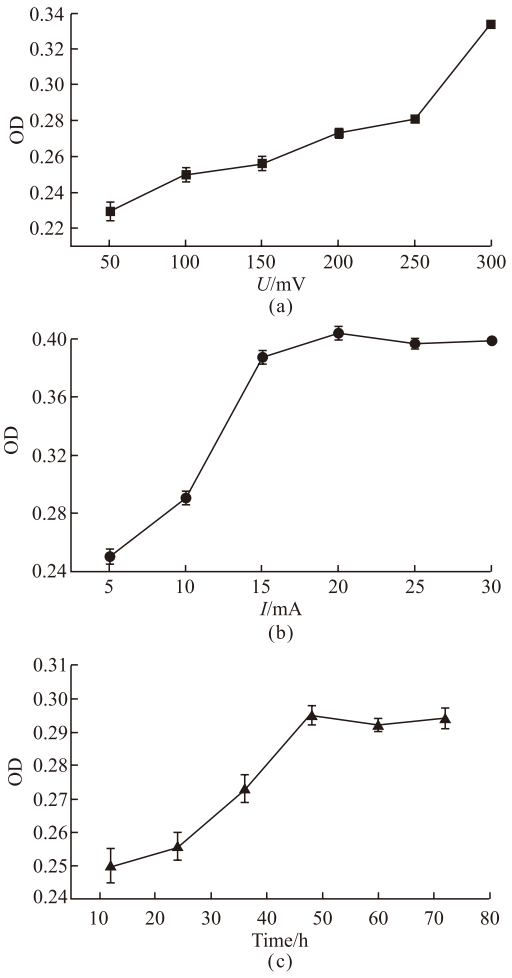


Fig.8 PC12 cells proliferation of under different conditions: (a) Proliferation of PC12 cells under different voltages; (b) Proliferation of PC12 cells under different currents; (c) Proliferation of PC12 cells under different time

Voltage, current and time are selected respectively to investigate the effect of single factor on the prolif-

eration of PC12 cells[36-38](Fig.8). Cells are cultured under different single condition, respectively. Based on the proliferation rate of PC12 cells, 200 mV voltage, 15 mA current and 36 h electric stimulation (ES) have been shown to exert the least influence on the PC12 cell proliferation. Then the effect of ES on GO in the PLA/0.5%GO and PLA/GO0.5%/NGF fiber membrane are investigated. Both ends of the PLA/0.5%GO and PLA/GO0.5%/NGF fiber membranes are received ES or not. ES exerts the least effect on the PLA/0.5%GO fiber membrane. There is no significant difference between PLA/0.5%GO with ES and PLA/0.5%GO without ES with regard to the proliferation rate of PC12 cells. However, ES can improve the proliferation rate of PC12 cells in the PLA/0.5%GO/NGF fiber membrane. The proliferation rate of PC12 cells in the PLA/0.5%GO/NGF fiber membrane with ES is significantly higher than that in the PLA/0.5%GO/NGF fiber membrane without ES (Fig.9). We speculate that the current might promote the cell growth through culture medium and contact guidance, moreover, GO can work collaboratively with NGF to promote the PC12 cells to proliferate.

PLA/0.5%GO fiber membrane samples are prepared under the same conditions as the control group 2mL of supernatant of each sample was taken out and tested on the 1st, 7th, 14th, and 21st day time points. PC12 cells are incubated with 1×10^5 /mL in

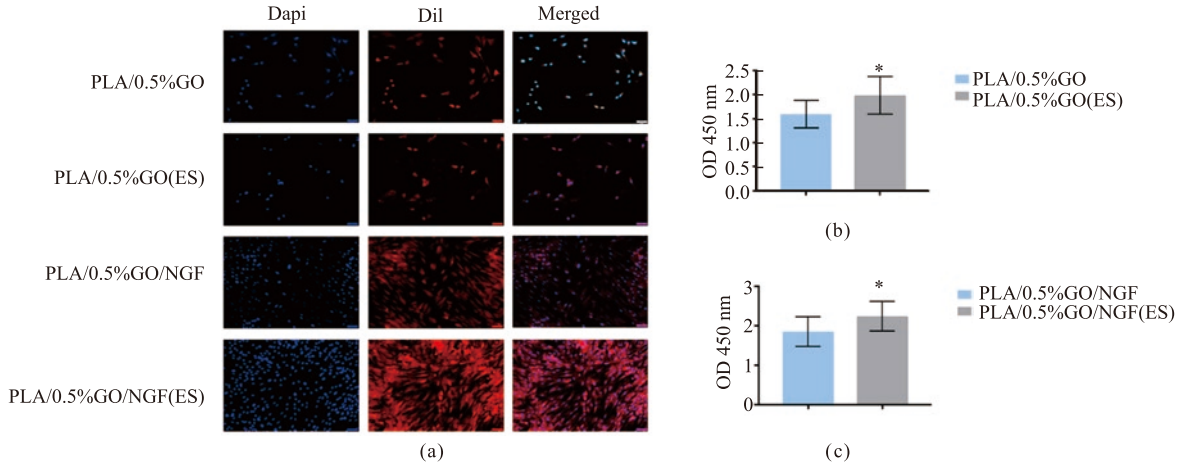


Fig.9 PC12 cells proliferation on the PLA/0.5%GO and PLA/0.5%GO/NGF fiber membrane with and without ES. (a) Fluorescence staining of PC12 cells on the surface of PLA/0.5%GO and PLA/0.5%GO/NGF fiber membrane; (b) Comparison of PC12 cells proliferation on the surface of PLA/0.5%GO with and without ES. $^*P<0.05$ represents PLA/0.5%GO (ES) *vs*. the control group; (c) Comparison of PC12 cells proliferation on the surface of PLA/0.5%GO/NGF with and without ES. $^*P<0.05$ represents PLA/0.5%GO/NGF (ES) *vs*. the control group. ES: electrical stimulation; PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor

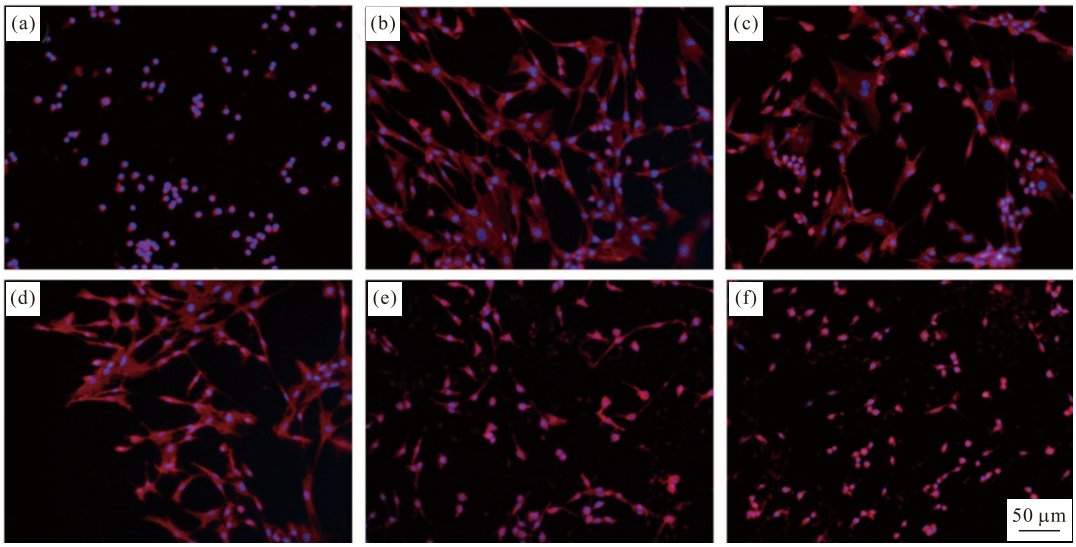


Fig.10 Differentiation of PC12 cells in PLA/GO/NGF release solution: (a) Negative control; (b) Positive control; (c) PC12 cells in PLA/GO/NGF release solution at 1-day; (d) PC12 cells in PLA/GO/NGF release solution at 7-day; (e) PC12 cells in PLA/GO/NGF release solution at 14-day; (F) PC12 cells in PLA/GO/NGF release solution at 21-day. Negative control: PLA/0.5%GO release solution; Positive control: NGF; PLA: Poly lactic acid; GO: Graphene oxide; NGF: Nerve growth factor

24 well plate. 1 L of PLA/0.5%GO fiber membrane slow release solution is added as the negative control (Fig.10(a)); 10 μL of 20 mg/L NGF and 990 μL serum-free RPMI 1640 culture medium are added as the positive control (Fig.10(b)); 500 μL of PLA/0.5%GO/NGF fiber membrane slow release solution and 500 μL serum-free RPMI 1640 culture solution are added as the experimental group (Figs.10(c)-10(d)); In the negative control group, PC12 cells maintain a normal spherical shape and are agglomerated without obvious axons in the PLA/0.5%GO sustained-release solution (Fig.10(a)). The PC12 cells have shown obvious axons and multiple somatic neuritis, which are connected into a network in the positive control group (Fig.10(b)) and experimental group (Figs.10(c)-10(d)). The cell with neuritis that are longer than twice the length of the cell is recognized as differentiated cells and measured. The release of NGF and axon differentiation of PC12 cells decrease gradually with the passage of time, as shown in Figs.10(c)-10(d). In conclusion, PLA/0.5%GO/NGF sustained-release drug delivery system can release NGF slowly and maintain its biological activity for at least 3 weeks.

4 Conclusions

Based on the results of this study, we can draw the following conclusions: GO could be incorporated successfully in PLA fiber membrane as supported by TEM diagram and Raman spectra. In addition, the incorporation of GO exerts no obvious effect on the chemical structure of PLA. Moreover, the incorporation of appropriate proportional GO can improve the porosity, hydrophilicity and mechanical properties of the electro spun scaffold, which is suitable for the engineering applications. The PLA/GO electro spun scaffold can achieve superior bio-mechanical properties when the incorporation ratio of GO is 0.5%.

When the ratio of BSA/NGF particles is 3: 1, the drug exerts the best release effect on the electro spun fiber membrane. Therefore, PLA/GO/NGF electro spinning sustained release drug delivery system can release NGF slowly. The degeneration study has shown that the PLA/GO/NGF scaffold can maintain its mechanical properties for at least 8 weeks. Moreover, the addition of GO basically maintains the stability of the pH value and provides a favorable microenvironment for the injured nerve repair.

Microenvironment provided by PLA/0.5%GO composite scaffold is beneficial for RSC96 cell adhesion. Moreover, ES promotes PC12 cell proliferation on the PLA/GO/NGF scaffold, indicating that ES may improve nerve regeneration. *In vitro* cell study has shown that PLA/0.5%GO/NGF sustained release drug delivery system can release NGF slowly and maintain its biological activity for at least 3 weeks in vitro experiment. This study demonstrates that the combination of PLA/GO/NGF sustained-release drug delivery system and combined with ES can overcome the shortcomings of the nerve conduit during repair. It is a promising material for applications in the field of neural tissue engineering. However，further studies need to be conducted to verify its mechanical and biocompatible properties *in vivo* in the future.

References

[1] Vijayavenkataraman S. Nerve Guide Conduits for Peripheral Nerve Injury Repair: A Review on Design, Materials and Fabrication Methods[J]. *Acta Biomater.*, 2020, 106: 54-69

[2] Grinsell D, Keating C P. Peripheral Nerve Reconstruction after Injury: A Review of Clinical and Experimental Therapies[J]. *Biomed Res. Int.*, 2014, 698256

[3] Manoukian OS, Baker JT, Rudraiah S, *et al.* Functional Polymeric Nerve Guidance Conduits and Drug Delivery Strategies for Peripheral Nerve Repair and Regeneration[J]. *J. Controlled Release*, 2020, 317: 78-95

[4] Tyler B, Gullotti D, Mangraviti A, *et al.* Polylactic Acid (PLA) controlled Delivery Carriers for Biomedical Applications[J]. *Adv. Drug Delivery Rev.*, 2016, 107: 163-175

[5] Dodla MC, Alvarado-Velez M, Mukhatyar VJ, *et al.* Peripheral Nerve Regeneration[J]. *Principles Regenerative Med.: Elsevier*, 2019, 1 223-1 236

[6] Zhou T, Wang N, Xue Y, *et al.* Electrospun Tilapia Collagen Nanofibers accelerating Wound Healing via Inducing Keratinocytes Proliferation and Differentiation[J]. *Colloids Surf., B*, 2016,143: 415-422

[7] Bhutto MA, Zhang J, Sun B, *et al.* Development of Poly (L-lactide-co-caprolactone) Multichannel Nerve Conduit with Aligned Electrospun Nanofibers for Schwann Cell Roliferation[J]. *Int. J. Polymer. Mater. Polymer. Biomater.*, 2016, 65(7): 323-329

[8] Cunha C, Panseri S, Antonini S. Emerging Nanotechnology Approaches in Tissue Engineering for Peripheral Nerve Regeneration[J]. *Nanomed. Nanotechnol. Biol. Med.*, 2011, 7(1): 50-59

[9] Sayanagi J, Tanaka H, Ebara M, *et al.* Combination of Electrospun Nanofiber Sheet Incorporating Methylcobalamin and PGA-Collagen Tube for Treatment of a Sciatic Nerve Defect in a Rat Model[J]. *J. Bone Joint Surgery*, 2020, 102(3): 245-253

[10] Chen SH, Li RQ, Li XR, *et al.* Electrospinning: An Enabling Nanotechnology Platform for Drug Delivery and Regenerative Medicine[J]. *Adv. Drug Delivery Rev.*, 2018, 132: 188-213

[11] Li XM, Zhao TX, Sun LW, *et al.* The Applications of Conductive

Nanomaterials in the Biomedical Field[J]. *J. Biomed. Mater. Res. Part A*, 2016, 104(1): 322-339

[12] Ning CY, Zhou ZN, Tan GX, *et al.* Electroactive Polymers for Tissue Regeneration: Developments and Perspectives[J]. *Progr. Polym*., 2018, 81: 144-162

[13] Bai RG, Ninan N, Muthoosamy K, *et al.* Graphene: A Versatile Platform for Nanotheranostics and Tissue Engineering[J]. *Progr Mater*., 2018, 91: 24-69

[14] Zhu T, Yu K, Bhutto MA, *et al.* A Facile Approach to Fabricate Nano-attapulgite/Poly(vinyl pyrrolidone)/Biopolymers Core-sheath Ultrafine Fibrous mats for Drug Controlled Release[J]. *RSC Adv.*, 2016, 49 817-49 823

[15] Sun M, Li J. Graphene Oxide Membranes: Functional Structures, Preparation and Environmental Applications[J]. *Nano Today*, 2018, 20: 121-137

[16] Bressan E, Ferroni L, Gardin C, *et al*. Graphene based Scaffolds Effects on Stem Cells Commitment[J]. *J. Translational Med*., 2014, 12(1): 296

[17] Kong Y, Zhao Y, Ji B, *et al.* Preparation and Characterization of Polyacrylamide/Silk Fibroin/Graphene Oxide Composite Hydrogel for Peripheral Nerve Regeneration[J]. *J. Biomater. Tissue Eng.*, 2016, 6(9): 682-689

[18] Vijayavenkataraman S, Kannan S, Cao T, *et al.* 3D-Printed PCL/PPy Conductive Scaffolds as Three-Dimensional Porous Nerve Guide Conduits (NGCs) for Peripheral Nerve Injury Repair[J]. *Frontiers Bioeng. Biotechnol.*, 2019, 7: 266

[19] Wang Y, Yin Y, Dai H, *et al*. Evaluation of a Novel Bioabsorbable PRGD/PDLLA/β-TCP/NGF Composites in Repair of Peripheral Nerves[J]. *J. Wuhan University Technol.-Mater. Sci. Ed*., 2009, 24(3): 409-414

[20] Ma WT, Kumar SR, Hsu CT, *et al.* Magnetic Field-assisted Alignment of Graphene Oxide Nanosheets in a Polymer Matrix to enhance Ionic Conduction[J]. *J. Membr. Sci.*, 2018, 563: 259-269

[21] Abdullah SI, Ansari MN. Mechanical Properties of Graphene Oxide (GO)/Epoxy Composites[J]. *Hbrc J*., 2015, 11(2): 151-156

[22] Rahmawati D, Aimon AH, Iskandar F, *et al.* Study on Graphene Oxide (GO) Supernatant Dilution to the Optical and Electrical Properties of TCF Based-reduced Graphene Oxide (RGO) Films[J]. *IOP Conference Ser. Mater. Sci. Eng.*, 2019, IOP Publishing

[23] Aldana AA, Abraham GA. Current Advances in Electrospun Gelatin-based Scaffolds for Tissue Engineering Applications[J]. *Int. J. Pharm.*, 2017, 523(2): 441-453

[24] Ye KQ, Kuang HZ, You ZW, *et al.* Electrospun Nanofibers for Tissue Engineering with Drug Loading and Release[J]. *Pharm*., 2019, 11(4): 182

[25] Remya K, Chandran S, Mani S, *et al.* Hybrid Polycaprolactone/Polyethylene Oxide Scaffolds with Tunable Fiber Surface Morphology, improved Hydrophilicity and Biodegradability for Bone Tissue Engineering Applications[J]. *J. Biomater. Sci. Polym. Ed*., 2018, 29(12): 1 444-1 462

[26] Olivera N, Rouf TB, Bonilla JC, *et al.* Effect of LAPONITE Addition on the Mechanical, Barrier and Surface Properties of Novel Biodegradable kafirin Nanocomposite Films[J]. *J. Food Eng.*, 2019, 245: 24-32

[27] Lins LC, Wianny F, Livi Sb, *et al.* Development of Bioresorbable Hydrophilic-hydrophobic Electrospun Scaffolds for Neural Tissue Engineering[J]. *Biomacromol*., 2016, 17(10): 3 172-3 187

[28] Ganesh B, Isloor AM, Ismail AF. Enhanced Hydrophilicity and Salt Rejection Study of Graphene Oxide-polysulfone mixed Matrix Membrane[J]. *Desalination*, 2013, 313: 199-207

[29] Perera VV, Fernando NL, Nissanka B, *et al.* In situ Real Time Monitoring of Hygroscopic Properties of Graphene Oxide and Reduced Graphene Oxide[J]. *Adsorpt*., 2019, 25(8): 1 543-1 552

[30] Zhang X, Geng B, Chen H, *et al.* Extraordinary Toughness Enhancement of Poly (Lactic Acid) by Incorporating very Low Loadings of Noncovalent Functionalized Graphene-Oxide via Masterbatch-based Melt Blending[J]. *Chem. Eng. J*., 2018, 334: 2 014-2 020

[31] Elsawy MA, Kim KH, Park JW, *et al.* Hydrolytic Degradation of Polylactic Acid (PLA) and Its Composites[J]. *Renewable Sustainable Energy Rev.*, 2017, 79: 1 346-1 352

[32] Saceda J, Isla A, Santiago S, *et al.* Effect of Recombinant Human Growth Hormone on Peripheral Nerve Regeneration: Experimental Work on the Ulnar Nerve of the Rat[J]. *Neurosci. Lett.*, 2011, 504(2): 146-150

[33] Liao Y, Loh C-H, Tian M, *et al.* Progress in Electrospun Polymeric Nanofibrous Membranes for Water Treatment: Fabrication, Modification and Applications[J]. *Progr. Polym. Sci.*, 2018, 77: 69-94

[34] Weng J, Wang YH, Li M, *et al.* GSK3β Inhibitor Promotes Myelination and Mitigates Muscle Atrophy after Peripheral Nerve Injury[J]. *Neural Regeneration Res*., 2018, 13(2): 324

[35] Xu H, Holzwarth JM, Yan Y, *et al.* Conductive PPY/PDLLA Conduit for Peripheral Nerve Regeneration[J]. *Biomaterials*, 2014, 35(1): 225-235

[36] Vijayavenkataraman S, Thaharah S, Zhang S, *et al.* Electrohydrodynamic Jet 3D-printed PCL/PAA Conductive Scaffolds with Tunable Biodegradability as Nerve Guide Conduits (NGCs) for Peripheral Nerve Injury Repair[J]. *Mater. Des*., 2019, 162: 171-184