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Abstract

Graphene (Gr) has been made of various forms used for repairing peripheral nerve injury with favorable electroactivity, however, graphene-based scaffolds in peripheral nerve regeneration are still rarely reported due to the difficulty of realizing uniform dispersion of graphene and electroactive materials at nanoscale as well as lacking biocompatibility. In this paper, graphene-silk fibroin (SF) composite nanofiber membranes with different mass ratios were prepared via electrospinning. Microscopic observation revealed that electrospun Gr/SF membranes had a nanofibrous structure. Electrochemical analysis provided electroactivity characterization of the Gr/SF membranes. The physiochemical results showed that the physiochemical properties of electrospun Gr/SF membranes could be changed by varying Gr concentration. Swelling ratio and contact angle measurements confirmed that electrospun Gr/SF membranes possessed large absorption capacity and hydrophilic surface, and the mechanical property was improved with increasing Gr concentration. Additionally, in-vitro cytotoxicity with L929 revealed that all the electrospun Gr/SF membranes are biocompatible. Moreover, the morphology and quantity showed that the membranes supported the survival and growth of the cultured Schwann cells. Collectively, all of the
results suggest that the electrospun Gr/SF membranes combine the excellent electrically conductivity and mechanical strength of the graphene with biocompatibility property of silk to mimic the natural neural cell micro-environment for nerve development.

Keywords: electrospinning; graphene; silk fibroin; electrical conductivity; schwann cells.

1. Introduction

Peripheral nerve injury remains a common clinical problem, which causes considerable morbidity about 2.8% in all trauma patients[1]. Unlike nerves in the central nervous system, peripheral nerves are capable of spontaneous regeneration to a certain degree[2]. However, peripheral nerve repair, especially in the cases related to long-distance nerve gaps, still has many barriers to overcome[3, 4]. Nerve tissue engineering is a promising approach that has shown potential to address this need with synthetic nerve conduits[5, 6]. There has been a significant effort dedicated to developing synthetic nerve conduits that have resulted in encouraging regeneration and functional recovery of peripheral nerve defects[5, 7]. These tubular scaffolds had interconnected pores and suitable mechanical properties for nerve regeneration, whereas, these scaffolds are not usually active and its efficacy is still not as good as autologous nerve in long nerve defects. Thus, in order to further control or tune the growth of neural cells, it remains a challenging task to improve the interaction between these cells and the scaffold.

Electrically conducting scaffolds induce specific cellular responses at molecular level. The need of an electrically conductive polymer for neural tissue engineering applications arises from the fact that uncharged surfaces are less than optimal for
promoting normal cellular phenotypic behaviors. Furthermore, the ability to deliver an electrical current to the cells via the scaffold surface may have several advantages. Current research has shown that physiologically relevant electrical stimulation can enhance nerve regeneration and help replenish neural cells at the injury site [8, 9, 10]. Therefore, designing scaffolds that not only have unique surface nanotopography but also have the ability to provide physiological levels of electrical stimulation for the neural cells is advantageous to modulate and enhance their cellular response.

Carbon-based materials, such as graphite, fullerenes, nanotubes, nanowires and nanoribbons have been used for various applications in tissue engineering and regenerative medicine [11]. Graphene (Gr) is an important new member to these carbon family materials recently, which is a two-dimensional, single-layer sheet of sp2 hybridized carbon atoms with a honeycomb lattice configuration [12, 13]. Due to its singularity molecular configuration, graphene shows outstanding properties, such as high electric and thermal conductivities, high mechanical strength, as well as excellent optoelectronic properties. Gr have also been developed in a considerable diversity of applications in biosensors, drug delivery, cell detection, and all sorts of electronic, optical and energy storage devices [14, 15]. However, due to the disadvantages such as highly hydrophobic, featureless surfaces, low aqueous solubility, and lack of biocompatibility, their potential in bio-nanotechnology remains undervalued to certain extent [16]. Apart from that, most approaches to obtain these materials were either complicated or hard to realize the uniform dispersion of Gr and electroactive materials at nanoscale, which largely weakened the advantages of Gr. To overcome these disadvantages, many natural biomacromolecules (e.g., DNA and proteins) have been bound to the surfaces of graphene [17].
Among naturally derived biomaterials, silk fibroin (SF) has been proved to possess favorable physicochemical and biological properties which has found increasing applications in the fabrication of tissue engineering scaffolds [18, 19]. Electrospinning technique is a versatile and effective method to prepare continuous fibers with large surface area to volume ratio, small pores and diameters range of several micrometers to several tens of nanometers, based on this technology, many polymers have been successfully prepared to nanofibers for several years [20]. In recent years, the electrospinning process has drawn much attention in manufacturing composite nanofibers for various technological applications [21, 22].

In this work, we obtained a well-combined Gr/SF based nanomembrane through a novel and efficient way. First, we prepared SF membranes which were further dissolved in formic acid. Then, nanoscale Gr nanopowder was added, followed by sonication treatment. A rather stable and homogeneous suspension spinning solution was harvested, and then Gr/SF based nanomembrane was obtained by electrospinning technique. The resulting Gr/SF based nanomembrane exhibits an excellent electrochemical performance. Further comparison of the electrochemical performance with different concentrations of Gr, the results showed that the optimum concentration of Gr was beneficial to the physical and chemical performance as well as to promote the biological activity of nerve cells. Therefore, this work provides a promising way to prepare a well combined electroactive Gr/SF nanomembrane for neural tissue engineering application.
2. Materials and methods

2.1 Synthesis of spinning solution

Raw silk fibers (from Bombyx mori cocoons) were bought from Xinyuan sericulture company, Hai’an, Jiangsu, China. The SF aqueous solution was prepared as previously described [23]. Degummed SF fibers were first dissolved in a tertiary solvent system of CaCl2/H2O/EtOH solution (mole ratio 1:8:2) at 80°C for 1 h, and then dialyzed against distilled water in a cellulose tube (molecular cutoff = 12,000-14,000) at room temperature for 3 days. The final SF aqueous solution spread on stainless steel dishes to generate the air-dried regenerated SF membranes which were further dissolved in 98 % (wt/wt) formic acid to obtain 13 % (wt/wt) SF spinning solution.

To make the composite films of graphene and SF, 5-20 wt% graphene/ SF spinning solution was prepared by dissolving appropriate amount of graphene nanopowder (TCI, G0441) in SF spinning solution at room temperature with a magnetic stirring apparatus. Then treated by an ultrasonic generator (FS-1200, Sonxi, Shanghai, China) for 60 min at a sonication power of 720 W and frequency of 20 kHz.

2.2 Contact Angle Measurement

The contact angle of spinning solutions were measured in a Goniometer (DSA20, Germany) which was tested on a glass slide [24]. The solutions were prepared at least 1 h prior to the measurements and sonicated by Ultra-sonicator for 60 min.

2.3 Preparation of the Gr/SF nanocomposite membrane and chemical treatment

Electrospinning was carried out by self-made equipment, which was made up of a high voltage supplier, a capillary needle serving as an anode, and a grounded collector serving as a cathode. A high electric potential (20 kV) was applied to the anode with a
needle tip (ID 0.9 mm), into which the droplet of spinning solution was loaded. The resulting electrospun membrane was formed on steel collector or on the glass slide. The distance between the needle tip and the collector ranged from 7 to 13 cm. A constant volume flow rate (0.3 mL/h) was maintained by a syringe pump, which kept the spinning solution in the needle.

After electrospun membranes were removed from the collector, they were inserted into absolute ethanol for 10 min to induce conformational transition, followed by wash with distilled water at 37°C for 72 h to remove residual formic acid.

2.4 Electric conductivity

Cyclic voltammetry (CV) experiments were conducted with a digital conductivity meter (CHI600E, Chenhua, Shanghai, China) at room temperature. A typical three-electrode system consisted of a platinum gauze counter electrode, Ag/AgCl (saturated KCl) reference electrode and glassy carbon working electrode. The measurements conducted in 0.1 M KCl containing 5.0 mM Fe(CN)$_6^{4-/3-}$ provided detailed electrochemical properties of each surface. The electrochemical performance of the electrospun Gr/SF was assessed through CV between 100 mV and 600 mV at a scan rate of 100 mVs$^{-1}$. The surface conductivity of various types of composite membarnes was measured using the four-probe (micromanipulator model 6000) at room temperature. The constant current applied to the surface created a differential voltage and then the sheet conductivity of the membarnes was calculated by correlating the surface resistance using the Van der Pauw equation. Measurements were performed four times in different directions from the center of the membarnes.
2.5 **Swelling ratio**

The swelling ratio was investigated by immersing SF and Gr/SF electrospun membranes into phosphate-buffered saline (PBS, pH=7) solution at 37°C for 24 h. Thereafter, the electrospun membrane was taken out, the excess water on the surface was removed by sandwiching the membrane between two paper towels, and then weighed immediately. The swelling ratio of the electrospun membrane in PBS was calculated using the following expression: \[ R_{sw} = \frac{W_s - W_d}{W_d} \times 100\% \], where \( W_d \) is the weight of the dried electrospun membrane, and \( W_s \) is the weight of the swollen electrospun membrane.

2.6 **Porosity rate**

The porosity rate of the SF membranes before and after adding different percentage of Gr was measured by a liquid displacement method reported by Rajesh R et al [25]. Briefly, the membrane was immersed in anhydrous ethanol with a known volume (\( V_0 \)), and then a series of vacuum-release cycles were performed to force the liquid into the pores of the membrane. Thereafter, the volume of the liquid-perfused membrane and liquid was recorded as \( V_1 \). Subsequently, the liquid-perfused membrane was taken out, and the remaining liquid volume was recorded as \( V_2 \). Finally, the porosity rate of the membranes was calculated using the following equation: \[ V_0 - V_2/V_1 - V_2 \times 100\% \].

2.7 **Mechanical Properties**

The prime objective of mechanical testing was to deduce electrospun membrane properties relevant for use [26]. Uniaxial tensile testing was performed to determine the mechanical strength of pure electrospun SF membranes and various Gr/SF composite electrospun membranes by an Instron 5943 (Norwood, MA, USA). Specifically, various electrospun membranes with an identical size of 10 mm (length) × 10 mm (width) × 1 mm (thickness) were subjected to a tensile load at a strain rate of 10 mm/min until
failure. The value of stress/strain was determined manually by calculating the ratio of fracture length and strain. Three parallel samples for each electrospun membranes were measured for obtaining statistical data.

2.8 In vitro cytotoxicity

According to ISO-10993, the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed to determine the in vitro cytotoxicity of the extracts of films. L929 cells were seeded into a 96-well plate at a density of 5000 cells per well and incubated with 100 μL culture medium containing either in the DMEM medium or the film extract fluid at 37 °C for various time periods. After the incubation, the culture medium in each well was removed and the cells were washed three times with PBS. 20 μL of MTT solution (5 mg/mL) was added to each well and cells were cultured for another 4 h. The supernatant was discarded and then 100 μL of DMSO was added to each well. The OD values of the plate were measured on an EIX-800 Microelisa reader at 570 nm (Bio-Tek Inc., USA).

2.9 Morphology Observation and Quantity Evaluation of SCs

Schwann cells (SCs) were harvested from lumbar dorsal root and sciatic nerves of 1-3 day old Sprague-Dawley (SD) rats, as described previously [27]. The lumbar dorsal roots and sciatic nerves were digested with 0.125% trypsin and incubated at 37 °C for 30 min, terminated the digestion with DMEM containing 10% FBS. The next day, 10 mM cytosine arabinoside was added and incubated for another 48 h to inhibit the growth of fibroblasts. Then the medium was changed every three days by DMEM containing 10% FBS, 2 mM forskolin and 2 ng/mL heregulin to provoke cell proliferation. After cells covered 80–90% of the plate, a purification process was performed. The cells were gently digested from plates by trypsin and incubated with
anti-Thy1.1 antibody (1:1000, AbD serotec, Raleigh, NC) on ice for 2 h, followed by incubated with complement for additional 1 h. After these procedures, the cells were cultured with complete medium. Then the medium was changed at the second day with complete medium containing growth factor. Thereafter, the medium was changed every other day until the cells were sufficient to seed on the materials.

For seeding the cells on the samples, the nanopatterned Gr/SF membranes were sterilized using 75% alcohol for 30 min and rinsing with PBS twice. Then the samples were transferred to a 24-well plate and 1mL cell suspension with a concentration of $5 \times 10^4$ cells/mL was added onto the samples. After culturing for designated time, the cells were washed with PBS and fixed for morphology evaluation. Then, the micromorphology of SCs was examined using SEM after coating with gold. And the cell morphology and distribution on various nanopatterned Gr/SF membranes were evaluated using TBO staining method.

For quantity evaluation, cell-counting kit 8 (CCK-8, BD Biosciences, San Jose, CA) was used to test the attachment and proliferation of SCs on nanopatterned samples and control samples. After SCs were cultured for 3 days, the medium was discarded and the samples rinsed with PBS. Then 500 µL of CCK-8 reagent (1:10 diluents in PBS) was added to the samples and incubated at 37 °C for 4 h. Subsequently, 150 µL of the supernatant was extracted and added to a 96-well plate. The optical density at 450 nm was obtained by a microplate reader.

The proliferation of Schwann cells was determined by 5-ethynyl-20-deoxyuridine (EdU) labeling according to the manual of an EdU labeling/detection kit (Ribobio, Guangzhou, China). Briefly, 50 mM EdU labeling medium was added to cell culture to allow incubation for 12 h at 37 °C under 5% CO$_2$. Afterward, cells were fixed with 4% (w/v) paraformaldehyde for 30 min and incubated with glycine for 5 min. Then washed
with PBS, stained with anti-EdU working solution for 30 min at room temperature, washed with 0.5% TritonX-100 in PBS, incubated with 5 mg/mL Hoechst 33342 dye for 30 min at room temperature in turn, followed by observation under a confocal laser scanning microscope (TCS SP2, Leica Microsystems, Germany). The percentage of EdU-positive cells was calculated from 10 random fields in 3 wells.

### 2.10 Statistical Analysis

Statistical significance of differences between groups was analyzed by unpaired \( t \) test or by one-way analysis of variance (ANOVA) when more than two groups were compared. Statistical significance was set at \( P < 0.05 \).

### 3. Results and Discussion

Five types of electrospun Gr/SF composite films, differing on the Gr percentage (Gr ~0%, Gr ~5%, Gr ~10%, Gr ~15%, and Gr ~20%), were prepared and analysed.

#### 3.1 Contact Angle Measurement

Hydrophobicity is one of the disadvantages of graphene, which limits its application in biomedical field [28]. The water contact angle data of all electrospun solutions were displayed in Figure 1. It could be seen that the contact angle of water on single silk fibroin is 32.4 due to the fact that silk fibroin has many hydrophilic groups such as –OH and –COOH. It has been well known that graphene was a hydrophobic material, and has no appreciable solubility in most solvents, thus the addition of graphene could obviously change the surface wettability of electrospun Gr/SF composite samples. The results indicated that, after being mixed with graphene, the water contact angle showed a gradual increase from 38.8 to 77.5 (Figure 1).
3.2 Physicochemical and Surface Characterization of Membranes

Morphological observation showed that the electrospun SF membrane was composed of a large number of randomly oriented fibers, which were interconnected to create a three-dimensional porous network structure (Figure 2 (A)). In the Gr/SF group, with the addition of graphene nanoparticle, it was found that Gr were uniformly distributed in silk fibers (Figure 2 (B-C)). But the graphene nanoparticle distributed as clump in Gr-15% and Gr-20% group (Figure 2 (D-E)). This phenomenon mainly due to the fact that with the increase in the concentration of Gr, the electrospinning solution is prone to block the needle and liquid droplets occur in the needle in the process of electrospinning.

Overall, the membrane fibers possessed circular cross-sections and smooth surfaces. And the nanofibrous structure of the membranes was confirmed by the scanning electron morphological observation. The electrospun Gr/SF membranes possess high pores with very small pore size, which can mimic the extracellular matrix and enhance the cell migration and proliferation, and are especially suitable for neural tissue applications.

Furthermore, different electrospun SF membranes were generated respectively through the adjustment of depositing the fibers on a rolling mandrel. The majority of the aligned microfibers were oriented along the longitudinal axis to form a unique aligned topography for the purified SF membrane (Figure 2 (G)). In contrast to SF group, the aligned topography of Gr-SF composite membrane need to be optimized (Figure 2 (H)).

3.3 The Electrochemical Performance of Gr/SF

The key component of neural communication in the human body is the action potential generated at the synapse. This implies that an ideal scaffold in neural tissue engineering should possess electrical conductivity to promote neurite outgrowth and thereby enhance nerve regeneration. The electrochemical conductivity of the scaffolds was measured by
cyclic voltammetry (CV) as a function of Gr content and the results are provided in Figure 3 (A). The CV curves of the four types of Gr/SF materials were shown in Figure 3 as well as the control sample (SF), which showed that differ from SF membranes, 10%-Gr/SF, 15%-Gr/SF % and 20%-Gr/SF % films exhibited a nearly rectangular shaped loop. As can be seen from Figure 3 (A), the output current of Gr/SF membranes appeared several fold higher than that of SF membranes, which implying that the electrochemical behavior of these carbon-based materials were attributed to the pseudo-capacitive effect of incorporated graphene. Indeed, the dispersion of graphene in the composites including causes a gradual increase in voltammetric response, indicating graphene activate the diffusion of ions and electrons on the surface of the electrodes. Fig. 3 (B) shows the surface electrical conductivity properties of electrospun graphene-silk fibroin composite membranes with increasing amount of Gr. In line with the CV property of composite membranes, the addition of Gr in the membrane resulted in an increase in conductivity. There was nearly a linear relationship between electrical conductivity and the content of Gr. This improvement is a direct result of a successful synthesis of the highly conductive Gr nanocomposite, its uniformly dispersion into Gr/SF matrix, and proper interaction between them.

3.4 Swelling Ratio

The swelling property of scaffold is very important for the nutrition and waste exchange in vitro and in vivo. The swelling ratio of electrospun SF and composite Gr/SF membranes after immersing in 37°C PBS for 24 h was shown in Figure 4. The swelling ratio of single silk fibroin membranes was about 5.3-fold, whereas significantly decreased to about 4.8-fold after mixing with 5% graphene. Then the swelling ratio further decreased to 3.5-fold after mixing with 20% graphene. It could be seen that the swelling ratio of all the Gr/SF composite membranes were significantly lower than
those of single silk fibroin membranes. As presented, the pure SF scaffold showed the highest swelling ratio in comparison with the other groups. This high water retention property might be attributed to the hydrophilic nature of silk fibroin [26]. By introducing Gr into the polymer matrix, water retention dropped, and this reduction continued as the Gr content increased. In other words, the water absorption capacity of a pure SF scaffold decreased as Gr was introduced, probably due to hydrophobicity nature of Gr [16].

3.5 Porosity Measurement

For nerve tissue regeneration, highly open porous polymer matrices are required for high-density cell seeding, as well as sufficient nutrient and oxygen supply to the cells [29]. Porosity allows nutrition for migration and proliferation of neurons and schwann cells, and aids in vascularization, thereby the more space and nutrition for the cells and tissue will be provided by the higher porosity of scaffolds than the lower one. The overall porosity of the prepared electrospun Gr/SF membranes were determined by the liquid displacement method, and all was found to be more than 65% (Table 1), which meets the requirements for cell migration and tissue growth.

3.6 Mechanical properties of the electrospun membranes

The value of stress/strain was used to characterize the mechanical properties of the prepared pure electrospun SF membranes and various Gr/SF composite membranes under uniaxial tensile loading (Data were shown in Figure 5). It can be seen that incorporation of graphene had significantly affected the mechanical properties of electrospun nanofiber membranes. The breaking strength of the pure electrospun SF membrane was 0.43 MPa and the elongation was 2.43%, while the breaking strength and the elongation of the Gr/SF composite electrospun membranes with 5% and 10%
graphene increased to 0.57 MPa and 0.82 MPa and 2.9% and 3.5%, respectively. The results indicate that the strength and flexibility of fiber membranes increased. But Gr/SF composite electrospun membranes with 15% and 20% graphene significantly smaller than Gr/SF composite electrospun membranes with 10% graphene. Graphene is a sheet of two-dimensional single layer sp2 hybridized carbon atoms in a honeycomb lattice configuration and has been found to have good mechanical properties and widely used in tissue engineering fields [30, 31]. Thus the use of graphene would give Gr/SF composite electrospun membranes relatively good mechanical properties and stress/strain value should be enhanced after graphene mixing in theory. In contrast, increasing the concentration of Gr from 10%-20% resulted in decrease of strength and flexibility instead of increasing. Yin et al [32, 33] reported that the droplet could arise when the concentration of electrospinning solution was inappropriate, and the mechanical property could be affected with the droplet. In our research, a similar phenomenon was observed, the Gr/SF composite electrospun membranes with 10% Gr displayed the largest stress/strain value, indicating the best mechanical properties.

3.7 In vitro cytotoxicity evaluation

The mouse fibroblast L929 cell is a routinely used cell line for cytotoxicity evaluation according to ISO-10993. Thus, in this study, the in vitro cytotoxicity tests of the prepared electrospun membranes against L929 cell were performed to determine their cell biocompatibility. The results of L929 cell culture on various electrospun membranes for 1, 4 and 7 days were shown in Figure 6 (A), respectively. As we can see that the viabilities of L929 cells on the various Gr/SF composite electrospun membranes was higher than pure electrospun SF membranes at the first day. After cultured for 4 and 7 days, cell viabilities in the groups with various membrane extract fluid showed some changes. However, there was no significant difference between
various membrane extract fluid group and control group (DMEM supplemented with 10% fetal calf serum) at 1st, 4th, and 7th days. The results indicated that the addition of Graphene had no obvious cytotoxic effect.

### 3.8 Schwann Cells affinity of electrospun membranes

It is well known that Schwann cells are involved in some peripheral nerve biological functions including salutatory conduction of nerve impulses along axon, providing neurotrophic factors support, producing nerve extracellular matrix for myelin sheaths formation and nerve development and regeneration. Therefore, Schwann cells were used to preliminary evaluate the feasibility of using Gr.

The viability and proliferation of Schwann cells after culture for 3 days were evaluated using CCK-8 assay and 5-ethynyl-20-deoxyuridine (EdU) labeling/detection kit assay in this study. CCK-8 assay showed that after 24 h culture, the cell viability of Schwann cells grown on the 10% Gr/SF electrospun membranes was significantly higher than that on each of other 4 substrates (Figure 6 (B)).

After Schwann cells were cultured on the electrospun Gr/SF membranes for 3 days, TBO staining was utilized to observe the cell behavior of SCs on all samples in Figure 7 (A). Schwann cells on most electrospun Gr/SF membranes showed obvious spindle shape with projections, a typical characteristic of the Schwann cell. Moreover, Schwann cells were homogeneously distributed on the surface with lower Gr concentration (5 and 10%), and there was no obvious morphology difference for the cells on these samples, whereas the number of Schwann cells on Gr-15/SF and Gr-20/SF membranes was much less than that on the others. In addition, SEM was used to further observe the micromorphology of SCs on the different composite membranes (Figure 7 (B)). It could be seen that the cultured SCs exhibited a spindle cell bodies with extending neurites, and the cells migrated along the surface of the membrane. There
were a few visible differences in cell behaviors between Gr-15/SF and Gr-20/SF membranes various and on the other membranes. Collectively, the results here indicated that the electrospun Gr/SF membranes with high Gr concentration could negatively affect Schwann cells growth behavior.

EdU incorporation provided the comparison in cell proliferation among Schwann cells grown on 5 substrates with different conductivity, and the quantitative data indicated that the 10% Gr/SF electrospun substrate led to prominent enhancement on cell proliferation among all substrates (Figure 8).

4. Conclusion

In order to overcome the drawbacks of SF-based materials, Gr/SF blend material with a narrow and uniform particle size distribution was prepared by an electrospinning technique. Surface morphology, electrochemical behavior, swelling ratio, porosity, tensile strength and contact angle for different ratios of Gr/SF nanofiber membranes were characterized. Comprehensive consideration of the above properties, Gr/SF (10%) was selected as the optimized one. Cell viability and cell attachment showed that electrospun Gr/SF (10%) membranes supported the survival and growth of Schwann cells cultured onto the membranes, and the membrane extract showed no significant cytotoxic effects on the proliferation of Schwann cells. These results suggested that electrospun Gr/SF (10%) nanofiber membranes might become a candidate membrane used for tissue engineered nerve grafts to promote peripheral nerve regeneration.

To sum up, in vitro characterization and evaluation of Gr–SF nanofiber membrane inspire us to improve the design of Gr–SF nanofiber membrane and develop their applications in peripheral nerve regeneration.
Disclosure statement

No potential conflict of interest was reported by the authors.

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nanosheets composite films with enhanced mechanical performances. *Carbohydr

Membranes Used as High Efficiency Filter Materials: Filtration Potential, Thermal

Figure Captions:

Table 1. Characteristics of Porosity of electrospun composite Gr/SF films.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Gr content (wt.%)</th>
<th>Porosity (%)</th>
</tr>
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<tbody>
<tr>
<td>SF</td>
<td>0</td>
<td>66 ± 1.15</td>
</tr>
<tr>
<td>Gr-5%</td>
<td>5</td>
<td>72 ± 0.98</td>
</tr>
<tr>
<td>Gr-10%</td>
<td>10</td>
<td>80 ± 1.23</td>
</tr>
<tr>
<td>Gr-15%</td>
<td>15</td>
<td>85 ± 1.37</td>
</tr>
<tr>
<td>Gr-20%</td>
<td>20</td>
<td>92 ± 1.52</td>
</tr>
</tbody>
</table>
Figure 1. Contact angles of various electrospun composite Gr/SF solutions. *$p < 0.05$ compared with pure electrospun SF samples (SF).
Figure 2. The light microscopy images of various random SF (A), 5%-Gr/SF (B), 10%-Gr/SF (C), 15%-Gr/SF (D), 20%-Gr/SF (E), and aligned SF (G), 5%-Gr/SF (H) electrospun composite Gr/SF membranes after drying. Scanning electron micrograph (F) is the magnified image of 10%-Gr/SF membrane interface. The scale bar for optical micrographs and scanning electron micrograph represents 100 µm and 10 µm, respectively.
Figure 3. Electrochemical properties of single electrospun SF membranes and electrospun Gr/SF membranes. The cyclic voltammogram (CV) curves of various electrospun composite Gr/SF films (A). The comparison of electrical conductance of electrospun SF membranes with different amounts of Gr (B).
Figure 4. Swelling ratio of various electrospun composite Gr/SF films in PBS at 37 °C after immersion for 24 h, *p < 0.05 vs. SF group.
Figure 5. Value of stress/strain of the pure electrospun SF membranes and Gr/SF membranes.
Figure 6. MTT assays of L929 cells cultured on the prepared different electrospun Gr/SF membranes at different time points (A). *p < 0.05 vs. Control group. The number of Schwann cells on different membranes by CCK-8 test for 3 day (B). * p < 0.05, compared with other samples. Data are presented as mean ± standard error (n = 4).
Figure 7. In vitro Schwann cell culture on single electrospun SF membranes and electrospun Gr/SF membranes for 3 days, respectively. Representative optical micrographs of TBO staining of Schwann cells after cultured on different membranes (A). Representative SEM images of Schwann cells (B).
Figure 8. The proliferation rate of primary cultured SCs was examined after grown on single electrospun SF membranes and electrospun Gr/SF membranes for 3 days, respectively. Error bars represent the standard deviation. *$p<0.05$ compared with single electrospun SF (SF) group. The scale bar, 50 μm.