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Preparation of graphene oxide/polyacrylamide composite hydrogel and its effect on Schwann cells attachment and proliferation

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ABSTRACT

Various hydrogel materials have been developed for improving the regeneration of peripheral nerve. Among which the graphene related hydrogels with excellent mechanical properties have attracted great attention. However, the effect of these hydrogels on peripheral nerve regeneration is still unclear. In the present study, the graphene oxide/polyacrylamide (GO/PAM) composite hydrogels were fabricated by in-situ free radical polymerization. The morphology, wettability, composition, swelling ratio, mechanical property and degradation behavior of the prepared GO/PAM composite hydrogels were separately characterized. The effect of GO/PAM hydrogel on the attachment and proliferation of Schwann cells was evaluated. Moreover, the release of biofactors by Schwann cells and adsorption of matrix proteins were further measured. The results showed that the color of the hydrogel became darker with the increased GO concentration, while the surface pore structure also displayed large variation when GO concentration was increased. The hydrophobicity and mechanical properties of hydrogel were increased with the ascending GO concentration. In addition, the variation of GO concentration displayed no obvious influence on the degradation of the composite hydrogel in different medium. The GO/PAM composite hydrogel with 0.4% GO (G0.4) could effectively enhance the attachment and proliferation of Schwann cells. Furthermore, the cells on G0.4 hydrogel displayed higher biofactors release and larger matrix adsorption than other samples. The results demonstrated that GO with suitable concentration in PAM hydrogel could effectively promote Schwann cell growth. The study may provide an important experimental basis for the design and development of new nerve grafts with potential application for peripheral nerve regeneration.

1. Introduction

Peripheral nerve injury (PNI), which is caused by traffic accidents, industrial accidents, war, earthquakes and natural disasters, is a global clinical problem. PNI not only seriously affected the quality of patients' life, but also increased their economic burden [1–3]. Currently, the peripheral nerve defects are mainly repaired using the traditional autologous nerve graft, which, however, is limited by the transplant neural source, new trauma formation, size mismatch, and the long-term denervation at transplant donor area, thus inhibiting the clinical application of autologous nerve graft. In the last two decades, a variety of artificial nerve grafts made from natural or synthetic biomaterials have been widely used for repairing PNI, including chitosan [4–7], carbon nanotubes [8], collagen/silica composites [9], etc. However, the effect of these implants on nerve regeneration is still not as good as that of autologous nerve graft.

Hydrogel material has received more and more attention in the fields of tissue engineering and regenerative medicine due to the good biocompatibility and structural stability [10]. In recent years, hydrogels were gradually applied in the field of peripheral nerve regeneration [11,12]. Hill et al. [13] found that keratin hydrogel scaffolds could repair peripheral nerve defects in rabbits. The hydrogel-enriched chitosan conduits containing engineered Schwann cells were also successfully used for peripheral nerve regeneration [14]. Polyacrylamide (PAM) hydrogel with good biocompatibility, softness and easy processing, has been widely studied in tissue engineering fields, indicating potential application for repairing PNI. However, the brittleness and poor mechanical properties are the main drawback of pure PAM hydrogel [15].

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For overcoming this problem, the bioactive nanoparticles [16], ion [17], clay [18], sodium alginate [19], silk fibroin [15], collagen [9], etc, have been used to form composite hydrogel with PAM. Vorobieva et al. prepared polyacrylic acid/PAM hydrogel [20], they found the addition of polyacrylic acid was beneficial for building hydrogen bonds and enhancing the mechanical property.

Graphene oxide (GO) is an important derivative of graphene, which has been widely used in reinforced composite materials [21–23]. The surface of GO contains abundant oxygen-containing groups, such as hydroxyl (—OH), carbonyl group (—C=O). These oxygen-containing groups greatly enhanced the surface ability of GO to bind with other materials [15]. GO has good biocompatibility and shows great potentials in biomaterials application [24–26]. Previous studies showed that the PAM hydrogel composited with GO displayed better mechanical properties than the pure PAM hydrogel [27]. GO was also found to obviously enhance the mechanical property after forming hydrogel with other materials, such as poly (*N*-isopropylacrylamide) [28], hyaluronic acid [29], and biological composite nanofibers [30], etc. but only a few of survey-based study referring to the hydrogel composed of GO and PAM [27,31–33]. From the above researches, it could be found that addition of GO can significantly improve the mechanical properties of the hydrogel. However, the effect of GO/PAM hydrogel on the peripheral nervous regeneration is rarely reported.

Herein, in the present study, the GO/PAM composite hydrogel was prepared by adding GO with different concentrations into the PAM solution via in-situ free radical polymerization. The morphology, composition, wettability, swelling ratio, mechanical property and degradation behavior of GO/PAM composite hydrogel were characterized. The effect of GO/PAM composite hydrogel on peripheral nerve regeneration was evaluated by Schwann cells culture. The influence of GO addition on PAM hydrogel and nerve regeneration was discussed.

2. Materials and methods

2.1. Materials and reagents

GO was prepared according to radical polymerization in situ as reported by literature [34]. Acrylamide, phalloidin were purchased from Sigma Aldrich, USA. *N*,*N*-methylene-bis-acrylamide was from Amresco, USA. Ammonium persulfate (AP) and bicinchoninic acid (BCA) protein assay kit were purchased from Beyotime, China. Bovine serum albumin (BSA), Phosphate buffer saline (PBS, pH 7) and Dulbecco's modified eagle medium (DMEM) were bought from Hyclone Co., Ltd. Rat nerve growth factor (NGF) assay kit, ciliary neurotrophic factor (CNTF) assay kit and brain-derived neurotrophic factor (BDNF) assay kit were purchased from Sigma Aldrich, USA. Sprague-Dawley rats (1 ~ 3d-old) were provided by Experimental Animal Center of Nantong University.

2.2. Preparation of GO/PAM composite hydrogel

Firstly, the 30 wt.% PAM solution was prepared by diluting 29 g of acrylamide and 1 g of *N*,*N*-methylene-bis-acrylamide in 100 mL of double distilled water (dH_2O). Then, the GO solution with a concentration of 2 mg/mL was prepared by dissolving GO powder in dH_2O completely. Thereafter, the GO/PAM mixture was prepared by adding various volume of GO solution to PAM solution as shown in Table 1. The total volume of the mixture was 5 mL. Subsequently, the mixture of GO/PAM was added with 50 μL AP (10% in dH_2O) as an initiator and mixed throughly. The mixture was then transferred to a home-made gel mold and heated at 60 °C for 2 h in a vacuum oven (DZF-6050, Shanghai Sanfa, China). Finally, the formed GO/PAM composite hydrogels were peeled off and washed with dH_2O for at least 24 h (Fig. 1). According to the variation of GO in the composite hydrogels, the prepared samples were denoted G0, G0.2, G0.4, G0.6,G0.8, G1.2, respectively.

Table 1
Preparation parameters of GO/PAM composite hydrogel.

Samples ID	PAM/mL	GO/mL	AP/μL
G0	5.0	0	50
G0.2	4.5	0.5	50
G0.4	4.0	1.0	50
G0.6	3.5	1.5	50
G0.8	3.0	2.0	50
G1.2	2.0	3.0	50

2.3. Morphological observation

The macro and micro morphology of GO/PAM composite hydrogel was observed by camera (Canon, Japan), optical microscope (OM, Leica, Gemany) and scanning electron microscope (SEM, JEOL, Japan), respectively. For camera and OM observation, the samples were directly observed under wet status. For SEM observation, the samples were firstly lyophilized using a freeze-drying machine (Beckman Co.). Then, the samples were sputtered with a gold layer (~50 nm thickness), fixed on an aluminum stage and observed at the vaccum condition of 1.4×10^{-4} bar.

2.4. Fourier transform infrared spectroscopy (FTIR)

The infrared absorption spectra of the prepared hydrogels were measured using a FTIR spectrometer (Nicolet5700, Madison, WI) in transmission mode and potassium bromide tablets. For each sample, a total of 64 scans were accumulated with 4 cm^{-1} resolution. Scanning was conducted in the range from 400 to 4500 cm^{-1}.

2.5. Contact angle measurements

The sessile drop method was used to measure the water contact angle of each sample by contact angle instrument (JY-PHa, China). The sample was first placed on the measure platform, and then a droplet of dH_2O was dropped on the sample surface, the shape image of droplet could be obtained by the microscope, and then the digital image was used to calculate the contact angle. Three to five different sites of each sample were taken to obtain an averaged contact angle value.

2.6. Swelling behavior

The swelling ratio of the samples was investigated by immersing the dried composite hydrogels in PBS (pH = 7), dH_2O and DMEM at 37 °C for 24 h to evaluate the dynamic swelling behavior, respectively. The time interval between the two measurement is 10 min. For each measurement, the samples were absorbed by filter paper to remove excess surface water. The swelling ratio was calculated using the following formula:

$$Rw = (W_2 - W_1)/W_1 \times 100\%$$

where W_1 is weight of the dry hydrogel, W_2 is the weight after the swelling of the hydrogel. Three parallel samples were used for each measurements.