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Strong and biocompatible three-dimensional porous silk fibroin/graphene oxide scaffold prepared by phase separation

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ABSTRACT

Silk fibroin (SF) is blended with graphene oxide (GO) to prepare the strong and biocompatible three dimensional porous SF/GO blended scaffold via phase separation. GO could be well dispersed in SF solution and GO could also be well distributed in the SF scaffold. Furthermore, the introduction of GO can lead to structural change in the bended scaffold. Higher concentration of GO resulted in more compact structure and smaller pore size of the composite scaffolds without decreasing their porosity. Scanning electron microscopy and energy dispersive spectrometry results also reveal that SF and GO are homogeneous blended together. Analysis of chemical structures of the scaffold shows that addition of GO do not affect the crystalline structure of SF and it is evenly blended with SF. The blended scaffold has significantly higher breaking strength than the pure SF scaffold. In vitro study indicates that both pure SF scaffold and SF/GO composite scaffold support growth and proliferation of MC3T3-E1 osteoprogenitor cells. However, the addition of GO contribute to the proliferation of MC3T3-E1 osteoprogenitor. The testing results show that the blended scaffold is an appropriate candidate for tissue engineering.

1. Introduction

Bombyx mori silk extracted from silkworm is a biopolymer fiber which has been extensively used in textiles for thousands of years due to its exceptional mechanical properties and luster [1, 2]. Native *Bombyx mori* silk is composed of silk fibroin (SF) coated with silk sericin proteins. Due to favorable biocompatibility, biodegradable and minimal inflammatory reaction [3, 4], SF has been fabricated into a variety of silk-based materials, such as gel, fiber, powder, sponge, film and tube, and so on [5–10], and these silk-based materials have been widely used for tissue engineering scaffold [11–16].

During the past three years, there are many reports about SF scaffolds prepared by phase separation, which are used for the tissue engineering [17–19]. As we known, tissue engineering scaffolds have high requirements for mechanical performance and biocompatibility of materials. However, in practical applications, there are problems that the strength of SF scaffolds is not high enough and the biocompatibility needs to be further improved. Therefore, SF scaffold is often modified to improve the strength and biocompatibility [20–21]. In our previous research, we have also explored the related research of improving the mechanical properties and biocompatibility of SF scaffold. One research is that the SF scaffold is crosslinked with organic alcohols to enhance the strength [22, 23], but the scaffold is hard and brittle after crosslinking with organic alcohols. Furthermore, organic alcohol treatment may decrease the biocompatibility of scaffolds. Another research, gelatin is blended with SF to improve the biocompatible of SF [24, 25], the results show that blending of gelatin does enhance the biocompatibility of SF scaffold. However, there is a problem that the mechanical strength of SF scaffold is not high enough.

Graphene oxide (GO) contains a large number of functional groups and an extremely large surface-to-volume, which gives some unique properties of the GO in reinforcement, biomaterials and other fields widely [26–28]. Recently, there are a few reports about the GO based biomaterials. However, till now, there has been no report on the SF/GO composite scaffold. In particular, there is no report on GO resulted in the improvement of both strength and biocompatibility of the scaffold. In this paper, the GO is prepared, and then GO is dispersed in SF solution. The three dimensional porous SF/GO blended scaffold is fabricated by phase separation. The fluid property of the SF/GO blended solution is investigated by rheometer. Macroscopic and microscopic morphology of the blended scaffold are observed by scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), X-ray diffraction (XRD). In particular, the mechanical property and biocompatibility

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are characterized by electronic strength tester and cell culture. The testing results show that the blended scaffold is an appropriate candidate for tissue engineering.

2. Results and discussion

2.1. Morphology and microstructure of GO

The as-prepared GO sheets have lateral dimensions of several micrometers and a thickness of about 0.8 nm (Fig. 1 a–b), which are characteristics of single layer GO sheets [29]. The chemical structure of GO was studied by Raman and XPS. The Raman spectrum has two prominent peaks at 1340 and 1580 cm^{-1} (Fig. 1 c) and they are assigned to the D-band and G-band of carbon, respectively. The G-band is related to graphitic carbon and the D-band is associated with the structural defects or partially disordered structures of graphitic domains [30]. The C/O atomic ratio of GO was measured to be about 2.2 by XPS examinations and the GO sheets have four types of carbon bonds: C—C/C═C (284.6 eV), C—O (286.6 eV), C═O (287.7 eV), and O—C═O (289.0 eV) (Fig. 1 d). These hydrophilic oxygenated groups of GO sheets make them dispersible in water to form a stable colloidal suspension [31]. It was also believed that the electrostatic repulsion between GO sheets caused by the ionization of carboxyl groups prevented their aggregation in aqueous medium [32]. The above results indicate that the GO sheets are prepared successfully.

2.2. Morphology and microstructure of the three-dimensional porous SF/GO scaffold

Fig. 2 a shows photo of a series of SF/GO blended aqueous solution with different GO concentrations. The concentration of the prepared aqueous SF solution was determined as 5 wt%. It can be seen that the pure aqueous SF solution appears to be transparent while the SF/GO blended aqueous solution is in a brown colour. By increasing the concentration of GO in the blended solution, the brown colour of the blended solution is gradually getting darker. Apparently, as the concentration of GO is lower than 5 wt%, GO is well dispersed in the SF solution without occurrence of precipitation or suspended solids, which shows that GO in a proper concentration can be added in the SF aqueous solution in an uniform dispersion. However, as the concentration of GO increases to 5 wt%, slight agglomeration of GO occurs, which indicates that the content of GO in the blended solution can't be increased further. As shown in Fig. 2 b, an increase in the viscosity of the solution can be observed with the addition of GO, and this increase is more clear upon increasing the content of GO. This may be the consequence of active groups such as hydroxyl (—OH), carboxyl (—COOH) and epoxy (—O—) in GO and nano-size effect of GO. The large surface area, nano-sheets structure and active groups of GO can increase the interaction between components of the solution system, which is favor to increase the viscosity of the solution [33]. Thus it is easier to prepare scaffolds with improved mechanical properties.

The SF/GO blended aqueous solution was poured into the Petri dish with a level about 2/3 of the height of the dish and freeze-dried to obtain scaffolds. Macrographs of those prepared scaffolds are shown in Fig. 3. It is observed that pure SF scaffold displays a white three dimensional structure while SF/GO composite three dimensional scaffolds show a homogeneous brown colour, indicating uniform distribution of GO in the composite scaffolds. The colour of the composite scaffolds turns gradually darker on increasing the amount of GO in the composite scaffolds.

Fig. 4 shows SEM micrographs of the SF and SF/GO composite scaffolds. It is noted that the pure SF scaffold exhibits an irregular loose and porous honeycomb structure, together with large pore sizes (average pore diameter is about 56 ± 9 μm), which can facilitate more cell growth, differentiation and proliferation. Beyond that, it can be seen that the surface of the pure SF scaffold is covered by small holes due to volatilization of *n* butyl alcohol. *N* butyl alcohol was used as porogen to achieve pore interconnectivity in the preparation process of composite scaffolds. Introduction of GO resulted in compact and porous honeycomb structure and small pore size in the composite scaffolds. At higher

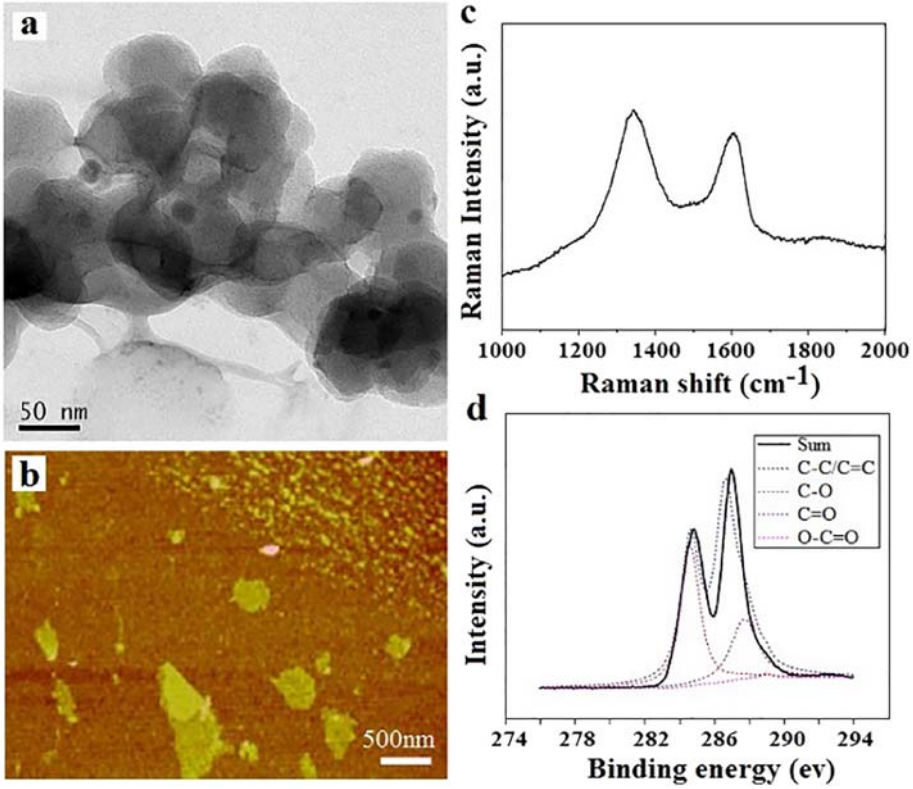

Fig. 1. Characterizations of the GO. (a)–(b) SEM and AFM images, (c)–(d) Raman and XPS spectra.