



Tunable green graphene-silk biomaterials: Mechanism of protein-based nanocomposites



Fang Wang^{a,b,1}, S.S. Jyothirmayee Aravind^{a,c,1}, Hao Wu^d, Joseph Forsys^a, Venkat Venkataraman^d, Kandalam Ramanujachary^c, Xiao Hu^{a,e,f,*}

^a Department of Physics and Astronomy, Rowan University, Glassboro, NJ 08028, USA

^b Center of Analysis and Testing, Nanjing Normal University, Nanjing 210023, China

^c Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08028, USA

^d Department of Cell Biology, Rowan University School of Osteopathic Medicine, Stratford, NJ 08084, USA

^e Department of Biomedical and Translational Sciences, Rowan University, Glassboro, NJ 08028, USA

^f Department of Biomedical Engineering, Rowan University, Glassboro, NJ 08028, USA

ARTICLE INFO

Article history:

Received 30 March 2017

Received in revised form 12 May 2017

Accepted 16 May 2017

Available online 17 May 2017

Keywords:

Silk

Graphene

Elastic modulus

Nanocomposite

ABSTRACT

Green graphene materials prepared by photoreduction of graphite oxide were first time blended with aqueous-based silk fibroin proteins to improve the mechanical and thermal properties of silk biomaterials, and their nano-composite interaction mechanism was illustrated. Powder X-ray diffraction (XRD) analysis confirmed the complete exfoliation of graphite oxide to graphene in presence of focused pulses of solar radiation. By varying the concentration of graphene (0.1 wt% to 10 wt%), a series of free standing graphene-silk films were prepared and were systematically characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and nanoindentation measurements. The homogeneity of graphene in silk as well as the thermal stability of the composite films was demonstrated by thermal gravimetric analysis (TGA) and temperature-modulated differential scanning calorimetry (TMDSC). Surprisingly, silk composite film containing only 0.5 wt% of graphene gives the highest Young's modulus of 1.65 GPa (about 5.8 times higher than the pure silk's modulus), indicating a nano-composite to micro-composite transition of silk-graphene structure occurred around this mixing ratio. This finding provided an easy approach to improve the elastic modulus and other physical properties of silk materials by adding a tiny amount of graphene sheets. Fibroblast cells studies also proved that these graphene-silk materials can significantly improve cell adhesion, growth and proliferation. This protein nanocomposite study provided a useful model to understand how to manipulate the hydrophobic-hydrophobic and polar-polar interactions between high-surface-area inorganic nanomaterials and amphiphilic protein materials, which has many emerging applications in the material science and engineering, such as bio-device fabrication, drug storage and release, and tissue regeneration.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Hybrid and inorganic materials have remarkable properties for delivery [1–3], therapy [4,5] and sensor applications [6–9]. Recently, two-dimensional graphene materials with only an atom thickness have paved novel ways for the development of various functional materials. Several studies have demonstrated fascinating attributes of graphene such as superior electrical and thermal transport properties as well as its high mechanical strength [10–12]. Furthermore, the high surface area of graphene makes it an ideal conducting matrix for

anchoring nanomaterials [13]. Different thermal [14], chemical [15], epitaxial growth [16] and photo reduction techniques [17–18] have been reported to produce single-layer graphene sheets and manipulate its properties for a variety of device applications. Graphene has also been investigated for varied applications in nanoelectronic and energy storage devices, biomedical sensors and was used as a critical component for nanocomposite materials recently [19–22].

There has been a great interest in developing graphene-polymer composites to completely exploit the superior properties of graphene in a flexible material. However, there are still many challenges associated with the development of graphene-polymer composites. For example, delicate morphological organization, fine interface control and uniform dispersion of graphene into polymers are crucial factors on determining the performance of the resulting composite. These requirements arise largely from the surface properties of graphene,

* Corresponding author at: Department of Physics and Astronomy, Rowan University, Glassboro, NJ 08028, USA.

E-mail address: hu@rowan.edu (X. Hu).

¹ Fang Wang and SS Jyothirmayee Aravind contributed equally to this manuscript.

and this was usually addressed mostly through chemical modifications of polymer or graphene for specific purposes. For instance, polar functionalization of graphene using concentrated acids has been reported to disperse properly in polyvinyl alcohol (PVA) polymer matrix [23]. As compared to such synthetic polymers, proteins are natural amphiphilic biomaterials that already having both hydrophobic and hydrophilic segments in backbone or side groups, enabling them good source for being adhesive to various hydrophilic and hydrophobic substrates such as graphene directly, without additional chemical modifications [24].

Regenerate silk fibroin (RSF) is a widely used, cost effective, water-soluble protein material. For a long time, it has been used in biomedical applications because of its notable mechanical properties, biocompatibility and long-term biodegradability [25,26]. Silk fibroin fiber is estimated to have 70–75% crystallinity with corresponding 25–30% amorphous region [27]. The fibroin molecular chains are composed of a complex of two components linked by disulfide bonds: a large protein fibroin chain which is relatively hydrophobic and can form anisotropic beta-sheet-rich nanocrystals; a second small protein fibroin chain which is hydrophilic and relatively elastic [27]. Different secondary structures including beta-pleated sheets, coiled coils, or twisted-helices were found in silk fibroin, which provide the biopolymer material unique physical properties, such as high extensibility, strength and elasticity [27,28]. Due to such excellent physical properties and versatility in diverse processing, silk proteins have been employed as substitutes for skin or bone scaffolds in tissue regeneration [29]. It was also found recently that silk can easily bind with graphene sheets by H-bonding and π - π stacking [30]. Hence, silk can be a promising natural polymer for dispersing graphene in solution and developing graphene-silk polymer composites. Ling et al. [31] reported the preparation of graphene-silk composite film, in which the graphite oxide (GO) was reduced to graphene using hydrazine hydrate as a reducing agent. In another study by Qu et al. [32], NaBH_4 is employed as a reducing agent after adding KOH under extensive reaction conditions and the synthesized graphene-silk nanosheets have been employed for sensor applications.

Although these chemical reduction methods of GO have attracted interest due to its simple procedure and low cost, the toxicity driven from these hydrazine-based reducing agents urged us to find new environmentally friendly reduction technique for the production of green graphene materials, which is specifically important in the biomedical applications. In addition, previous studies always tend to mix a large content of graphene (>10 wt%) into the silk matrix, which may bring many potential drawbacks for the biomedical applications of these composite materials- since human body cannot digest and absorb a large quantity of carbon nanomaterials in a short time. Skins or other organs may also have serious allergic or immune reactions to these materials if the graphene content is very high in the polymer matrix. Therefore, there is a need to produce fully green silk-graphene nanocomposite materials with only a small amount of graphene fillers (<10 wt%), while they can also dramatically improve the physical properties of silk materials. In this study, photo reduction method involving focused sunlight as the green reducing agent is investigated to generate the graphene sheets. Unlike previous toxic reagents or long processing hours reported in the synthesis of graphene sheets, focused pulses of natural solar radiation was employed in this rapid photothermal method. Herein, we report the first fabrication of green graphene-silk (G-S) composite films using varied amount of graphene fillers (0.1 wt% to 10 wt%) prepared from facile photo thermal reduction technique. It is demonstrated that these green graphene-silk composites are fully miscible in the nanoscale, which have great potential for biomedical applications in the future. Different graphene-silk composites were prepared by a simple aqueous solution casting technique, and the mechanism of their dramatically improved mechanical and thermal properties by adding very tiny amount of green graphene sheets (e.g. 0.5 wt%) was discussed in this study.

2. Experimental section

2.1. Synthesis of graphene

Graphite (crystalline, 300 mesh, 99%) was purchased from Alfa Aesar (Johnson Matthey Company, USA) and used without further purification. Graphite oxide (GO) was prepared from graphite by the Hummer's method [33–36]. Briefly, graphite was ground with NaCl and washed with DI water followed by filtration. After drying, the filtrate was stirred with concentrated H_2SO_4 for 8 h. 6 g of KMnO_4 was gradually added while keeping the temperature <20 °C. The mixture was stirred at 35 °C to 40 °C for 30 min and then 65 °C to 80 °C for 40 min. 92 mL of water was added to the above solution and heated to ~100 °C. This was diluted by adding 280 mL of water, followed by the addition of 30% H_2O_2 . The mixture was washed with repeated centrifugation (first by 5% HCl and then with water) and followed by filtration. The final product was washed and dried in vacuum for three days.

In addition, in our research, new method is environmentally benign, low temperature process and is fast as compared to conventional techniques. Incoming solar radiation was focused using a convex lens of 100 mm diameter towards GO [37]. The temperature increases rapidly (in 1–2 s) to 150–200 °C due to localized thermal excitation of GO by focused solar radiation, which causes the exfoliation of GO into graphene. Compared to the thermal exfoliation of GO [14], the heating rate was found to be more rapid in the solar exfoliation technique, and hence exfoliation took place at much lower temperature than thermal exfoliation (1050 °C). The details such as the effects of various experimental parameters including the diameter of the lens, wavelength of radiation and corresponding power and temperature were analyzed and reported in previous study by one of the authors [37].

2.2. Preparation of silk solution

Silk cocoons came from *Bombyx mori* Silkworm (China) were boiled for 25 min in an aqueous solution of 0.02 M Na_2CO_3 and rinsed thoroughly with Milli-Q water to remove the glue-like sericin proteins [38]. The extracted silk proteins were dried and dissolved in a 9.3 M LiBr solution at 60 °C for 4–6 h at a concentration of 20 wt%. The silk solution was dialyzed against Milli-Q water for at least 2 days using a dialysis cassette (Pierce Snake Skin MWCO 3500; Thermo Fisher Scientific, Waltham, MA). After centrifugation and filtration to remove insoluble residues, a 6 wt% silk fibroin aqueous solution was obtained.

2.3. Fabrication of graphene-silk film

The as-synthesized green graphene sheets were used for fabrication of graphene-silk (G-S) composite films. The synthesis of graphene-based silk thin films by a solution casting method can be briefly explained as follows: The required amount of graphene was first homogeneously dispersed into a 3 wt% silk water based solution with the help of a weak ultrasonication for nearly 20 min at room temperature. The composite solution was then transferred onto a polydimethylsiloxane (PDMS) substrate and kept in a hood under ambient conditions overnight to form the G-S films. G-S composite films were then peeled-off from the substrates and cut into required dimensions for further measurements. To avoid serious aggregation of graphene, low loading of graphene was preferred and the final graphene contents in the composite films were 0.1, 0.5, 1.0, 5.0 and 10.0 wt% (GS0.1, GS0.5, GS1.0, GS5.0, GS10) of the weight of total films, respectively. The film thickness was about 3–4 mm for each GS film.

2.4. Powder X-ray Diffraction (XRD) measurement

X-ray diffraction patterns were acquired using PANalytical Empyrean X-ray diffractometer with $\text{Cu K}\alpha$ radiation. Powder samples were spread over the glass slides and scanning was carried out with a step

Download English Version:

<https://daneshyari.com/en/article/5434780>

Download Persian Version:

<https://daneshyari.com/article/5434780>

[Daneshyari.com](https://daneshyari.com)