Materials Science and Engineering C 68 (2016) 798–804

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

One-step synthesis of soy protein/graphene nanocomposites and their application in photothermal therapy

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 4 July 2016
Accepted 16 July 2016
Available online 18 July 2016

Keywords:
Plant proteins
Carbon materials
Hybrid materials
Photothermal transformation
Biocompatibility

ABSTRACT

Photothermal therapy, due to its security and effectiveness, has recently become a promising cancer treatment after surgery, radiotherapy, chemotherapy, and biological therapy. In this article, soy protein isolate/reduced graphene oxide (SPI/rGO) nanocomposites are prepared via a simple and green process. That is, GO is reduced in situ and stabilized by SPI, an abundant, low-cost, sustainable natural material, and simultaneously forms SPI/rGO nanocomposites. The resulting SPI/rGO nanocomposites disperse in water very well and exhibit good biocompatibility due to the attachment of SPI to the surface of rGO. Such SPI/rGO nanocomposites demonstrate an excellent photothermal capacity and are able to kill HeLa cells efficiently with near-infrared irradiation (808 nm). The results in this work suggest that such a SPI/rGO hybrid material could be a promising candidate for future applications of photothermal therapy.

1. Introduction

In the past quarter century, although there were many explorations in cancer biology, such studies have not been translated into even remotely comparable advances in clinical practice [1,2]. Current cancer treatments still primarily focus on traditional surgical intervention, radiation and chemotherapeutic drugs, which often damage healthy cells and tissues, or even cause toxicity in patients. However, a new and non-invasive modality called photothermal therapy (PTT) has been increasingly recognized as a promising alternative to traditional cancer therapies, such as surgery, radiotherapy, and chemotherapy, due to its spatiotemporal selectivity and minimal invasiveness [3,4]. PTT usually utilizes photo-absorbing agents, including graphene oxide (GO) [5,6], carbon nanotubes [7,8], gold nanoparticles [9–12], and organic near-infrared (NIR) dyes [6,13] (such as cyanine) to generate a high localized temperature for ablating tumors. This advantage allows clinicians to save most of the healthy tissues close to the targeted lesions by using near infrared (NIR) light irradiation [13,14].

Graphene, which is one atom thick and has a two-dimensional (2D) single carbon layer, is emerging as a rising star in material science and condensed matter physics [15] due to its high specific surface area and excellent mechanical, electrical, and thermal properties [16–18]. These properties enable graphene to be considered an ideal material for a variety of applications. Recently, Yang [19], Kuilla [20], Meng [21] and their coworkers have explored the photothermal effect of graphene in polymer composites. They found that graphene had the potential to convert light to heat efficiently and that the conversion was even better than carbon nanotubes when graphene was used as a nanoscale heater and energy-transfer unit for infrared (IR)-triggered actuators. The perfect sp^2 carbon-network structure of graphene ensures that it efficiently absorbs and transforms IR light into thermal energy [22]. However, the potential of graphene in bionanotechnology is somehow underestimated due to its disadvantages, such as highly hydrophobic and featureless surfaces, low aqueous solubility, and poor biocompatibility [23]. To improve these drawbacks, several natural biomacromolecules (DNAs and proteins) have been applied to modify the surface of graphene nanosheets [24–26]. In our previous research, we demonstrated that silk fibroin was able to grow on the surface of graphene nanosheets in the form of nanofibrils by self-assembly. The graphene/silk nanofibril hybrid preserved the high electrical conductivity of pure graphene. In addition, the graphene/silk nanofibril hybrid film prepared via a vacuum-assisted filtering process showed good flexibility and biocompatibility [23,27].

Soy protein isolate (SPI), which is one of the most abundant natural polymers extracted from soybeans, has attracted comprehensive interests in the field of material science [28] due to its sustainability, low cost, functionality, biocompatibility, and tunable degradability. There have been several soy protein-based materials, such as plastics [29], gels [30], films [31,32], and biomedical materials [33] reported in the literature.

The synthesis of SPI/inorganic composites in previous research has mainly focused on improving the mechanical performance of SPI materials by using inorganic materials as strengthening agents. For example, Lee and his coworkers prepared SPI/montmorillonite (SPI/MMT)

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http://dx.doi.org/10.1016/j.msec.2016.07.034
0928-4931/

composite film with a melt extrusion method for drug release [34]. However, the preparation conditions were relatively harsh, and the structure of the obtained composite was not homogeneous at the nanoscale.

Zhuang and his coworkers have reported the preparation of SPI/graphene aerogel with a hydrothermal method, and the obtained composite was used as an adsorbent to remove antibiotics (ciprofloxacin and tetracycline) from aqueous solutions [35,36]. They found the SPI/graphene composites showed better hydrophilicity, improved biological compatibility, and lower cytotoxicity compared with pure graphene. During their preparation, they need add ascorbic acid as a reductant, and the application of such a SPI/graphene composite was for water treatment.

SPI is rich with sulfhydryl groups, and it also contains reducing amino acid residues such as tyrosine. Therefore, SPI may be able to reduce GO to prepare graphene, which we call reduced GO, i.e., rGO. In addition, SPI is an amphiphilic polymer (a nature for all proteins), which makes it well known for its adhesiveness to solid surfaces [37]. There are already several reports indicating that other proteins, such as bovine serum albumin (BSA) and silk fibroin, are able to attach to the surface of graphene by hydrophobic interaction and π-π stacking [24]. Thus, SPI may have the potential to reduce GO in situ and then attach to the surface of the resulting rGO nanosheets to ultimately form a hybrid nanocomposite.

In this article, we show evidence that we were able to obtain a SPI/rGO nanocomposite via a facile, mild, and one-step protocol. SPI was used as both the reducing and stabilizing agent, and it improved the biocompatibility of the resulting SPI/rGO nanocomposite. Then we demonstrated that a SPI/rGO nanocomposite could be a promising photothermal agent for cancer therapy.

2. Materials and methods

2.1. Materials

SPI powder (protein content >90%) was obtained from Shenyuan Food Co. Ltd., Shanghai, China. Dithiothreitol (99%) and 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Aladdin, Shanghai, China. Graphite powder, concentrated H_2SO_4 (98%), $KMnO_4$, NaOH and guanidine hydrochloride (99%) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All chemicals used in this work were analytical grade and used without further purification.

The preparation of an SPI aqueous solution followed a well-established procedure, as reported in our previous work [38]. Briefly, SPI powder was dissolved in aqueous solution containing 6 mol/L guanidine hydrochloride and 1 mmol/L dithiothreitol. After dialysis against NaOH aqueous solution (pH = 11.5) for two days and deionized water for another day at room temperature, the SPI solution was obtained. The resulting SPI solution was transferred into the freezer compartment of a refrigerator and then freeze-dried to obtain pure SPI powder.

GO was synthesized using a modified Hummers method [39]. Briefly, 3.7 g of graphite power were mixed into 120 mL concentrated sulphuric acid (98.5%) that was pre-cooled by an ice bath. Next, 2.5 g $NaNO_3$ and 11.6 g $KMnO_4$ were slowly added under continuous vigorous stirring such that the temperature was constantly maintained below 20 °C. The mixture was then stirred continuously at 35 °C for 7 h. After 9.5 g $KMnO_4$ was added, the reaction was left running uninterrupted at 35 °C for 14 h. The reaction was quenched by slowly pouring the mixture into 600 mL H_2O under stirring. Finally, 20 mL of 30% H_2O_2 was added, and the mixture turned bright yellow and bubbled. The mixture was filtered and washed with 1 L 5% HCl aqueous solution and 200 mL ethanol. The solid powder was dried at 70 °C.

2.2. Preparation and characterization of SPI/rGO nanocomposites

Desired quantities of SPI powder were added to the GO solutions under vigorous stirring to prepare a final SPI/GO mixture (10 mL) with a GO concentration of 0.1 wt% and solid mass ratios of 10:1, 50:1, 100:1 for SPI/GO. These samples were named SG10, SG50, and SG100, respectively, for both the SPI/GO mixtures and final SPI/rGO nanocomposites. Subsequently, the SPI/GO mixtures were incubated at 70 °C for 24 h to obtain SPI/rGO nanocomposites.

TEM images of the synthesized nanocomposites were observed with an FEI Tecnai G2 20 TWIN transmission electron microscope at 200 kV. The samples were prepared by drying the diluted nanocomposite solutions on a formvar/carbon-coated copper grid. AFM images were acquired using a Multi-mode 8 atomic force microscope in tapping mode. X-ray diffraction (XRD) data were recorded on an X'pert Pro X-ray diffractometer with Cu Kα radiation. Raman spectra were obtained with a Renishaw inVia Reflex spectrometer coupled to a Lieca microscope at a wavelength of 785 nm for a He-Ne laser. The energy of the laser was 0.6 mW, which is only 1/10 that used for a normal laser, because the graphene would be carbonized under high energy.

2.3. Measurement of free sulfhydryl with the Ellman method [40]

Ellman's reagent (10 mM DTNB) was prepared by suspending 198.2 mg DTNB in 50 mL of 50 mmol/L Na_2HPO_4 solution, and all DTNB was dissolved. Aliquots of all reagents were immediately frozen at −25 °C, and thawed portions were used within 1 day. Next, 100 μL 1 wt% of SG10 and SPI samples was diluted with Tris-HCl buffer (pH = 8) to a final volume of 1 mL. After adding 100 μL of 10 mmol/L Ellman reagent, the samples were immediately vortexed, and the A_{412} values were read against the samples using 100 μL Tris-HCl buffer instead of Ellman's regent as a blank after a 10-min incubation time (1 cm light path).

The amount of free sulfhydryl in the SPI sample was calculated as follows:

$$[\mathrm{SH}]\ (\mu\mathrm{mol/g\ soy\ protein}) = 1,000,000 \times \left([\mathrm{A}]_{\mathrm{SPI}} - [\mathrm{A}]_{\mathrm{SG10}}\right)/(\varepsilon \times \mathrm{b} \times \mathrm{c})$$

where the standard value ε_{412} = 14,150 L/mol·cm, b = 1 cm, and c refers to the concentration of SPI (g/L)

2.4. Cytotoxicity tests

The cytotoxicity of GO and SPI/rGO nanocomposites was determined by a CCK-8 assay on L929 cells. L929 cells were plated in a 96-well plate at a seeding density of 10,000 cells/well with 100 μL of DMEM media at various concentrations of GO and SPI/rGO. After incubating for 24 h at 37 °C, the solutions were removed from the well plates immediately prior to the addition of a colorimetric indicator. PBS was used to wash the residual nanocomposites to prevent any interference in the absorbance readings.

2.5. In vitro photothermal performance and photothermal therapy (PTT) effects on HeLa cells

The SPI/rGO nanocomposite solution samples were diluted to a desired concentration of 100 μg/mL with distilled water. Then, 1.2 mL SPI/rGO nanocomposite solution samples (i.e., SG10, SG50 and SG100), pure GO solution (100 μg/mL), pure SPI solution (0.1 wt%), and deionized water were continuously irradiated by an NIR laser (808 nm, 2.5 W) at a distance of 5 cm for 5 min in 1.5 mL vials. The photostability of the SPI/rGO nanocomposite samples was assessed by switching the laser on/off five times. Temperature was measured by an infrared thermal camera. All experiments were conducted at room temperature (~20 °C).

The PTT effect of the SPI/rGO nanocomposite was determined by a CCK-8 assay on HeLa cells. Digested HeLa cells were mixed with a desired amount of pure GO, SG10, SG50 and SG100 solution in 1.5 mL vials. The concentrations of graphene in each vial were set as 40, 60, 80, and 100 μg/mL, respectively. After 1 h of incubation, these HeLa