Full Length Article

# A general bio-inspired, novel interface engineering strategy toward strong yet tough protein based composites

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ABSTRACT

Biopolymers show a broad prospect as an effective alternative to petroleum-based materials. However, assembling biopolymers into the composites with integrated high strength and toughness still remains a great challenge. Herein, we developed a novel and versatile mussel-inspired modification design for tough and high-performance graphene oxide (GO)/soy protein isolate (SPI) nanocomposite films, where the GO nanosheets were modified with poly(dopamine) (PDA) to improve the dispersion of GO nanosheets in SPI matrix and enhance their interfacial adhesion. As expected, at 0.6 wt% of PDA-modified GO (PDG) loading, the tensile strength and toughness of the SPI/PDG films reached 8.87 MPa and 22.82 MJ m$^{-3}$, respectively, which simultaneously showed 86.34% and 263.95% higher than those of pristine film. The great enhancement of mechanical behaviors was due to the increased fracture line energy and the lack of significant coalescence of microcracks, as well as the strong interfacial adhesion force between peptide chains and PDG nanosheets. The resultant nanocomposite films also exhibited favorable vapor barrier behavior and water-resistance. The proposed method in this paper opens a new avenue for assembling two-dimensional nanosheets into the biopolymer-based composites with integrated high strength and great toughness for a series of innovative future applications.

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## 1. Introduction

Renewable biopolymers represent a necessary alternative to conventional petroleum-based polymers due to their recyclability and reutilization [1]. Among all the biopolymer-based materials available, soy protein isolate (SPI), a reproducible byproduct of the soy oil industry, has good biodegradability, processability, and film-forming capacity [2,3]. SPI-based films exhibit potential applications in the fields of bioscience and biotechnology [4]. However, unmodified SPI films have some drawbacks such as inferior mechanical strength, high moisture sensitivity, and poor process-ability, which limit their practical application [5,6]. Many attempts have been made to optimize the physico-chemical properties of SPI-based materials, including block copolymerization [7], chemical crosslinking [8], and enzyme treatment [9]. The combination of nanophase modification and bionic technique has been documented as one of the most effective ways for improving the performance of biopolymer nanocomposites [10].

Graphene oxide (GO) has aroused a wide range of research interests in recent years owing to its outstanding mechanical properties, high binding potential, large aspect ratio, light weight, and aqueous processability [11,12]. The rich oxygen-containing functional groups of GO nanosheets including hydroxyl, ketone, carboxylic acid, and epoxy, can integrate with the various polymer matrices by producing polar and ionic interactions [13–15]. These properties prompt GO to be one of the best candidates for achieving the high-performance composites through various interfacial interactions including π-π interaction, hydrogen bonding, covalent bonding, and ionic bonding [16]. Previous reports showed that GO nanosheets have been successfully built into an array of diverse biopolymer substrates such as cellulose acetate [17], poly(vinyl alcohol) [18], chitosan [19], and poly(methyl methacrylate) [20]. However, the unbalanced enhancement (e.g., low toughness) of biopolymer-GO nanohybrids properties remains problematic due to weak GO-matrix adhesion and lack of interlayer interaction between the adjacent GO nanosheets [21]. Thus, assembling GO to form macroscopic composites that exhibit the exciting properties of their nanoscale building blocks remains a key challenge. Tremendous efforts were made to functionalize GO nanosheets (including physical treatment and chemical cross-linking) to

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establish effective "glue" in the GO/matrix and adjacent GO nanosheets interfacial layers [22–24]. However, these approaches generally involve complicated multi-step processing and corrosive chemicals [25]. Therefore, a facile and eco-friendly approach is desired for high-efficiency enhancement of biopolymer-GO nanohybrids.

Dopamine (DOPA) has been selected as a specialized molecule mimic for the marine mussel adhesive protein [26]. DOPA has rich catechol and amino groups which confer its desirable multifunctional properties, namely, (1) it can be used as catalytic-reducing agent and stabilizer for GO or metal ions; (2) it will self-polymerize to form long chain polydopamine (PDA) that can strongly adhere to a wide range of substrates driven by physical and/or chemical cross-linking in a mild alkaline pH condition; and (3) PDA layer with functional catechol and amino groups can served as a reactive platform for Michael addition or Schiff base reaction with aldehyde or thiol groups [27–29]. Kang et al. reported that the use of DOPA-modified cellulose improved the mechanical properties of SPI films [30], but there was an obvious decrease in toughness. The mechanical properties of SPI nanocomposite films improved by integrating DOPA-modified GO were not addressed and reported. However, the unique characteristics in the DOPA layer are believed to build a strong covalent cross-linking between DOPA and GO nanosheets and sequentially yield flexible and strong interfacial adhesion to SPI chains.

In this study, PDA films were anchored on GO nanosheets in a mild alkaline solution to yield PDG nanosheets via the self-polymerization of DOPA. This process was accompanied by the reduction of GO. The approach of modifying GO did not involve toxic chemicals and was easy to operate, so the preparation method was green, mild and facile. Then the PDG nanosheets were incorporated into SPI matrix and the SPI-based nanocomposite film was fabricated using a casting method. A robust interfacial adhesion layer was generated between the PDG and SPI side chains via Schiff base or Michael addition reactions. The water resistance, opacity, thermostability, mechanical properties and microstructure of the resultant nanocomposite films were also examined.

## 2. Materials and methods

### 2.1. Materials

SPI (95% protein) was obtained from Yuwang Ecological Food Industry Co., Ltd. (Shandong, China). GO nanosheets with an average diameter of 50 um and thickness of 1.5 nm were supplied by Suzhou Carbon Feng graphene Technology Co., Ltd. (Jiangsu, China). Dopamine with 97% purity and tris(hydroxymethyl aminomethane) (Tris) were purchased from Lanyi Chemical Co., Ltd. (Beijing, China). Glycerol (99% purity) and analytical-grade sodium hydroxide were purchased from Beijing Chemical Reagents Co., Ltd. (Beijing, China).

### 2.2. Preparation of PDG nanosheets and nanocomposite films

GO nanosheets (1.0 g) were dispersed in 200 ml deionized water with a magnetic stirrer for 1.5 h and then ultrasonication for 0.5 h to obtain aqueous GO dispersion. Tris-HCl buffer solution (pH = 8.5) was prepared by dissolved Tris-HCl (0.5 g) in 200 ml deionized water with a magnetic stirrer for 15 min. Then the buffer solution and dopamine (1 g) were added to the pre-prepared aqueous GO dispersion and constantly stirred at 60 °C for 24 h. Thereafter, the mixture was poured into a glassware and dried at 45 °C for 48 h to obtain PDG nanosheets.

In this study, a two-step casting method was adopted to prepare SPI-based film. Firstly, 5 g of SPI power and 2.5 g of glycerol were added to 95 g of deionized water with stirring at 800 rpm for 30 min. Then, NaOH solution (10%, w/w) was used to adjust the pH of above solution to 9.0, and the ingredients were heated in a water bath at 85 °C for 30 min. Thereafter, GO (0.3% by weight of SPI) and PDA-modified GO (0.3%, 0.6%, 0.9% by weight of SPI) were dispersed in the above SPI solution with a magnetic stirrer for 1.5 h. After that, the resulting mixture was ultrasound-treated for 30 min to remove bubbles. Secondly, the above solution was cast on a Teflon-coated plate and vacuum dried at 45 °C. After drying for 24 h, films were stripped and placed in a saturate-$K_2CO_3$-regulated (50% relative humidity, 25 °C) desiccator before testing. A schematic of the preparation and proposed mechanism of nanocomposite films was illustrated in Scheme 1.

### 2.3. Characterization

The Atomic force microscopy (AFM) images were acquired using a Bruker Multimode 8 analyzer (Billerica, MA, USA) to observe the morphologies and thicknesses of GO and PDG.

The X-ray photoelectron spectroscopy (XPS) spectra were recorded in a PHI Quantera SXM spectrometer (Thermo Fisher Scientific Co., West Sussex, UK) with a monochromatic Al Kα radiation (1486.6 eV). Spectra were recorded with a pass energy of 50 eV and resolution of 0.1 eV.

The Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra were measured with a Nicolet 6700 (Thermo Scientific, Madison, WI, USA), in the wavenumber range from 650 to 4000 $cm^{-1}$ with 32 scans. The X-ray diffraction (XRD) patterns were collected using a D8 Advance diffractometer (Bruker AXS, Karlsruhe, Germany) scanning from 5° to 60° at a voltage of 40 kV.

The fracture surface morphologies of nanocomposite films were studied by using the scanning electron microscope (SEM) (SU8010, Hitachi, Tokyo, Japan) operating at 10 kV accelerating voltage.

The mechanical behaviors of SPI nanocomposite films, including elongation at break (EB), tensile strength (TS) and Young's modulus (E), were evaluated on a universal material testing machine (INSTRON 3365, Norwood, MA, USA) at a loading speed of 50 mm/min. Five rectangular strips (80 mm × 10 mm) for each formulation were performed to obtain the standard deviation.

The opacity of nanocomposite film was characterized by the TU-1901 ultraviolet–visible (UV–vis) spectrophotometer (Beijing Purkinje General, Beijing, China) within the range of 300–800 nm.

The thermal stabilities of different films were evaluated by a TGA Q50 device (TA Instrument, New Castle, DE, USA) under a nitrogen atmosphere. Film specimens were scanned from 30 to 600 °C at a liner heating rate of 10 °C/min.

The water contact angle (WCA) was tested on an OCA-20 instrument (Dataphysics Instruments GmbH, Filderstadt, Germany). The dimensions of films were cut into 20 mm × 80 mm. The GO and PDG were compressed into a circle with a diameter of 1.5 cm using a tablet press before the measurement of WCA. A drop of 3 μL deionized water was dropped onto the specimen surface. Six replicates were measured for each film and powder sample.

The water vapor permeability (WVP) tests were conducted using the TSY-T1 permeability analyzer (Labthink Instrument, Jinan, China) in accordance with ASTM E96 standard. All specimens were equalized in a $K_2CO_3$-regulated desiccator (25 °C, 50% relative humidity) before testing. The WVP values were calculated as follows:

$$WVP \ (g \ mm \ h^{-1} \ m^{-2} \ kPa^{-1}) = WVTR \cdot x/\Delta p \tag{1}$$

where WVTR represented the water vapor transmission rate (g $m^{-2}$ $h^{-1}$); x was the mean thickness (mm) of film; $\Delta p$ was the water vapor pressure difference (Pa) of both sides of the film.