Structural and thermal characteristics of photocrosslinked silk fibroin - PEG hydrogel

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Abstract

Hydrogels are crosslinked hydrophilic matrices for a variety of biomedical applications. Silk fibroin (SF), one of typical natural biomaterials, has been explored as base material for hydrogel. Photocrosslinked SF hydrogel containing poly(ethylene glycol) (PEG) was formulated through visible light initiated thiol-acrylate photopolymerization. The morphological, structural and thermal properties of SF – PEG hydrogel was investigated through scanning electron microscopy, X-ray diffractometry, thermogravimetry, and differential scanning calorimetry. The morphology of SF hydrogel showed dot and uneven surface with network cross-section. X-ray diffraction curves showed that the specific diffraction peaks of PEG were not changed by the intensity of the peaks were affected by sonication. Thermo-degradation behavior of SF – PEG hydrogel sonicated was significantly affected and became complex pattern compared to unsonicated ones. However, the melting endothermic temperature of SF – PEG hydrogel was not changed but the crystalline enthalpy was decreased by gelation and sonication.

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Introduction

Hydrogels are crosslinked hydrophilic matrices suitable for a variety of biomedical applications, including tissue engineering matrix, contact lenses, drug delivery carriers and cell therapy (Kweon et al., 2014; Lin and Anseth, 2009; Kweon et al., 2001; Hanawa et al., 1995). A variety of natural and synthetic polymers have been explored as the base materials for hydrogel crosslinking. Silk fibroin (SF) produced by Bombyx mori silkworm can be formulated into physical hydrogels or be modified to display reactive motifs (e.g., methacrylate) for chemical crosslinking (Yecel et al., 2014; Kim et al., 2014; Kasoju and Bora, 2012; Kweon et al., 2001; Kweon et al., 2000). SF can also form β-sheets structure, which can self-assemble into higher order structures with enhanced stability. On the other hand, hydrogels prepared from derivatives of poly(ethylene glycol) (PEG) chemically crosslinked with limited degradability (Lin, 2015; Lin and Anseth, 2009; Lutolf and Hubbell, 2005).

Among the various physical and chemical methods for hydrogel crosslinking, we are particularly interested in visible light induced gelation (Hao and Lin, 2014; Hao et al., 2014; Kweon et al., 2001). Especially, thiol-acrylate photopolymerization not only permits rapid and efficient crosslinking, but also yields hydrolytically degradable hydrogels.
without the need to synthesize degradable macromers (Hao and Lin, 2014; Hao et al., 2014).

Gelation of SF depends on a number of parameters including concentration, temperature, mechanical strength, chemical treatment, ultrasound sonication, and composition (Kim et al., 2015; Samal et al., 2013; Wang et al., 2008; Kweon et al., 2000; Kang et al., 2000; Ayub et al., 1994). Among them, sonication induced rapid gelation by reduction of molecular weight and β-sheet conformation (Samal et al., 2013).

The authors reported the modulating properties of SF-PEG hydrogels were reported (Bragg et al., 2016). In this study, we examined the structural characteristics of SF-PEG hydrogel. Aqueous SF was mixed with PEG macromer solution containing PEG-diacrylate (PEGDA), dithiothreitol (DTT), and N-vinylpyrrolidone (NVP) to form hydrogel. Visible light mediated thiol-acrylate photopolymerization was performed to make SF hydrogel with chemically crosslinked and hydrolytically labile thiol-acrylate. We also examined the effect of sonication on the SF solution on the SF-PEG hydrogel.

**Materials and methods**

**Materials**

SF was purified from *Bombyx mori* silkworm as described previously (Kim et al., 2014; Kweon et al., 2008). PEGDA (3.4kDa) was synthesized following an established protocol (Hao and Lin, 2014). Eosin-Y disodium salt was purchased from MP Biomedical and used without purification. All other chemicals and reagents were obtained from Thermo Fisher Scientific unless noted otherwise.

**Preparation of SF aqueous solution**

SF was dissolved in ethanolic calcium chloride solution (CaCl₂ : H₂O : ethanol = 1 : 8 : 2 in molar ratio). The solution was heated to 90°C for 4 h, cool down to room temperature, and subsequently dialyzed against ddH₂O using dialysis membrane with MWCO 6-8 kDa (Fisher) for 2 d to remove the salts. After dialysis, the SF solution contained in the dialysis membrane was placed in a bath of dry PEG (10kDa) to concentrate the SF solution. The final concentration (wt/vol.%) of SF aqueous solution was determined from lyophilization of a small portion of the dialyzed SF solution (5 – 6 wt%). Sonication of SF solution was achieved using a Branson 450 Sonifier with a converter, an externally threaded disruptor horn, and 1/8” diameter-tapered microtip. To avoid forming, SF solution was sonicated for 1 s at 10% amplitude and then sonicated at 20% amplitude for 20 s (Wang et al., 2008). The silk was used for hydrogel formation within 30 min after sonication.

**Hydrogel formation initiated by visible light**

Hydrolytically degradable PEG hydrogels were formed by visible light initiated thiol-acrylate photopolymerization using PEGDA and dithiol liker. A typical pre-polymer solution was prepared in pH 7.4 PBS and contained macromer PEGDA (10 wt%), photosensitizer eosin-Y (0.1 mM), bi-functional co-initiator DTT (7.5 mM), and co-monomer NVP (0.1 vol%). All concentrations indicated were final concentrations in the pre-polymer solutions. Aliquots of the pre-polymer solution were subjected to halogen cold light (400-700 nm, AmScope, Inc.) exposure for 5 min (10mW/cm² at 550nm or 70k Lux) between glass slides with spacers. In some experiments, soluble SF was added at different weight contents.

**Instrumental analysis**

Surface morphology of SF hydrogel was observed using scanning electron microscope (S-3500N, Hitachi, Japan). The hydrogels were coated with platinum and observed. To investigate the molecular structure of the hydrogels, X-ray diffraction (XRD) was measured on X’Pert-Pro (PANalytical, Netherlands) using Cu-Kα radiation. The X-ray source was operated at 40 kV and 40 mA. Differential scanning calorimetry was analyzed using Differential Scanning Calorimeter (Mettler Toledo, Australia) at a scanning rate of 10°C/min over 50-350°C. Thermogravimetric analysis was done using Mettler Toledo ThermoGravimetric Analyzer (DSC 823e, Australia) from 35°C to 800°C at the heating rate 10°C/min with a nitrogen flow rate of 50 mL/min.

**Results and discussion**

In this study, SF-PEG hydrogel crosslinked from PEGDA,
showed that relatively even surface morphology with network structure. This results means that SF and PEGDA were phase-separated and results in that SF was distributed in the PEG matrix.

Crystalline structure

XRD curves of SF-PEG hydrogels are shown in Fig. 2 SF showed a typical amorphous diffraction pattern (data not shown), whereas PEGDA hydrogel showed sharp diffraction peak at 20=19.1, 23.5, and 31.7° (Kweon et al., 2001; Priola et al.,

Morphology of SF-PEG hydrogel

To observe the surface and fractured section of SF hydrogel, SEM is a powerful method. As Fig. 1, Surface of SFPEGDA showed dot and uneven surface morphology with network cross-section structure. Sonicated SFPEGDA shows some big and small islets with network cross section. However, PEGDA showed that relatively even surface morphology with network structure. This results means that SF and PEGDA were phase-separated and results in that SF was distributed in the PEG matrix.

Fig. 1. SEM photographs of SF-PEG hydrogels.

co-monomer NVP, di-thiol containing linker DTT, and photosensitizer eosion-Y were evaluated by instrumental analysis on morphological, structural, and thermal properties.
of PEGDA did not change by semi-interpenetrating polymer networks composed of SF and PEG. Liang et al. (1995) also reported that the crystalline structure of PEG did not change by blending with cellulose. However, some diffraction peaks were changed by sonication; it means that the crystalline structure was affected by molecular agitation induced by sonication.

**Thermogravimetric analysis**

Fig. 3 showed the thermogravimetric curves of PEGDA, SFPEGDA and sonicated SFPEGDA. PEGDA showed slightly two step mass loss around 230-350°C and 350-390°C. SFPEGDA showed two step weight losses around 220-360°C and 360°C and 390°C. On the other hand, sonicated one showed more complex degradation behavior from 180-420°C.

Decomposition of pure PEG were occurred in one step around 1993). The diffraction pattern of SFPEG (Fig. 2(B)) is consistent with that of the PEGDA itself, regardless of SF entrapment. However, by sonication, the relative diffraction intensities at 20°= 31.7° and 27.4° were increased.

SF was entrapped in crosslinked PEG networks. XRD curves show that entrapped SF does not affect the crystal structure of PEG. Kweon et al. (2001) reported that the diffraction pattern

Fig. 2. X-ray diffraction patterns of PEGDA(A), SFPEGDA(B), and sonicated SFPEGDA(C).

Fig. 3. Thermogravimetric curves of PEGDA(A), SFPEGDA(B), and sonicated SFPEGDA(C).
with 9.5 J/g. Sonicated SF hydrogel (Fig. 4(c)) also shows two endothermic peaks at 55.8°C with 99.6 J/g and weak peaks around 290°C.

The melting temperature of PEG crystalline observed at 50-60°C (Icart et al., 2016; Kweon et al., 2001). So, the sharp endothermic peak around 60°C in Fig. 4, $T_m$ of PEG crystalline region, was slightly shifted to higher temperature by entrapment with SF. The degree of crystalline is related to the melting enthalpy $\Delta H_m$. The melting enthalpy of SFPEGDA 108.2 J/g was lower than that of PEGDA, means that the degree of crystalline was decreased with a decrease of melting enthalpy. By sonication, the degree of crystalline region of PEG was significantly decreased. On the other hand, the endothermic peak around 287°C is considered as the degradation of SF itself. Many researchers reported that SF degraded around 270-380°C with structural characteristics (Kweon et al., 2001; ). These results are corresponding with the TGA results (Fig. 3).

**Acknowledgments**

This study was supported by an international research collaboration agreement between the Indiana University and the Rural Development Administration (RDA) of the Republic of Korea (PJ0120862016).

**References**


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