

# Preparation of photo-crosslinkable silk sericin hydrogel

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## Abstract

A photo-crosslinkable sericin hydrogel has been prepared by introducing methacrylate groups on sericin. Lysine was the amino acid for methacrylation and the degree of methacrylation was increased with the amount of methacrylic anhydride added. When the concentration of methacrylic anhydride was 20% of sericin, 0.635 mmol/g of methacrylate group could be introduced. The storage modulus of sericin hydrogel was increased proportionally with the degree of methacrylation. However, the swelling ratio of sericin hydrogel decreased with the increase of methacrylation.

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## Introduction

Hydrogels are one of suitable material form for various biomedical applications such as drug delivery and tissue engineering. Hydrogels can be prepared by physical or chemical cross-linking of hydrophilic polymers, which allow high water retention in their polymeric network (Kapoor et al., 2016). While most of the synthetic polymers require chemical cross-linker to make a hydrogel, many natural polymers can be fabricated into hydrogel without any chemical cross-linker. For example, gelatin hydrogel can be prepared only by cooling after dissolving gelatin powder in hot water. The inherent ability of gelatin to form triple helices will induce the gelation of gelatin solution (Gilsenan et al., 2000). The lack of chemical cross-linker will eliminate any toxic problem in the future biomedical application.

Silk sericin (SS) hydrogel can be also prepared by inducing  $\beta$ -sheet structure (Teramoto et al., 2005). When the SS solution is

incubated at room temperature for 2-3 days, a structural transition from random coil structure to  $\beta$ -sheet structure will occur and result in hydrogel formation. SS hydrogel has been used for various applications such as drug delivery (Nishida et al., 2011). However, the gelation time of SS is too long and unpredictable for practical applications. Based on our experience, the gelation of SS took place usually within 2 days but, in some cases, it took more than 4 days. Therefore, for practical application, a controllable gelation mechanism should be introduced. Recently, photo-crosslinkable gelatin has been introduced and widely adopted for cell encapsulation. Gelatin is modified with a methacrylate group, which allows photo-crosslinking of gelatin in the presence of photo-initiator upon UV radiation (Van Den Bulcke et al., 2000). In addition, photo-crosslinkable fibroin hydrogel has been also reported (Jo et al., 2016; Ki et al., 2016). This photo-crosslinkable hydrogel system has advantages of controllable and instantaneous gelation.

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In this study, we tried to prepare a photo-crosslinkable SS hydrogel by introducing photo-crosslinkable methacrylate groups. We report a preparation method of methacrylate modified SS, and the effect of initial methacrylic anhydride concentration on the degree of modification and physical properties of SS hydrogel will be reported.

## Materials and Methods

### Materials

SS solution was prepared by hot-water extraction as described in the previous article (Yun et al., 2016). Briefly, silkworm cocoons were boiled for an hour at 120°C. SS solution was recovered after removing undissolved silk fiber by filtration. The final concentration of SS solution was 1 wt%. Methacrylic anhydride and other chemicals were purchased from Sigma-Aldrich.

### Methods

**Preparation of methacrylated SS:** Pre-defined amount of methacrylic anhydride was dropped under stirring at 50°C into SS solution which was adjusted to pH 8 with phosphate buffer, and the reaction was carried out for 3 hours in a dark chamber. The reaction was terminated by cooling the solution to room temperature. Unreacted methacrylic anhydride was removed by dialysis with semi-permeable membrane tube (Spectra/Por<sup>®</sup>, USA) having MWCO of 6 kDa. The dialysis was performed in a dark chamber at 50°C in order to prevent pre-gelation. After 3 days of dialysis, the solution was freeze-dried to get a solid powder. The methacrylate modified SS (SS-MA) was wrapped with aluminum foil in order to prevent any exposure to light and kept in a desiccator until use.

**Preparation of SS-MA hydrogel:** SS-MA hydrogel was prepared by dissolving SS-MA in 1M LiCl/DMSO solution at a concentration of 10 wt%. Irgacure 2959 (8 wt% to SS-MA) was added to the solution as a photo-initiator. The final solution was injected in between two slide glasses having a 1 mm gap. The gelation of SS-MA was induced by radiating the solution with UV light source (365nm) for 30 min. After gelation, LiCl and DMSO were removed by 5 times washing with distilled water. The hydrogel was kept in distilled water for 24 hours to reach

maximum swelling.

**Characterization of SS-MA and its hydrogel:** Attenuated total reflection (ATR) FT-IR was performed to confirm the reaction between methacrylate and SS. The ATR-FT-IR spectra of SS and SS-MA powder were acquired in between 4,000-650 cm<sup>-1</sup> with 64 scans having 4 cm<sup>-1</sup> resolution. The degree of modification was determined by <sup>1</sup>H-NMR (DSX-400, Bruker, Germany) analysis. The SS-MA (2 mg) was dissolved in 1 ml of D<sub>2</sub>O, and the peak of 1.26 ppm which was originated from valine, isoleucine, and leucine was used as a reference peak. The following equation was used to calculate the degree of methacrylation.

$$\text{Degree of methacrylation} \left( \frac{\text{mmol}}{\text{g}} \right) = \frac{I_{5.5\text{ppm}}}{I_{1.26\text{ppm}}} \times (\text{known composition of (Val + Leu + Ile)mmol in 1g sericin}) \div 2$$

The shear modulus of SS-MA hydrogel was measured with a rheometer (MARSIII, Thermoscientific, USA). The size of SS-MA hydrogel was 8 mm in diameter and 1 mm in height. Oscillatory rheometry was performed in a strain-sweep mode (0.1-5%) using a parallel plate geometry (diameter = 8 mm). The average G' was obtained from the linear viscoelastic region. The swelling ratio of SS-MA hydrogel was calculated from the weight of fully swollen hydrogel and fully dried hydrogel using following equation;

$$\text{Swelling ratio}(q) = \frac{m_{\text{swollen}}}{m_{\text{dry}}}$$

## Results and Discussion

The reaction scheme of methacrylate modification of SS is shown in Figure 1. The methacrylic anhydride predominantly react with a ε-amino group of lysine (Wang et al., 2017). After introducing the methacrylate group, a water-soluble photo-initiator crosslink the methacrylate groups under UV light, and finally, a hydrogel is formed.

In this study, we added 10, 15 and 20% of methacrylic anhydride relative to the amount of SS, and it was designated as SS-MA10, SS-MA15 and SS-MA20, respectively. Figure 2 shows the <sup>1</sup>H-NMR spectra of SS before and after methacrylation. A clear doublet peak of -C=CH<sub>2</sub> (5.5 ppm, red circle) can be observed after modification. The peak intensity at 3.2 ppm and 2.9 ppm decreased at the same time indicating

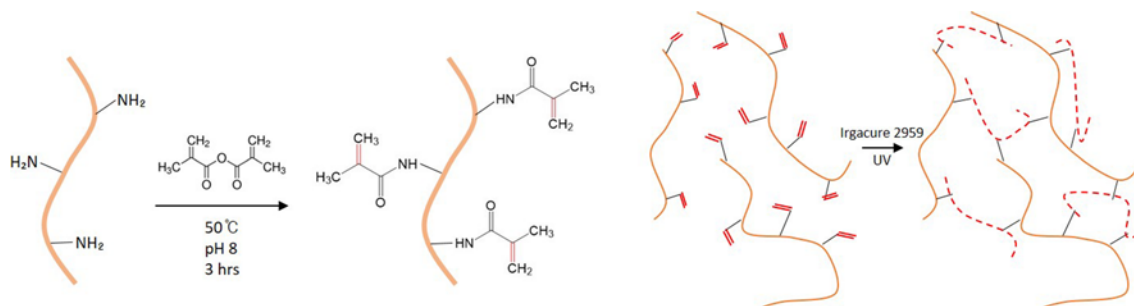


Fig. 1. Reaction scheme of SS and methacrylic anhydride.

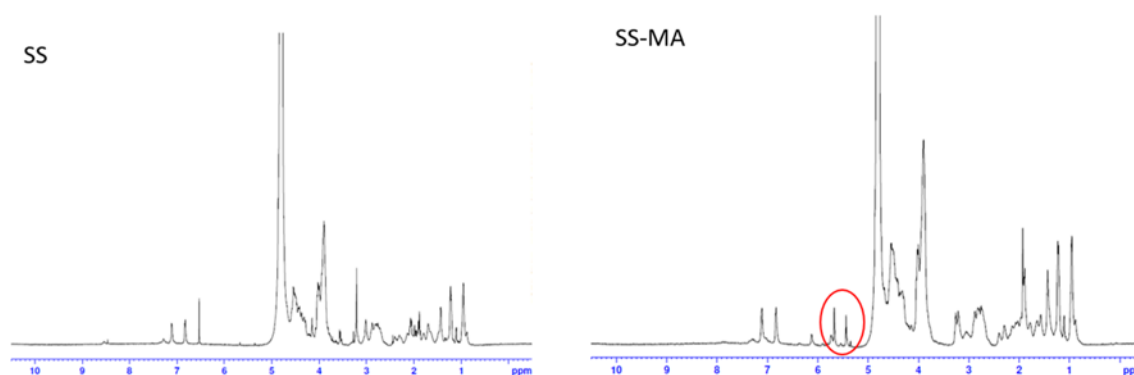


Fig. 2.  $^1\text{H-NMR}$  spectra of SS and SS-MA15.

lysine is the modification site (Wang *et al.*, 2017). It has been known that histidine and arginine can be another reaction site of methacrylic anhydride but the complex  $^1\text{H-NMR}$  spectra of protein hinder an exact analysis.

Table 1 shows the degree of methacrylation according to the amount of methacrylic anhydride added relatively to SS. With the increase of the concentration of methacrylic anhydride from 10 to 20 % of SS, the amount of methacrylate introduced to SS increased from 0.448 to 0.635 mmol/g. It is well known that the degree of methacrylation increases proportionally with the amount of methacrylic anhydride. The degree of methacrylation of 0.635 mmol/g corresponds about 75.55% of lysine of SS has been modified.

After introducing the methacrylate functional group, however, the solubility of SS-MA in water reduced significantly, which also implies that the modification took place at the hydrophilic lysine group. This was unexpected result since methacrylated gelatin has good water solubility. In order to solve the problem, we dissolved SS-MA in 1 M LiCl/DMSO solution. This solvent system has been used to dissolve SS at a high concentration (Oh *et al.*, 2007). As shown in Figure 3, SS-MA dissolved at 10 wt% forms a gel after 30 min under UV radiation. However, at 1 wt%,

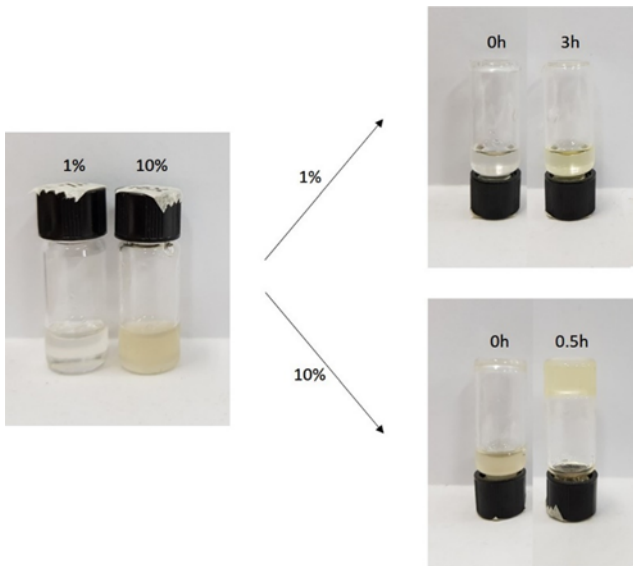
Table 1. Degree of methacrylation of SS-MA samples

Sample	Degree of methacrylation (mmol/g)
SS-MA10	0.448
SS-MA15	0.503
SS-MA20	0.635

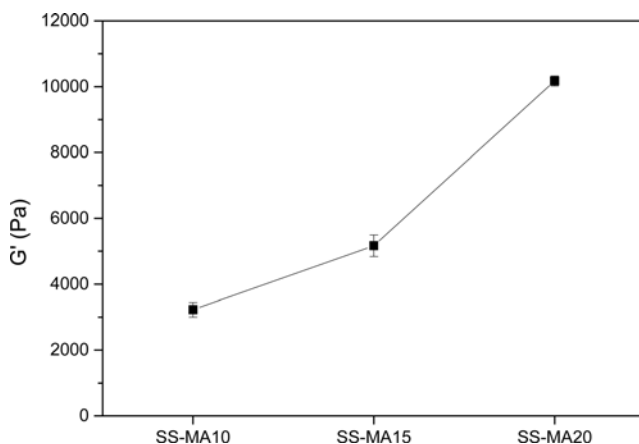
gel cannot be formed even after 3 hours of UV radiation. Due to the concentration dependence of SS-MA gel formation, the use of LiCl/DMSO solvent is unavoidable. Nonetheless, by introducing the methacrylate groups to SS, we could prepare an SS hydrogel within 30 min, which is much faster than conventional methods.

The LiCl and DMSO were removed after gelation and the storage modulus of SS-MA hydrogel was measured. The SS-MA hydrogel with a high degree of methacrylation had the higher storage modulus (Fig. 4). The storage modulus of 10wt% of SS-MA10, SS-MA15 and SS-MA20 was 3,225, 5,167 and 10,174 Pa, respectively. Since the introduced methacrylate groups on SS undergo radical polymerization in the presence of photo-initiator, the cross-linking density is increased with the degree of methacrylation.

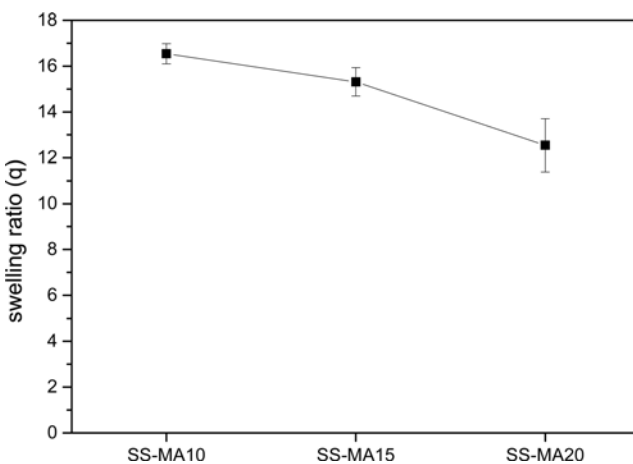
The increased cross-linking density can be also confirmed



**Fig. 3.** Effect of SS-MA15 concentration on the gelation behavior.



**Fig. 4.** Storage modulus of SS-MA hydrogel.



**Fig. 5.** Swelling ratio of SS-MA hydrogel

by measuring the swelling ratio of SS-MA. Figure 5 shows the swelling ratio of SS-MA hydrogel. As the degree of methacrylation increases, the swelling ratio of SS-MA decreased. From Figure 4 and 5, the storage modulus and swelling ratio of SS hydrogel can be controlled by the degree of methacrylation.

In this study, we introduced methacrylate groups on SS and prepared photo-crosslinkable SS hydrogel. However, the use of organic solvent will limit the application of SS-MA hydrogel to drug delivery only. Drugs can be loaded by absorption of drug solution into the dried SS-MA hydrogel. The drug release from SS-MA hydrogel is an ongoing project and will be reported in the future.

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