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Modulating properties of chemically crosslinked PEG hydrogels via physical entrapment of silk fibroin

John C. Bragg,[1] Haeyong Kweon,[2] YouYoung Jo,[2] Kwang Gill Lee,[2] Chien-Chi Lin[1]

[1]Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis Indiana

[2]Sericultural and Apicultural Materials Division, Department of Agricultural Biology, National Academy of Agricultural Science, Rural Development Administration, Republic of Korea

Correspondence to: C.-C. Lin (E-mail: lincc@iupui.edu)

ABSTRACT: A variety of polymers of synthetic origins (e.g., poly(ethylene glycol) or PEG) and macromolecules derived from natural resources (e.g., silk fibroin or SF) have been explored as the backbone materials for hydrogel crosslinking. Purely synthetic PEG-based hydrogels are often chemically crosslinked to possess limited degradability, unless labile motifs are designed and integrated into the otherwise non-degradable macromers. On the other hand, SF produced by *Bombyx mori* silkworm can be easily formulated into physical hydrogels. These physical gels, however, are less stable than the chemically crosslinked gels. Here, we present a simple strategy to prepare hybrid PEG-SF hydrogels with chemically crosslinked PEG network and physically entrapped SF. Visible light irradiation initiated rapid thiol-acrylate gelation to produce a network composed of non-degradable poly(acrylate-co-NVP) chains, hydrolytically labile thioether ester bonds, and interpenetrating SF fibrils. We evaluated the effect of SF entrapment on the crosslinking efficiency and hydrolytic degradation of thiol-acrylate PEG hydrogels. We further examined the effect of adding soluble SF or sonicated SF (S-SF) on physical gelation of the hybrid materials. The impacts of SF or S-SF inclusion on the properties of chemically crosslinked hybrid hydrogels were also studied, including gel points, gel fraction, equilibrium swelling ratio, and mesh size. We also quantified the fraction of SF retention in PEG hydrogels, as well as the influence of remaining SF on moduli and degradation of chemically crosslinked thiol-acrylate PEG hydrogels. This simple hybrid hydrogel fabrication strategy should be highly useful in future drug delivery and tissue engineering applications. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43075.

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INTRODUCTION

Hydrogels are a class of crosslinked and hydrophilic matrices suitable for a variety of biomedical applications, ranging from contact lenses to carriers for protein delivery and cell therapy.[1] A variety of synthetic polymers and macromolecules derived from natural resources have been explored as the base materials for hydrogel crosslinking. The characteristics of these base materials determine, to a large degree, the properties of the resulting hydrogels. For example, hydrogels prepared from derivatives of poly(ethylene glycol) (PEG)[1–3] and silk fibroin (SF)[4,5] have enjoyed great success in various biomedical applications. PEG-based hydrogels are purely synthetic and the vast majority of PEG hydrogels are chemically crosslinked with limited degradability unless labile motifs are designed and integrated into the otherwise non-degradable PEG macromers.[6–9] On the molecular level, hydrogels prepared from chemical crosslinking of PEG-based macromers are mesh-like and have tunable mechanics and permeability. On the other hand, 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.[4,5] SF can also form peptide β-sheets, which can self-assemble into higher order structures with enhanced stability.

Among the various physical and chemical methods for hydrogel crosslinking, we are particularly interested in visible light initiated thiol-acrylate photopolymerization as it not only permits rapid and efficient crosslinking, but also yields hydrolytically degradable hydrogels without the need to synthesize degradable macromers.[10,11] The mechanical properties of thiol-acrylate PEG hydrogels can be tuned by adjusting concentration of macromer (e.g., PEG-diacrylate or PEGDA), di-thiol linker (e.g., dithiothreitol or DTT), or co-monomer (e.g., N-vinylpyrrolidone or NVP). Furthermore, by tuning the ratio of PEG-based macromer and multifunctional thiol linker or the concentration of comonomer, thiol-acrylate PEG hydrogels can be degraded hydrolytically in a predictable fashion.[10,11] To fabricate thiol-

acrylate hydrogels with high crosslinking density and high modulus, the easiest method is to increase the concentration of co-monomer NVP in the prepolymer solution.[10,12] However, this approach simultaneously decreases the degradation rate of the resulting hydrogels. This was attributed to the formation of dense and non-degradable poly(acrylate-*co*-VP) chains in the crosslinked hydrogel network.[12]

To decouple the initial gel modulus from the degradation rate of the hydrogels, an approach different from adjusting macromer content is required. We hypothesize that physical entrapment of SF into chemically crosslinked hydrogels will result in different initial gel moduli due to the formation of higher order structure of SF fibrils in the mesh-like PEG hydrogel. Furthermore, it is possible that, at appropriate conditions, the entrapment of SF will not lead to significant changes in gel degradation rate because SF does not take part in the hydrolysis of thioether ester bonds. In this contribution, we prepared SF-incorporated visible light cured thiol-acrylate hydrogels to test these hypotheses. SF was prepared in aqueous solution and mixed with PEG macromer solution containing PEGDA, DTT, and NVP to examine the effect of PEG-based macromers on physical gelation of SF hydrogel. We also utilized visible light mediated thiol-acrylate photopolymerization to entrap SF fibrils within the chemically crosslinked and hydrolytically labile thiol-acrylate hydrogels. Because it has been shown shear force (vortex or sonication) accelerates the formation of SF hydrogel,[13–16] we prepared and incorporated sonicated SF (S-SF) solution into the thiol-acrylate hydrogels and examined the effects of S-SF entrapment on thiol-acrylate hydrogel properties, SF retention, and hydrolytic degradation of the hybrid hydrogels.

MATERIALS AND METHODS

Materials

SF was purified from *Bombyx mori* silkworm as described previously.[17,18] PEGDA (3.4kDa) was synthesized following an established protocol.[10] 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

Extracted SF was dissolved at 20 wt % in ddH_2O composed of 9.3*M* $CaCl_2$ and 20% (v/v) of absolute ethanol ($CaCl_2$:H_2O: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 ddH_2O using dialysis membrane with MWCO 6-8 kDa (Fisher) for 2 days to remove the salts. After dialysis, the SF solution contained in the dialysis membrane was placed in a bath of dry PEG (10 kDa) 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. The SF solution was sonicated at 20% amplitude for 20 s.[14]

In Situ Photo-Rheometry

In situ gelation and real time photo-rheometry studies were conducted on a digital rheometer (Bohlin CVO 100) to determine gel points, which were the time at which storage moduli (G') surpasses loss moduli (G''). Briefly, prepolymer solution was placed in a light cure cell and was irradiated through a quartz plate using a flexible visible light guide. Time-sweep photo-rheometry was operated at 10% strain, 1Hz frequency, and 90-μm gap size. Visible light was turned on 30 s after starting the time-sweep measurement. Hydrogel shear moduli were also measured to reveal gel stiffness and hydrolytic degradation as a function of time.

Visible Light Initiated Thiol-Acrylate Gelation and Characterization of Gel Properties

Hydrolytically degradable PEG hydrogels were formed by visible light initiated thiol-acrylate photopolymerization using PEGDA and dithiol linker (e.g., dithiothreitol or DTT). A typical prepolymer solution was prepared in pH 7.4 PBS and contained macromer PEGDA (10 wt %), photosensitizer eosin-Y (0.1 m*M*), bi-functional co-initiator DTT (7.5 m*M*), and co-monomer NVP (0.1 vol %).[10,11] All concentrations indicated were final concentrations in the prepolymer solutions. Aliquots of the prepolymer solution were subjected to halogen cold light (400 − 700 nm, AmScope) exposure for 5 min (10 mW/cm^2 at 550 nm or 70 k Lux). In some experiments, soluble SF was added at different weight contents.

For gel fraction characterization, hydrogels were dried immediately following thiol-acrylate photopolymerization. After 2 days, the dried polymer weights were measured gravimetrically and denoted as $W_{dry\text{-}1}$. The dried gels were then incubated in ddH_2O at 37°C on an orbital shaker for 24 h to remove all unreacted macromers, followed by a second drying process for another 24 h to obtain the second dried gel weight ($W_{dry\text{-}2}$). Gel fraction, an index of gelation efficiency, was defined as $W_{dry\text{-}2}/W_{dry\text{-}1} \times 100$. For swelling ratio characterization, hydrogels were swollen for 2 days in ddH_2O at 37°C on an orbital shaker. Hydrogel swollen weights were measured gravimetrically and denoted as $W_{swollen}$. The swollen gels were then dried *in vacuo* for 24 h to obtain dried gel weight, which was denoted as W_{dry}. The mass swelling ratio (q) was defined as $W_{swollen}/W_{dry}$. The mass swelling ratios were used to calculate hydrogel mesh size as described elsewhere.[19]

Characterization of Gel Properties and Hydrolytic Degradation

Storage moduli (G') and loss moduli (G'') were determined using a digital rheometer (CVO 100, Bohlin Instruments). Hydrogels for rheometrical characterization were formed in between two glass slides separated by 1 mm thick Teflon spacers. Hydrogel discs with 8 mm in diameter were punched out from the gel slabs and incubated in pH 7.4 PBS at 37°C. Storage (G') and loss (G'') moduli of the hydrogels were measured at predetermined time periods for characterizing hydrolytic degradation as a function of time. Rheometry was performed in oscillatory strain-sweep (0.1 − 5%) mode using 8 mm parallel plate geometry.

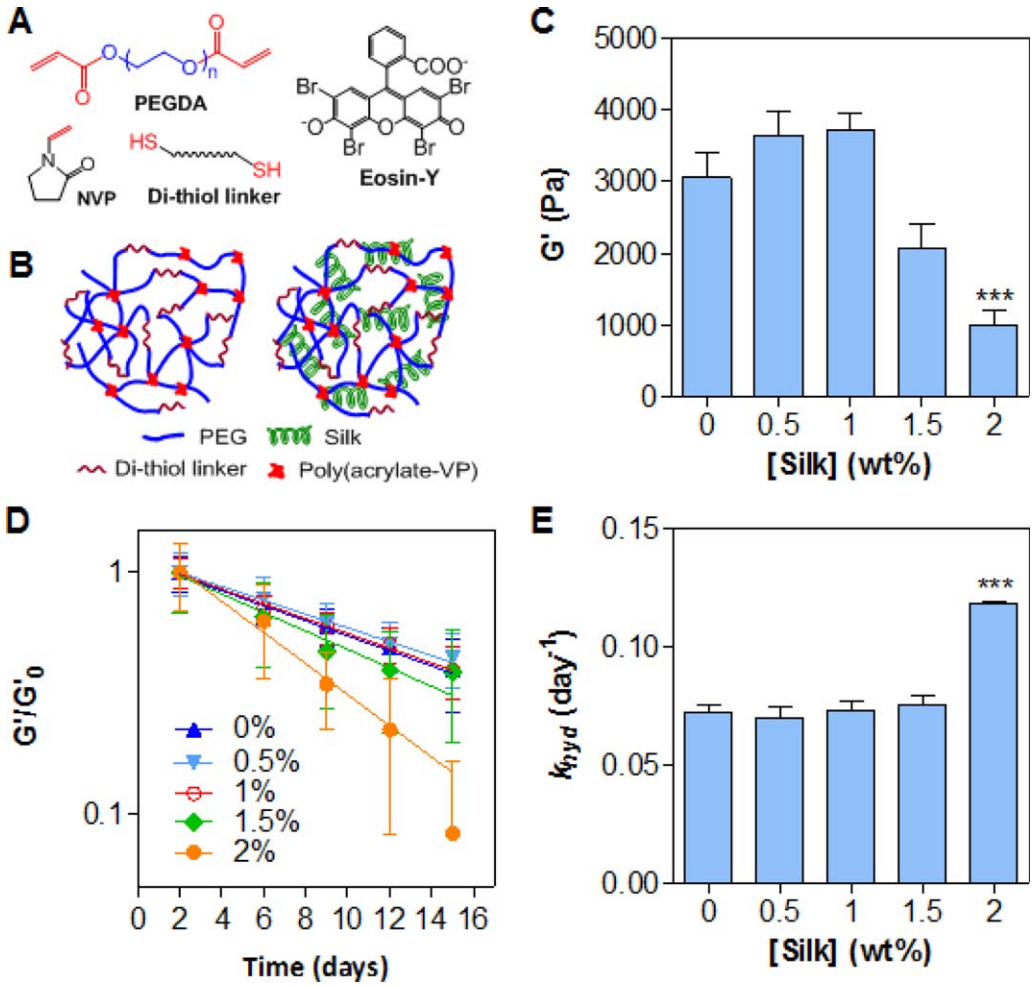


Figure 1. (A) Components used in visible light cured thiol-acrylate hydrogels. (B) Schematics of thiol-acrylate hydrogel without or with silk fibroin (SF) entrapment. (C) Effect of SF entrapment on shear modulus (G') of thiol-acrylate PEG hydrogels composed of 10 wt % PEGDA, 7.5 m*M* DTT, 0.1% NVP, and 0.1 m*M* eosin-Y. Gels were formed by visible light exposure for 5 min. (D) Effect of SF entrapment on hydrolytic degradation of visible light cured thiol-acrylate hydrogels. Pseudo-first order degradation kinetics was used for the curve fittings, which represent exponential decay of gel moduli as a function of time (note the log scale on the Y-axis). (E) Hydrolytic degradation rate constants abstracted from the pseudo-first order degradation curve fitting in D. Data represent Mean ± SEM; $^{***}p < 0.0001$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

SF Retention

PEG/SF or PEG/S-SF hybrid hydrogels (1 wt % SF or S-SF) were prepared as described above and incubated in 2 mL pH 7.4 PBS at 37°C for 48 h. Aliquots (500 μL) of the buffer solution were sampled at 1 and 24 h. After solution sampling, equal amount of fresh buffer was added to maintain the total volume. SF release from the gels was quantified using a Micro-BCA protein assay kit (Thermo-Scientific). A series of SF solutions with known concentrations were used as standards to determine the amount of SF leached out to the sampling buffer. SF retention was obtained by subtracting the amount of released SF from the total SF in PEG hydrogels.

Statistics

All statistical analyses and curve fittings were conducted using GraphPad Prism 5 software. Gel modulus, gel point, gel fraction, swelling ratio, and mesh size data were analyzed by One-Way ANOVA followed by Turkey post-hoc test. All experiments were conducted independently for at least three times and data presented were Mean ± SEM. Single, double, and triple asterisks represent $p < 0.05$, 0.001, and 0.0001, respectively. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Influence of SF Entrapment on the Modulus of Thiol-Acrylate PEGDA Hydrogels

In this study, hydrolytically degradable PEG-based hydrogels crosslinked from PEGDA, co-monomer NVP, di-thiol containing linker (e.g., DTT), and photosensitizer eosin-Y [Figure 1(A)] were used to evaluate the influence of SF entrapment on hydrogel properties. Following visible light exposure, a crosslinked hydrogel network formed with two types of crosslinkers: non-degradable poly(acrylate-*co*-NVP) chains and hydrolytically labile thioether ester bonds [Figure 1(B), left]. The content of non-degradable poly(acrylate-*co*-NVP) chains affects the mechanical properties of the hydrogels, whereas the presence of thioether ester bonds gives rise to the hydrolytic degradability

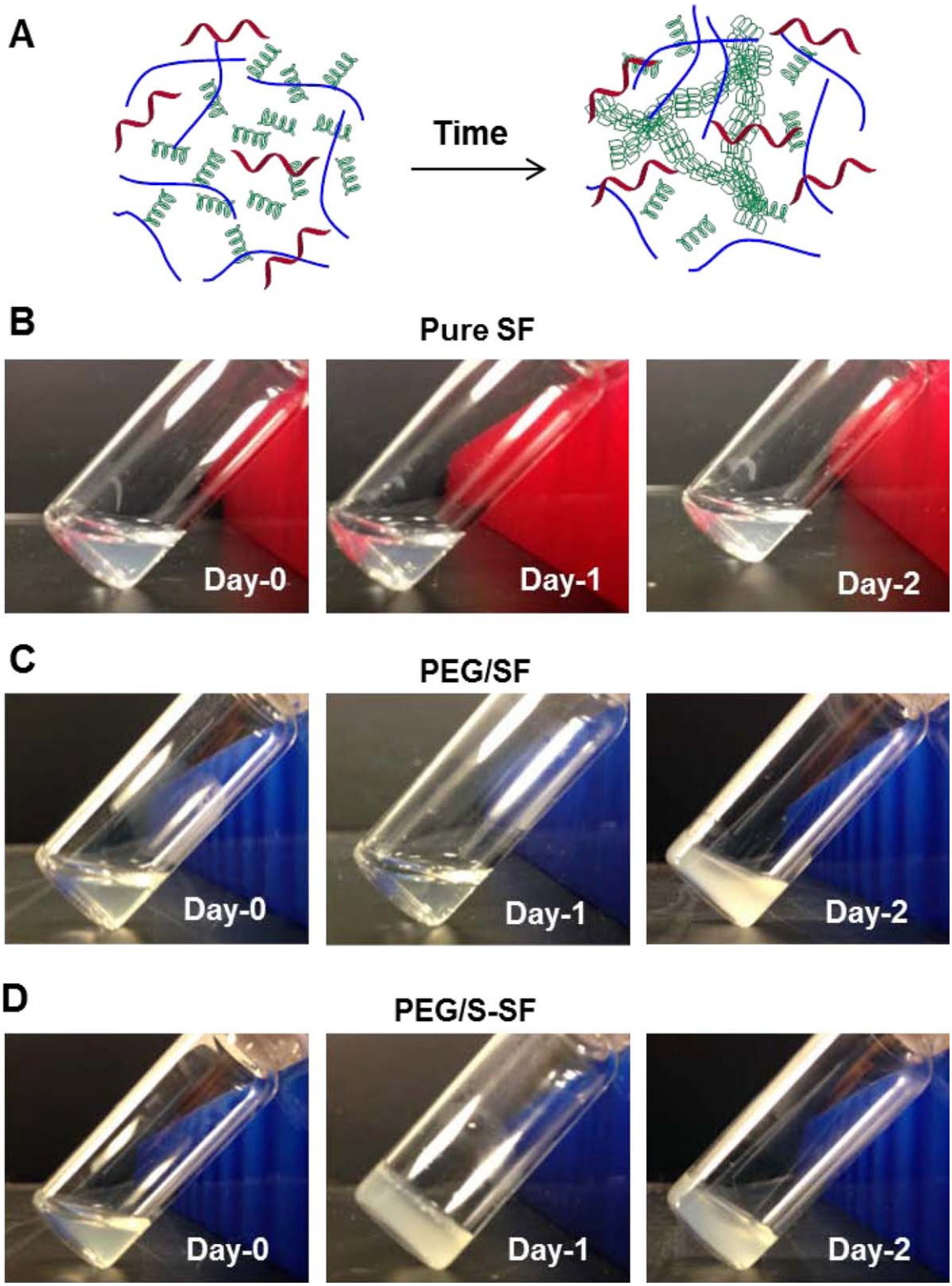
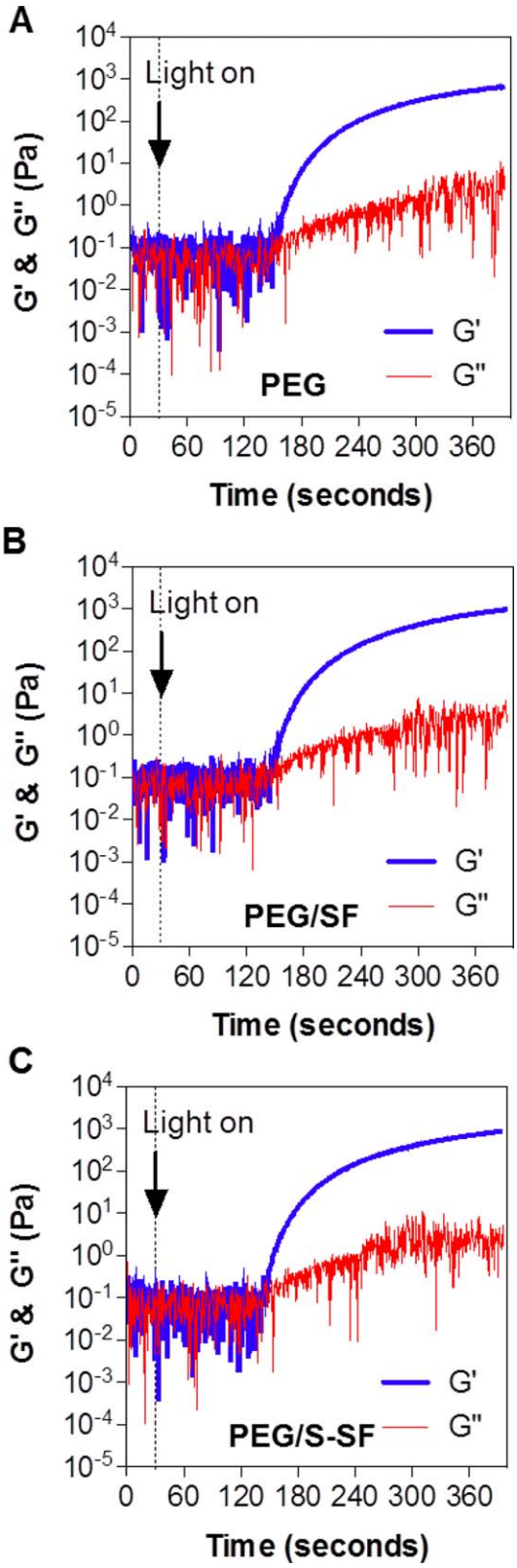


Figure 2. (A) Schematic of accelerated β-sheet formation and physical gelation of SF mixed in PEGDA/DTT solution. Photographs of (B) 1 wt % pure SF solution, (C) 1 wt % SF mixed in macromer solution, and (D) 1 wt % sonicated SF mixed in macromer solution. Compositions of the macromer solution are: 10 wt % PEGDA, 7.5 m*M* DTT, and 0.1% NVP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the gels. In a previous work, we have shown that the hydrolytic degradation rate of a thiol-acrylate hydrogel depends on the absolute concentration of thioether ester bond and the concentration of co-monomer NVP.[10,11] Hydrogels formed at a higher crosslinking density usually degrade slower due to the presence of higher density of poly(acrylate-*co*-NVP) chains in

the network. To decouple the mechanical properties of thiol-acrylate hydrogels from their hydrolytic degradation rate, we physically entrapped soluble SF during the photocrosslinking of thiol-acrylate hydrogels [Figure 1(B), right]. Aliquots of SF solution (~5 wt %) was mixed with the PEGDA/DTT/NVP/eosin-Y pre-polymer solution to yield final concentrations of 0, 0.5, 1, 1.5, and 2 wt %. The mixture solutions were subjected to visible light exposure for 5 min. The moduli of the PEG/SF hybrid hydrogels were evaluated using strain-sweep rheometry [Figure 1(C)]. Hydrogels with 0, 0.5, and 1 wt % of SF entrapment exhibited moderate but not statistically significant increase in shear moduli. As the SF concentration was further increased to 1.5 and 2 wt %, significant drop in shear moduli were observed, indicating that the presence of SF at high concentrations hindered the thiol-acrylate gelation efficiency. When compared with thiol-acrylate PEG hydrogels without SF entrapment, this reduction in gel moduli reached statistical significance for gels with 2 wt % of SF entrapment (30% reduction in G'). This phenomenon could be attributed to an increased turbidity in the concentrated SF solution, which decrease light penetration in the solution. It was also possible that the SF at high concentration physically interrupted the formation of thiol-acrylate crosslinks.



We further examined the hydrolytic degradation of these hybrid hydrogels [Figure 1(D,E)]. Hydrogel shear moduli were monitored as a function of time and the data were fitted with exponential decay equation as reported previously.[20,21] We have previously shown that the degradation rate of thiol-acrylate hydrogels followed pseudo-first order degradation kinetics[10,11] and this correlation was used here to obtain information regarding the influence of SF entrapment on thiol-acrylate hydrogel degradation. As shown in Figure 1(C–E), the entrapment of SF at high concentration (i.e., 2 wt %) not only decreased gel initial modulus, but also accelerated the hydrolytic degradation rate of the hydrogel. The increased degradation rate was reasonable as we have previously reported that thiol-acrylate hydrogels formed with lower crosslinking density would degrade faster than the gels formed with higher crosslinking density. Since the purpose of SF entrapment was to modulate thiol-acrylate hydrogel stiffness but not the hydrolytic degradation rate, we chose 1 wt % of SF entrapment in the subsequent experiments.

Physical Gelation of PEG-SF Hydrogels

It is known that silk fibroin self-assembles into β-sheet and forms physical hydrogel in a concentration and temperature-dependent manner [Figure 2(A)].[4,22] We investigated the potential physical gelation of PEG/SF mixture as a way to prove that the PEG/SF hybrid hydrogels formed were indeed due to the light-mediated crosslinking process. As shown in Figure 2(B), SF at 1 wt % did not gel after 2 days of incubation at 37°C.

Figure 3. *In situ* photo-rheometry of thiol-acrylate PEGDA hydrogels without SF entrapment (A), with 1 wt % SF entrapment (B), and with 1 wt % sonicated SF entrapment (C). In all experiments, visible light was turned on at 30 s ($n = 3$). Data shown were representative of three independent experiments in each condition. Compositions of the macromer solution are: 10 wt % PEGDA, 7.5 m*M* DTT, 0.1% NVP, and 0.1 m*M* eosin-Y. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

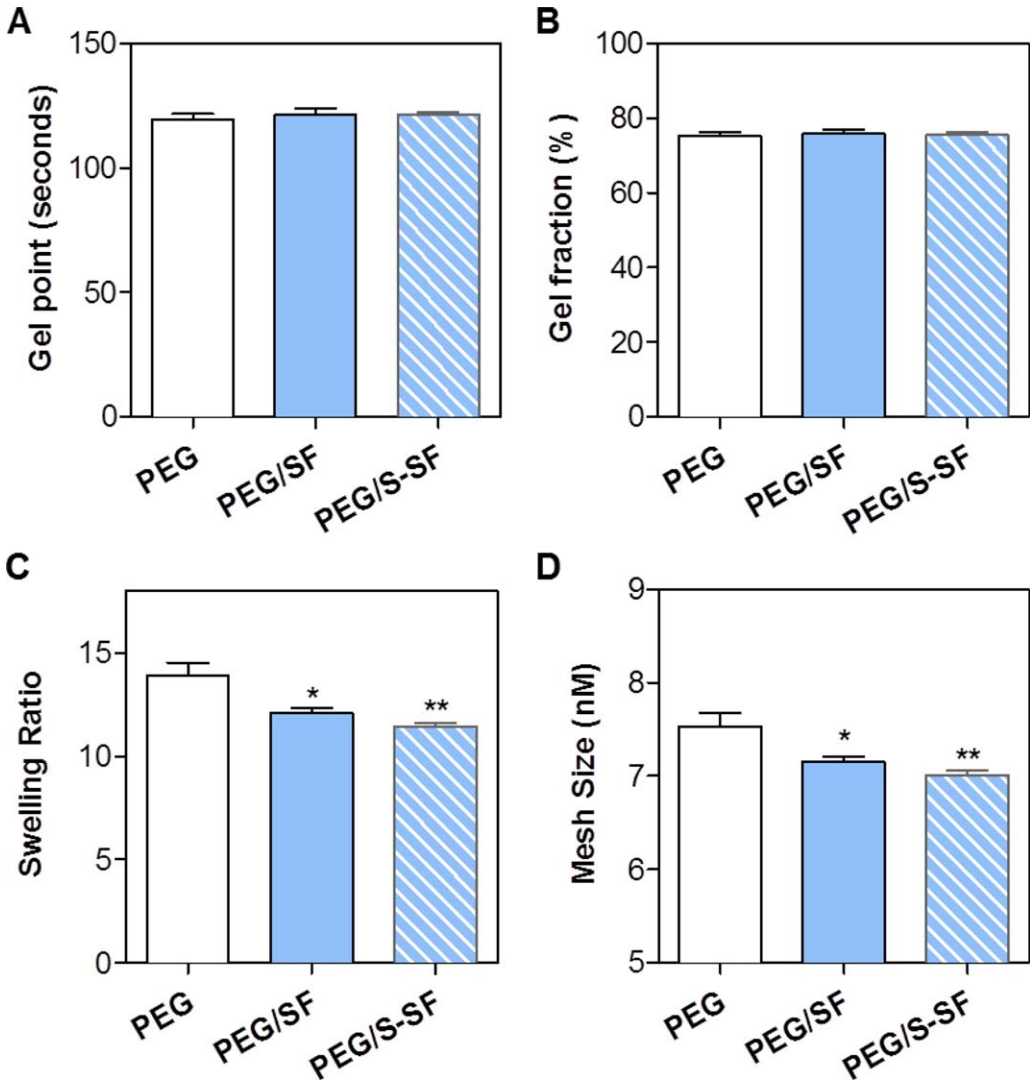


Figure 4. Characterization of thiol-acrylate PEGDA hydrogels formed without (PEG), with nonsonicated (PEG/SF), or with sonicated SF solution (PEG/S-SF). (A) Gel points determined by *in situ* photo-rheometry; (B) Gel fraction; (C) Equilibrium swelling ratio; and (D) Mesh size ($^{*}p < 0.05$; $^{**}p < 0.001$ compared to PEG group). SF or S-SF added at 1 wt %. Data represent Mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

However, when SF was mixed with PEGDA precursor solution (10 wt % $PEGDA_{3.4kDa}$, 7.5 m*M* DTT, 0.1% NVP) at 1 wt %, partial gelation occurred at 37°C after two days [Figure 2(C)]. This partial gelation was accompanied with increased turbidity in the solution. It is likely that the presence of PEGDA at 10 wt % served as a crowding agent to accelerate SF β-sheets formation and self-assembly into SF fibrils. This phenomenon agrees with a recent report where SF β-sheets and physical gelation was accelerated by low molecular weight PEG entrapment.[16]

In addition to using unprocessed SF solution, we also subjected some SF solution to sonication as studies have shown that sonicated or vortexed SF solution accelerates the formation of physical silk hydrogels.[14–16] We adopted a similar sonication strategy to prepare sonicated SF solution (i.e., S-SF), which was incorporated into the prepolymer solution in the same fashion as nonsonicated SF solution. Interestingly, the physical gelation of PEG/S-SF (1 wt % SF) mixture occurred after just 1 day [Figure 2(D)]. This experiment suggests that the presence of PEGDA, DTT, and NVP did not interrupt physical gelation of SF. Furthermore, the physical gelation process was accelerated when sonicated SF (S-SF) was used. It is worth noting that all physical gelation occurred after at least overnight incubation whereas the gels studied in Figure 1 were obtained after only 5 min of visible light exposure. However, it is possible that self-assembly of SF fibrils could still happen within a chemically crosslinked hydrogel.

Influence of SF Entrapment on Gelatin Kinetics of Thiol-Acrylate PEGDA Hydrogels

To assess the potential influence of SF on gelation kinetics of thiol-acrylate PEGDA hydrogels, we conducted *in situ* photo-rheometry using prepolymer solution mixed with 1 wt % of SF or S-SF. Except for the silk component (SF or S-SF), other macromer compositions were identical for all thiol-acrylate hydrogels (i.e., 10 wt % $PEGDA_{3.4kDa}$, 7.5 m*M* DTT, 0.1% NVP, and

0.1 m*M* eosin-Y). *In situ* gelation was performed immediately after mixing PEGDA and associated components with SF or S-SF solution to prevent the complications contributed by the potential self-assembly of SF β-sheets. Figure 3 shows the gelation kinetics of the three sets of hydrogels, including pure thiol-acrylate PEGDA hydrogels [i.e., PEG, Figure 3(A)], hydrogels with nonsonicated SF solution [i.e., PEG/SF, Figure 3(B)], and hydrogels with sonicated SF solution [PEG/S-SF, Figure 3(C)]. Visible light was turned on 30 s after the onset of the measurement. For all conditions tested, gelation did not occurred until roughly 2 min after the initiation of photopolymerization. The gel points (the time at which storage modulus, or G', surpasses loss modulus, G'') were 120 ± 4, 121 ± 6, and 121 ± 2 s for PEG, PEG/SF, and PEG/S-SF hydrogels, respectively [Figure 4(A)]. Although shear moduli of all groups rose rapidly after the initiation of thiol-acrylate photopolymerization, no statistical significance was found between the any two of the three gel groups. *In situ* gelation results show that the incorporation of 1 wt % SF, either sonicated or not, does not impact thiol-acrylate gelation kinetics. These gel points were similar to what we reported previously for visible light cured thiol-acrylate hydrogels formed by linear PEGDA and bi-functional crosslinkers.[10,11]

Influence of SF or S-SF Entrapment on Hydrogel Properties

Since incorporating 1 wt % nonprocessed SF solution does not yield significant difference in gel modulus [Figure 1(D)] and degradation rate [Figure 1(E)], we were interested to examine the impact of incorporating sonicated SF solution on the properties of thiol-acrylate hydrogels. If sonication induces and accelerates β-sheet formation in the thiol-acrylate hydrogels, we should be able to observe strengthening in gel stiffness in the hybrid hydrogels. Figure 4(B) shows that gel fractions (~78%) remained unaffected by SF entrapment, indicating that gel crosslinking efficiency was similar among the three gel formulations. This level of gel fraction was similar to the numbers reported previously.[10,11] Although gel fraction was not affected by SF entrapment, there was a significant reduction in equilibrium swelling ratios [Figure 4(C). 13.9 ± 1.1, 12.1 ± 0.43, and 11.4 ± 0.31 for PEG, PEG/SF, and PEG/S-SF hydrogels, respectively]. Correspondingly, the calculated hydrogel mesh size [Figure 4(D)] also decreased with SF entrapment (7.5 ± 0.14, 7.0 ± 0.04, and 7.1 ± 0.06 for PEG, PEG/SF, and PEG/S-SF hydrogels, respectively). It is worth noting that hydrogel equilibrium swelling ratio experiments involved with incubating the gels in water for 2 days. During this time, SF or S-SF might have sufficient time to form β-sheets or higher order structures that subsequently affected the dried weight of the hydrogels. More importantly, the entrapment of S-SF caused significantly more reduction in gel swelling ratio and mesh size, indicating that gel physical properties are affected by the potential β-sheets formed in the chemically crosslinked hydrogels. Note that the mesh sizes obtained from these hydrogels were between 6 and 8 nm, a size range similar to or slightly larger than many growth factors.[19] Therefore, this class of hybrid hydrogels should be useful in sustained release of growth factors.

In addition to causing reducing in gel swelling, the incorporation of SF also led to slight turbidity in the resulting thiol-acrylate hydrogels [Figure 5(A)]. This phenomenon was especially apparent in the S-SF group, further signifying the potential self-assembly of SF fibrils in the chemically crosslinked thiol-acrylate PEG hydrogels. We also evaluated the fraction of SF being retained in the PEG hydrogels [Figure 5(B)]. Notably, there was a significant decrease in SF retention in PEG hydrogels after 24 h of incubation. On the other hand, the fractions of S-SF retained in the hydrogels were similar ($64 \pm 1.4\%$ vs. $73 \pm 3.5\%$, no statistical significant difference) after 1 h and 24 h of incubation, further suggesting the potential formation of higher order structure (potentially self-assembled SF fibrils) within the chemically crosslinked PEG hydrogels.

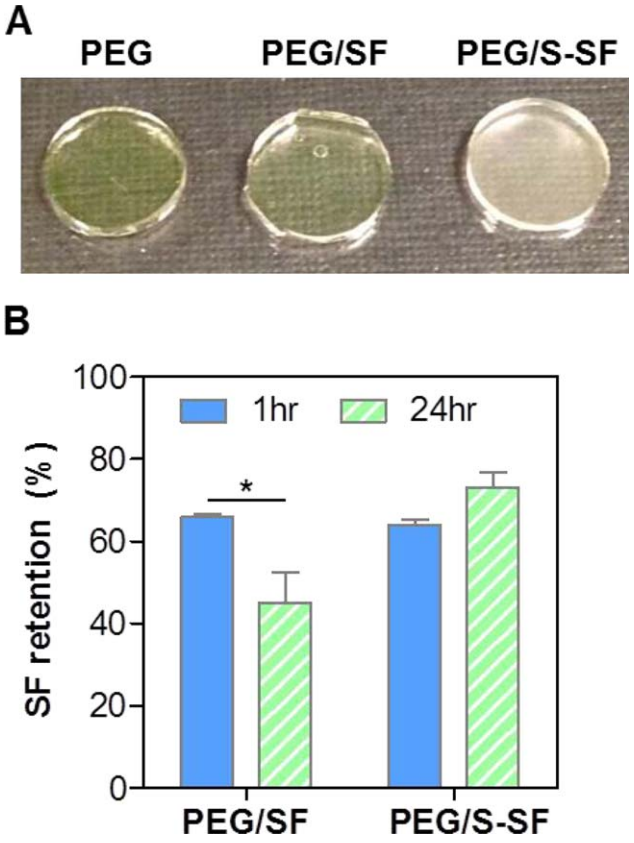


Figure 5. (A) Photograph of a thiol-acrylate PEGDA hydrogels (PEG), PEGDA hydrogel mixed with 1 wt % SF (PEG/SF), and PEGDA hydrogel mixed with sonicated SF (PEG/S-SF). (B) Retention of nonsonicated or sonicated SF in thiol-acrylate PEG hydrogels 1 h and 24 h after gelation ($^*p < 0.05$). Data represent Mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Influence of SF or S-SF Entrapment on Hydrolytic Degradation of Thiol-Acrylate Hydrogels

It has been shown that SF forms β-sheets gradually and sonication or vortex accelerates this process and leads to faster sol−gel transition.[13–16] When physically incorporated in a chemically crosslinked thiol-acrylate PEGDA hydrogels, the formation of SF β-sheets will entangle with the chemically crosslinked polymer network and result in increased gel stiffness [Figure 6(A)]. To test this hypothesis, we examined the influence of SF or S-SF entrapment on hydrogel shear moduli as a function of time. While no statistically significant difference was found between

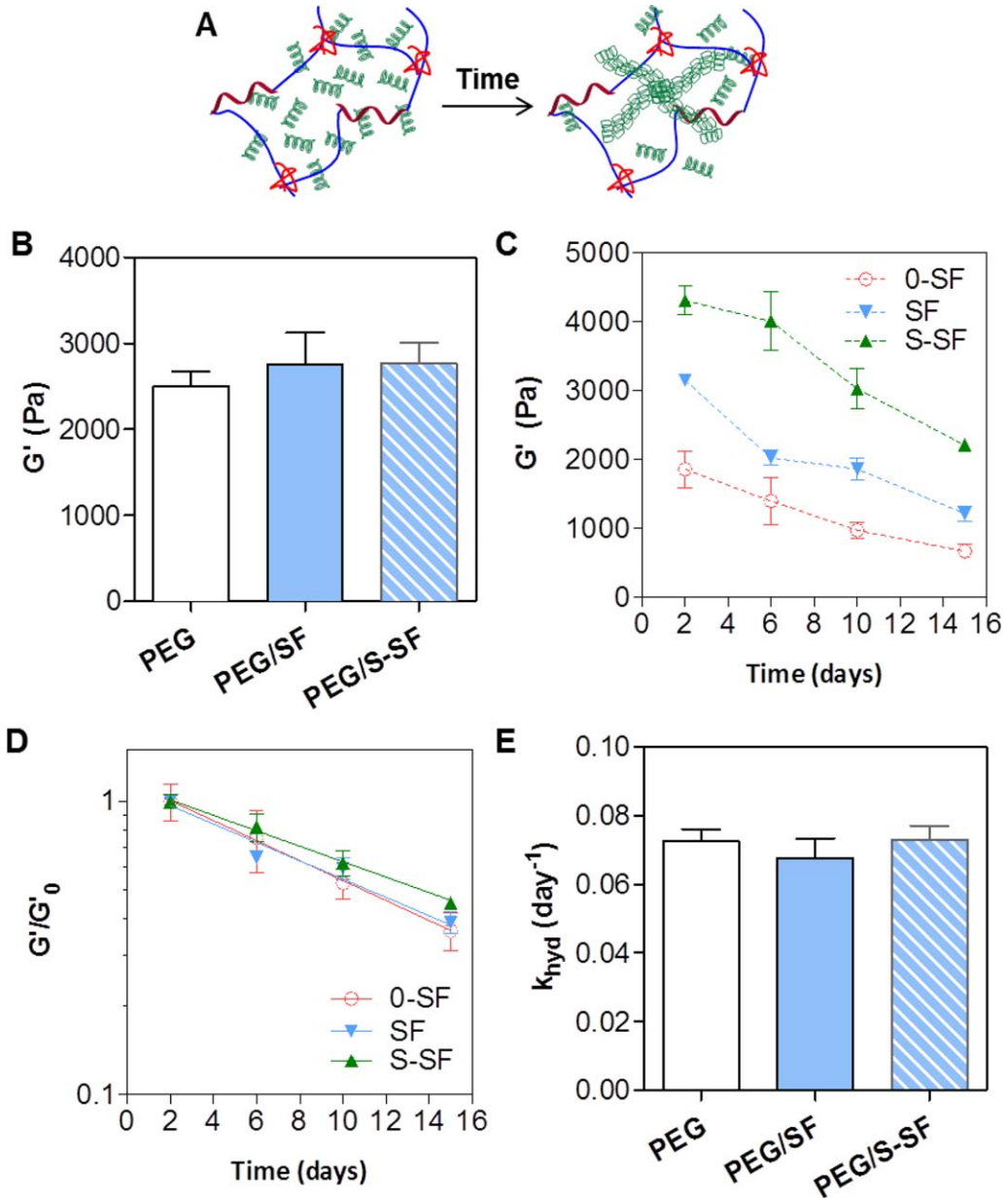


Figure 6. A: Schematic of SF β-sheets formation in thiol-acrylate PEGDA hydrogels. B: Gel moduli at day 0. C: Gel moduli as a function of time. D: Pseudo-first order analysis of gel hydrolytic degradation rate as a function of time (G'_0 = shear modulus in respective group before significant degradation has occurred, i.e., at day-2). E: Hydrolytic degradation rate constants abstracted from the pseudo-first order degradation curve fitting in C. SF or S-SF added at 1 wt %. Data represent Mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PEG, PEG/SF, and PEG/S-SF hydrogels following thiol-acrylate photopolymerization [Figure 6(B)], hydrogels containing sonicated SF (PEG/S-SF) exhibited significantly higher moduli after 2 days of incubation when compared with PEG or PEG/SF hydrogels [Figure 6(C)]. We found that the shear moduli of gels containing SF and S-SF increased from ~2000 Pa to ~3000 Pa and ~4200 Pa, respectively [day 2, Figure 6(C)]. The increases in gel shear moduli suggest that SF formed secondary structure, most likely SF fibrils, in the chemically crosslinked gel network. It could be noted that the moduli of pure PEG hydrogels presented in Figure 1(C) (i.e., ~3000 ± 341 Pa) is different from that in Figure 6(B) (~2500 ± 180 Pa). This might be caused by experimental variations from two independent experiments. To verify this, we performed additional statistical analysis (*t*-test) to compare the two independent experiments and did not find statistical significance in the differences ($p > 0.08$).

To assess the influence of SF entrapment on hydrolytic degradation of thiol-acrylate PEGDA hydrogels, we tracked the shear moduli of these gels periodically for two weeks. As shown in Figure 6(C), the moduli of all gels decreased as a function of time, which was attributed to the hydrolysis of thioether ester bonds formed following thiol-acrylate photopolymerization. We analyzed the moduli data using the pseudo-first order degradation

assumption as reported previously for thiol-acrylate hydrogels.[10] Upon plotting of G'/G'_0 (in log-scale) against time, we found that the pseudo-first order hydrolytic degradation kinetics applies to all three groups of hydrogels and there was no statistically significant difference in hydrolysis rate constants in these hydrogels [Figure 6(D)]. These results show that the entrapment of SF or S-SF did not change the degradation rate of the hybrid hydrogels [Figure 6(E)], suggesting that the hydrolysis rate of thioether ester bonds was not affected due to the potential formation of SF fibrils.

The major implication for this type of material control is for controlled delivery of therapeutically relevant molecules without the potential complications from altering gel crosslinking density. The delivery rate of drugs from a chemically crosslinked hydrogel is often determined by the mesh size of the gel. To reduce the rate of drug delivery, one can simply increase the crosslinking density of the gel, hence reducing gel mesh size and imposing hindrance on molecular transport. The increase of gel crosslinking density, however, leads to higher gel modulus and potentially lower gel degradation rate. By physically entrapping SF or S-SF in chemically crosslinked thiol-acrylate PEG hydrogels, we successfully decouple hydrogel degradation rate from their initial moduli. This implies that future studies can be performed to independently control the delivery rates of therapeutically relevant drugs and the moduli of chemically crosslinked PEG/SF hydrogels.

Another potential application for this type of hybrid hydrogels is to create local heterogeneity in material properties in a mesh-like hydrogel. One disadvantage of PEG-based hydrogel is that the material on a macroscopic level exhibits homogeneous or isotropic properties. A hybrid hydrogel system such as the one presented here might have the potential to provide a strategy to create an anisotropic material for directing alignment of cells or for providing heterogeneous mechanical cues for the encapsulated cells.

CONCLUSIONS

Through physical entrapment of silk fibroin (SF) or sonicated silk fibroin (S-SF), we have developed a simple strategy to modulate the properties of visible light cured thiol-acrylate PEG hydrogels. The entrapment of SF at higher contents (>1.5 wt %) significantly reduced the crosslinking efficiency of thiol-acrylate PEG hydrogels and accelerated its hydrolytic degradation. On the other hand, SF entrapment at 1 wt % minimally affected gel crosslinking, mechanics, and degradation. Adding sonicated SF (S-SF) in the PEG precursor solution induced rapid physical gelation even at low weight content. When hybrid hydrogels were prepared through light-mediated thiol-acrylate photopolymerization, the entrapment of SF or S-SF did not alter gel points or gel fraction but significantly reduced equilibrium gel swelling and mesh size. Furthermore, S-SF was retained more in the hybrid hydrogels, which increased gel moduli but had minimal effect on hydrolytic degradation rate of the hybrid hydrogels. This simple hybrid hydrogel fabrication strategy should be highly useful in future drug delivery and tissue engineering applications.

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