A Robust, Resilient, and Multi-Functional Soy Protein-Based Hydrogel

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Supporting Information

ABSTRACT: Soy protein is one of the most abundant plant protein in nature. Despite numerous studies on soy protein-based materials, the applications of soy protein hydrogel are still extremely limited because of its cumbersome gelation process and poor mechanical properties. Herein, we present a facile method to prepare robust soy protein hydrogels by the introduction of oxidized dextran, a natural polysaccharide derivative, as a macromolecular cross-linking agent. The preparation conditions such as pH value of reaction system and the mass ratio of soy protein to oxidized dextran have been extensively investigated. The resultant soy protein hydrogels exhibit better mechanical properties than the previously reported soy protein-based hydrogels. The best formulated hydrogel shows the compressive modulus of 4.3 kPa and fracture stress of 401 kPa at 87% compression. Also, the hydrogels demonstrate an excellent flexibility to withstand high compression with minimal hysteresis. Owing to the protein and polysaccharides nature of soy protein and dextran, the soy protein hydrogels maintain a good biocompatibility along with excellent mechanical properties. Finally, we show the possibility to design such a soy protein hydrogel as a tolerant multifunctional platform for fluorescence imaging, photothermal therapy, drug carrier, and antibacterial agent. The developed soy protein-based hydrogel is natural, sustainable, robust, and biocompatible and hence has great potential as a multifunctional material for various applications.

KEYWORDS: Proteins, Polysaccharides, Soybean, Dextran, Mechanical properties, Multifunctional platform

INTRODUCTION

Hydrogels are three-dimensional networks consisting of cross-linked hydrophilic polymer chains, which only swell but do not dissolve in water. Due to their remarkable properties, hydrogels have shown promising applications in the areas of sensors, adsorbents, catalysts, energy storage devices, and biomaterials. Specifically, in the biomedical field, hydrogels are used in tissue engineering, drug and protein delivery, imaging, and injectable fillers. A significant progress has been made in the fabrication of hydrogels from both synthesized and natural resources. Hydrogels formulated from synthetic materials have provided researchers with great flexibility in characteristic designing. For instance, poly-(ethylene glycol), poly(vinyl alcohol), poly(acrylic acid), and polyacrylamide, have all been fabricated into hydrogels with variable mechanical strengths and controlled degradation. However, there are still significant challenges, including their lack of biocompatibility and the possible toxic degradation products. Hydrogels made from nature derived polymers, such as collagen, silk fibroin, chitosan, and hyaluronic acid have also been thoroughly investigated, due to an interest in their intrinsic properties including biocompatibility, biodegradability, and cell-interactivity. However, in several cases, potential applications of these hydrogels are often limited by their low mechanical properties. Therefore, it is of vital importance to fabricate hydrogels which combine excellent mechanical properties with good biocompatibility and biodegradability.

Soy protein is one of the most abundant plant proteins which people have harvested from nature. After separation and purification, soy protein isolated (SPI) can be obtained with over 91% of protein content. Because of its abundance, low cost, and sustainability, SPI has been regarded as an ideal environmentally friendly material. Till date, a variety of SPI-based materials, namely gels, plastics, films, adhesives, and biomedical materials have been reported, among which SPI hydrogel has drawn significant attention. For the preparation of SPI hydrogel, various cross-linking
approaches, including physical and chemical, have been explored. Physically cross-linked SPI hydrogels, such as heat-induced hydrogels,\textsuperscript{33} cold-set hydrogels,\textsuperscript{32} and high pressure hydrogels,\textsuperscript{36} were formed primarily by hydrogen bonding and hydrophobic interaction. However, these hydrogels have poor mechanical properties and are unstable.\textsuperscript{36,37} In the case of the chemically cross-linked hydrogels, the addition of cross-linking agent, such as organic molecular aldehyde (glutaraldehyde and glyceraldehyde),\textsuperscript{38,39} biological active enzymes (microbial transglutaminase),\textsuperscript{40} and natural cross-linker (genipin),\textsuperscript{34} improved the mechanical properties of these SPI-based hydrogels to some extent. However, these hydrogels are still not strong enough and hence the improvement is far from satisfactory. Several of the chemically cross-linked SPI hydrogels cannot be self-supported and hence their mechanical properties can only be estimated from the storage modulus from rheological tests.\textsuperscript{34,38–42} For instance, the storage modulus of the SPI hydrogel cross-linked by glutaraldehyde was only about 1.7 kPa,\textsuperscript{38} while for the hydrogels that were cross-linked by transglutaminase or genipin, the storage modulus was even worse.\textsuperscript{35,41} As mentioned above, a small molecular aldehyde has a high probability to introduce cytotoxicity, limiting the applications of the related chemically cross-linked hydrogels in biomedical fields.\textsuperscript{43} Furthermore, in order to improve the mechanical properties of the final SPI hydrogel, some pretreatments for SPI such as acetylation and high temperature heating, are cumbersome, complicated,\textsuperscript{38} and relatively simple and practical preparation procedure.

Herein, we present a practical way to prepare a novel kind of elastic soy protein isolate/oxidized dextran (SPI/Odex) hydrogels with exceptional mechanical behavior. Odex is a derivative of dextran, a natural polysaccharide which is biodegradable in concert with its biocompatibility and has been extensively used in the biomedical field.\textsuperscript{44} It has already shown its merit when enhancing the strength of collagen hydrogels.\textsuperscript{20} The gelation conditions were intensively investigated, adjusting the pH of the reaction system and the mass ratio of SPI and Odex. Furthermore, the biocompatibility of the resultant SPI/Odex hydrogels was evaluated to check its value as pure SPI. In addition, the as-prepared SPI/Odex hydrogels were utilized as flexible matrix to prepare specific composite materials for potential applications in fluorescence imaging, photothermal therapy, drug release, and antibacterial area.

\section*{Experimental Section}

\textbf{Materials.} SPI powder (protein content >90\%) was obtained from Shenyuan Food Co., Ltd., Shanghai, China. Dithiothreitol (99\%) and chloroauric acid trihydrate were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), hydrochloric acid, guanidine hydrochloride (99\%), and polyethylene glycol (PEG, M\textsubscript{w} 20 000) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dextran (M\textsubscript{w} 70 000), sodium periodate, and tetracycline hydrochloride (TCH) were purchased from Aladdin Chemical Reagent Co., Ltd., Shanghai, China. Dioxurubicin hydrochloride (DOX) was obtained from Dalian Meilun Biotech Co., Ltd., China. Dialysis tube (MWCO: 12–14 kDa) was purchased from Blue Bird Co., Ltd., Shanghai, China. All chemicals used in this work were of analytical grade and used without further purification.

\textbf{Preparation of SPI and Odex Aqueous Solutions.} The SPI solution was prepared following a well-established procedure reported in our previous work.\textsuperscript{5,6,46} Briefly, raw SPI powder was first dissolved in 6 mol L\textsuperscript{–1} guanidine hydrochloride aqueous solution and then stirred for 3.5 h at room temperature. In order to break disulfide bonds, 25 mmol L\textsuperscript{–1} dithiothreitol was added, followed by continuous stirring for 2 h. After dialysis against NaOH solution (pH 10) for 2 days and deionized water for another day. Thereafter, the solution was centrifuged at 9000 \texttimes \text{g} for 10 min to remove the insoluble components. The supernatant was concentrated to 6 wt % by reverse dialysis against 10 wt % PEG solution and then lyophilized to obtain pure SPI powder. The as-prepared pure SPI powder was completely dissolved in deionized water to get a SPI solution with desired concentration.

Odex was prepared by oxidizing dextran with sodium periodate to introduce aldehyde groups following typical protocols described in the literature.\textsuperscript{6,7,42} Briefly, 4 g dextran and 3.3 g sodium periodate were dissolved in 50 mL deionized water and stirred in dark for 24 h at the room temperature. Thereafter, 1 g glycol was added to terminate the reaction. Finally, the Odex solution was dialyzed against deionized water for 3 days to remove the untreated sodium periodate and lyophilized to obtain the final product. The as-prepared Odex powder was dissolved in deionized water to obtain Odex solution.

\textbf{Preparation of SPI/Odex Hydrogels.} The SPI solutions (10 wt \%) with different pH adjusted by 2 mol L\textsuperscript{–1} NaOH were mixed with Odex solution (10 wt \%) at different mass ratios under room temperature. After simple mixing, the mixed solution was poured into a mold, and the SPI/Odex hydrogel was readily formed via Schiff-base reaction. By using different molds, the hydrogels with different shapes (cylindrical, rectangular, and spherical) can be obtained correspondingly.

To study the effect of pH, SPI powder was dissolved in pH solutions at 8.0, 8.5, 9.0, 9.5, and 10.0, respectively, with a fixed mass ratio of SPI to Odex of 70/30. To study the effect of reactant proportion, SPI solution was mixed with Odex solution at mass ratios of SPI to Odex of 95/5, 90/10, 80/20, 70/30, 60/40, and 50/50, respectively, while using the SPI solution by dissolving SPI powder in pH solution at 9.

The gelation time was determined using a vial inverting method. No visible flow within 60 s when the vial was vertically inverted was regarded as the criteria for gel formation.

\textbf{Preparation of Functionalized SPI/Odex Hydrogels.} The functionalized SPI/Odex hydrogels such as gold nanocluster-contained SPI/Odex (AuNCS@SPI/Odex) hydrogel, gold nanoparticle-contained SPI/Odex (AuNPs@SPI/Odex) hydrogel, DOX-loaded SPI/Odex (DOX@SPI/Odex) hydrogel, and TCH-loaded SPI/Odex (TCH@SPI/Odex) hydrogel, were prepared simply by adding functional ingredients into SPI aqueous solution while other steps remained the same. The details can be found in the Supporting Information (SI).

\textbf{Mechanical Testing of SPI/Odex Hydrogels.} The mechanical properties of SPI/Odex hydrogels were evaluated via Instron 5565 universal testing machine at 25 ± 5 °C and 45–50% relative humidity. In the uniaxial compression tests, cylindrical hydrogel samples with the height of 11.5 mm and diameter of 15.5 mm were used and the compression rate was 1 mm/s. For the tensile tests, hydrogels were made into a rectangular shape with 60 mm in length, 10 mm in width, and about 2 mm in thickness. The gauge length was 20 mm and the cross-head speed was 10 mm/min. For each measurement, at least five hydrogel samples were tested.

\textbf{Cytotoxicity Tests.} The biocompatibility evaluation of SPI/Odex hydrogels was carried out by testing the metabolic viability of mouse fibroblast cells (L929) cultured with hydrogel extracts. After sterilization with 75% ethanol, the hydrogels were immersed in DMEM medium at 37 °C for 24 h to obtain the hydrogel extracts. The L929 cells were seeded into 96-well plates at a density of 3 \times 10\textsuperscript{3} cells/well. After 24 h of incubation, the pristine DMEM medium was removed and the hydrogel extracts were added into the wells. Then after culturing for 24, 48, 72, and 96 h, respectively, the cell viability was measured by using a CCK-8 assay. Each measurement was repeated at least by triplicate. Images of L929 cells incubated with hydrogel extract were also taken using phase
Contract imaging mode by an inverted microscope (Nikon, Eclipse Ti2, Japan). The cell viability with regards to the control sample incubated with pristine DMEM was calculated as follows:

$$\text{cell viability(%) = } \frac{[A]_{\text{test}} - [A]_{\text{blank}}}{([A]_{\text{control}} - [A]_{\text{blank}}) \times 100\%}$$

where $[A]_{\text{test}}$ is the absorbance of the test sample, $[A]_{\text{control}}$ is the absorbance of the control sample, and $[A]_{\text{blank}}$ is the absorbance of the sample without any cells.

Cell Culture on Surface and Inside SPI/Odex Hydrogels.

After sterilization with 75% ethanol, the hydrogel samples were placed at the bottom of 35 mm glass bottom culture dishes, 2 mL of cells suspension containing $2 \times 10^5$ L929 cells was gently dropped on the surface of hydrogel samples. The cells were cultured in a CO$_2$ (5%) incubator at 37 °C for 3 days. Some samples were stained using a Live/Dead Viability/Cytotoxicity Kit (Molecular Probe, U.S.) according to the manufacturer’s instruction. Other samples were fixed with 4% paraformaldehyde in PBS for 20 min before permeabilization with 0.1 vol % Triton X-100 for 5 min all at room temperature. Phalloidin diluted solution was then incubated with the cells for 20 min in the dark, followed by the addition of 4′,6-diamidino-2-phenylindole (DAPI) and a further 5 min incubation. The samples were then washed with fresh PBS for 3 times and the images of L929 cells were obtained by a Nikon C2 laser scanning confocal microscope.

On the other hand, the hydrogel samples with the height of 1 mm and diameter of 15.5 mm were first frozen at −20 °C and then thawed under ambient temperature. After sterilization with 75% ethanol, the frozen–thawed hydrogel samples were placed at the bottom of 35 mm glass bottom culture dishes. Two mL of cells suspension containing 2 × 10^5 L929 cells was gently dropped on the frozen–thawed hydrogel samples. The cells were cultured in a CO$_2$ (5%) incubator at 37 °C for 3 days before staining using the same methods described above. The fluorescence images were recorded by a Nikon C2 laser scanning confocal microscope.

RESULTS AND DISCUSSION

Mechanical Properties of SPI/Odex Hydrogels. In our previous work, Odex has been used as a macromolecular cross-linker to fabricate collagen/Odex hydrogel with enhanced mechanical properties, improved thermal stability, and excellent biocompatibility. Herein, we have used Odex as a macromolecular cross-linking agent to cross-link SPI with the same mechanism. Generally, SPI/Odex hydrogels can be easily prepared by mixing SPI and Odex aqueous solutions under room temperature via Schiff-base reaction between the amino groups in SPI and aldehyde groups in Odex at the desired concentration and different cross-linking ratio. As shown in Figure 1A, the as-prepared SPI/Odex hydrogels were translucent and smooth-faced, maintaining the exact shape of the mold. As far as we know, the SPI hydrogels reported before have been plagued by their fragile mechanical properties and is difficult to be molded into different shapes, while our hydrogels can be fabricated into different shapes according to the shape of the mold. We also prepared a SPI hydrogel cross-linked by glutaraldehyde (GA), which is a common way reported in the literature. Although we chose the optimal GA concentration (64 mmol/L) used in the literature to prepare the SPI hydrogel with the best mechanical performance, the cylindrical SPI/GA hydrogel sample with the height of 11.5 mm and diameter of 15.5 mm still cannot fully support its own weight. Figure 1B shows a direct impression of the difference between the SPI/GA and SPI/Odex hydrogel with the same solid content (10%). It clearly showed that the SPI/Odex hydrogel kept the shape of the mold very well, while the SPI/GA hydrogel had collapsed a little from its original shape already. When bearing a 200 g weight, the SPI/GA hydrogel collapsed totally and could not
recover to its initial state; in contrast, the SPI/Odex sample only went through great deformation and recovered to its original shape immediately after the removal of the weight. In addition, the hydrogel presented great elasticity, as the video shows that the ball-shape SPI/Odex hydrogel with a diameter of 1 cm could bounce repeatedly with a ping-pong bat (see video in the SI). These phenomenon give us an intuitive impression of the strength and elasticity of the SPI/Odex hydrogels, which undergoes a qualitative leap as compared to other SPI hydrogels reported.33,34,38 Afterward, we conducted a quantitative analysis of the mechanical properties of the SPI/Odex hydrogel by both compression and tensile tests. Figure 1C is a typical stress-compression curve of the SPI/Odex hydrogel with 70/30 mass ratio (solid content = 10%, pH 9.0). Such a hydrogel showed a compressive elastic modulus of approximately 4.3 kPa, a fracture stress of 401 kPa at 87% compression, and an elastic region up to 18% (see Figure 1C inset). To the best of our knowledge, this is the best mechanical performance reported in SPI-based hydrogels.33,34,38,42

Figure 2. (A) Compressive modulus and (B) stress at 50% compression of SPI/Odex hydrogels fabricated at different pH conditions (SPI/Odex = 70/30, solid content = 10%). (C) Compressive modulus and (D) stress at 50% compression of SPI/Odex hydrogels with a different mass ratio of SPI and Odex (solid content = 10%, pH 9.0). Data were expressed as mean ± standard deviations (n = 5).

During the experiment, we discovered that pH of the reaction system has a crucial impact on the formation of SPI/Odex hydrogels. Thus, we further investigate the gelation time of SPI/Odex hydrogel under different pH conditions and the results are shown in SI Table S1. With the increase of pH, the gelation time was significantly shortened. For instance, when the pH of SPI solution increased from 7.0 to 10.0, the gelation time was reduced from 2.5 to 0.3 min. However, if the pH was too high (>10), the gelation process was found to be too rapid to get a homogeneous hydrogel. The acceleration of the gelation process is primarily attributed to two reasons: first, high pH enhanced the nucleophilicity of amino group and thus greatly accelerated the reaction progress.49,51 Second, under alkaline conditions, the SPI chains were more easily to extend and exposed to more amino groups, facilitating the cross-linking process.52 Moreover, in most cases, even "strong" hydrogels can hardly be stretched, but our SPI/Odex hydrogels were strong enough to prepare specimens for tensile tests. Figure 1D is a representative stress—strain curve of the same SPI/Odex hydrogel for a compression test, showing a breaking stress of nearly 8.0 kPa and a breaking strain of 133%. Similar to compression, SPI/Odex hydrogel also showed a large elastic region (up to 80%) with an elastic modulus of 5.2 kPa.

Another critical factor that influences the mechanical properties of SPI/Odex hydrogels is the reacting mass ratio. Figure 2C and D compare the compressive modulus and compressive stress at 50% compression of hydrogels with different SPI/Odex ratios. When the proportion of Odex was increased, the compressive modulus witnessed an increase and reached a maximum of 3.72 at SPI/Odex = 70/30, which was the same with the compressive stress at 50% compression. As is well-known that the increase in the amount of cross-linking agent increases the cross-linking ratio which improves the mechanical strength of the hydrogels. However, if the amount of cross-linking agent increased by a certain amount, on one hand, the amount of cross-linkable amino groups in SPI decreased. On the other hand, the increase of relatively low molecular weight (∼28 kDa) component Odex instead of the high molecular weight (∼300 kDa) component SPI is clearly

33,34,38,42
not favorable to the mechanical properties of SPI/Odex hydrogels. Therefore, when the Odex content in the hydrogel was extremely high (e.g., SPI/Odex = 50/50), the excessive Odex deteriorated the strength of the hydrogel instead of increasing it. In the meantime, changes in gelation time also confirmed this inference (SI Table S2). With the increase of the Odex proportion, the gelation time first decreased from 4 to 1.5 min and then increased again to 5 min thereafter. The longer reaction time at SPI/Odex = 50/50 is a strong evidence that reduction of the amount of SPI leads to fewer amino groups for reaction. Therefore, owing to the best mechanical performance and adequate gelation time, we can conclude that SPI/Odex = 70/30 is the optimal ratio for the SPI/Odex hydrogel.

In order to thoroughly investigate the mechanical properties, loading and unloading compression tests were conducted to study the deformation response and hysteresis behavior of the SPI/Odex hydrogels. As shown in Figure 3A, no compression fracture was observed even at a high compression of 80%, indicating an excellent compression-resistant property. As the term “elastic” implies not only to the ability to deform in response to large strains with little force, but also to the ability to deform reversibly without a loss of energy (i.e., high resilience),

Biocompatibility of SPI/Odex Hydrogels. Since one of the most attractive characteristics of protein-based hydrogels is its biocompatibility, it is of vital importance to find out whether this natural advantage remains in our SPI/Odex hydrogels. We first cultivated L929 mouse fibroblasts cells in the extracts from SPI/Odex hydrogels (SPI/Odex = 90/10 and SPI/Odex = 70/30) for 24 to 96 h. As shown in Figure 4A, B, and SI Figure S2, L929 cells grow quite well, showing no significant differences with those cultured with DMEM. Meanwhile, SI Figure S1 clearly demonstrates the significant proliferation of L929 cells with the incubation time from 24 to 96 h. These results were further supported by the in vitro cytotoxicity test on L929 cells. After 24 and 48 h of incubation with the extract of SPI/Odex hydrogel, for all SPI/Odex hydrogel samples studied (i.e., SPI/Odex = 90/10 to 50/50), the cell viability was more than 95% (Figure 4C), suggesting the good biocompatibility of our SPI/Odex hydrogels. In addition, we chose two hydrogels with different composition (i.e., SPI/Odex = 90/10 and SPI/Odex = 70/30) and extended the incubation time to 96 h to test their in vitro cytotoxicity with L929 cells (SI Figure S2). The results showed the same conclusion as we can see the cell viability was still more than 95% even after 96 h of incubation. As a note, the phenomenon that the cell viabilities in some SPI/Odex hydrogel extracts exceed 100% may because some dextran was extracted from the hydrogel and became the extra nutrition for the cells.

In order to get the supplementary evidence to support the good biocompatibility of SPI/Odex hydrogels shown above, we also tried to culture L929 cells on the surfaces and inside the hydrogels, and used two staining methods (Live/Dead Viability/Cytotoxicity Kit and phalloidin/DAPI assay) to compare the results. As can be seen from Figure 4D and SI Figure S3, most of the cells are alive and proliferate on the
surface of SPI/Odex hydrogels well. In addition, as we want to culture cells inside the hydrogel, so we first used a freezing-thawing method to make the hydrogel into a scaffold (the morphology is shown in SI Figure S4). Figure 4E and SI Figure S5 shows that L929 cells penetrate into the hydrogel scaffold and are alive inside it. Figure 4D, 4E, SI Figures S3, and S5 indicate that L929 cells can live on the surface and inside the hydrogel, demonstrating the possibility of the SPI/Odex hydrogel as a good candidate for biomedical material. All the results shown above imply that the SPI/Odex hydrogels exhibit a good biocompatibility and have a great potential in biomedical applications.

SPI/Odex Hydrogels As Multifunctional Platform. To make the SPI/Odex hydrogel qualify as a tolerant and multifunctional material, various kinds of nanomaterials or drugs were added in it during the gel formation process. In our previous work, we have synthesized gold nanomaterials with different sizes and shapes using SPI as reductant, template, and capping agent. In other words, by adjustment of the pH conditions, we are able to synthesize either gold nanoclusters (AuNCs) or gold nanoparticles (AuNPs) in SPI aqueous solutions (see details in the SI). Therefore, we first used Odex to cross-link these SPI solutions with as-prepared AuNCs and AuNPs to fabricate AuNCs@SPI/Odex hydrogel and AuNPs@SPI/Odex hydrogel. The optical image of the resulting AuNCs@SPI/Odex hydrogel under visible light and 365 nm UV light are shown in Figure 5A and B. We observed that the addition of AuNCs did not affect the formation of SPI/Odex hydrogel. More importantly, the homogeneous pink light under UV irradiation indicated that AuNCs were uniformly dispersed in the SPI/Odex matrix and the fluorescence performance was not jeopardized by the SPI-based substrate. In this regard, SPI/Odex hydrogels can be proposed as an attractive matrix for stabilizing AuNCs for further processing and utilization in the field of fluorescence imaging.

Thereafter, we fabricated AuNPs@SPI/Odex hydrogels to test their photothermal transformation properties. As a minimally or even noninvasively therapeutic treating protocol, photothermal therapy (PTT) has been suggested as an alternative method to the conventional approaches (surgery, chemotherapy, and radiotherapy) to treat cancer. Based on photoabsorbing nanomaterials, PTT uses near-infrared (NIR)
light in the range of 700–1100 nm to induce hyperthermia that can ablate the tumor tissues. Various amounts of AuNPs were mixed with SPI and Odex to form different AuNP@SPI/Odex hydrogels as shown in Figure 5C. Then, the photothermal performance of these AuNP@SPI/Odex hydrogels was evaluated by measuring the temperature changes under NIR laser (808 nm, 2.5 W) for 5 min. As depicted in Figure 5D, the temperature of the control groups (pure water and pure SPI/Odex hydrogel) only showed a minor change (about 4 °C). In contrast, the temperature of AuNP@SPI/Odex hydrogels increased clearly, namely 38.8, 32.4, 29.7, and 25.9 °C after 5 min of irradiation at AuNP concentration of 6, 5, 4, and 3 mg/mL, respectively. These results suggest that the temperature increase of AuNP@SPI/Odex hydrogels can be easily controlled by adjusting the loading amount of AuNPs. In addition, to embed AuNPs in SPI/Odex hydrogel may help AuNPs maintain higher concentration and longer residence time in tumor site to conduct a repeated photothermal therapy. After the successful incorporation of AuNCs and AuNPs in hydrogels, we tested the efficacy of SPI/Odex hydrogel as a drug carrier. Hence, we loaded an anticancer drug, DOX, into SPI/Odex hydrogels. By decreasing the solid content to 7%, the DOX-loaded SPI/Odex hydrogel could be easily injected from a syringe (inset of Figure 5E). The drug release experiments of the DOX@SPI/Odex hydrogels were carried out in PBS (pH 7.4) at 37 °C over a period of 200 h. As presented in Figure 5E, the release curve of DOX from SPI/Odex hydrogel shows a good controllable and sustainable behavior. These results suggest that the DOX@SPI/Odex hydrogel can be employed as an injectable hydrogel for the anticancer drug delivery. Finally, to further explore the potential of SPI/Odex hydrogels as a wound dressing material, we incorporated 0.2 wt % TCH into the SPI/Odex hydrogel to endow it with antibacterial properties. Antibacterial properties of TCH-loaded SPI/Odex hydrogels were tested against two common bacterial strains using zone of inhibition (ZOI) assay. After 24 h incubation with Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus, an evident inhibition zone could be seen around the TCH-loaded hydrogels in both the groups, indicating an effective inhibition of bacteria growth, which makes the TCH-loaded SPI/Odex hydrogel a potential candidate for wound dressings.

**CONCLUSION**

In this work, we present a simple method to synthesize robust SPI-based hydrogels by using Odex as a macromolecular cross-linking agent through Schiff-base reaction between amino groups in SPI and aldehyde groups in Odex. Extremely different from other SPI-based hydrogels reported previously, the introduction of macromolecular cross-linking agent endows the SPI/Odex hydrogels with excellent flexibility to withstand high compression with negligible hysteresis. By adjusting the pH value in the reaction system and the mass ratio of SPI to Odex, we were able to control the gelation time and the mechanical properties of the resulting SPI/Odex hydrogels. We found that the SPI/Odex ratio at 70/30 was the optimal condition to conduct the best mechanical performance. As SPI is a protein and Odex is a derivative of polysaccharides, the SPI/Odex hydrogels maintained good biocompatibility, testifying by the proliferation of L929 cells both on the surface and inside the hydrogels. In addition, after bearing Au nanomaterials or loading with drugs, the SPI/Odex hydrogels showed great ability to serve as a tolerant multifunctional platform for fluorescence imaging, photothermal therapy, drug carrier, and antibacterial agent. Thus, we believe that such a natural, sustainable, robust, and biocompatible SPI-based hydrogels held great promise as a flexible matrix in various application areas.

**ASSOCIATED CONTENT**

Supporting Information

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Experimental details of the preparation of AuNCs@SPI/Odex, AuNP@SPI/Odex, DOX@SPI/Odex, and TCH@SPI/Odex hydrogel. Experimental details of the photothermal performance of AuNP@SPI/Odex hydrogel, in vitro drug released measurement of DOX@SPI/Odex hydrogel, and in vitro antibacterial activity assay of TCH@SPI/Odex hydrogel. Comparison of gelation time of SPI/Odex hydrogel under different pH condition and with different mass ratio of SPI to Odex. SEM photograph of SPI/Odex hydrogel scaffold. Microscopic images of L929 cells cultured with hydrogel extract, on the surface of the hydrogel, and inside the hydrogel (PDF). Demonstration of great elasticity of SPI/Odex hydrogel ball (AVI).

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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