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An *in situ* forming tissue adhesive based on poly(ethylene glycol)-dimethacrylate and thiolated chitosan through the Michael reaction

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In this paper, a novel biocompatible and biodegradable tissue adhesive composed of poly(ethylene glycol)-methacrylate (PEGDMA) and thiolated chitosan (CSS) was prepared. PEGDMA and CSS cross-linked rapidly under physiological conditions through the Michael addition reaction *via* UV lamp irradiation. The chemical structures of PEGDMA and CSS were confirmed *via* FTIR and ^{1}H NMR. The equilibrium swelling ratio and biodegradation of the hydrogels were tunable by varying the component ratios of the hydrogels. The compression strength and adhesive strength of the resulting hydrogels were measured with a tensile tester, and the adhesion strength of the hydrogel was higher than the fibrin glues. Moreover, the cytotoxicity of the PEGDMA/CSS hydrogels for L929 cells was evaluated by the MTT assay, and the results indicate that the photocured hydrogels are biocompatible and less cytotoxic towards the growth of L929 cells. These findings imply that the obtained hydrogel adhesives are a potential bioadhesive for clinical application in the future.

1. Introduction

Hydrogels are three-dimensional polymeric networks, which are analogous to the structure of the extracellular matrix (ECM).[1] They are widely utilized in various applications including tissue adhesive,[2] drug release loaders,[3] protein or RNA delivery vehicles, and extracellular matrix for tissue engineering and surgical dressing.[4] Hydrogel adhesives are used for tissue adhesive and have been developed for many years considering their advantages,[5] especially in recent decades. Compared with the biological adhesives, such as cyanoacrylates and fibrin glue, which are the leading products of commercially available tissue adhesive,[6] hydrogel adhesives are safer than cyanoacrylates and have higher adhesive strength than fibrin glue. Moreover, the degradation products of hydrogel adhesives are nontoxic.[7] However, cyanoacrylates generate formaldehyde, which is harmful to patients when degraded in the body,[8] whereas fibrin glue has a weakness in its material source for industrial production and risks infection and transmission of zoonotic diseases.[9] Researchers have proposed that the ideal adhesive should fulfill the following criteria: strong and rapid adhesive, no or low immunogenicity, not expensive, biocompatible, facilitate storage, and biodegradable.[10] There is no report on adhesives that meet all the criteria for an ideal biological adhesive or sealant.

Chitin is the second abundant polysaccharide after cellulose and is widely distributed in nature. Chitosan is the partially *N*-deacetylation product of chitin and has been used as a biomedical material.[11] Chitosan is biodegradable and nontoxic, can accelerate wound healing and is conducive to hemostasis. It has been observed that chitosan has antibacterial,[12] superior tissue adhesive properties[13] and immunological activity.[14] Chitosan is considered to be a candidate that can be applied for tissue adhesive, hemostasis and medical dressing. Chitosan can be modified with functional groups,[15–17] such as thiol groups and maleimide groups, in order to improve its solubility at the physiological pH. Thiol-modified chitosan is gaining popularity lately since the thiol–acrylate reaction (Scheme 1c) is a Michael additional reaction which is a mild, nontoxic reaction, with no side reaction. Besides, hydrogels composed of thiol-modified polymers and polymers containing acrylates or methacrylates are formed *in situ* under physiological conditions without byproducts.[18–20] Michael addition crosslinking can avoid the

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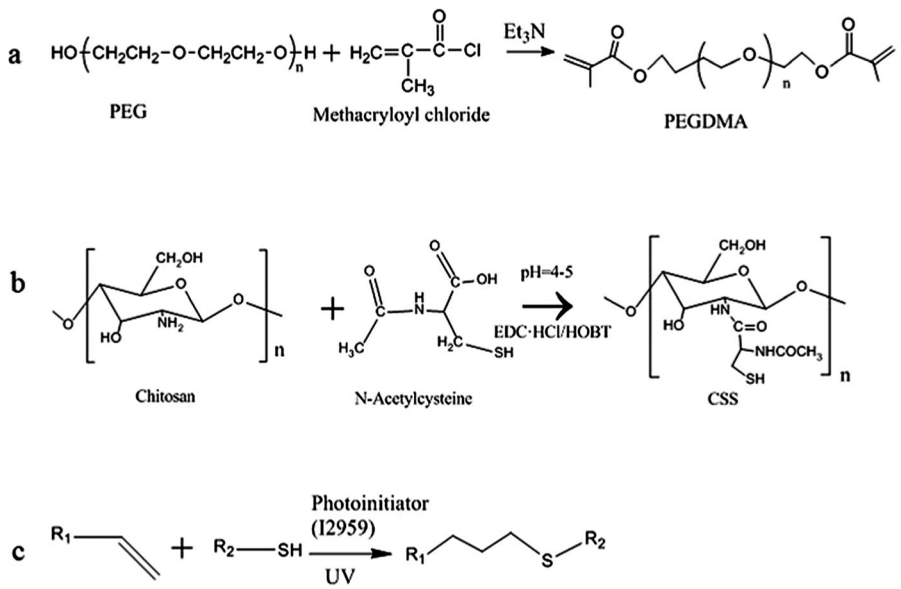

Scheme 1 Schematic of (a) synthetic route for PEGDMA; (b) synthetic route for CSS and (c) preparation of hydrogels *via* the thiol-acryl reaction.

use of cytotoxic free-radicals, H_2O_2,[21] photoinitiators, and UV light, however its crosslinking rate is not fast enough to meet the standard of clinics. Recently, degradable thiol–acrylate polymers have been regarded as a new class of material that is capable of quickly polymerizing *via* UV irradiation.[22] Amber E. Rydholm[23] *et al.* reported that thiol–acrylate polymers afford a much higher degree of control over its properties because of the versatility of their chemistry.

There have been many efforts to prepare suitable alternatives for the available bioadhesives. Researchers have developed many tissue adhesives, for example, nanocomposite hydrogels,[24] mussel inspired polymers,[25] and nanoparticle solutions.[26] The aim of our research is to develop a photo-crosslinkable CSS/PEGDMA hydrogel adhesive, which is formed *in situ* rapidly upon exposure to a UV lamp (Scheme 1). FTIR and ^{1}H NMR were used to confirm the chemical structure of the functional polymers. The swelling ratios, compressive strength, adhesion strength and degradable properties of the prepared hydrogels are also investigated. Furthermore, the cell compatibility *in vitro* of the hydrogels was tested on L929 cells, and the result suggests that the hydrogel prepared is nontoxic to L929 cells. This paper provides an alternative route for the development of tissue adhesive used for wound healing and hemostasis.

2. Experimental

2.1 Materials and methods

2.1.1 Materials. Medium molecular weight chitosan with a nominal degree of deacetylation, 85%, *N*-acytyl-L-cysteine (NAC), and the photoinitiator, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, namely Irgacure 2959 (I2959) were purchased from Sigma-Aldrich. 1-Hydroxybenzotrizole hydrate (HOBT) was purchased from Shanghai WoKai Chemical Reagent Co., Ltd. Methacryloyl Chloride was purchased from Energy Chemical. Poly(ethylene glycol) (PEG, 2 kDa) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. Fibrin sealant (Tisseel®) was purchased from Baxter Healthcare Ltd, 3-[4-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, (MTT) was purchased from Biosharp, Fetal bovine serum (FBS) and 0.25% Trypsin-EDTA was purchased from Gibco, and Dulbecco's modified eagle medium (DMEM, high glucose) was purchased from Hyclone.

2.1.2 Thiolation of chitosan. The modification of chitosan with thiol groups was done according to a previous paper.[27] Typically, 0.5 g of chitosan powder was dispersed in 50 mL ultrapure water in a flask with stirring. After 20 minutes, HOBT (2.58 mmol) was added, and the solution became clear under stirring, then NAC (5.16 mmol) was added to the mixture solution followed by the addition of a solution of EDC·HCl (10.32 mmol). The reaction proceeded for 5 hours at room temperature and the pH of the reaction solution was kept at 4–5 by the addition of 1 M HCl solution during the reaction. After 5 hours, the reaction solution was dialyzed in ultra-pure water and lyophilized. The lyophilized product was CSS, and the degree of substitution of thiol groups was determined using the Ellman's test.

2.1.3 Synthesis of PEGDMA. The liner PEG (2 kDa) was dissolved in toluene in a flask and the PEG concentration was 8 wt%, then the water contained in the PEG was removed by azeotropic distillation using a Dean–Stark trap. After cooling to room temperature, the solution was degassed with N_2 for 30 min. Triethylamine (TEA) was dissolved in dichloromethane (DCM) at the volume ratio of 1 : 9, and then added to the PEG solution with stirring. The reaction began when methacryloyl chloride was added to the mixture solution dropwise in an ice bath. The reaction proceeded for 24 h in the dark under an

Table 1 Ratio of the components for the photo-crosslinking CSS/PEGDMA hydrogels and the gelation time

Hydrogels	Concentration of CSS (wt%)	Concentration of PEG-DMA (wt%)	Concentration of I2959 (wt%)	Gelation time (s)
CSS/PEGDMA-1	0	20	0.1	30
CSS/PEGDMA-2	0.5	20	0.1	60
CSS/PEGDMA-3	1	20	0.1	130
CSS/PEGDMA-4	0.5	10	0.1	480
CSS/PEGDMA-5	1	10	0.1	530

N_2 atmosphere. The obtained reaction solution was filtered, and then concentrated by rotary evaporation at 60 °C. PEGDMA was precipitated from the concentrated solution by the addition of cold diethyl ether and obtained by filtration. The precipitate was washed with diethyl ether three times and then dried in a vacuum oven at room temperature. The reaction molar ratio of PEG : TEA : methacryloyl chloride was 1 : 2 : 2.

2.1.4 Preparation of CSS/PEGDMA gels. CSS/PEGDMA gels were prepared by irradiated mixed solutions of I2959, CSS and PEGDA, and the concentration of the three components in the mixed aqueous solution is shown in Table 1. The gelation time is defined as the time the solution in the vial stopped flowing when the vial is tilted and irradiated by a UV lamp. The solutions prepared, as described in Table 1, were pipetted into 48-well cell culture plates and the volume in each well was 200 μL. Then, disk-shaped gels (diameter = 12 mm) were obtained by UV irradiation at the wavelength of 365 nm for a period specified in each experiment.

2.2 Characterization methods

2.2.1 Fourier transform infrared reflection (FTIR) spectroscopy. The FTIR spectra of CSS and PEGDA were recorded using a Nicolet 6700 instrument equipped with an Attenuated Total Reflection (ATR) (Thermo Company, USA) attachment in the wavenumber range of 4000 to 650 cm^{-1}.

2.2.2 Nuclear magnetic resonance spectroscopy (^{1}H NMR) analysis. ^{1}H NMR analysis spectroscopy was carried out on an Advance 400 (400 MHz) Bruker NMR equipped with the MestReC processing software. The solvent used was D_2O for CSS and $CDCl_3$ for PEGDMA.

2.2.3 Measurement of the swelling ratio. The swelling ratio of the prepared gels was obtained by the weighing method. In detail, the disc-shaped hydrogels were allowed to swell at 37 °C after the application of 10 mL phosphate buffered solution (PBS, pH = 7.2) in triplicate. After 24 hours, the liquid on the surface of the samples was softly wiped off with a filter paper, then weighed and the weight (W_s) recorded. The samples then were dried in a vacuum oven at 37 °C overnight, and then weighed and the weight recorded (W_d). The swelling ratio was calculated as follows:

$$\text{Swelling ratio (wt\%)} = (W_s - W_d)/W_d$$

where W_s is the weight of the swelling equilibrium hydrogels, and W_d is the weight of the dried hydrogels.

2.2.4 Degradation study. The *in vitro* degradation experiment of the prepared samples (n = 3) was performed at 37 °C in PBS (pH = 7.2) solution, and the degradation property was assessed by the percentage of weight loss. The time point that the experimental swollen hydrogels began to lose weight is the onset of degradation, and the weight of the swelling equilibrium hydrogels was denoted as W_o. After the PBS solution on the samples' surface was removed at a predetermined time point, the samples were weighed and the weight recorded, and these values were set as W_t. The samples were immersed into PBS in an incubator set at 37 °C, and the PBS solution was refreshed at every point after the testing hydrogels were weighed. The weight loss ratio of the hydrogel samples was calculated as follows:

$$\text{Weight loss ratio (wt\%)} = (W_o - W_t)/W_s$$

where, the W_o is the weight of the swelling equilibrium hydrogels when the hydrogels initially lost weight and W_t is the weight of the hydrogels at time t.

2.2.5 Adhesion strength measurement. The adhesion strength of adhesives is generally represented by the lap shear strength. Gelatin coated glass and SD rat skin were used as the adherent for the measurement of the adhesion strength. The lap shear strength was measured according to the previous method described by Ai Yufei *et al.*[28] Firstly, rectangle glass slides (10 mm × 30 mm) were coated with 20 wt% gelatin solution on one side to mimic living tissue. Secondly, the solutions that were prepared, as shown in Table 1, were dropped and spread uniformly on the gelatin coated glass, then the gelatin coated glass was overlapped with another gelatin coated glass, and the adhesion area was 10 mm × 10 mm. The overlapped glass slides were clamped tightly and then irradiated for 10 min under UV light at the light intensity of 15 mW cm^2. The photocured samples were stored in an incubator at 37 °C for 3 h to ensure that the reaction was completed. Three hours later, the samples were measured using a testing machine (HY-940FS, Shanghai Hengyu Co., Ltd) with a crosshead speed of 5 mm min^{-1} at room temperature.

Furthermore, the adhesion strength was also tested in SD rat skin, which was used as living tissue adherent. SD rats were sacrificed for the test, then their hair was removed with a shaver and the rat skin was peeled off for the adhesion strength measurement. The rat skins were cut into strips (10 mm × 25 mm), the prepared solutions were added onto a strip, and then the strip was covered with another strip. All the samples for adhesion measurement were irradiated by a UV lamp for 10 minutes and then stored in an incubator at 37 °C for 1 h. After 1 h incubation, the lap shear strength was measured with the testing machine at a speed of 5 mm min^{-1}. For each group the two experiments were repeated five times and finally the average values were recorded.

2.2.6 Compression test of hydrogels. Hydrogel discs (diameter = 12 mm, thickness = 2.5 mm) were prepared according to the method described in Section 1.1.4 in this paper. Unconfined, uniaxial compression testing was carried out using a materials testing system (WDW 3230, Changchun Kexin testing machine, China). The hydrogels were compressed

at a rate of 5 mm min^{-1} until they were fractured. The maximum compressive stress and maximum compressive strain were obtained from the stress–strain curve and compressive elastic modulus (E) was taken from the slope of the stress–strain curve at a strain range of 0.1–0.3.

2.2.7 *In vitro* cell compatibility. The cytotoxicity of the photo-crosslinking hydrogels was evaluated based on a procedure adapted from the ISO 10993-5 standard test method. The prepared hydrogels were fumigated and sterilized in 75% ethanol vapor for 5 hours, and then transformed to centrifuges tube containing 20 mL DMEM at a concentration of 1.0 mg mL^{-1} after washing 3 times with sterilized PBS. The centrifuge tubes were cultured for 48 hours in an incubator set at 37 °C and 100 rpm. The extract was obtained after the hydrogels were removed, and subsequently stored in a refrigerator at −20 °C. Mouse fibroblasts, L929, were cultured in DMEM supplemented with 10% FBS and 1% pen per step under standard culture conditions at 37 °C and 5% CO_2. The L929 cells were seeded into 48-well cell culture plates at a concentration of 10×10^4 cells per well. After 24 h incubation, the culture medium was removed and replaced with the stored extraction medium, and then incubated for another 24 h, 48 h, and 72 h under the same conditions. 30 μL of 5 mg mL^{-1} MTT assay solution and 200 μL of DMEM were added to each well at a predetermined time after the culture medium was removed, and then returned back to incubate for 4 h. The medium containing unreacted MTT was removed. Then 200 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals, and after incubation for 15 min at 37 °C, the optical absorbance values of the solutions were measured in an ELISA reader (Multiscan GO, Thermo Scientific) at a wavelength of 570 nm to determine the number of living cells. L929 cells were seeded in a fresh culture medium as the negative control for comparison.

2.2.8 Statistical analysis. Statistical analysis was performed using the Origin Pro software 8.0 software (OriginLab Corporation). One-way analysis of variance (ANOVA) followed by a Bonferroni test was conducted. A p-value <0.05 was considered statistically significant.

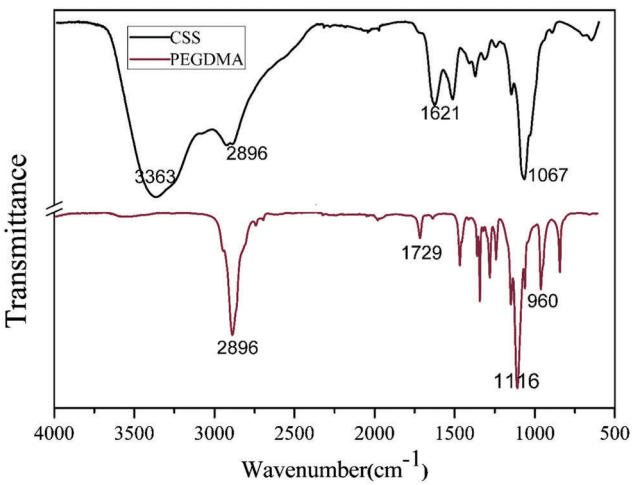

Fig. 1 FTIR spectra of CSS and PEGDMA.

3. Results and discussion

3.1 The structures of CSS and PEGDMA

Fig. 1 illustrates the FTIR spectra of CSS and PEGDMA. The main spectral features of CSS (black line) show a broad band at 3363 cm^{-1}, which is attributed to the O–H and N–H stretching vibration of CSS molecules, a peak at 1067 cm^{-1}, which is attributed to the C–O–C stretching vibration of the backbone, and a peak at 1621 cm^{-1}, which is assigned to the C═O stretching vibration of the ester bond produced by the reaction between chitosan and NAC. Furthermore, the peak at 2896 cm^{-1} is wide and broad, and it is believed that the S–H of NAC and the C–H of the chitosan contribute to this signal peak. The spectrum of PEGDMA (blue line) has a sharp single peak, which is assigned to the $-CH_2$ stretching vibration of the PEGDMA main chain. The strong peak at 1116 cm^{-1} is assigned to the C–O stretching vibration of PEG, and the C–O bond is the ether bond of PEGDMA. The peak at 1729 cm^{-1} is assigned to the C═O stretching vibration of the ester bond, and the peak at 843 cm^{-1} is assigned to the C═C double bond. These two peaks are attributed to the esterification between PEG and methacryloyl chloride (Scheme 1b).

CSS was synthesized by grafting NAC to chitosan (Scheme 1a), and NAC is a derivative of L-cysteine which is an amino acid containing thiol group. The structure of CSS was confirmed by ^1H NMR. From Fig. 2, the peak at 1.87 ppm is attributed to the

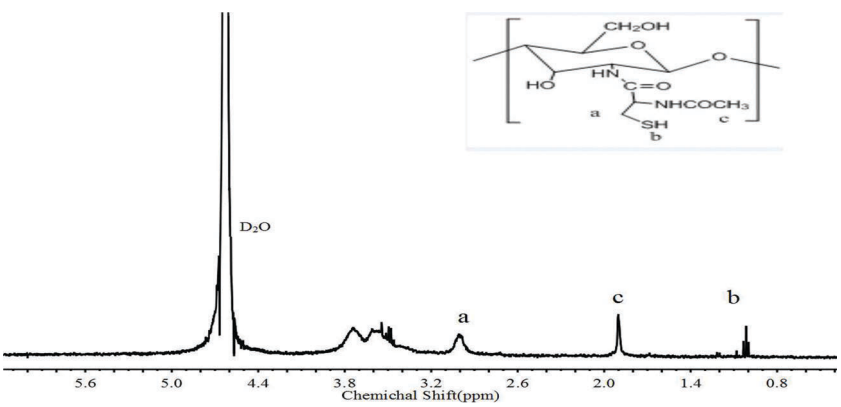

Fig. 2 ^1H NMR spectrum of CSS dissolved in D_2O.

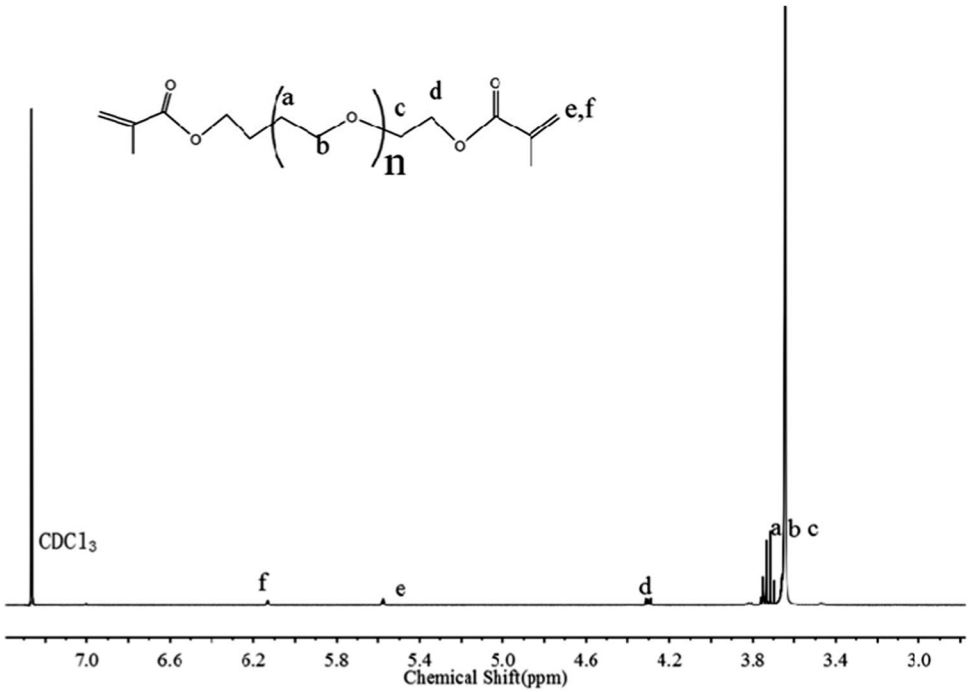

Fig. 3 ^{1}H NMR spectrum of PEGDMA dissolved in $CDCl_3$.

N-acetyl methyl proton of CSS and the peaks at 3.12 ppm and 1.12 ppm are believed to correspond to the side chain methylene and thiol group of CSS, respectively. According to the Ellman's method, we calculated that the content of thiol groups on CSS is 312.6 μmol g^{-1}.

The typical ^{1}H NMR spectrum of PEGDMA in $CDCl_3$ is shown in Fig. 3. The peaks for PEGDMA are 3.63 ppm (114.2H, –H of PEG main chain), 4.26–4.31 ppm (t, 2.21H, $CH_2C\underline{H}_2$–$OCOC(CH_3)$═CH_2), 5.55–5.60 ppm, and 6.10–6.13 ppm (dd, 1.0H, $C\underline{H}_2$═$C(CH_3)COO$–). The peak at 5.55–5.60 ppm (double bond protons) shows that the double bonds (C═C) of methacryloyl chloride are attached to the molecular chain.

3.2 *In situ* formation and characterization of the hydrogels

Four different photo cross-linked hydrogels were prepared by UV light irradiation. The gelation time and mechanical properties were significantly influenced by the content of the initiator, I2959, and the concentration of the polymers. Biocompatible initiators have not been discovered to the best of our knowledge. Obviously, the reaction would be fast when the initiators added much, but the initiator I2959 is not harmless. Stephanie J Bryant reported that I2959 decreases cell activity when its content exceeds 0.1 wt%. Hence, the concentration of the initiator I2959 was controlled at 0.1 wt% in this paper. Even with a little initiator (0.1%), thiol–acrylate photopolymerization occurred quickly, and the gelation time of the hydrogels was between 30 seconds to 550 seconds. This is changeable since the concentration of CSS and PEGDMA significantly affects the gelation time of the hydrogels. When the PEGDMA and I2959 concentration was kept constant, the CSS content added was between 0.5 to 1%, the gelation time increased from 60 s (CSS/PEGDMA-2) to 120 s (CSS/PEGDMA-3). When the concentration of PEGDMA was decreased from 20 wt% to 10 wt%, the gelation time significantly decreased, for example, the gelation time of CSS/PEGDMA-2 was 60 s, whereas the gelation time of CSS/PEGDMA-4 was 480 s. Compared to CSS/PEGDMA-1, the gelation time of CSS/PEGDMA-4 and CSS/PEGDMA-5 increased to ~450 s and ~500 s, respectively. These results indicate that the gelation time is influenced significantly by the weight ratio of CSS and PEGDMA in the hydrogels.

The equilibrium swelling ratio and degradation of the hydrogels are shown in Fig. 4a and b, respectively. The swelling and degradable properties are important indicators for hydrogels that are applied as biomaterials. Fig. 4a shows that the sample CSS/PEGDMA-4 (1 wt% CSS and 20 wt% PEGDMA) has the largest swelling equilibrium ratio of all the prepared hydrogels, and the swelling ratio of CSS/PEGDMA-2 (0.5 wt% CSS and 20% PEGDMA) was 300%, which is the smallest of the prepared samples. The swelling ratio of the hydrogels decreased as the CSS content added increase from 0 to 0.5 wt%, and then increased. It is believed that with an increase in the concentration of CSS, the high weight molecular CSS would have a "big" space to stretch its long chain when the hydrogels were dipped into PBS solution. The degradation of the hydrogels was evaluated by the weight loss of the two samples that had different contents of PEGDMA and a constant amount of CSS. The PEGDMA/CSS hydrogels degraded slowly in PBS solution, which were incubated in an incubator set at 37 °C. Fig. 4b shows that the degradation rate of the hydrogels increased when the concentration of PEGDMA in the hydrogels was reduced. It is supposed that the entanglement and involvement of the chains of CSS and the molecule interaction forces contributed to the degradation of the hydrogels.

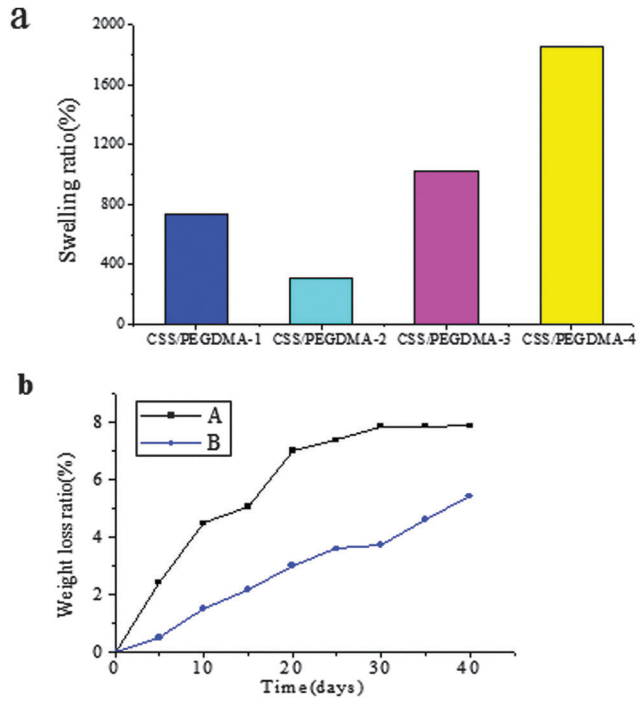

Fig. 4 Swelling ratio of the prepared hydrogels (a) and weight loss of the hydrogels (b): (A) 20% PEGDMA–1% CSS (black squares) and (B) 25% PEGDMA–1% CSS (blue dots). The photo initiator I2959 of the two hydrogels was controlled at 0.1 wt%.

3.3 Evaluation of adhesion strength

The adhesion strength of the bioadhesive is represented by the lap shear adhesion in this paper. The lap shear adhesion of the hydrogels and the appearance of adherents after the lap shear strength was measured are shown in Fig. 5. As seen in Fig. 5A, the adhesion strength of CSS/PEGDMA-3 (252.36 ± 3.8 kPa) was the highest of all the samples, and the hydrogels without CSS (CSS/PEGDMA-1) did not bond to the adherent tightly (15.6 ± 6.5 kPa). Its lap shear strength was much smaller than the fibrin adhesive (49.53 ± 2.1 kPa), which was regarded as the control group, whereas the hydrogels containing CSS were bigger than the fibrin adhesive. Therefore, the adhesion strength increased with an increase in the content of CSS in the CSS/PEGDMA hydrogels, whereas the concentration of PEGDMA in the hydrogels slightly influenced the bond strength of the adhesive. For example, the adhesive strength of CSS/PEGDMA-2 was 182.6 ± 7.35 kPa and CSS/PEGDMA-4 was 175.4 ± 1.16 kPa, and comparing CSS/PEGDMA-2 with CSS/PEGDMA-4, the adhesion strength of CSS/PEGDMA-2 was not dramatically higher than CSS/PEGDMA-4 although the concentration of PEGDMA in the two hydrogels was different. The results indicate that the thiol group (–SH) was the key to the adhesion of the thiol–acrylate adhesives since it is biologically reactive. The thiol group can react with –COOH, –OH, –SH or other groups, and these active groups mostly exist in proteins, saccharides and fat which are the main components of biological tissue. The –SH group of CSS would react with the active group of the gelatin that coated the glass slides and then hold the two glass slides together, hence the adhesion strength of the hydrogels is mostly dependant on the quantity of CSS.

The lap shear strength of the adhesive was also measured by using a SD rat skin model as the living tissue adherent. As shown in Fig. 5B, the lap shear strength of CSS/PEGDMA-3 was 48.73 ± 0.56 kPa, which was the highest of all the tested samples. The adhesion strength of CSS/PEGDMA-1 was 16.32 ± 0.76 kPa, and obviously, it was significantly lower than the adhesion strength of CSS/PEGDMA-2, CSS/PEGDMA-4 and CSS/PEGDMA-5. These results indicate that the thiol group (–SH) plays an important role in the adhesion of the thiol–acrylate adhesives, even if the adhesion strength decreased when the adherent was changed from gelatin coated glass to SD rat skin. Fig. 5C and D show the appearance of the gelatin coated glass slides and SD rat skin, respectively, after adhesion strength was measured. It is observed that the adhesion failed between the gelatin coated glass slides and SD rat skin. There were visible hydrogels left on the two pieces of glass (Fig. 5C), which show that the adhesives bonded to the glass slides when they was photocured. There is nothing visible left on the SD rat skin after adhesion strength was measured, which indicates that the interface between the rat skin and hydrogels was thin and CSS and PEGDMA were cross-linked with the rat skin through chemical bonds or physical interactions (intermolecular forces and hydrogen bond).

3.4 Compression properties

The hydrogels that were used for the compression test were prepared as described in Section 1.1.4. Without any other treatment, the samples (n = 3) were tested in a mechanical testing instrument equipped with a compression fixture directly to determine their mechanical properties. The uniaxial unconfined compression testing results are shown in Table 2.

The calculated elastic modulus (E) of the gels increased when the concentration of the precursor solution and the content of CSS was increased. Additionally, CSS/PEGDMA-3 exhibited the highest maximum compressive stress and elastic modulus (380 ± 10.97 kPa and 1317.85 ± 277.23 kPa, respectively). The result of the uniaxial unconfined compression reveal that the hydrogels prepared *via* the thiol–acrylate reaction were likely to have excellent mechanical properties. Despite the high maximum stress of the CSS/PEGDMA hydrogels, the strain was significantly decreased as the CSS content increased, and it is believed that the physical interaction forces between PEGDM and CSS would be the main force to support the hydrogel structure, and that the hydrogel is loosely porous. This indirectly explains the fact that the swelling ratio of CSS/PEGDMA-3 was higher than CSS/PEGDMA-2 when the concentration of CSS increased from 0.5 wt% to 1 wt%. Furthermore, the maximum stress of CSS/PEGDMA-4 (69.6 ± 6.54 kPa) and CSS/PEGDMA-5 (130 ± 14.14 kPa) was weaker than CSS/PEGDMA-2 (176 ± 6.92 kPa) and CSS/PEGDMA-3 (380 ± 10.97 kPa), where the PEGDMA concentration of the former is dilute to double of the latter. Researchers have found that the concentration of the constituents is an important factor to consider for the compressive performance of hydrogels, in which a higher concentration usually means a higher compressive

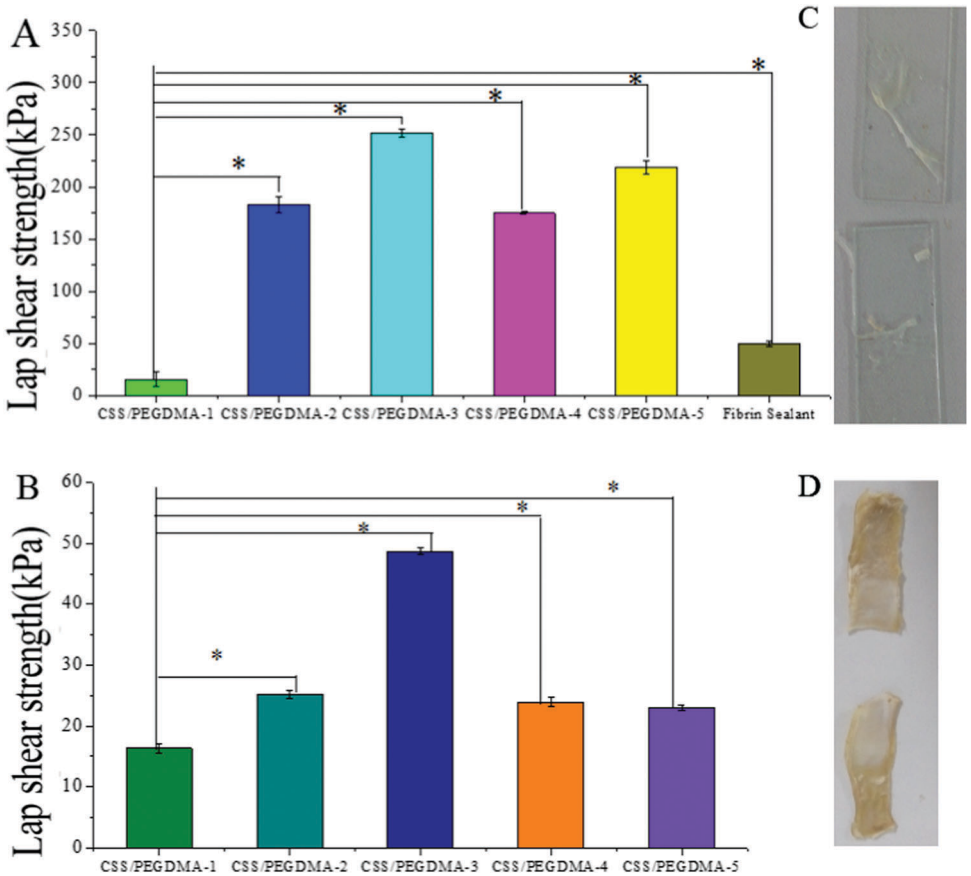

Fig. 5 Lap shear strength of the prepared hydrogels using different adherents: (A) gelatin coated glass and (B) SD rat skin. (C and D) The appearance of the gelatin coated glass slides and SD rat skin, respectively, after the adhesion strength was measured. ''*'' Indicates statistically significant differences ($p < 0.05$) compared to CSS/PEGDMA-1.

Table 2 Results of uniaxial unconfined compression testing on the prepared hydrogels

Hydrogels	Max stress (kPa)	Max strain	Elastic modulus (E, kPa)
CSS/PEGDMA-1	175 ± 7.07	24.34 ± 1.89	214.48 ± 21.40
CSS/PEGDMA-2	176 ± 6.92	22.36 ± 2.33	235.20 ± 41.95
CSS/PEGDMA-3	380 ± 10.97	8.29 ± 0.43	1317.85 ± 277.23
CSS/PEGDMA-4	69.6 ± 6.54	6.59 ± 0.36	508.60 ± 45.39
CSS/PEGDMA-5	130 ± 14.14	6.61 ± 0.24	653.39 ± 3.93

stress. Therefore, the compressive results of the prepared hydrogels are consistent with previous works.

3.5 Cytotoxicity assays

The cytotoxicity assay is an important and basic property of biomaterials, and the quantitative MTT cytotoxicity assay was performed by exposing L929 fibroblast cells to the extraction medium (10% FBS and 1% pen per step) of the prepared CSS/PEGDMA hydrogels. The absorbance illustrating of the viability of the L929 cells is shown in Fig. 6. The average optical absorbance values of the testing samples ($n = 4$) increased with the time running. Compared to the negative control, which was incubated in DMEM containing 10% FBS and 1% pen per step under standard culture conditions at 37 °C and 5% CO_2,

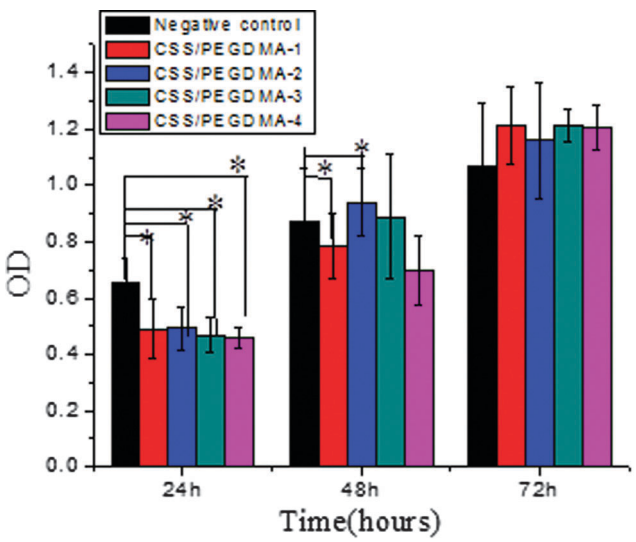

Fig. 6 MTT test of the prepared hydrogels at 24, 48, and 72 h. The data represent the mean and standard deviations of five samples (*: statistically significant differences ($p < 0.05$), which were compared to the negative control at the same incubation time).

statistically significant differences ($p < 0.05$) were discovered at 24 h for the extraction medium of the prepared hydrogels.

However, after 48 hours, no statistically significant differences ($p < 0.05$) were found in the extraction media of CSS/PEGDMA-2 and CSS/PEGDMA-3, and all of the extraction media did not show statistically significant differences ($p < 0.05$) after 72 h. This result indicates that the extraction media of the prepared hydrogels impact L929 cell proliferation in a short time (24 h). These results strongly suggest that the CSS/PEGDMA hydrogels are less toxic to L929 cells.

4. Conclusion

In this study, a photocurable bioadhesive hydrogel based on PEGDMA and CSS was prepared and investigated. PEGDMA and CSS were synthesized and characterized *via* FTIR and ^{1}H NMR. The adhesive hydrogel was formed *in situ* through a thiol–acrylate addition reaction by UV irradiation. The equilibrium swelling ratio, degradation behavior, compression properties and adhesive strength of the hydrogel adhesive were studied. We also evaluated the cytotoxicity of the prepared hydrogels by incubating L929 fibroblast cells with the extraction medium of the hydrogels.

Our findings demonstrate that PEGDMA was successfully synthesized and the thiol group was grafted to chitosan by the reaction between NAC containing a thiol group and chitosan. The compressive experiment of the hydrogels showed that the hydrogels have superior mechanical properties, and the result of the degradation test showed that the hydrogels are degradable. The adhesion strength of the CSS/PEGDMA adhesive was significantly larger than the common commercially available fibrin glues, and it was indicated that the adhesive force of the adhesive can be attributed to the reactive thiol group of CSS. The MTT assays showed that the CSS/PEGDMA hydrogel is biocompatible and less toxic to L929 fibroblast cells. In conclusion, the photocrosslinkable hydrogel has strong adhesion strength and compression strength and it is biodegradable and biocompatible. The method used to prepare the hydrogel adhesive *via* the thiol–acrylate reaction may provide a route for the development of bioadhesives.

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