Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

An in Situ Forming Tissue Adhesive Based on Poly(ethylene glycol)-Dimethacrylate and Thiolated Chitosan Through Michael Reaction

Zhiwen Zeng [a], Xiu-mei Mo [a, b, f, †], Chuanglong He [b], Yosry Morsi [c], Hany EI-Hamshary [d, e], Mohamed El-Newehy [d, e]

[a] *State Key Laboratory for Modification of Chemical Fibers and Polymer Materials，College of Materials Science and Engineering, Donghua University, Shanghai 201620,China.*

[b] *College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, China*

[c] *Faculty of Engineering and Industrial Sciences, Swinburne University of Technology, Hawthorn, Vic 3122, Australia*

[d] *Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia*

[e] *Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt*

[f] *Shandong International Biotechnology Park Development Co, Ltd*

[†] Correspondence should be addressed to Xiumei Mo, *E-mail: xmm@dhu.edu.cn.*

Abstract: In this paper, a novel biocompatible and biodegradable tissue adhesive composed of poly (ethylene glycol) –dimethacrylate (PEGDMA) and thiolated chitosan (CSS) was prepared. PEGDMA and CSS cross-linked rapidly under physiological conditions through Michael addition reaction by UV lamp irradiation. The chemical structures of PEGDMA and CSS were confirmed by FTIR and ^{1}HNMR. The equilibrium swelling ratio and biodegradation of the hydrogels were tunable through varied the components ratio of the hydrogels. The resulting hydrogels were measured for the compression strength and adhesive strength with tensile tester, and the adhesion strength of the hydrogel was higher than the fibrin glues. Moreover, the cytotoxicity of PEGDMA/CSS hydrogels for L929 cells was evaluated by MTT assay, the result was indicated the photocured hydrogels were biocompatible and less cytotoxicity toward the growth of L929 cells. These findings implied that the obtained hydrogel adhesives would be a potential bioadhesive for clinical application in the future.

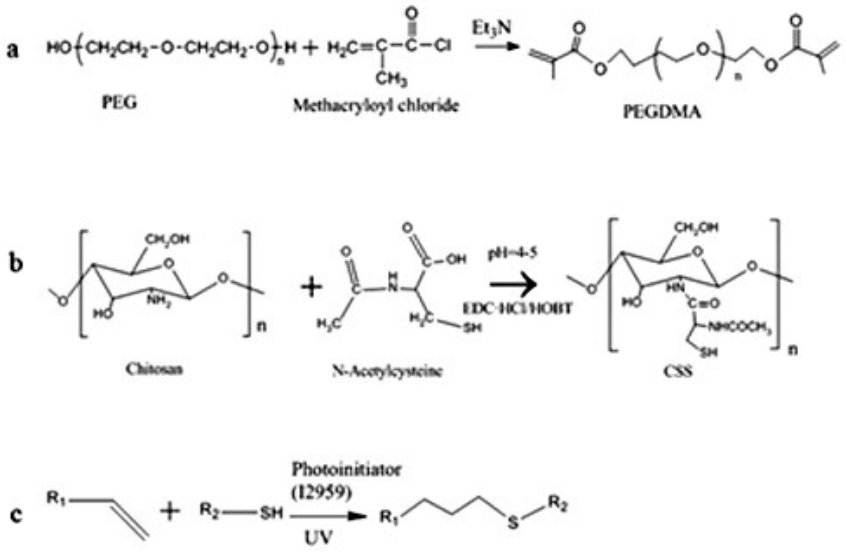
Key words**:** *poly (ethylene glycol) (PEG); hydrogels; bioadhesive; poly (ethylene glycol); chitosan*

Introduction

Hydrogels are three-dimensional polymeric networks that are analogy to the structure of extracellular matrix (ECM) [1]. They are broadly utilized in various applications including tissue adhesive [2], drug release loader [3], protein or RNA delivery vehicles, extracellular matrix for tissue engineering and surgical dressing [4]. Hydrogel adhesives are used for tissue adhesive and have been developed in many years considering their advantages [5], especially in recent decades. Compared with the biological adhesives such as cyanoacrylates, fibrin glue, which are the leading product of commercially available tissue adhesive [6], the hydrogel adhesives are more safe than

the cyanoacrylates and have higher adhesive strength than fibrin glue. The degradation products of the hydrogel adhesives are nontoxic. [7] However cyanoacrylates generate formaldehyde harm to patients when they are degraded in body, [8] while fibrin glue has a weakness in its material source for industrial production and it is risk to infect and transmit zoonotic disease. [9] Researchers proposed the ideal adhesive should fulfill following criterion: strong and rapid adhesive, no or low immunogenicity, less expensive, biocompatibility, facility storage, and biodegradability. [10] There is not any report about the adhesives meet all the criteria for an ideal biological adhesive or sealant.

Chitin is the second abundant polysaccharide after cellulose and widely distributed in nature. Chitosan is the partially N-deacetylation product of chitin and has been used as biomedical material [11]. Chitosan is biodegradable and nontoxic, and can accelerate wound healing and 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 applied for tissue adhesive, hemostasis and medical dressing. Chitosan can be modified with functional groups, [15-17] such as thiol group, maleimide group, to improve its solubility at physiological pH. The thiol-modified chitosan is gaining popular lately as the thiol-acrylate reaction ((Scheme 1c) is one of Michael additional reaction which is a mild reaction, nontoxic and no side reaction. Besides, the hydrogels composed of thiol-modified polymers and the polymers containing acrylates or methacrylates were formed in situ under physiological conditions without byproducts. [18-20] Michael additional crosslinking can avoid the utility of cytotoxic free-radicals H_2O_2, [21] photoinitiators, and UV light, but the crosslinking rate is not fast enough to meet the standard of the clinics. Recently the degradable thiol-acrylate polymers are regard as a new class of materials that capable of quickly polymerizing via UV irradiation. [22] Amber E. Rydholm [23] et al reported that thiol-acrylate polymers afford a much high degree of control over its properties because of the versatility of their chemistry.



Scheme 1. Schematic of a: the synthesis route of PEGDMA; b: the synthesis route of CSS; c: preparation of hydrogels via thiol-acryl reaction.

Many efforts have been done to prepare suitable alternatives of the available bioadhesive. Researchers have developed a lot of tissue adhesives, for example, nanocomposite hydrogels [24], mussel inspired polymers [25], and nanoparticles solutions [26], and so on. The aim of our research is to develop a photo-crosslinkable CSS/PEGDMA hydrogel adhesive, which formed in situ rapidly exposure to UV lamp (Scheme 1). FTIR and ^{1}HNMR was used to confirm the chemistry structure of the functional polymers. the swelling ratios, compressive strength, adhesion strength and degradable properties of the prepared hydrogels have been investigated. Furthermore, the cell compatibility in vitro of the hydrogels was test on L929 cells, and the result suggested that the hydrogel prepared here is nontoxic to the L929 cell. The paper provided an alternative route for develop tissue adhesive used for wound healing and hemostasis.

1. Experimental

1.1 Materials and methods

1.1.1 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 K Da) 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]-2,5diphenyltetrazolium bromide, (MTT) was purchased from Biosharp, Fetal bovine serum (FBS) and 0.25% Trypsin-EDTA was purchased from Gibco, dulbecco's modified eagle medium (DMEM, high glucose) was purchased from Hyclone.

1.1.2 Thiolation of chitosan

The modification of chitosan with thiol group was prepared referring to a previous paper [15]. Typically, 0.5 g of chitosan powder was dispersed in 50 mL ultrapure water on a flask under stirring. After 20 minutes, HOBT (2.58mmol) was added, and the solution became clear under stirring, then NAC (5.16mmol) was added to the mixture solution followed by the addition of a solution of EDC•HCl (10.32mmol). The reaction was proceeded for 5 hours at room temperature and the pH value of the reaction solution was keeping 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 group was determined using the Ellman's test.

1.1.3 Synthesis of PEGDMA

The liner PEG (2 K Da) was dissolved in toluene on 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 degased with N_2 for 30 min. Triethylamine (TEA) was dissolved in dichloromethane (DCM) at the volume ratio of 1:9, then

added to the PEG solution with stirring. The reaction begun with the Methacryloyl chloride added into the mixture solution dropwise at the condition of ice bath. The reaction proceeded for 24 h in the dark under N_2 atmosphere. The obtained reaction solution was filtered, and then concentrated by rotary evaporation at 60 °C. The PEGDMA was precipitated from the concentrated solution by addition of cooled diethyl ether and obtained by filtration. The precipitate was washed with diethyl ether for three times and then dried in vacuum drying oven at room temperature. The react molar ratio of the PEG: TEA: Methacryloyl chloride was 1:2:2.

1.1.4 Preparation of CSS/PEGDMA gels

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

1.2 Characterization methods.

1.2.1 Fourier transform infrared reflection (FTIR) spectroscopy

The FTIR spectra of CSS and PEGDA were recorded by using a Nicolet 6700 instrument equipped with Attenuated Total Reflect (ATR) (Thermo Company, USA) over the wave number range from 4000 to 650 cm^{-1}.

1.2.2 Nuclear magnetic resonance spectroscopy (^{1}HNMR) analysis

^{1}HNMR analysis spectroscopy was carried out on Advance 400 (400 M Hz) of Bruker NMR equipped with MestRec processing software, the testing solvent was D_2O for the CSS and it was $CDCl_3$ for PEGDMA.

1.2.3 Measurement of 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 applied 10 mL phosphate buffered solution (PBS, pH=7.2) in triplicates. Followed after 24 hours, the liquid on the surface of the samples was softly wiped off with filter paper and then weighed and recorded the weight(*Ws*). The samples then were dried in a vacuum oven at 37 °C overnight，and then weighed and recorded(*Wd*). The swelling ratio was calculated as follows:

$$\text{Swelling ratio (wt. \%)} = (Ws - Wd) / Wd$$

Where *Ws* is the weight of the swelling equilibrium hydrogels, and the *Wd* is the weight of the dried hydrogels.

1.2.4 Degradation study

The in vitro degradation experiment of the prepared samples(n=3) were 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 experimental swollen hydrogels begun to lose weight is the onset of degradation, and the weight of the swelling equilibrium hydrogels was denoted as *Wo*. After removed the PBS solution on the samples' surface at predetermined time point, the samples weighed and recorded, and the values were set as *Wt*. The samples were immersed into the PBS in

an incubator set at 37 °C, and the PBS solution was refreshed at every point after the testing hydrogels weighed. The weight loss ratio of the hydrogel samples was calculated as follows:

$$\text{Weight loss ratio (wt. \%)} = (Wo - Wt) / Ws$$

Where the *Wo* is the weight of the swelling equilibrium hydrogels when the hydrogels initial lost weight and *Wt* is the weight of the hydrogels at the time *t*.

1.2.5 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 previous method described by Ai Yufei et al [28]. Firstly, the rectangle glass slides (10mm×30 mm) were coated with 20 wt% gelatin solution on one side to mimic the living tissue. Secondly, the solutions which 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 light intensity of 15 mW/cm^2. The photocured samples were stored in the incubator at 37 °C for 3 h to ensure reaction completed. Three hours later, the samples were measured by using a testing machine (HY-940FS, Shanghai Hengyu Co., Ltd) with a crosshead speed of 5 mm/min at room temperature.

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

1.2.6 Compression test of hydrogels

Hydrogel discs (diameter=12mm, thickness=2.5mm) were prepared according to the method described in 1.1.4 in this paper. Unconfined, uniaxial compression testing was carried out using a materials testing system (WDW3230, Changchun Kexin testing machine company, China). Hydrogels were compressed at a rate of 5 mm/min until it was fractured. 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.

1.2.7 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 the 75% ethanol vapor for 5 hours, and then transformed to the centrifuge tube containing 20mL DMEM at a concentration of 1.0mg/mL after washed 3 times with the sterilization PBS. The centrifuge tubes were cultured for 48 hours in the incubator that set 37 °C and 100rmp. The extraction was obtained after the hydrogels were removed, and subsequently stored in refrigerator at -20 °C. Mouse fibroblasts L929 were cultured in DMEM supplemented

with 10% FBS and 1% pen/step under standard culture conditions at 37 °C and 5% CO_2. 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 24h, 48h, and 72h under the same conditions. 30 μL of 5 mg/mL MTT assay solution and 200 μL of DMEM were added to each well at the predetermined time after removed the culture medium, and then returned back to incubate for 4h, the medium containing unreacted MTT was removed. Then 200μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystal, after incubated 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 to a fresh culture medium as the negative control for comparison.

Table 1. The ratio of 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

1.2.8 Statistical Analysis

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

2 Results and Discussion

2.1 The structures of CSS and PEGDMA

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

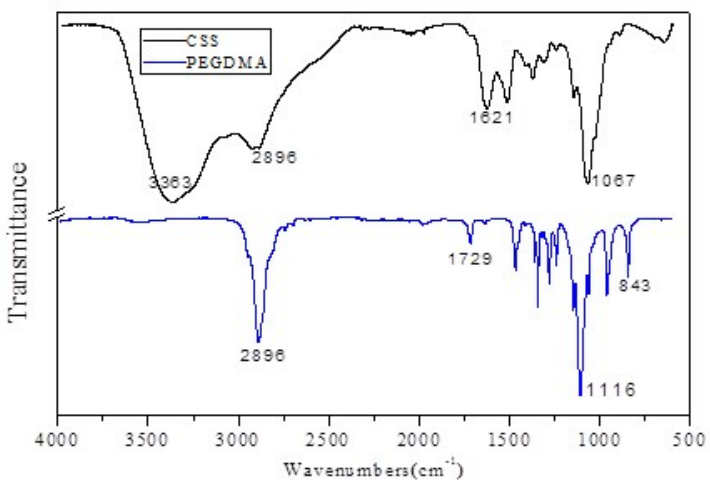


Fig 1. FTIR spectra of CSS and PEGDMA.

CSS was synthesized by grafting NAC to the chitosan (Scheme 1a), and NAC is a derivative of L-cysteine that is an amino acid containing thiol group. The structure of CSS was confirmed by ^{1}HNMR. From Fig 2, the peak at 1.87 ppm was attributed to the N-acetyl methyl proton of CSS and the peak at 3.12 ppm and 1.12 ppm was believed to correspond to the side chain methylene and thiol group of CSS, respectively. According to the Ellman's method, we have calculated that the content of the thiol groups on CSS was 312.6 μ mol/g.

The typical ^{1}HNMR spectra of PEGDMA in $CDCl_3$ was shown in Fig 3. For PEGDMA: 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, 6.10-6.13ppm (dd, 1.0H, $C\underline{H}_2$=C (CH_3) COO-). The peak at 5.55-5.60 ppm (double bond protons) represented that the double bonds (C=C) of methacryloyl chloride were attached to the molecular chain.

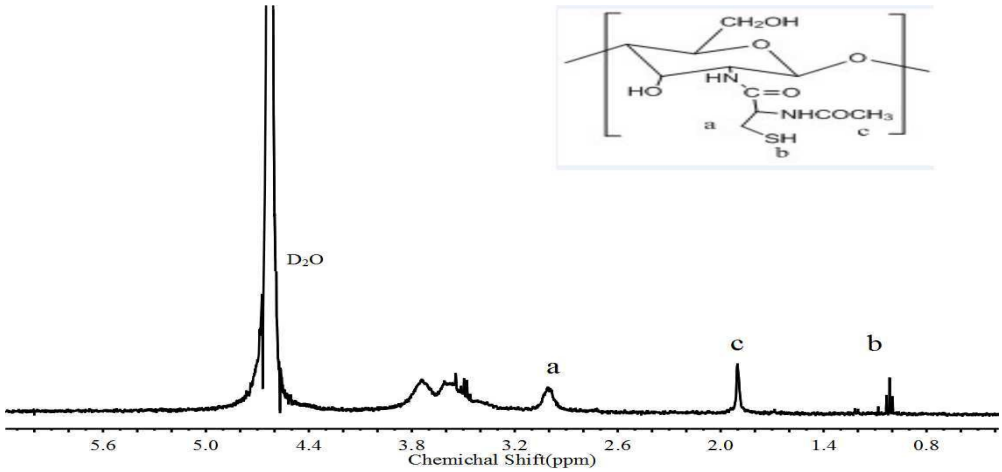


Fig 2. ^{1}HNMR spectra of CSS which was dissolved in D_2O.

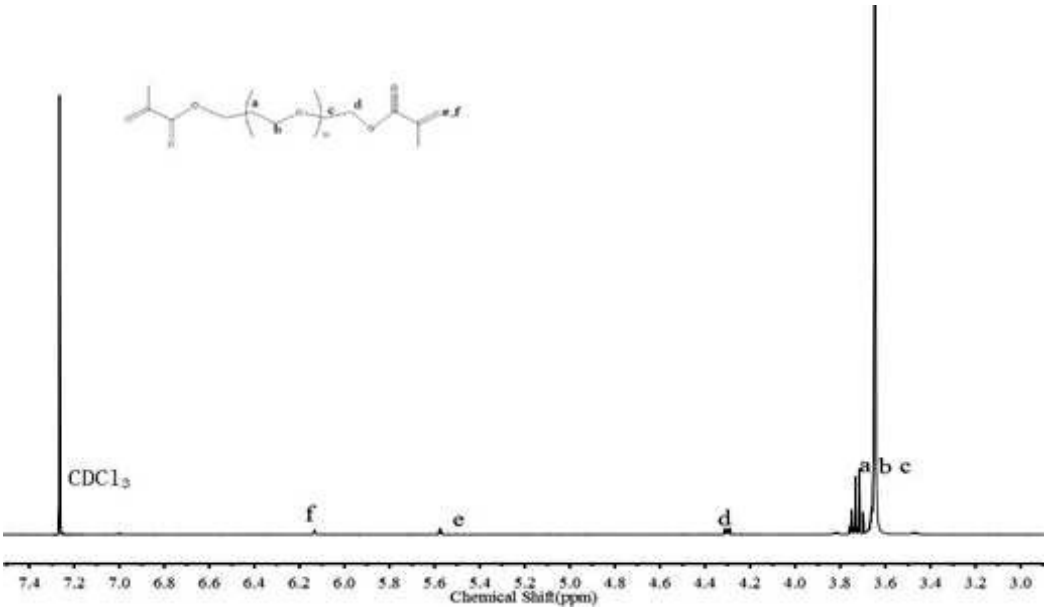


Fig 3. ^{1}HNMR spectra of PEGDMA which was dissolved in $CDCl_3$.

2.2 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. The biocompatible initiators have not been discovered in our knowledge. Obviously, the reaction would be fast when the initiators added much, but the initiators I2959 is not harmless. Stephanie J Bryant reported that I2959 would decrease the cells activity when its content was exceeded 0.1 wt%. So the concentration of the initiator I2959 was controlled at 0.1 wt% in this paper. Even with a little initiator (0.1%), Thiol-acrylate photopolymerizations reacted fast, the gelation time of the hydrogels was from 30 seconds to 550 seconds. It was changeable as the concentration of the CSS and PEGDMA would significantly impact the gelation time of the hydrogels. When the PEGDMA and I2959 was keeping constant, the CSS added from 0.5 to 1%, the gelation time would be increased from 60 s (CSS/PEGDMA-2) to 120 s (CSS/PEGDMA-3). When the concentration of the PEGDMA was decreased from 20 wt% to 10 wt%, the gelation time was significantly decreased, for example, the gelation time of CSS/PEGDMA-2 was 60 s while the gelation time of CSS/PEGDMA-4 was 480 s. Compared to the CSS/PEGDMA-1, the gelation time of CSS/PEGDMA-4 and CSS/PEGDMA-5 was increased to ~450 s and ~500 s, respectively. These results indicated that the gelation time would influenced significantly by the weight ratio of CSS and PEGDMA in the hydrogels.

The equilibrium swelling ratio and degradation of the hydrogels was shown in Fig 4a and Fig 4b, respectively. The swelling and degradable properties are important indicators to the hydrogels that are applied for biomaterials. Fig 4a showed that the sample CSS/PEGDMA-4(1wt%CSS and 20 wt% PEGDMA) have the largest swelling equilibrium ratio of all the prepared hydrogels, and the swelling ratio of CSS/PEGDMA-2(0.5wt%CSS and 20% PEGDMA) was 300%, which is the smallest of the prepared samples. The swelling ratio of the hydrogels was decreased as CSS added from 0 to 0.5wt%, and then increased, it was believed that with the concentration of CSS

increasing, the high weight molecular CSS would hold a "big" space to stretch its long chain when the hydrogels were dipped into the PBS solution. The degradation of the hydrogels was evaluated by the weight loss of the two samples which at different content of PEGDMA and constant of CSS. The PEGDMA /CSS hydrogels were degraded slowly in PBS solution which was incubated at incubator that set 37 °C. Fig 4b showed that the degradation rate of the hydrogels increased when the concentration of PEGDMA in the hydrogels was reduced. It was supposed that the entanglement and involvement chains of the CSS and the molecules interaction forces were contributed to the degradation of the hydrogels.

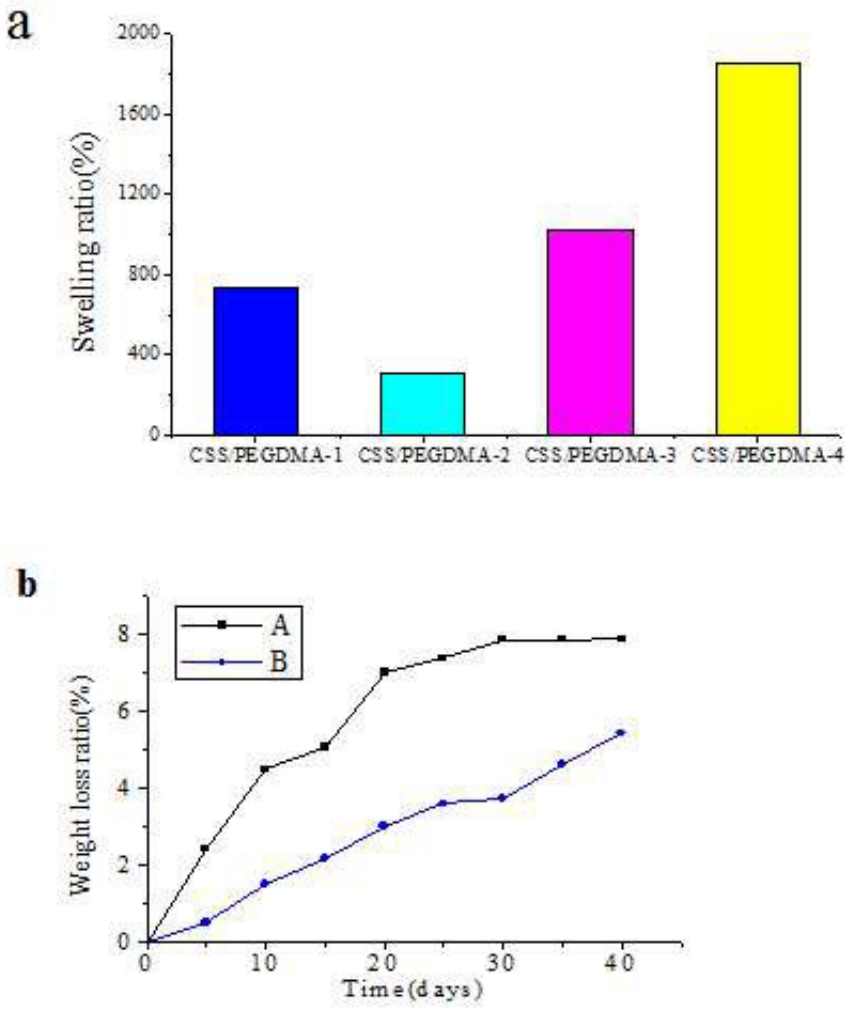


Fig 4. The swelling ratio of the prepared hydrogels (a) and the weight loss of the hydrogels (b): A: 20%PEGDMA-1%CSS (black, square), B: 25%PEGDMA-1%CSS (blue, dot), and the photo initiator I2959 of the two hydrogels was controlled at 0.1 wt%.

2.3 Evaluation of adhesion strength

Adhesion strength of the bioadhesive was represented by the lap shear adhesion in this paper. The lap shear adhesion of the hydrogels and the appearance of the adherents after lap shear

strength measured were shown in Fig 5. As seen in Fig 5A, the adhesion strength of CSS/PEGDMA-3(252.36±3.8 KPa) was highest of all the samples, and the hydrogels without CSS (CSS/PEGDMA-1) was 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 a control group, while the hydrogels containing CSS were bigger than the fibrin adhesive. Therefore, the adhesion strength increased with increasing the content of the CSS in the CSS/PEGDMA hydrogels, while the concentration of PEGDMA in the hydrogels would be 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, compared the CSS/PEGDMA-2 with CSS/PEGDMA-4, the adhesion strength of the CSS/PEGDMA-2 was not dramatically more than CSS/PEGDMA-4 though the concentration of the PEGDMA in the two hydrogels was different. The results indicated that the thiol group (-SH) was the key to the adhesion of the thiol-acrylate adhesives as it has biological reaction active. The thiol group can react with the –COOH, -OH, –SH or other groups, and these active groups are mostly existed in the proteins, saccharides and fat that are the main composes of biological tissues. The –SH of CSS would react with the active group of the gelatin that coated in glasses and then hold the two glasses together, so the adhesion strength of the hydrogels is depend mostly on the quantity of the CSS.

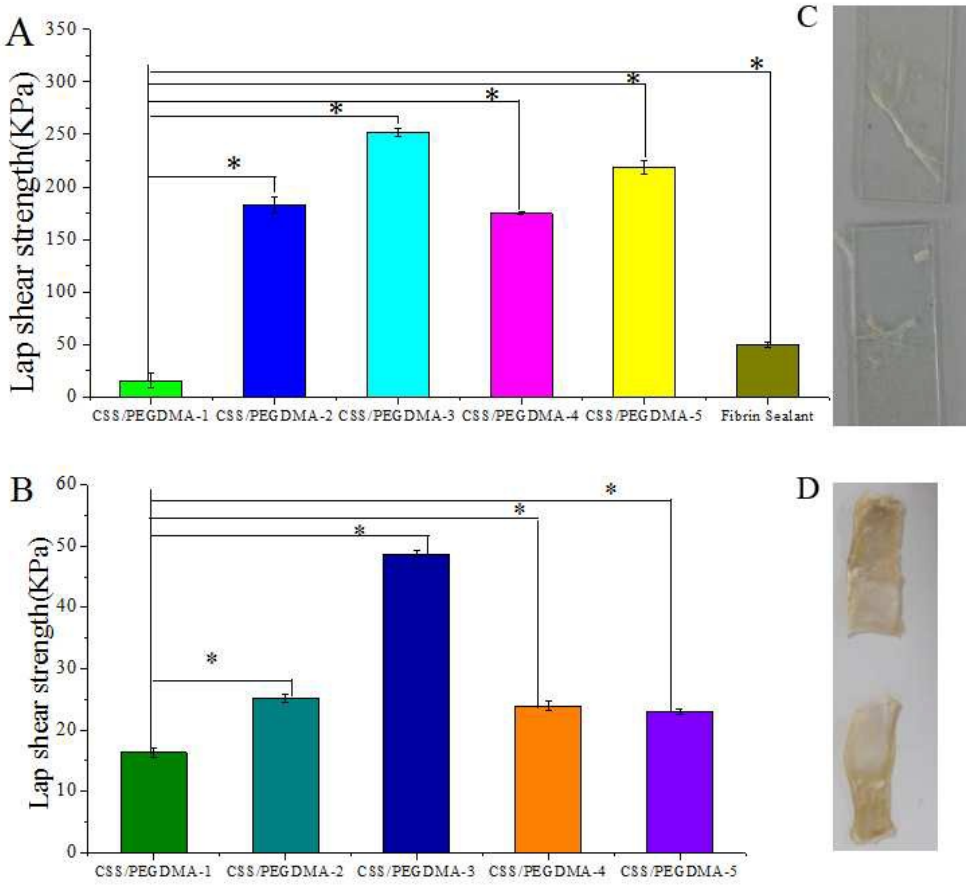


Fig 5. Lap shear strength of the prepared hydrogels used different adherents: A was gelatin coated glass, B was the SD rat skin; C and D was the appearance of the gelatin coated glasses and SD rat skin after adhesion strength measured, respectively, the symbols "*" indicated statistically

significant differences ($p<0.05$) compared to the CSS/PEGDMA-1.

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

2.4 compression properties

Hydrogels that used for compression test were prepared as 1.1.4 described. Without any other treatment, the samples(n=3) were tested in a mechanical testing instrument equipped with compression fixture directly for determining its mechanical properties. The uniaxial unconfined compression testing results were showed in Table 2.

Table 2 Result 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

The calculated elastic modulus(*E*) of the gels increased when the concentration of the precursor solution and the content of the CSS increased. Additionally, the CSS/PEGDMA-3 exhibited the highest maximum compressive stress and elastic modulus (380±10.97 KPa, 1317.85±277.23 KPa, respectively). The result of the uniaxial unconfined compression revealed that the prepared hydrogels by 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 increased, it was believed that the physical interaction force between the PEGDM and CSS would be the main force to support the hydrogel structure, and the hydrogel is loose porous. It indirectly explained the swelling ratio of the 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 the CSS/PEGDMA-4(69.6±6.54 KPa) and CSS/PEGDMA-5(130±14.14 KPa) were weaker than the CSS/PEGDMA-2(176±6.92 KPa) and CSS/PEGDMA-3(380±10.97KPa), which the PEGDMA concentration of the former is dilute to double of the latter. Researchers have founded that the concentration of the constituents are an important considering factor to the compressive performance of the hydrogels, the higher

concentration usually means the higher compressive stress, the compressive results of the prepared hydrogels was consistent with the previous works.

2.5 Cytotoxicity assays

Cytotoxicity assay is an important and basic property of the biomaterials, and the quantitative MTT cytotoxicity assay was performed by exposing L929 fibroblast cells to the extraction medium (10% FBS and 1% pen/step) of the prepared CSS/PEGDMA hydrogels and the absorbance illustrating of the viability of L929 cells was showed 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 the DMEM containing of 10% FBS and 1% pen/step under standard culture conditions at 37 °C and 5% CO_2, statistically significant differences ($p<0.05$) were discovered at 24h for the extraction medium of the prepared hydrogels, but after 48 hours, statistically significant difference ($p<0.05$) were not found in the extractions mediums of CSS/PEGDMA-2 and CSS/PEGDMA-3, and all of the extraction mediums were not show statistically significant differences ($p<0.05$) after 72h. The result indicated that the extraction mediums of the prepared hydrogels have impacted the L929 cells proliferation in a short time (24 h). The results were strongly supported that the CSS/PEGDMA hydrogels were less toxicity to L929 cells.

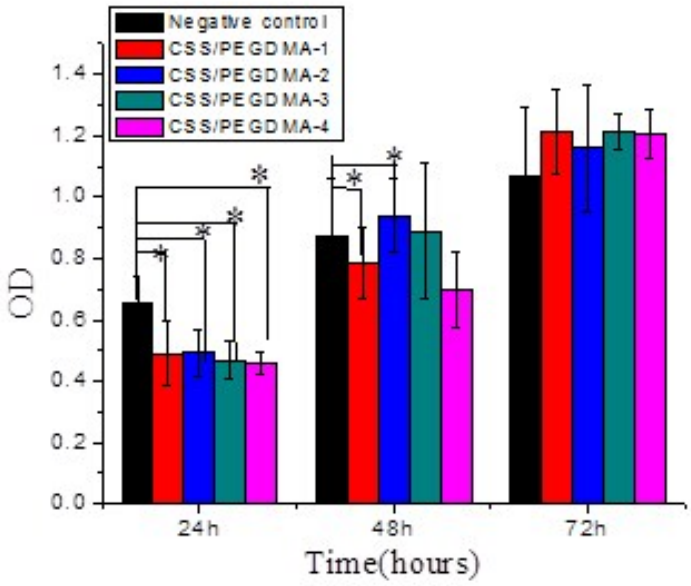


Fig 6. MTT test of the prepared hydrogels at 24, 48, and 72h.The data represented mean and stand deviations of five samples (∗: statistically significant differences ($p<0.05$), which compared to the negative control at the same incubation time).

3 Conclusion

In this study, a photocurable bioadhesive hydrogel based on PEGDMA and CSS was prepared and investigated. PEGDMA and CSS were synthesized and characterized by FTIR and ^{1}HNMR. The adhesive hydrogel formed in situ through thiol-acrylate additional reaction by the UV irradiation. The equilibrium swelling ratio, degradation behavior, compression properties and adhesive strengths 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 demonstrated that PEGDMA was succeed to synthesized and the thiol group was grafted to the chitosan by the reaction between the NAC containing thiol group and the chitosan. The compressive experiment of the hydrogels showed that the hydrogels have superior mechanical properties, and the result of degradation test showed the hydrogels was degradable. The adhesion strength of the CSS/PEGDMA adhesive was significantly larger than the common commercially available fibrin glues, it was indicated that the adhesive force of the adhesive can be attributed to the reactive thiol group of the CSS. The MTT assays showed that the CSS/PEGDMA hydrogel was biocompatible and less toxic to the L929 fibroblast cells. In conclusion, the photo-crosslinkable hydrogel had strong adhesion strength and compression strength and it was biodegradable and biocompatible, the method to prepare hydrogel adhesive by thiol-acrylate reaction may provide a route for developing bioadhesives.

Acknowledgement

This research was supported by National Nature Science Foundation of China (No.31470941, 31271035), Science and Technology Commission of Shanghai Municipality (No.15JC1490100, 15441905100), Yantai Double Hundred Talent Plan, and "111 Project" Biomedical Textile Materials Science and Technology, China (No. B07024). The authors extend their appreciation to the International Scientific Partnership Program ISPP at King Saud University for its funding research through the research group project (No. ISPP-0000).

.References

[1] Biji Balakrishnan and R. Banerjee, *Chemical reviews*, 2011, **111**, 4453.

[2] Morgan Cencer, Yuan Liu, Audra Winter, et al, *Biomacromolecules*, 2014, **15**, 2861.

[3] Jing Yu, Xu Xu, FuLin Yao et al, *International Journal of Pharmaceutics*, 2014, **470**, 151.

[4] Masayuki Ishiharaa, Kuniaki Nakanishia, Katsuaki Ono et al, *Biomaterials*, 2002, **23**, 833.

[5] Burhan Ates, Suleyman Koytepe, Merve Goksin Karaaslan et al, *International Journal of Adhesion & Adhesives*, 2014, **49**, 90.

[6] Takahito Inoue, Tetsushi Taguchi, and Shinji Imade, *Science and technology of advanced materials*, 2012, **13(6)**, 06429.

[7] Michael C. Giano, Zuhaib Ibrahim, Scott H. Medina et al, *Nature communications*, 2014, **5**,4095.

[8] D. G. Wallace, G. M. Cruise, W. M. Rhee et al, *Journal of Biomedical Materials Research*. 2001, **58**, 545.

[9] Ugur Erdemir, Hande Sar Sancakli, Batu Can Yaman et al, *Journal of dentistry*, 2014, **42**,149.

[10] Hoyong Chung and Robert H. Grubbs, *Macromolecules*, 2012, **45**, 9666.

[11] Kazuo Azuma, Masahiro Nishihara Haruki Shimizu et al, *Biomaterials*, 2015, **42**, 20.

[12] Jae-Soon Ahn, Hoo-Kyun Choi, Chong-Su Cho, *Biomaterials*, 2001, **22**, 923.

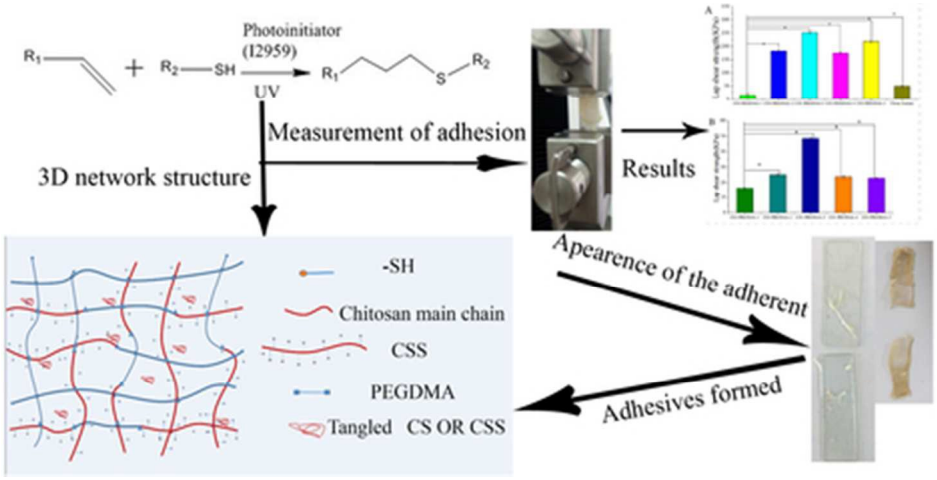
[13] Mi Kyung Kim, Ji-Soo Lee, Kwang Yup Kim et al, *Colloids and Surfaces B: Biointerfaces*, 2013, **103**, 391.

[14] Katsuaki Ono, Masayuki Ishihara, Yuichi Ozeki, et al, *sugery*, 2011, **130(5)**, 844.

[15] Kazunori Yamada, Tianhong Chen, Guneet Kumar et al, *Biomacromolecules*, 2000, **1**, 252.

[16] Wei Nie, Xiaoyan Yuan, Jin Zhao et al, *Carbohydrate Polymers*, 2013, **96**, 342.

[17] Katsuaki Ono, Yoshio Saito, Hirohumi Yura et al, *Journal of biomedical materials research*, 2000, **49(2)**, 289.
[18] Xuanyue Gao,Yingshan Zhou, Guiping Ma et al, *Carbohydrate Polymers*, 2010, **79**, 507.
[19] Devatha P. Nair, Maciej Podgórski, Shunsuke Chatani Et al, *Chem. Mater*, 2014, **26**, 724.
[20] Zong-Chun Wang, Xiao-Ding Xu, Chang-Sheng Chen et al, *Applied materials & interfaces*, 2010, **2(4)**, 1009.
[21] Eugene Lih, Jung Seok Lee, Kyung Min Park, et al, *Acta Biomaterialia*, 2012, **8**, 3261.
[22] Cramer NB and Bowman CN, *Journal of Polymer Science Part A: Polymer Chemistry*, 2001, **39(19)**, 3311.
[23] Amber E. Rydholm, Christopher N. Bowman, Kristi S. Anseth, *Biomaterials*, 2005, **26**, 4495.
[24] Akhilesh K. Gaharwar, Nicholas A. Peppas, Ali Khademhosseini, *Biotechnology and Bioengineering*, 2014, **11(3)**, 441.
[25] Carrie E. Brubaker and Phillip B. Messersmith, *Biomacromolecules*, 2011, **12**, 4326.
[26] Severine Rose, Alexandre Prevoteau, Paul Elziere, *Nature*, 2014, **505(7483)**, 382.
[27] Da-yong Teng, Zhong-ming Wu, Xin-ge Zhang et al, *Polymer*, 2010, **51**, 639.
[28] Yufei Ai, Jun Nie, Gang Wu et al, *Journal of applied polymer science*, 2014, **131**, 41102.



39x19mm (300 x 300 DPI)

A novel tissue adhesive composed of CSS and PEGDMA based on Michael addition reaction.