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Dextran and gelatin based photocrosslinkable tissue adhesive

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ABSTRACT

A two-component tissue adhesive based on biocompatible and bio-degradable polymers (oxidized urethane dextran (Dex-U-AD) and gelatin) was prepared and photocrosslinked under the ultraviolet (UV) irradiation. The adhesive could adhere to surface of gelatin, which simulated the human tissue steadily. The structures of above Dex-U-AD were characterized by FTIR, ^{1}H NMR spectroscopy and XRD. The adhesion property of result products was evaluated by lap-shear test. The maximum adhesion strength could reach to 4.16 ± 0.72 MPa which was significantly higher than that of fibrin glue. The photopolymerization process of Dex-U-AD/gelatin was monitored by real time infrared spectroscopy (RTIR). It took less than 5 min to complete the curing process. The cytotoxicity of Dex-U-AD/gelatin also was evaluated which indicated that Dex-U-AD/gelatin gels were nontoxic to L929 cell. The relationship between all the above-mentioned properties and degree of oxidization of Dex-U-AD was assessed. The obtained products have the potential to serve as tissue adhesive in the future.

1. Introduction

Nowadays, bioadhesives have been remarkably expanding in the field of medical applications with the properties of providing a range of potential therapeutic benefits, e.g. to assist surface lacerations and abrasions repair, reduce operative risk through anastomotic stabilization and promote wound healing between disjoints. In the clinical medical field, fibrin glue and cyanoacrylates have been commercially applied. Fibrin glue, consisting of fibrinogen and thrombin, contained an antiproteinase, which impaired the ingrowth of vascular granulation tissue and accelerated the healing of surgical wounds. However, it degraded fast and holds insufficient adhesion to tissue. Cyanoacrylate adhesives possessed high bonding strength but they were cytotoxic and harmful to tissues. Besides, cyanoacrylates were not absorbable and thus inhibited endogenous bone repair (Montanaro et al., 2000). Commercially available adhesives, such as fibrin glue and cyanoacrylates, often require a choice between degree of adhesion and biocompatibility.

A number of adhesives (Takaoka et al., 2008) based on natural products like gelatin, collagen, polysaccharide and biomimetic mussels monomer like L-3,4-dihydroxylphenylalanine (DOPA) and its derivatives have been developed to overcome these disadvantages in commercial surgical adhesives.

Multipolysaccharides composed of chitosan and modified starch were relevant for the design of bioadhesives for tailor-made biological applications. Laurent David's group investigated the properties of chitosan based adhesive with modified starch (oxidized maltodextrin), including viscoelastic behavior, adhesion strength correlation between rheological behavior and adhesion, etc. (Serrero et al., 2011, 2010). Bruce P. Lee et al. (Dalsin, Hu, Lee, & Messersmith, 2003; Lee, Lee, & Messersmith 2007) added biomimetic functional groups such as DOPA and obtained flexible organic nanoadhesive known as 'geckel'. As a result, it could enhance the adhesion of materials to surfaces and tissues through fully reversible, noncovalent interactions. Unlike the most other polysaccharides without clear physical structure and properties, dextran has received considerable attention because of its ample natural resources, nontoxicity and good water-soluble ability. Dextran (Nowakowska, Zapotoczny, Sterzel, & Kot, 2004) was a polymer of glucose in which the glucosidic linkages were predominantly of the α-(1–6) type. Structurally, there are abundant pendant hydroxyl groups in its anhydroglucose unit; dextran could be chemically modified to form hydrogels via cross-linking. It has been used in the medical and biomedical fields for more than 60 years, such as plasma volume expansion and carrier for drugs and proteins (Wondraczek, Elschner, & Heinze, 2011). In recent studies, synthetic dextran hydrogels were used in vascular tissue engineering regeneration and tissue adhesive (Liu & Chan-Park, 2009, 2010; Sun et al., 2011).

Guoming Sun (Sun, Chen, & Chu, 2009; Sun & Chu, 2010; Sun et al., 2011) synthesized a series of dextran-based macromers by incorporating various functional groups, including allyl isocyanate (Dex-AI), ethylamine (Dex-AE), chloroacetic acid (Dex-AC), or maleic-anhydride (Dex-AM) into dextrans. The dextran-based

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hybrid hydrogels were developed by integrating polyethylene glycol diacrylate (PEGDA). Differences in physical and biological properties of the hydrogels are found. Natalie Artzi used a series of amine-PEG (linear and star) and aldehyde dextran to get composite materials acting as a controlled biocompatible tissue adhesive to meet the varying demands of a wide range of tissue composition and mechanical loading conditions (Artzi, Shazly, Baker, Bon, & Edelman, 2009; Artzi, Shazly, Crespo, et al., 2009). Although these natural biological polymers possessed good biocompatibility and biodegradability, inadequate mechanical properties and the long gelation time still were an issue that could not be ignored.

By contrast, the process of photoinitiating crosslinking was relatively simple, fast and mild. For in situ curing bioadhesives, these characteristics were one of the remarkable advantages. Since the adhesive gels could be prepared from aqueous liquids, they could be formed in situ following injection or similar in vitro invasive delivery. Furthermore, the gels could be controlled both temporally and spatially by altering monomer concentration, photoinitiator concentration, light intensity and exposure time to light source. Recently, photocuring process has gained great attention in medical fields. Our team (Li, Niu, Yang, Nie, & Yang, 2011) had successfully developed an attractive photocrosslinkable dextran based bioadhesive system. The bioadhesive completed crosslinking within five minutes and demonstrated good adhesive strength that is significantly superior to the commercially available fibrin. Because of high swelling ratios of dextran based bioadhesive, cells were difficult to adhere to the surface of gels. While gelatin (Mo, Iwata, Matsuda, & Ikada, 2000) was capable of promoting cell adhesion and proteolytic degradation and was relatively inexpensive compared to collagen or fibrinogen.

The current study aimed to develop a tissue adhesive based on photocrosslinking aldehyde dextran and gelatin. Introducing photocrosslinkable vinyl group with 2-isocyanatoethyl methacrylate (IEMA) by chemical modified on to dextran backbone (Scheme 1). Aldehyde groups introduced in dextran by oxidizing reaction between sodium periodate and vicinal diol and gelatin could be incorporated into dextran hydrogel not only to form intermolecular network but also to increase tissue crosslinking by aldehyde groups. The synthesized products called Dex-U-AD were characterized by XRD, FTIR and hydroxylamine hydrochloride method. The physical properties of the adhesive system (i.e. adhesion strength, swelling ratio) were also measured. Cell adhesion and proliferation on hydrogels were observed.

2. Methods

2.1. Materials

Dextran (Mw 20 kDa) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 2-Isocyanatoethyl methacrylate (IEMA) was purchased from Ginray Chemical Reagent Co. Ltd. (Shanghai, China). Dibutyltion dilaurate (DBTL) as catalyst was purchased from Aladdin Reagent Co. (Shanghai, China). Photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Darocur 2959) was supplied from Ciba-Geigy Chemical Co. (Tom River, NJ, USA). Dimethyl sulfoxide (extra dry DMSO, water <50 ppm) and other reagents were all obtained from Beijing Chemical Agent Co. (Beijing, China).

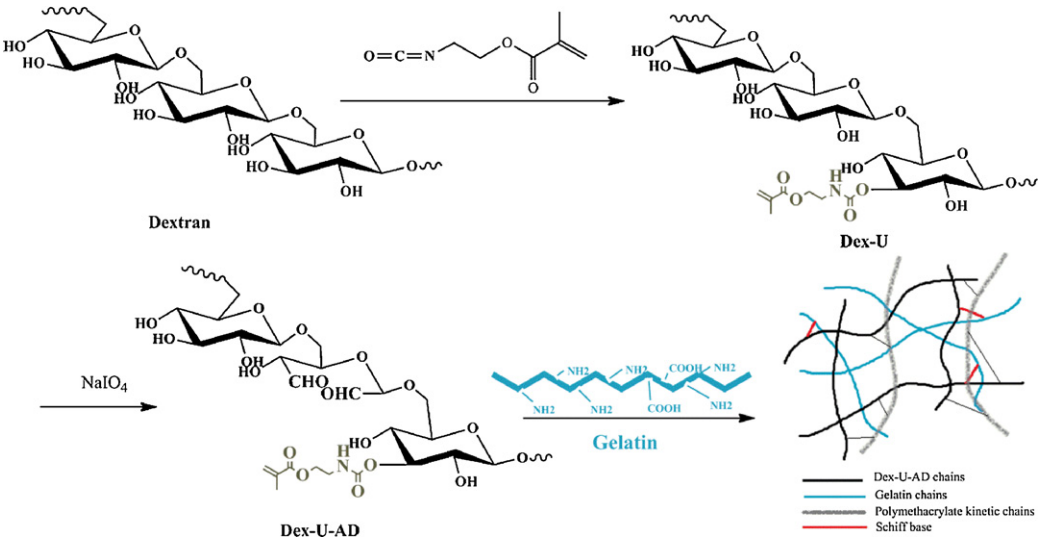
2.2. Preparation of urethane dextran derivatives (Dex-U)

8 g of dextran and 0.05 g of DBTL were dissolved in 250 mL of DMSO after adding into a three-necked flask at 40 °C and under nitrogen protection. 7.5 g of IEMA dissolved in 80 mL DMSO was dropped into the above mixture within 3 h. The solution was poured into 800 mL of saturated sodium chloride solution with vigorous stirring. The white precipitate was filtered and washed with distilled water for three times to remove DMSO and catalyst DBTL. Then the Dex-U was obtained after lyophilization. The different degrees of substitution (DS) of Dex-U could be obtained by adjusting molar ratio of IEMA to the hydroxyl groups of dextran. As reported (Li et al., 2011), when the DS > 0.5, the Dex-U undissolved in water. In order to get hydrogel system, the urethane dextran of DS = 0.5 was used.

The DS of Dex-U, which was adjusted by molar ratio of IEMA to the hydroxyl groups of dextran, according to ^{1}H NMR spectra was calculated as A_1/A_a, where A_a was the area of proton of double bond (Ha), and A_1 was the average area of proton of anomeric carbon of the α-1,6 linkages proton (H_1) (Yin, Wang, Han, & Nie, 2010)

2.3. Preparation of oxidization of urethane dextran (Dex-U-AD)

Dex-U was further oxidized by sodium periodate to introduce aldehyde groups. Briefly, 250 mg Dex-U was dissolved in 10 mL distilled water. 60 mg sodium periodate was added to the solution. The mixture was stirred in ice bar in the dark for 4 h. The Dex-U-AD solution was dialyzed against distilled water; using Ultraviolet



Scheme 1. Synthesis of Dex-U-AD and gel networks formed by Dex-U-AD/gelatin.

Spectrometer to diagnose the vanish of periodate (λ = 223 nm), and then lyophilized. The degree of substitution aldehyde was determined by using the hydroxylamine hydrochloride titration assay. In this article, Dex-U-16-AD, Dex-U-8-AD, Dex-U-4-AD and Dex-U-2-AD were referring to the degree of oxidization at 4.8 ± 0.6%, 11.0 ± 1.6%, 22.8 ± 1.3% and 41.6 ± 3.0% respectively.

The degree of oxidization of Dex-U-AD was measured by following steps: (Zhao & Heindel, 1991) Dex-U-AD (0.1 g) with different degrees of oxidization (DO) was dissolved in 25 mL of 0.25 mol/L hydroxylamine hydrochloride–methyl orange solution. Each mixture was allowed to stand for 2 h and was titrated by standardized sodium hydroxide solution until the red to yellow end point was achieved by matching the color of the sample solution with that of a blank one. Thereby, the degree of substitution of —CHO groups was calculated based on the sample weights. Three samples were measured for each experiment, and the average of these values was recorded.

The degree of aldehyde substitution was calculated by

$$\frac{(V_1 - V_0) \times M \times M_w}{1000w} \times 100\%$$

where V_1 and V_0 are the volume of samples and blank sample consumed respectively, M is the molar concentration of sodium hydroxide, M_w stands for the molar weight of repeating units and w is the weight of Dex-U-ADs to be measured.

2.4. Preparation of photocrosslinkable gel

Hydrogels were fabricated by photocrosslinking of precursor solutions. The precursor solutions were prepared with 5 wt% gelatin and a range (15, 25, 35 and 45 wt%) of Dex-U-AD concentrations in PBS to produce Dex-U-AD/gelatin hydrogels respectively. The detailed procedure is as follows: the solution was vigorously stirred under 40 °C water bath for 10 min followed by the addition of 1% the photoinitiator Irgacure 2959 (I-2959). The final concentration of photoinitiator was kept at 1 wt%. After homogenizing, the solution was injected into the mold. The system was irradiated under UV light source (320–480 nm, EXFOlite, EFOS Corporation, Mississauga, Canada), and the distances between the dip of the light guide and the sample was fixed at 44 mm, and the light intensity was adjusted to 5, 10, 15, 30 and 50 mW/cm^2.

2.5. ^{1}H NMR

^{1}H NMR spectra of Dex-U solved in deuterated DMSO were obtained with a Bruker AV (Bruker, Germany) spectrometer at 400 MHz at room temperature.

2.6. FTIR spectra measurement

FTIR spectra were recorded on a Nicolet 5700 instrument (Nicolet 5700, Thermo Electron, USA, equipped with an extended range KBr beam-splitter and an MCT/A detector). Samples were prepared as KBr pellet from 4000 to 650 cm^{-1} with resolution of 4.0 cm^{-1}. The photocrossing process was monitored by using series

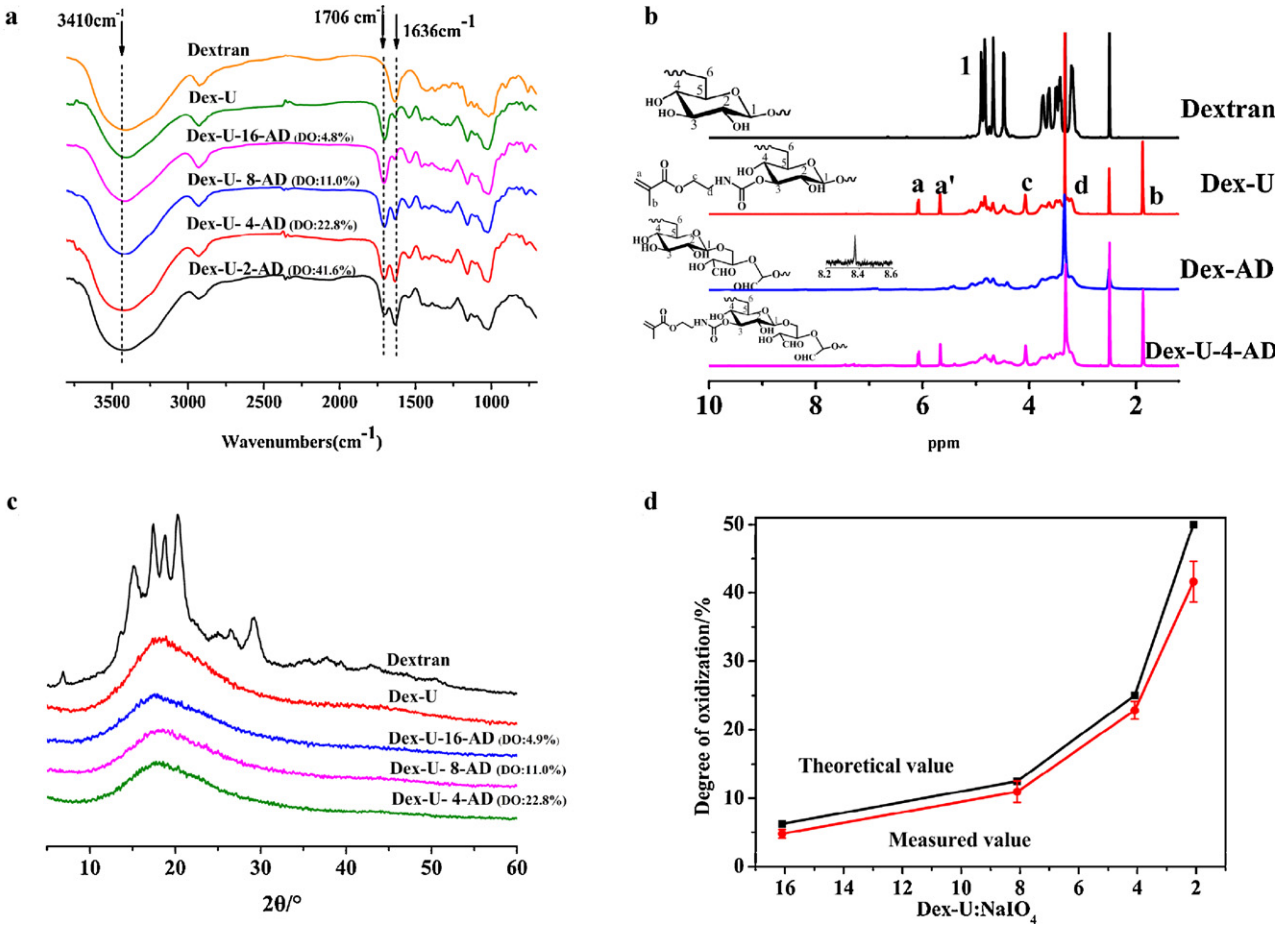


Fig. 1. The spectra of dextran, Dex-U and Dex-U-ADs; (a) FTIR; (b) ^{1}H NMR; (c) XRD; (d) DO of different aldehyde content.

real time infrared spectroscopy. The double bond conversion (DC) of the gels was monitored by real-time near FTIR with the resolution of 4 cm^{-1}. A horizontal transmission accessory (HTA) was designed to enable mounting of samples in a horizontal orientation for FTIR measurements. The change of C—H absorbance peak area attribute to C=C double bond from 6110 to 6210 cm^{-1} in the near-IR range was correlated to the double bond conversion. For each sample, the series FTIR runs were repeated three times, and in most cases, the error of double bond conversion was less than 2%.

2.7. X-ray diffraction (XRD) study

X-ray diffraction (XRD) patterns of the samples were recorded on an X-ray diffractometer (D/Max 2500VB2+/Pc, Rigaku, Japan) with Cu K characteristic radiation (wavelength 0.154 nm) at a voltage of 40 kV and a current of 50 mA. The scanning rate was 10°/min and the scanning scope of 2θ was from 5° to 90° at room temperature.

2.8. Measurement of adhesion strength

Spread uniformly on the surface of pieces of glass, 20 wt% gelatin solutions were used to mimic the living tissue. The dimension of one piece of glass was 5 mm × 20 mm × 50 mm. After drying at 70 °C for 20 min, there was a homogenous sheet of gelatin on the surface of glass. Then, the pieces of glass were overlapped in 10 mm in which the Dex-U-AD solution was spread and the area of bonding was 20 mm × 10 mm. They were irradiated by UV light under light intensity 30 mW/cm^2 for 10 min. The Dex-U was crosslinked between the gelatin sheets. The glass samples after UV curing were tested by using a universal testing machine (Model 1185, Instron, USA) with a crosshead speed of 5 mm/min at room temperature. For each experiment, five samples were measured.

2.9. Measurement of swelling kinetics

The dry gels (weight W_0) were immersed in PBS at 37 °C with constant shaking at certain time intervals. After removal of the excess superficial water, the weight of the swollen hydrogels (W_s) was measured until no further weight change was detected. Swelling ratio of hydrogel was calculated by the following equation (Kim, Won, & Chu, 1999):

$$\text{Swelling ratio } (\%) = \frac{W_s - W_0}{W_0} \times 100.$$

2.10. Scanning electron microscopy (SEM)

The morphology of cross-linked Dex-U-AD gels was examined by an SEM. Lyophilized gels were fractured in liquid nitrogen to expose the structure inside the gels. Samples were mounted and sputter coated with gold/palladium using Hitachi S4700.

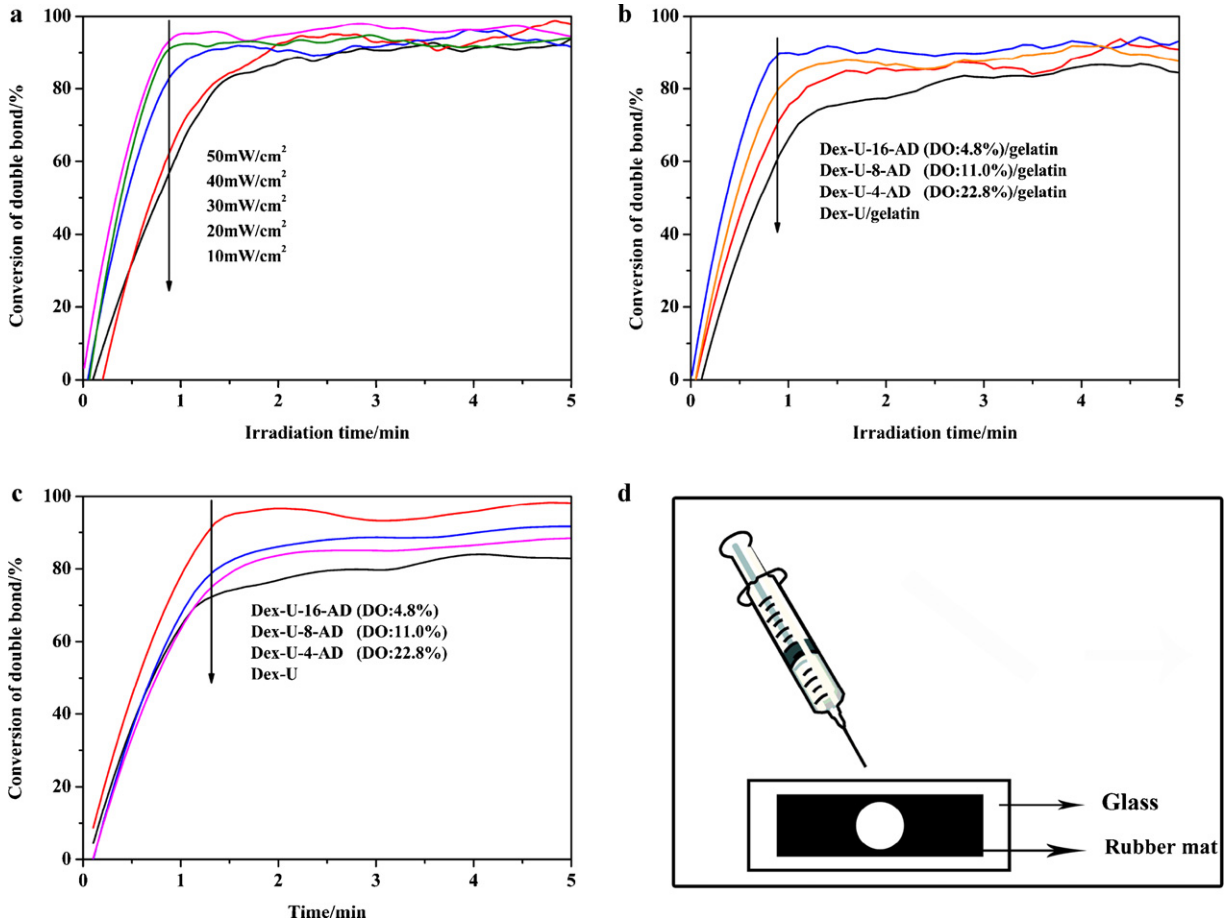


Fig. 2. Photocrosslinking kinetics of Dex-U in PBS (pH 7.4) solution under different conditions: (a) different light intensity in Dex-U-4-$AD_{(DO:22.8\%)}$/gelatin solution of 30 wt%; (b) different degrees of oxidization of Dex-U/gelatin solutions; (c) different degrees of oxidization of Dex-U without gelatin solutions; (d) the schematic of preparation of sample.

2.11. Cytotoxicity assays

The cytotoxicity of the gel samples was evaluated based on a procedure adapted from the ISO10993-5 standard test method. Mouse fibroblasts (L929) were cultured in DMEM medium supplemented with 10% fetal bovine serum, together with 1.0% penicillin–streptomycin, and 1.2% glutamine. Culture was maintained at 37 °C in a wet atmosphere containing 5% CO_2. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1×10^3 M ethylene diaminetetraacetic acid (EDTA).

The viabilities of cells were determined by the MTT (3-[4-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) assay. For the MTT assay, the films (without acetic acid) with ~0.2 mm thickness were sterilized with highly compressed steam for 15 min and placed in wells of 24-well culture plate. The samples were then incubated in 1 mL DMEM medium at 37 °C for 24 h. The extraction ratio is 100 mg/mL. At the end of this period, the films were removed, and the so-called extracts were obtained. L929 cells were seeded in wells of 96-well plate at a density of 10^3 cells per well. After incubation for 24 h, the culture medium was removed and replaced with the as-prepared extraction medium and incubated for another 24 h, and then 100 μL MTT solution was added to each well. After 4 h incubation at 37 °C, 200 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. The dissolved solution was swirled homogeneously about 10 min by the shaker. The optical density of the formazan solution was detected by an ELISA reader (Multiscan MK3, Labsystem Co., Finland) at 570 nm.

After 48 h of culturing of the prepared circular samples, cellular constructs were harvested, rinsed twice with PBS to remove non-adherent cells and subsequently fixed with 3.0% glutaraldehyde at 4 °C for 4 h. After that the samples were dehydrated through a series of graded ethanol solutions and air-dried overnight. Dry samples were sputtered with gold for observation of cell morphology on the surface of the scaffolds by fluorescence microscope and SEM.

3. Results and discussion

3.1. The structure of bifunctional Dex-U-AD

Synthesis of bifunctional dextran was synthesized by coupling IEMA to dextran followed by oxidation of Dex-U with sodium peri-

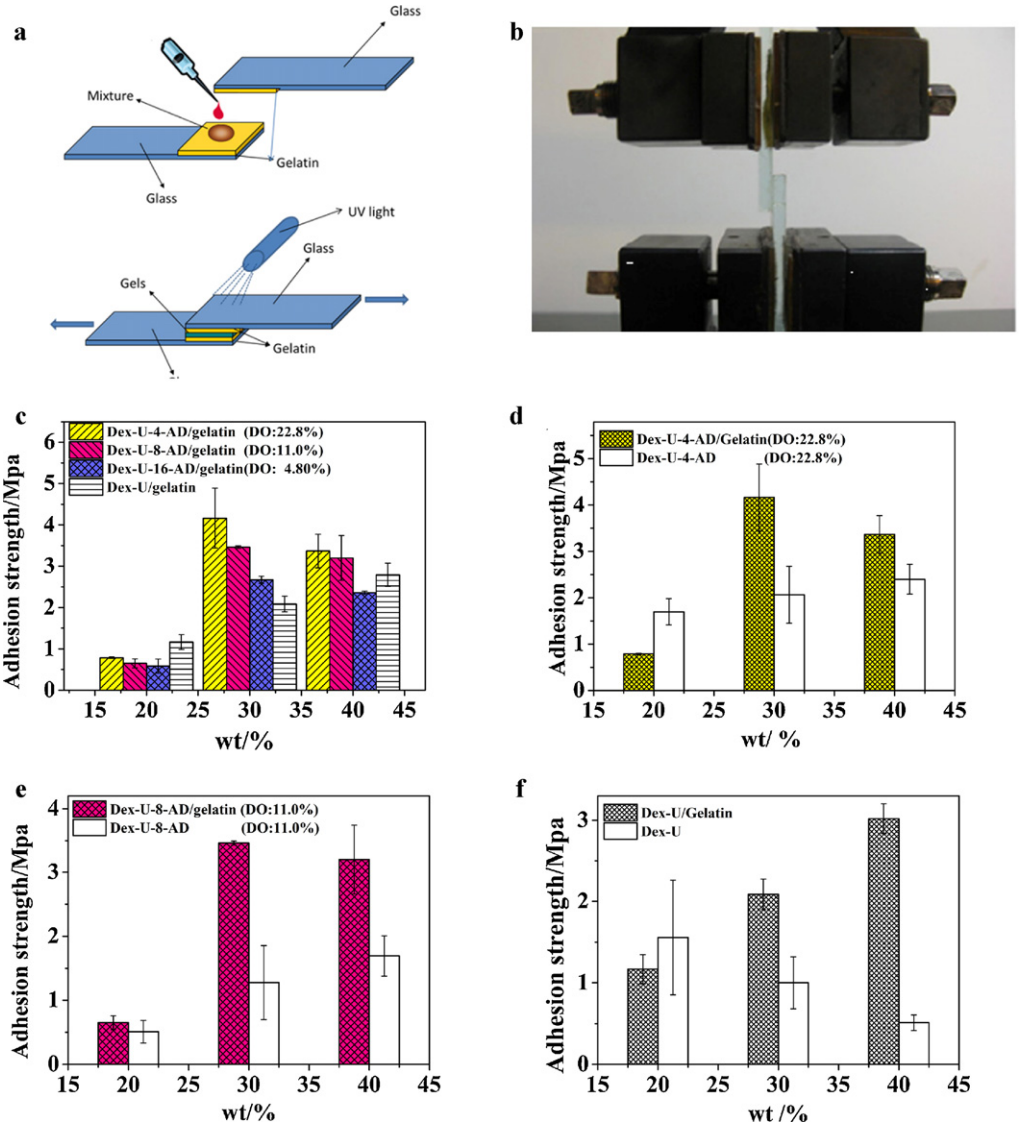


Fig. 3. Adhesion strength test of Dex-U-AD/gelatin solution: (a) Dex-U-AD/gelatin with different DO. (b) Dex-U-AD/gelatin solutions compared to Dex-U-AD; (c) Dex-U-8-$AD_{(DO:11.0\%)}$/gelatin solutions compared to Dex-U-8-$AD_{(DO:11.0\%)}$; (d) Dex-U-4-$AD_{(DO:22.8\%)}$/gelatin solutions compared to Dex-U-4-$AD_{(DO:22.8\%)}$; (e) the schematic of preparation of lap-shear sample; (f) the device of lap-shear test.

odate, as shown in Scheme 1. The successful synthesis of Dex-U-AD was confirmed by FTIR and ^{1}H NMR.

As shown in Fig. 1(a), compared with the spectrum of dextran, the spectrum of Dex-U showed the new peaks at 1706 cm^{-1}, 1636 cm^{-1}, 1528 cm^{-1} and 810 cm^{-1}. Typical double bond absorption bands were observed at 1636 cm^{-1} and 810 cm^{-1}. Peaks at 1706 cm^{-1} and 1528 cm^{-1} are the characteristics of the C=O and C—N stretching and N—H bending of urethane groups of Dex-U. These peaks were obtained by the reaction between IEMA and the hydroxyl groups of dextran. With the increase of the DO, the relative intensity of peak of the C=O at 1706 cm^{-1} decreased and the relative intensity of peak of the C=C at 1636 cm^{-1} increased. Aldehyde groups of dialdehyde polysaccharides were generally thought as being due to the formation of hemiacetal and hydrated functionalities in water solutions. It has been reported that this is valid only for a narrow pH range (4.0–5.2) and the formation of the enol and enolate anion is in fact a main feature at least at higher pH. The absorption at 1636 cm^{-1} in the spectra of alkaline dextran solutions may arise from aldo–enol transitions. Because of dialysis against distilled water, the oxidized dextran transfer to formation of the enol so that with the higher degree of oxidization, the peak intensity of the C=C at 1636 cm^{-1} increased (Drobchenko, Isaeva-Ivanova, Kleiner, & Eneyskaya, 1996; Neishlos, Novikova, Passet, & Moskvin, 2004).

The ^{1}H NMR spectra of the samples were represented in Fig. 1(b). A singlet at 4.89 ppm assigned to anomeric carbon of the α-1,6 linkages proton (H_1). The typical multiplet peaks range from 3.1 to 3.8 ppm was assigned to the other protons of the glucopyranosyl ring. In the spectrum of Dex-U, new signals at 5.65, 6.05 ppm (H_a, the double bond protons) and 1.87 ppm (H_b, methyl protons) were clearly observed. No obvious aldehyde protons peak was detected (position 9, expected at about 8.3 ppm) (Wang et al., 2007), and this may be due to the low extent of oxidation (Li et al., 2011).

The XRD pattern evaluated the crystalline degree of dextran and Dex-U with different DO (Fig. 1(c)). The dextran showed series characteristic peaks at 2θ = 15.1°, 17.5°, 18.8°, 20.5° and 29.5°, which indicated a high crystalline degree. The XRD patterns of Dex-U were significantly different from the neat dextran. For Dex-U, the DS above 0.5 were almost amorphous, which illuminated that the crystalline structure of dextran were completely destroyed. The introduction of IEMA hindered the formation of inter- and intra-molecular hydrogen bonds after isocyanate groups reacted with hydroxyl groups. As the oxidization occurred, the crystalline structure of dextran was completely destroyed.

Fig. 1(d) illustrated the actual oxidized degree of sodium periodate on Dex-U (DS = 0.5). DO could be controlled by the variation of the feed ratios. When the ratio of sodium periodate to Dex-U increased, DO of Dex-U enhanced. The DO of Dex-U reached 41.6% as molar ratio of sodium periodate to Dex-U was 1:2. We have tried to continue the increase of the feed ratios, but the products Dex-U-AD transfer into water were insoluble.

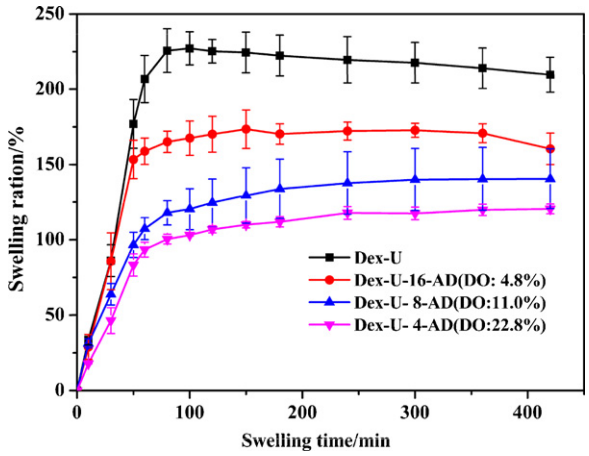


Fig. 4. Swelling kinetics of hydrogels of Dex-U-AD and 5 wt% gelatin.

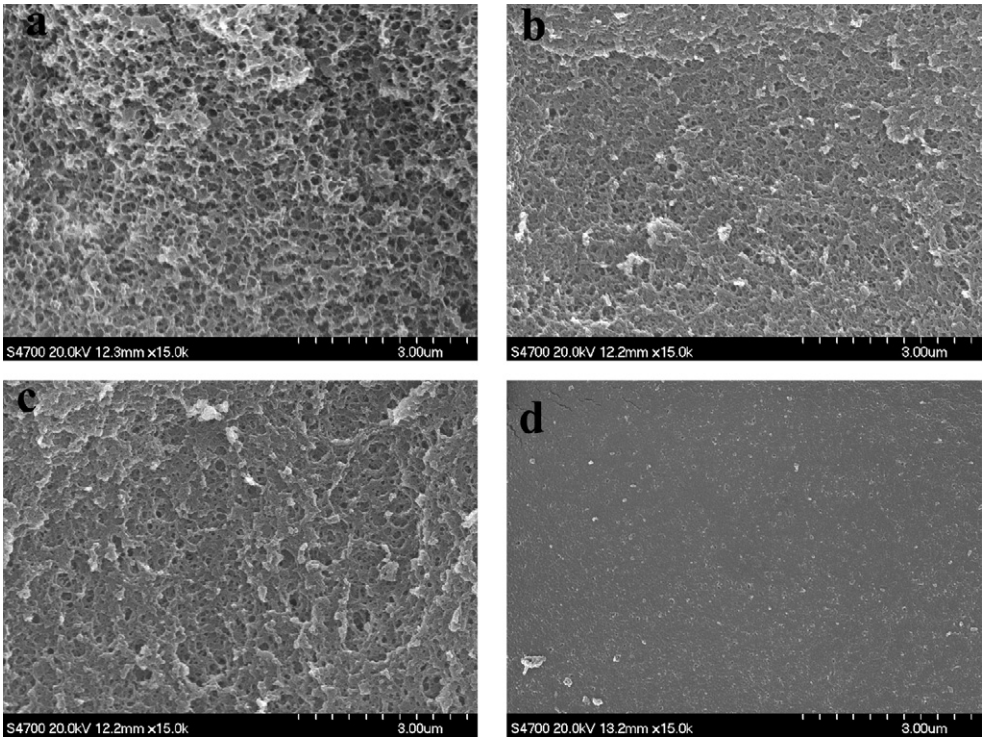


Fig. 5. SEM of interior morphology of gels: (a) Dex-U-4-AD$_{(DO:22.8\%)}$/gelatin; (b) Dex-U-4-AD$_{(DO:22.8\%)}$; (c) Dex-U/gelatin; (d) Dex-U.

It could be found that the actual DO was close to the theoretical DO calculated in accordance with the feed ratio. There was a side reaction for sodium periodate oxidizing Dex-U, and sodium iodate could be oxidized by sodium periodate to form unattached iodine which hindered the reaction between Dex-U and sodium periodate.

3.2. Photocrosslinking of Dex-U-AD/gelatin

When exposed to UV light, the unsaturated C=C of Dex-U-AD in the system could be cross-linked. As shown in Fig. 2(a), when the intensity of UV light increased from 10 mW/cm^2 to 50 mW/cm^2, we could get both the maximum conversion rate and eventual con-

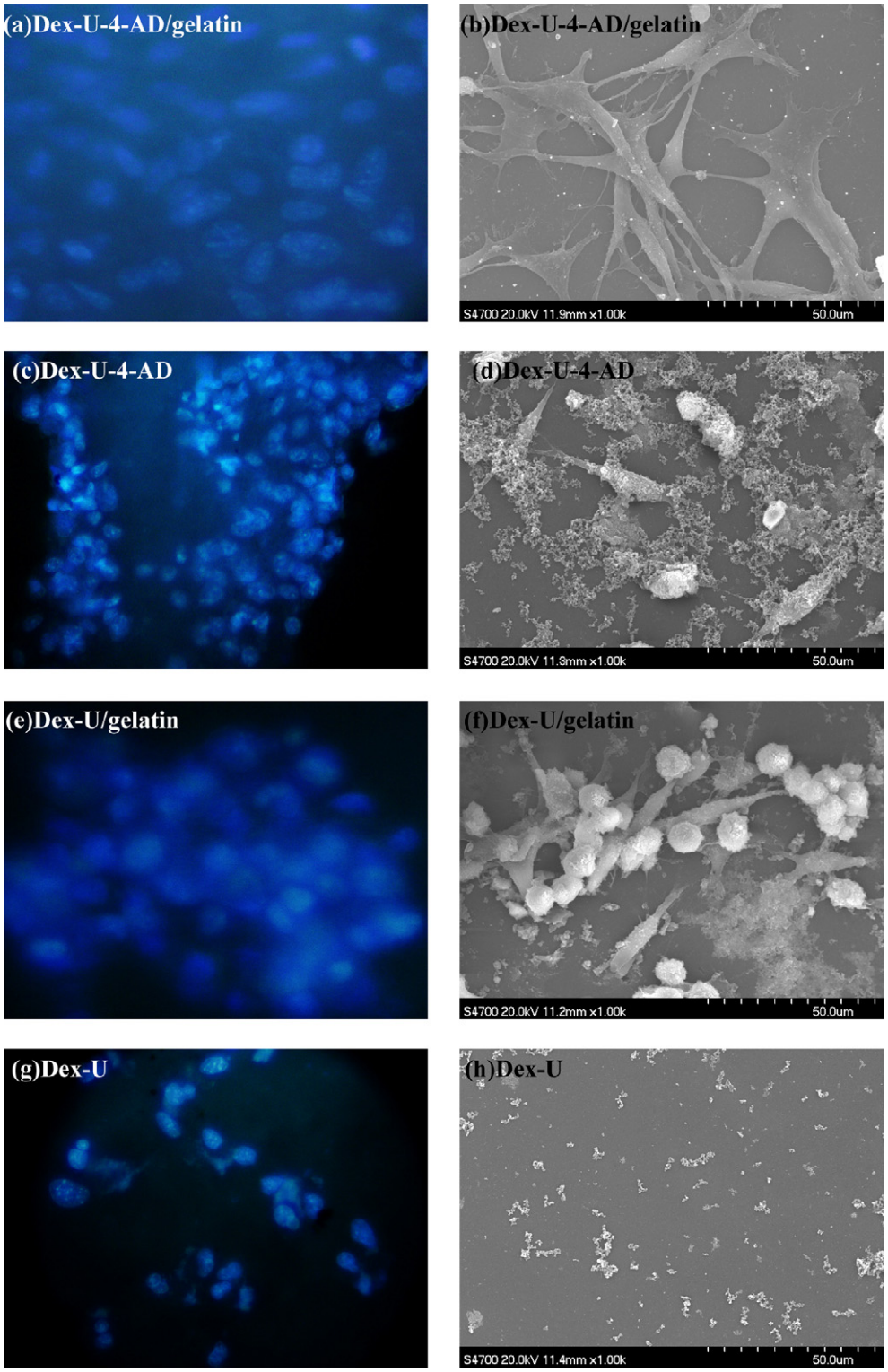


Fig. 6. Fluorescence microscopy images and selected SEM images of L929 cell seeded on the surface of the dextran hydrogels: (a and b) Dex-U-4-AD$_{(DO:22.8\%)}$/gelatin; (c and d) Dex-U-4-AD$_{(DO:22.8\%)}$ (e and f) Dex-U/gelatin; (g and h) Dex-U.

version because of the more free radicals by D-2959 under higher UV light intensity. Considered the balance cell injury and polymerization rate by high light intensity, light intensity of 30 mW/cm^2 was selected to insure the rapid polymerization and low cell injury. Fig. 2(b) exhibited the maximum and eventual conversion of double bonds of the Dex-U-AD/gelatin systems. All eventual conversion of Dex-U/gelatin solution reached above 80% within 5 min. When the DO of Dex-U increased which was equivalent to the higher double bonds density for both the C=C double bond and —CHO participated in crosslinking, the eventual conversion decreased due to the gel effect in radical polymerization. The higher density of crosslinkage hindered the movement of residual methacrylate double bonds in late stage of photocrosslinking, so as to decrease the eventual of methacrylate double bonds. The trends of conversion of double bonds in Fig. 2(c) were the same as Fig. 2(b) that meant gelatin had no obviously influence on the photocrosslinking.

3.3. Analysis of adhesion strength

Fig. 3(a) indicated that with the DO increase, the adhesive strength of gels significantly improved. The aldehyde groups could not only increase the density of crosslinkage between Dex-U-AD and gelatin, but also improve the cohesive force of adhesive. The adhesion strength of gels eventually reached saturation with increasing aldehyde content. This saturation was characterized by a threshold of 30 wt% (25% Dex-U-AD + 5% gelatin) Dex-U-AD/gelatin at the DO of 41.6%; the highest fracture strength obtained was 4.16 MPa which was twice as much as the adhesion strength at the same content of the Dex-U system.

Fig. 3(b)–(d) showed that 5% gelatin could help to promote the adhesive strength. It significantly promoted, especially when the solid content of gels reached to 30% (25% Dex-U-AD). Dex-U-4$_{(DO:22.82\%)}$/gelatin gels at 4.16 ± 0.72 MPa and Dex-U-8-AD$_{(DO:10.95\%)}$/gelatin gels at 3.46 ± 0.032 MPa were 83 times higher than that of Tisseel (0.05 MPa) (Alston, Solen, Broderick, Sukavaneshvar, & Mohammad, 2007)

3.4. Swelling kinetics

Unfortunately, the Dex-U-AD hydrogels without adding gelatin broke into pieces in varying sizes because of its high swelling ratio and poor mechanical strength. It could not be weighed accurately in irregular shapes.

Fig. 4 showed the swelling kinetics of Dex-U-AD/gelatin hydrogels, when incubated in PBS at pH 7.4. The maximum swelling ratio of Dex-U-AD/gelatin hydrogels decreased with increase in aldehyde content. For a fixed total solid content of 25 wt% Dex-U-4-AD$_{(DO:22.82\%)}$ and 5 wt% gelatin, the ratio was significantly decreased from 227 ± 11% to 103 ± 0.77% in the Dex-U-4-AD$_{(DO:22.82\%)}$/gelatin hydrogel at 100 min.

Crosslinking density, gel composition and charge density were generally affected by the swelling ratio. The declining swelling ratio was attribute to the Schiff based reaction with the additional interpenetrating gelatin. Therefore, the higher amount of aldehyde groups, the higher the crosslink densities attributed to the lower equilibrium swelling ratio.

3.5. Interior morphology of the hydrogels

Fig. 5 demonstrated the interior morphology of Dex-U-AD/gelatin hydrogels. The pores were formed by water dissociating after lyophilization. In fixed amount of urethane groups, the higher aldehyde content was important to interior structure of these gels. Bifunctional dextran mixing with gelatin formed gels having large quantity of regularly distributed pores as is shown in Fig. 5(a). Uniform size pores indicated stable internal structure, ensured all ingredients mixing homogeneously reacting sufficiently.

3.6. Cytotoxicity assays

Fig. 6 shows fluorescence microscopy images and selected SEM images of L929 cell seeded on the surface of the dextran hydrogels. The L929 cells spread quite well on the surface of all hydrogels indicating good cell–surface interaction and good cell viability on the Dex-U-AD gels surface especially on the surface of the Dex-U-4-AD/gelatin gel. Oxidization and gelatin both help improve the crosslink density of the dextran hydrogels which influence the cell adhesion.

Toxicity test is an important aspect of biomaterials. An ideal bioadhesive should not release toxic products or produce adverse reactions, which can be evaluated through in vitro cytotoxic tests. It is a basic property of a biomaterial. Fig. 7 showed the absorbance obtained from an MTT assay of mouse fibroblast cells (L929) which were cultured with the extraction media from various types of specimens. For L929, it could be seen that, no statistically significant differences ($p < 0.05$) were observed in the cell activity within 72 h in the presence of the most of the hydrogels extracts in comparison with negative control except Dex-U-AD/gelatin (40 wt%). When crosslinked hydrogels of Dex-U-AD/gelatin (40 wt%) extract

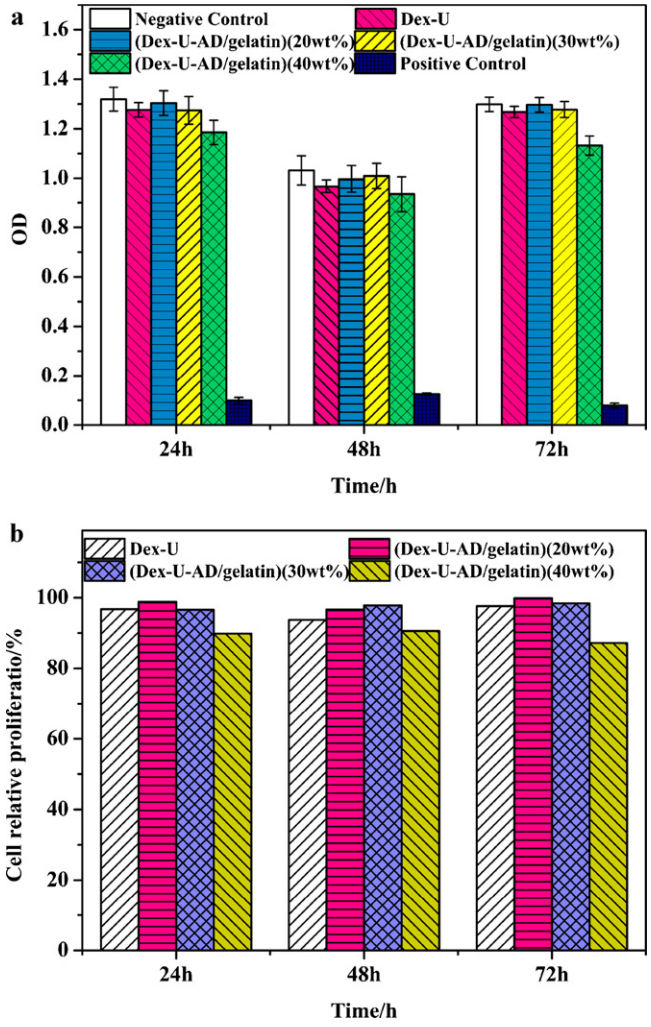


Fig. 7. Cytotoxicity test of gels: (a) MTT test of Dex-U gels with positive and negative controls, $^*p > 0.05$ predicted no statistically significant differences when compared to the negative control of indirect cytotoxicity. The data represented mean and standard deviations of six samples; (b) cell relative proliferation.

was used, no statistically significant differences ($p > 0.05$) were observed in the cell activity in comparison with control within 72 h, but the viability of the cell still reached about 85% of the negative control. This indicates that the Dex-U-AD/gelatin was less toxic to L929 cells.

4. Conclusion

In this study, an attractive photocrosslinkable bioadhesive system consisting of dextran derivatives (Dex-U-AD) and gelatin was successfully prepared. The degree of oxidization of Dex-U-AD and the addition of gelatin, as important factors, were related to the product properties. The adhesion strength and the cytotoxicity were improved significantly. The Dex-U-AD/gelatin bioadhesive not only showed adhesive strength that was superior to the commercially available fibrin based adhesives but also showed no cytotoxicity toward growth of L929 cell and had good in vitro biocompatibility. These films have the potential to be used as bioadhesive material.

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