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Strong Collagen Hydrogels by Oxidized Dextran Modification

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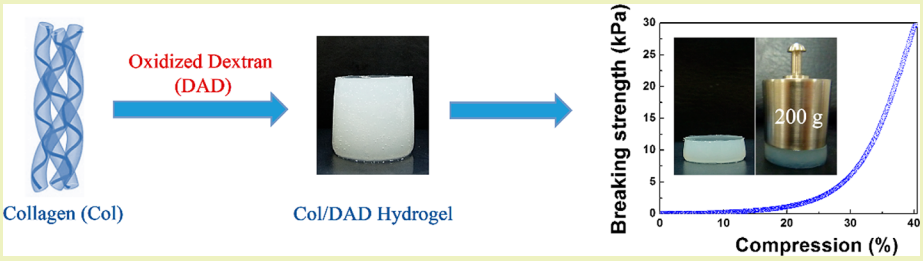
Supporting Information



ABSTRACT: Collagen hydrogel has been regarded as an excellent biomaterial because it is an abundant and sustainable resource and has good biocompatibility and controllable cell-based biodegradability. However, the poor mechanical properties of collagen hydrogel are the main disadvantage preventing it from having wide applications. In this communication, we use aldehyde-functionalized dextran, which is prepared from the oxidation of another natural polymer dextran, as a macromolecular cross-linker to enhance the strength of the collagen hydrogel. The resulting collagen/aldehyde-functionalized dextran (Col/DAD) hydrogels are much stronger and show better thermostability than the pristine collagen hydrogel, as expected. The maximum compressive strength of the Col/DAD hydrogel is 32.5 ± 1.6 kPa, which is about 20 times more than that of the pristine collagen hydrogel. We also prove that our method maintains the good biocompatibility of the collagen hydrogel and does not bring the cytotoxicity often observed from conventional chemical cross-linking in the product. Therefore, the strong collagen hydrogel made by oxidized dextran modification may have a great potential in tissue engineering and other biomedical fields.

KEYWORDS: *Proteins, Polysaccharides, Hydrogels, Mechanical properties, Biocompatibility*

INTRODUCTION

Tissue engineering normally contains three inseparable aspects, such as cells, polymer scaffolds mimicking the extracellular matrix (ECM) in tissues, and other growth inducing factors.[1−3] Among them, the key aspect is to build a biocompatible/biodegradable three-dimensional polymer scaffold to bring cells together, regulate their functions, and allow the diffusion of nutrients, metabolites, and growth factors.[4,5] In other words, the cells are put into three-dimensional polymer scaffolds that act as the analogues to the natural ECM.[3,6] Hydrogels, which can be processed under relatively mild conditions, are well known to be similar to the macromolecule-based ECM and thus have attracted much attention for tissue engineering application accordingly.[7] In order to make the hydrogels suitable for tissue engineering, a variety of synthetic and naturally derived materials have been investigated.[6,8−10]

Due to the advantage of biocompatibility and biodegradability, natural polymers such as collagen, gelatin, fibrin, agarose, alginate, chitosan, and hyaluronic acid have been extensively studied as the potential matrix for tissue engineering application.[9,11−13] Among them, collagen is the most widely used as it is the main component of ECM of mammalian tissues itself, showing good attachment/adhesion to different kinds of cells.[14−18] However, naturally formed collagen hydrogel (normally physically cross-linked) shows poor thermal stability and mechanical properties;[10] therefore, different kinds of chemical cross-linking methods (for example, the use of glutaraldehyde, formaldehyde, and carbodiimide as cross-linking agents)[15,19−22] were logically thought to be used to improve their properties. In addition, other attempts like physical treatments (for example, UV irradiation, freeze-drying, and heating)[23−25] and blending with other natural/synthetic polymers (for example, hyaluronic acid, chitosan, poly(ethylene oxide), polylactic acid, and polyglycolic acid)[10,26−29] were also considered. Unfortunately, the problems seemed not totally to be solved with these methods because blending with other polymers generally cannot improve the strength of the collagen hydrogels much and chemical cross-linking often has adverse effects on the biocompatibility or even adds cytotoxicity to the hydrogels.[10,30] In addition, though researchers have thought to use the natural product genipin to cross-link collagen, it still introduced cytotoxicity when genipin concentration was more than 5 mmol/L, and the improvement of the mechanical properties was regrettably not significant.[31]

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In the present work, we tried a new approach by cross-linking collagen with a polysaccharide derivative, which at the same time improved the mechanical properties of the hydrogels and kept its good biocompatibility.

EXPERIMENTAL SECTION

Preparation of Collagen/Aldehyde-Functionalized Dextran (Col/DAD) Hydrogels. The method for the preparation of type I collagen (Col) is well-documented in the literature.[32] Briefly, fresh bovine tendons were treated with 0.5 mol/L acetic acid at 4 °C for 72 h under mechanical stirring, and then sodium chloride was used to salt out the protein. After dialyzing with deionized water for another 3 days, the collagen was lyophilized and stored at 4 °C before use. SDS-PAGE analysis showed the molecular weight of collagen was about 300 kDa, and the electrophoresis pattern displayed two α1 bands and one α2 band, indicating the collagen we obtained from the tendons was type I. The CD spectrum further confirmed the prepared collagen was an unspoiled triple-helical structure.

Aldehyde-functionalized dextran (DAD) was prepared by oxidizing dextran with sodium periodate to introduce aldehyde groups with a common recipe reported in the literaure.[33,34] Briefly, 4 g of dextran and 3.3 g of sodium periodate were dissolved in deionized water, and then the mixture was stirred in the dark for 24 h at room temperature. After adding ethylene glycol to end the reaction, the resulted DAD solution was dialyzed against deionized water to remove unreacted sodium periodate. The final DAD solution was stored at 4 °C for further use, and its concentration was determined by a weight method. The aldehyde substitution degree of DAD was determined with *tert*-butyl carbazate (tBC) via carbazone formation, and the molecular weight was analyzed by GPC.

To prepare Col/DAD hydrogels, the lyophilized collagen was fully dissolved in 0.25 mol/L acetic acid solution at 4 °C and then neutralized by the addition of 3 mol/L sodium hydroxide. The neutralized collagen solution and DAD solution were mixed with different mass ratios under continuous stirring at 4 °C. If not specified, the mixed Col/DAD solution was incubated at 37 °C for 24 h to form hydrogels, and the Col content in the mixture was set to 6 mg/mL. The pristine Col and Col/dextran (Col/Dex) hydrogels were prepared with a similar method.

Chemical Structure of DAD. The chemical structure of synthesized DAD was determined by a DMX 500 NMR spectrometer (Bruker, Switzerland). ^{1}H NMR spectra were recorded in D_2O (150–200 mg DAD in 0.5 mL D_2O) with the a 90° pulse length of 11.2 μs. The delay before the application of pulse was 6 s, and the acquisition time was 1.0 s. The spectral width was 16 ppm with the number of data points of 32 k. A total of 16 scans were co-added for ^{1}H NMR spectra.

Oxidized Degree of DAD. The oxidized degree of DAD was measured according to a method reported in the literature.[35] In brief, DAD (0.5 mL, 0.01 mol/L), tBC (0.5 mL, 0.025 mol/L), and trichloroacetic acid solution (1%) were mixed and allowed to react for 24 h at room temperature. Then, 200 μL of the final solution was transferred to a disposable centrifuge tube containing 2 mL of a 4 mmol/L aqueous trinitrobenzenosulfonic acid (TNBS) solution (in 0.1 mmol/L borate buffer, pH 8). After a 30 min reaction at room temperature, the mixture was diluted with 0.5 mol/L of an aqueous hydrochloric acid solution, and the absorbance at 334 nm was measured. Aqueous tBC solutions with different concentrations were used as standards to obtain a calibration curve for determination of the unreacted tBC in DAD samples, and then the oxidized degree of DAD can be calculated by the amount of tBC consumed in the reaction.

Rheology Measurements. The rheological behaviors of Col/DAD systems were performed in a strain-controlled mode using a Physica MCR 301 rheometer (Anton Paar GmbH, Austria) with a cone-and-plate geometry of 1° incline and 60 mm diameter (CP 60/1). To minimize evaporation, a solvent trap was employed, and low viscosity mineral oil was applied around the sample. To test the effect of incubation temperature on the rheological behavior of the pristine Col solution and Col/DAD mixtures, the storage moduli (G') and loss moduli (G'') were monitored as a function of time for 24 h at 1 Hz and 0.01 strain under a constant temperature (from 25 to 40 °C). The nonisothermal tests were performed at 1 Hz with a small deformation (1%) from 37 to 70 °C with a heating rate of 1 °C/min. Before testing, the samples were incubated on the rheometer at 37 °C for 5 h to ensure the formation of hydrogels. The temperatures of the sample and atmosphere were controlled using a Peltier temperature control device.

Morphology Observations. AFM observations were performed on a Dimension 3100 Nanoscope IV equipped with silicon cantilevers (TESP) in a noncontact (taping) mode. Both dilute pristine Col and Col/DAD (ratio of 50/50) solutions were dropped onto a fresh mica substrate and allowed to fully dry in a desiccator for 24 h at room temperature. SEM observations were carried out on a TS 5136MM SEM at 20 kV. Both pristine Col and Col/DAD (ratio of 50/50) hydrogels were frozen by liquid nitrogen and then lyophilized. Then, the test samples were transferred onto a substrate covered with carbon tape and coated with a 2 nm layer of gold to ensure the conductivity of all samples.

Mechanical Property Measurements. The mechanical properties of the hydrogels were measured by a uniaxial compression test method using an Instron 5565 universal testing machine. Measurements were performed at a crosshead speed of 1 mm/s at 25 °C until the breakdown of the hydrogel structures. The hydrogel samples were cylindrical with a diameter of 26 ± 1 mm and height of 9 ± 1 mm. The result was averaged by at least three samples.

Cytotoxicity Tests. The L929 mouse fibroblasts, COS-7 monkey fibroblast, and Hela cells were used to study the cytotoxicity of Col/DAD hydrogels. L929 cells were seeded on the surface of the formed hydrogel, or in another way, the cells was first mixed with pristine Col and Col/DAD solutions and then formed hydrogels. Afterward, these Col/DAD hydrogels with L929 cells were incubated in culture medium (Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin) at 37 °C in a CO_2 incubator containing 5% CO_2 for 24 h. To assess cell attachment and proliferation on the hydrogel samples, calcein-AM and ethidium homodimer (EthD) were used as staining dyes. Green-labeled cells represent viable cells with no membrane disruption (live cells), while red-labeled nuclei indicate cell necrosis (dead cells).[36] Images were obtained with a fluorescence microscope (Olympus IX71, Japan) and a confocal laser scanning microscope (Zeiss Ism710, Germany). The quantitative analysis was performed by using a CCK-8 assay according to the ISO standard (ISO 10993.12–2004). The pristine Col and Col/DAD hydrogels were placed into a culture plate with fresh culture medium added at 1.25 cm^2/mL under sterilized conditions. The L929, COS-7, and Hela cells were cultured at a density of 1.0×10^4 cells/mL on 96-well plates (with 200 μL per well) in a CO_2 (5%) incubator at 37 °C. After incubation for 24 h, the extracts of the hydrogels were added to replace the culture medium (with 200 μL per well) of the cells. In the meantime, the fresh culture medium was added to the control group. After incubation for 24 and 72 h, the culture medium was replaced with fresh serum-free DMEM. Then, CCK-8 was added in the dark according to the protocol, and the absorbance of the solution at 450 nm was tested using an Elx 800 instrument (Biotek, U.S.A.) after incubation for 4 h. The relative cell viability (%) was calculated as follows

$$\text{Cell viability (\%)} = [A]_{\text{test}}/[A]_{\text{control}} \times 100\%$$

where $[A]_{\text{test}}$ is the absorbance of the test sample, and $[A]_{\text{control}}$ is the absorbance of the control sample.

RESULTS AND DISCUSSION

Characterization of Col/DAD Hydrogels. First of all, we used NMR to confirm whether we obtained the desired DAD structure. Figure 1 is the ^{1}H NMR spectra of both pristine dextran (curve a) and oxidized dextran (curve b). This shows that besides the peaks (1, 2, 3, 4, 5, and 6) assigned to the protons from the glucose unit both appearing in pristine and

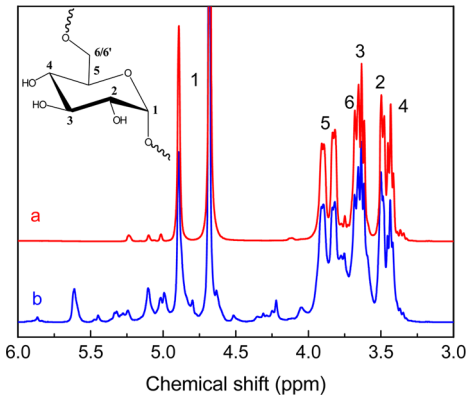


Figure 1. ^{1} H NMR spectra of pristine dextran (a) and oxidized dextran (b).

oxidized dextran, the spectra of oxidized dextran presents several additional peaks in the range of 4.2−5.8 ppm, which were assigned to the protons from the hemiacetal structures.[37] This confirmed that oxidation of dextran was successful, and the aldehyde groups were readily formed in dextran molecular chains. Then, from GPC analysis, we can obtain the weight-average molecular weight and polydispersity index (PDI) of the as-prepared DAD as 2.8×10^4 and 1.88, respectively. Moreover, the oxidized degree of the as-prepared DAD was determined as 13.8%.

Both pristine Col and Col/DAD mixture solutions can form hydrogels after 24 h of incubation at 37 °C. The appearance of pristine Col hydrogel and Col/DAD hydrogel was almost identical (Figure S1, Supporting Information). However, after lyophilization, the morphology of the two hydrogels was different. The SEM image of pristine Col hydrogel shows the existence of irregular large pores (Figure 2a). For Col/DAD

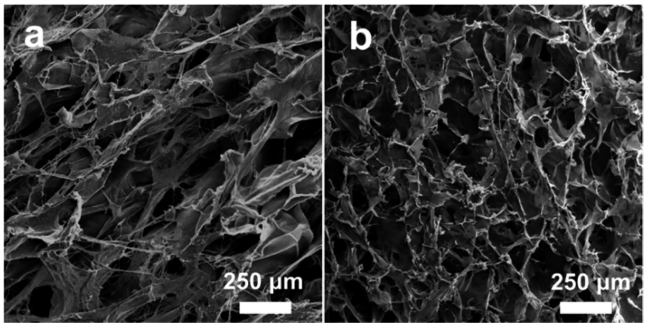


Figure 2. SEM images of the cross-section of lyophilized pristine Col hydrogel (a) and Col/DAD hydrogel (b). Col/DAD = 50/50.

hydrogel, the pores are obviously small and homogeneous (Figure 2b), which is favorable for improvement of the strength. The AFM image from the pristine Col solution shows the the Col fibrils formed randomly on the mica and simply overlapped with each other (Figure 3a), in line with the previous reports.[38,39] On the contrary, the AFM image from Col/DAD solution clearly indicates the interconnection of the fibrils, forming a quite uniform network (Figure 3b). Such a phenomenon is supposed to be caused by the interconnection of Col molecular chains, which may be attributed to the chemical cross-linking by DAD.

Mechanical Properties of Col/DAD hydrogels. The SEM and AFM observations imply that DAD seems serve as a

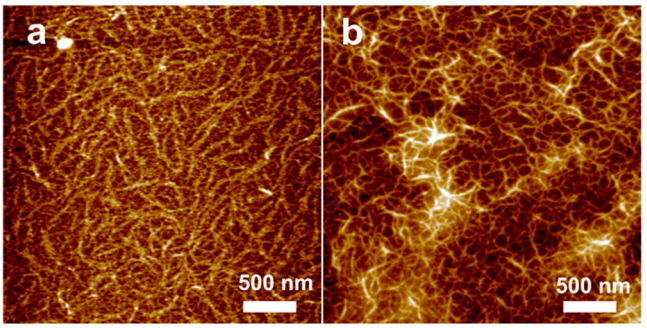
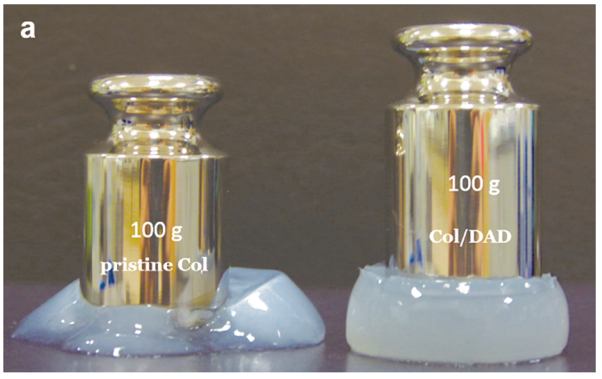


Figure 3. AFM images after the pristine Col solution (a) and Col/DAD solution (b) were dropped on the mica substrate and dried. Col/DAD = 50/50.

macromolecular cross-linker to cross-link Col in the Col/DAD hydrogels. Thus, the strength of the Col/DAD hydrogels is expected to be enhanced compared to the pristine Col hydrogel. Figure 4a shows the direct impression on the



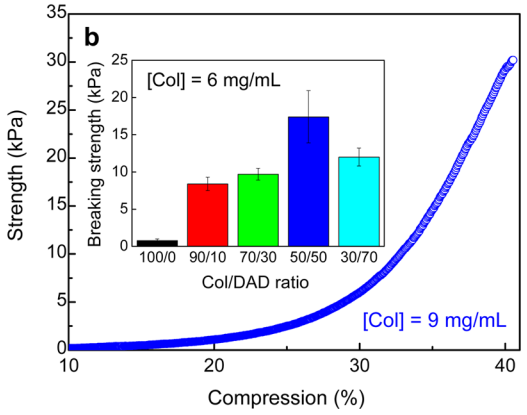


Figure 4. (a) Digital photo of pristine Col hydrogel and Col/DAD hydrogel subjected to compression by 100 g weight. (b) Representative stress−compression curve of the Col/DAD hydrogel (Col/DAD = 50/50, [Col] = 9 mg/mL). Inset is the comparison of the breaking compressive strength of the hydrogels with different Col/DAD ratios ([Col] = 6 mg/mL).

difference between two kinds of hydrogels. When we put a 100 g weight on both hydrogels, the pristine Col sample collapsed immediately, but the Col/DAD sample sustained it firmly. Figure 4b is a typical stress−compression curve of the Col/DAD hydrogel (Col/DAD = 50/50, [Col] = 9 mg/mL) and the comparison of the breaking strength of the hydrogels with different Col/DAD ratios (inset, in which [Col] = 6 mg/mL).

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It is found that no matter what ratio was used (Col/DAD ratio from 90/10 to 30/70), the Col/DAD hydrogel was much stronger than the pristine Col hydrogel. Among the hydrogels with different Col/DAD ratios, the one in which Col/DAD = 50/50 exhibited the largest breaking strength (17.4 ± 3.5 kPa), which was more than 20 times higher than the pristine Col hydrogel (0.8 ± 0.2 kPa). It is worth noting that if we increased the Col content from 6 mg/mL to 9 mg/mL, the breaking strength can be further enhanced to 32.5 ± 1.6 kPa when keeping the Col/DAD ratio unchanged (50/50).

A maximum value of breaking compressive strength at Col/DAD = 50/50 is a good explanation of the interactions between DAD and Col. In Figure 4b, we kept the Col content constant in the hydrogel, so with a decrease in the Col/DAD ratio (i.e., from 90/10 to 30/70), the DAD content increased, and the total solid content in the hydrogel also increased. Therefore, if there was only a simple blending, the breaking compressive strength should linearly increase with an increase in solid content in the hydrogel. We also added unmodified dextran to the hydrogel in order to make the total solid content constant and then tested the breaking compressive strength again. The result showed that although the individual breaking compressive strength increased a little, the whole tendency did not change at all. The Col/DAD hydrogels were still much stronger than Col/Dex hydrogels, and the maximum value still appeared at Col/DAD = 50/50 (Figure S2, Supporting Information). This further supports our assumption that DAD acts as a macromolecular cross-linker to interact with Col and thus forms a network. When the DAD content in the hydrogel was too high (for instance, Col/DAD = 30/70), the excess DAD did not contribute but contarily deteriorated the strength of the hydrogels because of its low molecular weight (~28 kDa) compared with Col (~300 kDa).

In order to better understand the formation process of the Col/DAD hydrogels, we applied rheology tests for further investigation. Figure 5 shows that temperature has a great effect

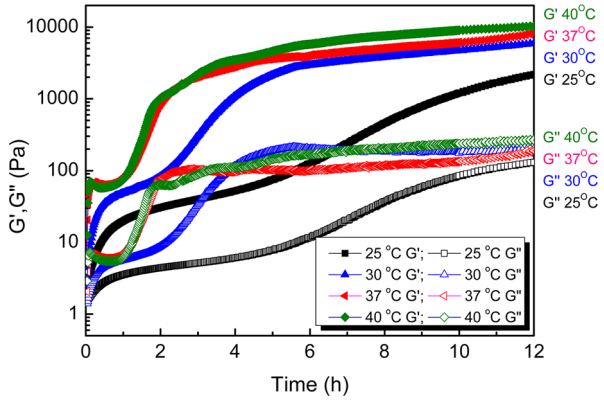

Figure 5. Change of storage modulus (G') and loss modulus (G'') of the Col/DAD hydrogel (Col/DAD = 50/50) as a function of time under different incubation temperatures.

on hydrogel formation. At relatively high temperatures (37 or 40 °C), the G' of the Col/DAD system started to increase significantly at about 1 h after mixing, from ~100 Pa at 1 h to ~1000 Pa at 2 h and then ~2500 Pa at 3 h. If the temperature was 30 °C, the increase in G' started at 2 h after mixing, reaching ~1000 Pa at 4h, and then ~2500 Pa at 5.5 h. Obviously, the rate of hydrogel formation at 30 °C was much slower than those at 37 or 40 °C. As expected, the formation of the hydrogel was slower still 25 °C, taking more than 12.5 h to reach a G' of ~2500 Pa (Figure 5 and Figure S3, Supporting Information). Although an increase in incubation temperature speeded the hydrogel formation process of Col/DAD system, it seemed have little effect on the final mechanical property of the resulting Col/DAD hydrogel. It showed that after 24 h incubation, the final G' of the Col/DAD hydrogel was almost the same (about 20 kPa), no matter whether at 30, 37, or 40 °C (Figure S3, Supporting Information). In addition, the G' of the Col/DAD hydrogel was about 1 order of magnitude large than that of the pristine Col hydrogel (Figure S4, Supporting Information), which agreed well with the result of compressive strength shown above. This further demonstrates that DAD has interacted with Col by its aldehyde groups as a macromolecular cross-linker, just like the ordinary protein cross-linking by aldehydes (for example, glutaradehyde). Obviously, an increase in temperature is favorable to such a cross-linking reaction.

In addition, when we gradually heated the pristine Col, Col/Dex, and Col/DAD hydrogels, it was found that only the G' and G'' of the Col/DAD hydrogel was kept unchanged or even increased a little until the temperature was increased to 70 °C (Figure 6). However, the G' and G'' of both the pristine Col

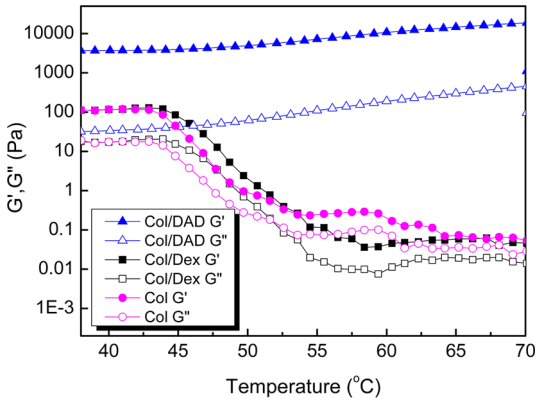

Figure 6. Change of G' and G'' for pristine Col, Col/Dex, and Col/DAD hydrogels with an increase in temperature (ratios of Col/Dex and Col/DAD are all 50/50).

and Col/Dex hydrogels declined sharply when the temperature increased to 45 °C. This means that the network in the Col/DAD hydrogel maintained very well when the temperature reached 70 °C, but the networks in the pristine Col and Col/dextran hydrogels were broken at 45 °C. Such a phenomenon further supports the assumption that DAD cross-linked Col, so the Col/DAD hydrogel had a better thermostability than the pristine Col and Col/Dex hydrogels.

Biocompatibility of Col/DAD Hydrogels. The best advantage of the Col hydrogel is its excellent biocompatibility, so it is rather important that such a merit is not affected by the modification. In this research, we first cultivated L929 mouse fibroblasts both on the surfaces and inside the hydrogels to have a direct impression on the biocompatibility of Col/DAD hydrogels. The optical microscopic observation indicated that L929 cells were well spread on the surface of the hydrogels with abundant cytoplasmic extensions, even when the Col/DAD ratio was as low as 30/70 (Figure 7a,c,e,g). The corresponding fluorescence microscopic images show that almost all the cells are alive on the surface of the Col/DAD hydrogels, having no difference compared to the pristine Col hydrogel (Figure 7b,d,f,h). In the meantime, the confocal laser scanning

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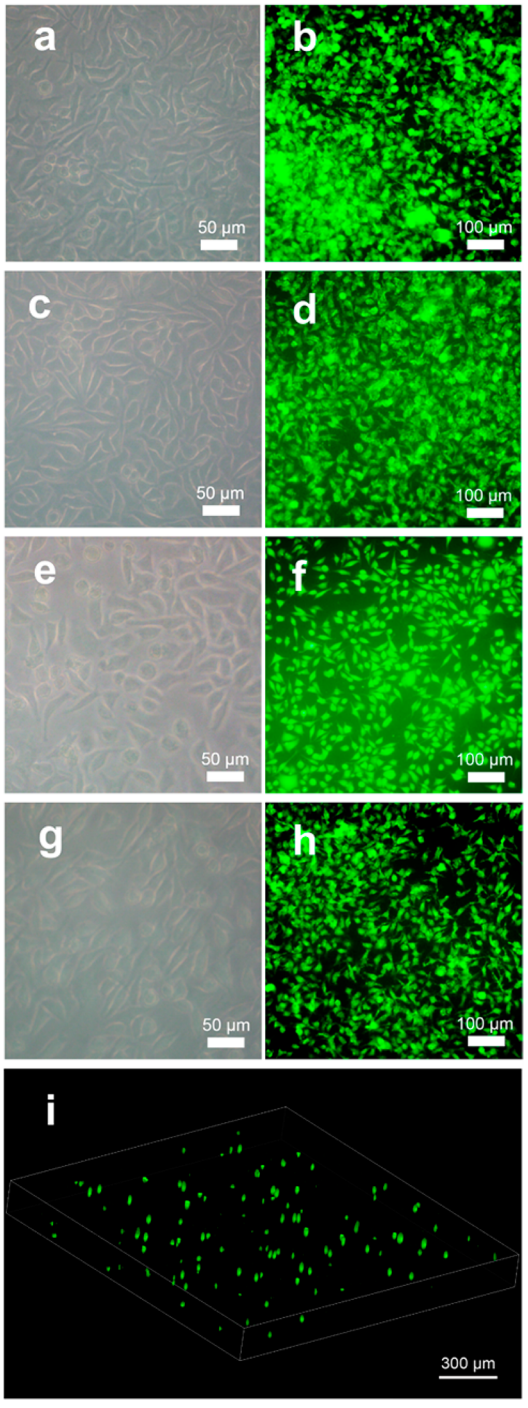

Figure 7. Optical microscopic images (a, c, e, g) and fluorescence microscopic images (b, d, f, h) of L929 cells cultivated on the surface of pristine Col (a, b) and Col/DAD (c, d: Col/DAD = 70/30. e, f: Col/DAD = 50/50. g, h: Col/DAD = 30/70) hydrogels, and 3D reconstructed confocal laser scanning microscopic image of L929 cells cultivated inside the Col/DAD hydrogel (i: Col/DAD = 50/50).

microscopic image also shows that L929 cells can disperse and grow well inside the hydrogel (Figure 7i). Then, we tested the cytotoxicity of the Col/DAD hydrogel by the CCK-8 assay not only with L929 cells but also with COS-7 and Hela cells. The results shown in Figure 8 further prove there is almost no cytotoxicity for Col/DAD hydrogels. All of these observations confirmed that DAD had no adverse effect on the biocompatibility of the Col hydrogel as designed because

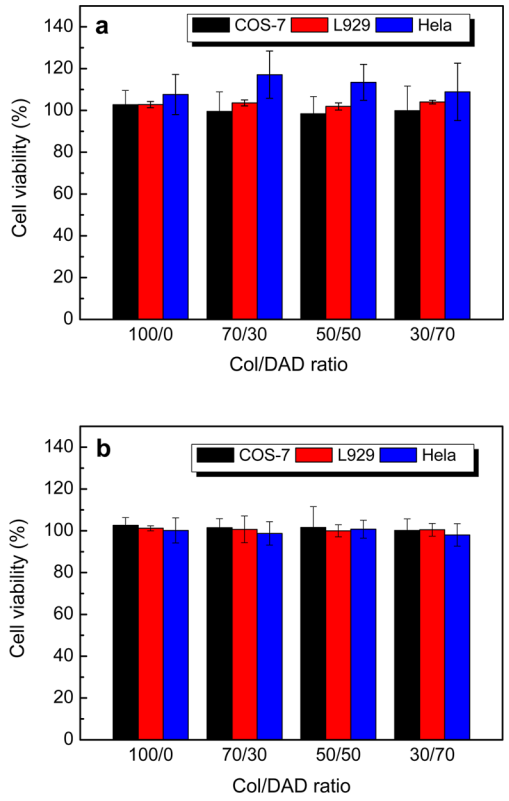

Figure 8. Cytotoxicity of pristine Col and Col/DAD hydrogels with COS-7, L929, and Hela cells after incubation for 24 h (a) and 72 h (b).

DAD is a polysaccharide derivative, which should have good biocompatibility.

DISCUSSION

The principle of the method reported in this communication is actually rather simple as it is well known that aldehyde (such as formaldehyde, glutaradehyde, and dialdehyde starch) can cross-link proteins. We know that Col has about 3.1% of amino acid residues with free amino groups (mainly, lysine and hydroxylysine),[40] so DAD should cross-link Col by creating covalent bonds between its aldehyde groups and amino groups in lysine and hydroxylysine residues. Lin et al. reported that the decrease in amide A and B bands of Col in the FTIR spectrum of Col/DAS (dialdehyde starch) cryogel could be the evidence for the reaction of the amino groups of lysine and hydroxylysine in Col and the aldehyde groups in DAS,[41] and we also can observe such a change in the FTIR spectrum of our Col/DAD hydrogel (Figure S5, Supporting Information). In addition, we put both pristine Col and Col/DAD hydrogels into an 8 mol/L guanidine hydrochloride solution, which is strong enough to break hydrogen bonds in physically cross-linked hydrogel. The results showed that the pristine Col hydrogel dissolved within 30 min, but the Col/DAD hydrogel stably existed at least for 24 h (Figure S6, Supporting Information). Therefore, we have enough reason to say that DAD acts as a macromolecular cross-linker in Col/DAD hydrogel and attribute the significant improvement of the mechanical properties to the corresponding cross-linking. Normally, the Col hydrogels are weak, so the researchers often have to compare G' from rheological tests to evaluate the improvement of the mechanical properties of the cross-linked Col hydrogels. Sundararaghavan et al. used natural

product genipin to cross-link Col and found that the G' of Col was increased from 0.1 to 1.0 kPa when the genipin concentration was 10 mmol/L.[31] However, under such a genipin concentration, the cross-linked Col hydrogel showed some cytotoxicity. In another report, the authors used chemical cross-linking agents 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC), malic acid derivative, and glutaradehyde to cross-link Col hydrogels.[42] Despite the risk of inducing cytotoxicity (especially for EDC and glutaradehyde), the G' of the cross-linked Col hydrogels only increased to 2.0, 4.0, and 7.5 kPa, respectively. In our case, the G' of the Col/DAD hydrogel was about 20 kPa with almost no cytotoxicity. In addition, although few reports on the compressive strength of Col hydrogel can be found in the literature, the one by Zhang et al. showed there was only about a 16% improvement of compressive strength by cross-linking with genipin,[43] which is much less than we reported in this work.

Thus, we believe DAD is a good candidate to cross-link proteins and further enhance their mechanical properties. We think such a method could be used on other protein materials to make various strong protein hydrogels. We are working to apply this method to modify another protein material studied in this laboratory, soy protein, which has much more amino acid residues with free amino groups (6.3% lysine and 6.5% arginine),[44] to hopefully develop another strong and biocompatible protein-based hydrogel.

CONCLUSIONS

In this work, we demonstrate our attempt to use DAD, a natural polysaccharide derivative, as a macromolecular cross-linker to modify weak Col hydrogel. The results show that the strength of Col hydrogel is remarkably increased when incorporated with DAD. The compressive strength of the Col/DAD hydrogel is about 20 times more than that of the pristine Col hydrogel with the same Col content. Specifically, it can reach 32.5 kPa when Col/DAD = 50/50 and [Col] = 9 mg/mL. In addition, the Col/DAD hydrogels show a better thermostability than the pristine Col and Col/Dex hydrogels, supporting that there is a chemical cross-linking in Col/DAD hydrogels. Both SEM and AFM observations also show that the Col/DAD hydrogels have a better and more regular three-dimensional structure than the pristine Col hydrogel. Finally, we prove that the excellent biocompatibility of the Col hydrogel is not affected by the addition of DAD. Therefore, on the basis of the remarkable mechanical property and biocompatibility, the Col/DAD hydrogel could be an excellent candidate for wide application in tissue engineering.

ASSOCIATED CONTENT

Supporting Information

Digital photos of pristine Col hydrogel and Col/DAD hydrogel as prepared, comparison of the breaking compressive strength of the hydrogels with different Col/DAD ratios when adding dextran to keep the total solid content as a constant, change of storage modulus (G') and loss modulus (G'') of the Col/DAD hydrogel (Col/DAD = 50/50) as a function of time under different incubation temperatures for 24 h, comparison of storage modulus (G') and loss modulus (G'') changes of the pristine Col and Col/DAD hydrogel at 37 °C, FTIR spectra of the pristine Col and Col/DAD hydrogel (Col/DAD = 50/50), and digital photos of pristine Col and Col/DAD hydrogel (Col/DAD = 50/50) immersed in 8 mol/L of guanidine hydrochloride solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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

Notes

The authors declare no competing financial interest.

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