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# Transglutaminase-catalyzed grafting collagen on chitosan and its characterization

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ABSTRACT

Collagen grafted chitosan was prepared with microbial transglutaminase (MTGase) as biocatalyst which showed high efficiency, selectivity, mild reaction condition and environmental friendliness. The reaction conditions that influenced the degree of substitution (DS) were optimized, which included the reaction time, the reaction temperature, the mass ratio of collagen to chitosan and the mass ratio of MTGase to chitosan. In this study, the water-solubility collagen–chitosan could serve not only to reduce the loss of moisture but also to absorb the moisture. And the moisture absorption and moisture retention abilities were closely related to the DS values. In addition, in vitro antioxidant activity was evaluated in terms of DS values and concentration. Furthermore, L929 mouse fibroblasts were cultured with collagen–chitosan, and methylthiazol tetrazolium (MTT) assay exhibited that collagen–chitosan with DS of 0.660 displayed pronounced cell viability at 2.5 mg/ml. Therefore, the water-soluble collagen–chitosan showed the potentiality to repair skin in cosmetic, biomedical and pharmaceutical fields.



## 1. Introduction

Skin is a dynamic and integrated organ with the largest surface area in human body. Its normal functions can be gradually decreased by many factors, such as aging, health status, environmental pollutants and wounds (Lee et al., 2012). UV irradiation and burns also disturb the balance between the formation of reactive oxygen species and the capacity of antioxidant system in several ways (Frisman, Racz, & Chmelarova, 2013). At high levels of oxidative stress, the significant imbalance between free radicals and antioxidant defense system can lead to cell injury (Lin et al., 2013). Over the past few decades, natural biomaterials such as collagen, silk and chitosan had received increasing attention in food, cosmetic, biomedical and pharmaceutical fields due to their unique properties, such as biodegradability, biocompatibility and non-toxicity (Aramwit, Siritientong, Kanokpanont, & Srichana, 2010; Li et al., 2013). It was also reported that some natural polysaccharides and peptides had the ability to promote cell attachment, proliferation and tissue regeneration (Chen, Wang, Chen, Ho, & Sheu, 2006; Koide, 2007; Ueno et al., 1999).

Collagens, characterized by a unique triple-helical structure, are the cardinal component of extracellular matrices existing in all multicellular animals (Koide, 2007). Collagen has favorable biodegradability, biocompatibility, antioxidant activity, reparative ability to skin, and weak immunogenic reactions (Fujii et al., 2013; Sangeetha, Ramamoorthy, Sreeram, & Nair, 2012; Sun et al., 2009). And collagen is extensively utilized in the cosmetic industry as a water-retain and moisturizing agent. Recently, the use of fish collagen is remarkable because of its high availability and it could reduce the potential for disease transmission (Shen et al., 2008). Therefore, fish scale collagen is acknowledged as one of the most promising biomaterials in wound healing application (Gopinath, Kumar, Selvaraj, & Jayakumar, 2005).

Chitosan is a natural hydrophilic biomacromolecule abundantly found in crustaceans (Muzzarelli et al., 2012). Structurally, it is the linear (1-4)-2-amino-2-deoxy-β-D-glucan, randomly acetylated to a minor extent (Francis Suha & Matthewb, 2000). As the only cationic polysaccharide in nature, chitosan has attracted the attention of researchers owing to biocompatibility, biodegradability, antimicrobial activity, and peculiar biological properties in wound healing (Muzzarelli, 2009). However, chitosan is normally insoluble in aqueous solution above pH 7 because of the stable, crystalline structure (Chen, Wang, Liu, & Park, 2002). So improving

*Abbreviations:* MTGase, Microbial transglutaminase; $R_a$, Moisture-absorption; $R_h$, Moisture-retention; DS, The degree of substitution; MTT, Methylthiazol tetrazolium; Vc, Ascorbic Acid; HA, Hyaluronic acid.

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the solubility of chitosan is the key step to exploit its application. PEG-grafting, hydroxylation, sulfonation, quaternarization and carboxymethylation are the common methods to modify chitosan. However, chemical crosslinking agents frequently induce toxic side effects (owing to residual agents) or to secondary reaction with unwanted products (Lin, Liang, Chung, Chen, & Sung, 2005). And compared with 1-ethyl-(dimethylaminopropyl) carbodiimide and *N*-hydroxy sulfosuccinimide crosslinking method (Fan et al., 2013), the enzyme-catalyzed reaction time is distinctly shortened and the degree of substitution (DS) of product is higher. Therefore, a great interest is devoted to focus on enzyme catalyzed reactions to modify the chitosan. Enzyme-catalyzed reactions show a variety of benefits, including the high efficiency, selectivity, mild reaction condition and environmental friendliness (Bertoni, Barbani, Giusti, & Ciardelli, 2006).

As a biocatalyst, microbial transglutaminase (MTGase) is an extracellular enzyme with a remarkable characteristic of calcium-independent catalytic property, furthermore, exhibits a wide substrate specificity being also active over a wide range of temperature and pH values (Porta, Mariniello, Pierro, Sorrentino, & Giosafatto 2011). The MTGase is used to catalyze macromolecular grafting and crosslinking of proteins (Wu, Bentley, & Payne, 2011). And it catalyses the formation of covalent linkages between γ-carboxyamide groups of peptide-bound glutamine residues and ϵ-amino groups of lysine or primary amino groups of a number components (Chen, Embree, Brown, Taylor, & Payne, 2003; Kołodziejska, Piotrowska, Bulge, & Tylingo, 2006). For the sake of integrating mutual advantages, researchers have tried to conjugate chitosan with variety of proteins or peptides (Sang, Zhou, Yun, & Zhang, 2010).

In this paper, collagen–chitosan was synthesized with MTGase as biocatalyst, and the reaction conditions were optimized. Then the ability of moisture absorption and retention was measured in different humidity. The antioxidant activity of collagen–chitosan was discussed in terms of the hydrogen peroxide scavenging activity and DPPH radical scavenging activity. In addition, the impact of collagen–chitosan on proliferation of L929 fibroblasts was investigated.

## 2. Materials and methods

### 2.1. Materials

Chitosan (Mw 520,000) with a 92% degree of deacetylation was supplied by the Golden-Bell (Cochin, India). Fish scale collagen (Mw 3000) was purchased from Sichuan Mingrang Biological Technology Co. Ltd., Sichuan, China, without further purification. Microbial transglutaminase (MTGase) from Streptoverticillium mobaraensis were purchased from Huashun Biological Technology Co. Ltd., Wuhan, China. Dubecco's Modified Eagle Medium (DMEM) was purchased from Thermo Co. Ltd, Beijing, China. Fetal bovine serum (FBS) from Sijiqing Co. Ltd., Zhejiang, China. Methylthiazol tetrazolium (MTT) was purchased from Sigma, Deisenhofen, Germany. Acetic acid, sodium hydroxide and other reagent used in this investigation were of analytical grade and without further purification. They were purchased from Sinopharm Group Chemical Reagent Corp.

### 2.2. Purification of MTGase

MTGase was first poured into 0.2 mol/l $Na_2HPO_4/NaH_2PO_4$ buffer solution (PBS, pH 5.0). The liquid was centrifuged at the speed of 3000 rpm for 10 min. Then the supernatant was purified by dialysis through the 8000–10,000 molecular weight cut-off dialysis tubing for three days. The dialyzed MTGase was lyophilized for 24 h to obtain the MTGase lyophilized power.

### 2.3. Preparation of collagen–chitosan

In a typical reaction procedure, chitosan (1 g) was dissolved in 1% acetic acid and the solution was adjusted to pH 6.0. Collagen (1 g) was dissolved in PBS (0.2 mol/L, pH 6.0). Then they were mixed together to form homogeneous solution. Thereafter, MTGase powder (0.1 g) was also dissolved in PBS solution, and then it was added to the above mixture to catalyze the reaction between chitosan and collagen. Magnetic stirring was continuous for 1 h at 40 °C. Then the solution was treated in boiling water for 10 min and later cooled to room temperature. The collagen–chitosan solution was obtained after filtering with qualitative filter paper. Subsequently, the solution was neutralized with 20% (w/w) aqueous NaOH and then purified by dialysis through the 8000–10,000 molecular weight cut-off dialysis tubing for three days. The dialyzed product was finally freeze-dried to obtain the purified collagen–chitosan. The dried products were stored in vacuum desiccators over $P_2O_5$ for further analysis.

### 2.4. One-factor-at-a-time experiment

Four independent variables were investigated, including the reaction time (0.5, 1–4 h), the reaction temperature (20–60 °C), the mass ratio of collagen to chitosan (0.6, 0.8, 1.0, 1.2 and 1.4) and the mass ratio of MTGase to chitosan (0.06, 0.10, 0.14, 0.18 and 0.22). Their variables were fixed at a certain value, while changing only one variable value (Lin et al., 2013).

### 2.5. Fourier transforming infrared spectroscopy (FT-IR) analysis

FT-IR spectra of collagen–chitosan samples and chitosan were performed with a Nicolet 5700 Fourier transform infrared spectrometer (USA) in the wavenumber ranging from 400 to 4000 $cm^{-1}$. The samples were prepared by the KBr-disk method.

### 2.6. Measurement of the degree of substitution

The degree of substitution (DS) is defined as the number of amine groups substituted per repeating structural unit of the chitosan. In this work, the DS was measured according to the method of Fan (Fan et al., 2013), the concentration of collagen between 0.001 g/l and 0.05 g/l was liner relation with absorbance at 200 nm by UV spectrophotometer. And the standard curve for the liner relation was described as Eq. (1). The chitosan and collagen–chitosan were also measured with absorbance at 200 nm by ultraviolet spectrophotometry with the concentration of 0.05 g/l. The chitosan sample was the blank control. The DSs of collagen–chitosan were determined by Eq. (2).

$$\begin{aligned} A &= 34.05C + 0.030 \\ R^2 &= 0.994 \end{aligned} \quad (1)$$

$$\mathrm{DS} = \frac{1526.45C}{150 - 2999C} \quad (2)$$

where $A$ is the absorbance of collagen, $C$ is the concentration of collagen.

### 2.7. Water solubility testing (Mao, Shuai, Unger, Wittmar, Xie & Kissel, 2005)

Solubility of collagen–chitosan was measured at different pH values at room temperature. Briefly, the samples were dissolved in a 0.25% acetic acid solution (2 mg/mL), the pH value of the solution was adjusted using NaOH and the transmittance of the solution at

600 nm as a function of pH was recorded on an UV spectrophotometer. The samples were considered insoluble when the transmittance was less than 90%, compared to that of distilled water.

### 2.8. Moisture absorption and retention test (Wang, Jin, Hou, Niu, Zhang & Zhang, 2013; Ying, Yang, Yi, & Xu, 2007)

Prior to the moisture-absorption testing, the samples were dried over $P_2O_5$ in vacuo for 24 h. The water-absorption ability was evaluated by the percentage of weight increase of dry sample ($R_a$):

$$R_a(\%) = \frac{W_n - W_0}{W_0} \times 100$$

where $W_0$ and $W_n$ are the weights of samples before and after putting it into a saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and in a saturated $K_2CO_3$ desiccator (43% relative humidity) at 20 °C for 48 h.

In the moisture-retention test, wet samples were prepared by adding 10% water to each sample. The moisture-retention ability was evaluated by the percentage of residual water of wet sample ($R_h$):

$$R_h(\%) = \frac{H_n}{H_0} \times 100$$

where $H_0$ and $H_n$ are the weights of water in sample before and after putting in the silica gel and in a saturated $K_2CO_3$ desiccator (RH 43%) at 20 °C for 48 h.

### 2.9. Antioxidant properties

#### 2.9.1. Hydrogen peroxide scavenging activity

Samples of 1.0 ml were mixed with 6.0 ml of PBS solution (0.1 mol/l, pH 7.4) and 1.0 ml of 40 mmol/l $H_2O_2$. After 10 min, the absorbance of mixture was measured at 230 nm with UV spectrophotometer. And ascorbic acid (Vc) was used as positive control. The $H_2O_2$ scavenging activity of samples which were expressed as percentage inhibition of $H_2O_2$, were calculated according to the following equation:

$$H_2O_2 \text{ scavenging rate}(\%) = \left[\frac{1 - (A_s - A_b)}{A_c}\right] \times 100$$

where $A_s$ is the absorbance value of the sample; $A_b$ is the absorbance value of blank sample without $H_2O_2$; $A_c$ is the absorbance value of control sample without collagen–chitosan.

#### 2.9.2. DPPH radical scavenging activity (Bamdad & Chen, 2013)

Samples were mixed 1:1 v/v with 0.1 mM DPPH in anhydrous ethanol. After incubation at 25 °C in the dark for 30 min, the reduction of DPPH free radicals was determined by measuring the absorbance at 517 nm with a UV spectrophotometer. Ascorbic acid (Vc) was used as positive control. DPPH radical scavenging activity of collagen–chitosan was calculated according to the following equation:

$$\text{DPPH scavengingrate} = \left[\frac{1 - (D_s - D_b)}{D_c}\right] \times 100$$

where $D_s$ is the absorbance value of the sample; $D_b$ is the absorbance value of blank sample without DPPH; $D_c$ is the absorbance value of control sample without collagen–chitosan.

### 2.10. Cell culture of mouse fibroblast cell line L929

The mouse connective tissue fibroblast cell line L929 was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). Cells were incubated at 37 °C in a 5% $CO_2$ incubator and the medium was renewed every 2 days. Cells were harvested using 0.25% trypsin when they reached the state of congestion. Then the fresh culture medium was added to create a new single cell suspension for further inoculation (Sun, Dai, Zhao, & Chen, 2007).

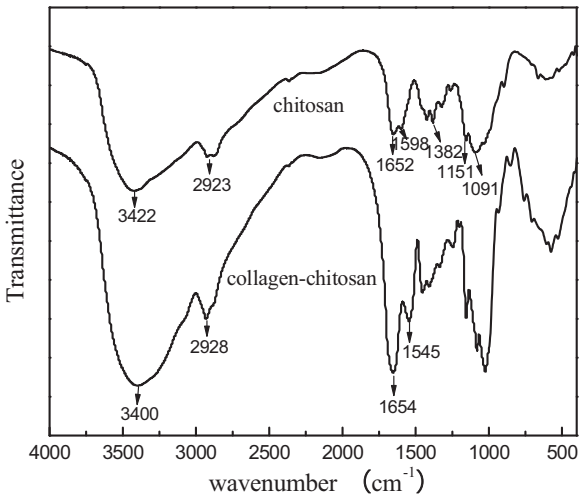


**Fig. 1.** FT-IR spectra of chitosan and collagen–chitosan.

### 2.11. MTT assay

The cell viability was examined by MTT assay (Deng, Zhao, Zhang, Hu, & Chen, 2002; Fischer, Li, Ahlemeyer, Krieglstein, & Kissel, 2003). Briefly, L929 cells at a density of 6000 cells per well were seeded into 96-well microtiter plated in DMEM supplemented with 10% FBS. After 24 h incubation, cells were washed with phosphate buffered saline (PBS) and the fresh culture medium was added. Then samples dissolved by PBS with different parameters were added to each well. Cells added with the same volume PBS acted as control group. After incubation for 22 h, the culture was washed with PBS twice and the culture medium was renewed. Then MTT solution (5 mg/ml in PBS) were added to each well and the plates were incubated for 4 h at 37 °C, followed by removal of the media and addition of dimethylsulfoxide (DMSO) to each wall to dissolve the formazan crystals. The dissolvable solution became homogenous after about 10 min of shaking. The optical density (OD) at 492 nm was measured using microplate reader. The cell viability rate was calculated by the following equation:

$$\text{Cell viability}(\%) = \frac{OD_{sample}}{OD_{control}} \times 100$$

where $OD_{sample}$ was obtained in the presence of collagen–chitosan, and $OD_{control}$ was obtained in the absence of collagen–chitosan.

## 3. Results and discussion

### 3.1. FT-IR spectroscopy

The FT-IR spectra of chitosan and collagen–chitosan are shown in Fig. 1. It shows the absorption bands at 1652 $cm^{-1}$ (C=O stretch), 1598 $cm^{-1}$ (N—H bend), 1382 $cm^{-1}$ (C—H bend), 1151 $cm^{-1}$ (bridge—O—stretch), and 1091 $cm^{-1}$ (C—O stretch) on the IR spectra of chitosan. Obviously, the typical amide I and II bands at 1654 $cm^{-1}$ and 1545 $cm^{-1}$ are observed in the spectrum of collagen–chitosan. It indicates the formation of amido bond at $-NH_2$. FT-IR spectra have given an evidence of the modification of chitosan with collagen.

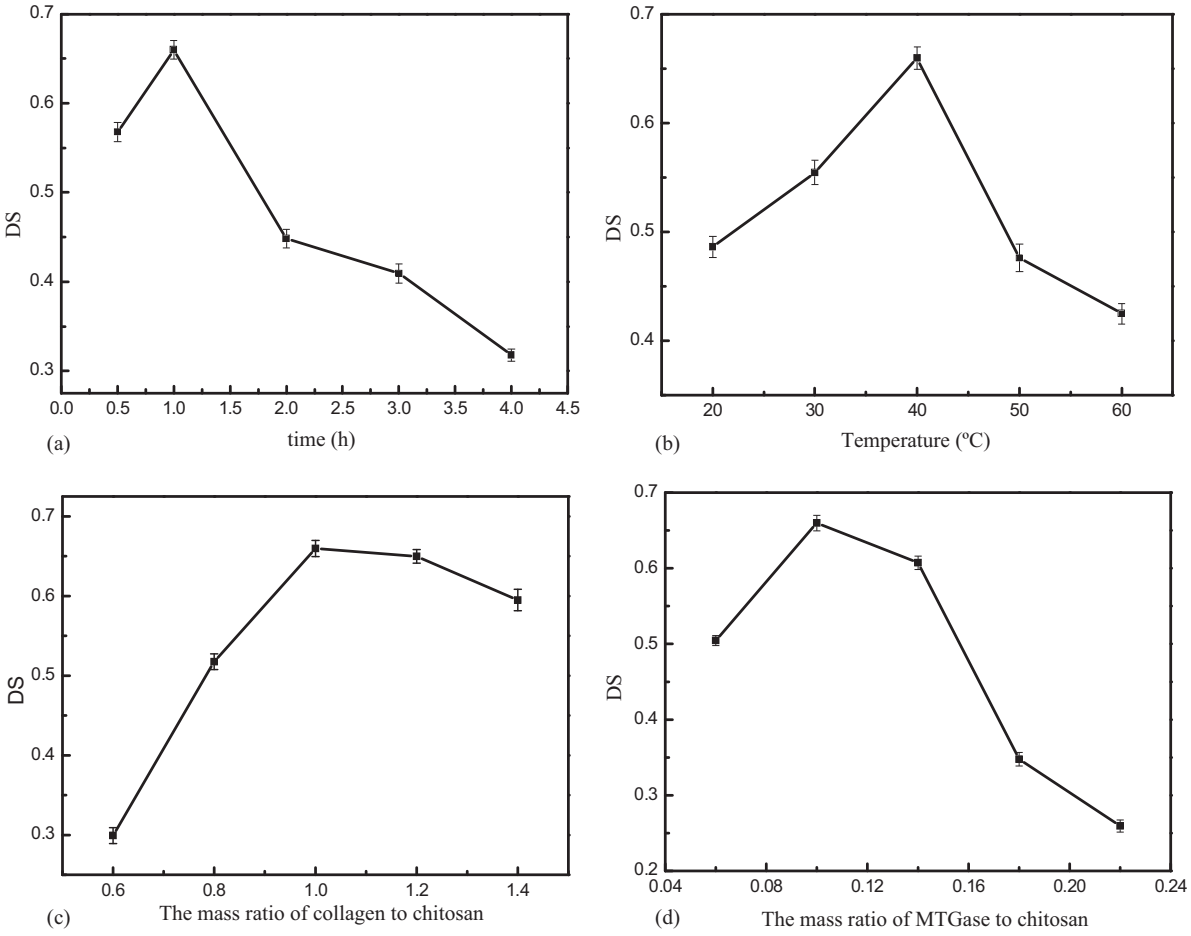


**Fig. 2.** The effect of reaction conditions on DS: (a) The effect of reaction time on DS ($T$ = 40 °C, $m_{collagen/chitosan}$ = 1.0, $m_{MTGase/chitosan}$ = 0.1); (b) The effect of reaction temperature on DS ($t$ = 1 h, $m_{collagen/chitosan}$ = 1.0, $m_{MTGase/chitosan}$ = 0.1); (c) The effect of the mass ratio of collagen to chitosan on DS ($t$ = 1 h, $T$ = 40 °C, $m_{MTGase/chitosan}$ = 0.1); (d) The effect of the mass ratio of MTGase to chitosan on DS ($t$ = 1 h, $T$ = 40 °C, $m_{collagen/chitosan}$ = 1.0).

### 3.2. Optimization of reaction conditions of synthesizing collagen–chitosan

#### 3.2.1. The effect of reaction time on DS

Fig. 2(a) shows that the DS of collagen–chitosan increases from 0.567 to 0.660 when the reaction time increases from 0.5 h to 1 h. However, with the further increase of reaction time from 1 h to 4 h, the DS sharply decreases to 0.318. This can be explained as follows: at first stage, the longer duration of reaction enhances the speed of chitosan and collagen diffusing to the active centre of MTGase. The enzyme becomes saturated with substrates at 1 h. The DS reaches the highest level. Nevertheless, the reversible reaction is accelerated with the further extended period of reaction. And the intermediate reacts with $H_2O$ instead of collagen. That leads the DS to be on the decrease obviously. Therefore, it reveals that the optimal time is 1 h with the highest level of DS 0.660.

#### 3.2.2. The effect of reaction temperature on DS

As shown in Fig. 2(b), the DS increases from 0.486 to 0.660 at the temperature between 20 and 40 °C, but then decreases to 0.425 over 40 °C. It can be seen that temperature affects the movement of these molecules. Thus, with the increase of the temperature, the activity of the reactants and the rate of reaction are accelerated gradually. However, when the temperature is reached, the DS decreases with the further elevation of temperature due to the inactivation of enzyme at a higher temperature. And also the reversible reaction is accelerated because the enzyme-catalyzed reaction is exothermic reaction. So the optimal temperature is 40 °C.

#### 3.2.3. The effect of the mass ratio of collagen to chitosan on DS

According to Fig. 2(c), the DS increases at the ratio between 0.6 and 1.0. At higher ratios, the DS has the tendency of slight reduction. From the enzymatic catalytic reaction equation, this may be as the result of the stereohindrance effect and electrostatic repulsion between macromolecule chitosan and collagen. And when the mass ratio of collagen to chitosan is over 1.0, there is a lack of amine group. MTGase catalyzes the hydrolysis of the γ-carboxamide group of the glutaminyl residue, resulting in deamidation. So the further increase of mass ratio makes some difference on DS. As the overall consideration, the optimal mass ratio of collagen to chitosan is 1.0.

#### 3.2.4. The effect of the mass ratio of MTGase to chitosan on DS

The effect of the mass ratio of MTGase to chitosan on DS is described in Fig. 2(d). As presented, the DS value increases to 0.660 as the mass ratio of MTGase to chitosan increases. However, along with the further increase of the MTGase, the DS drops sharply. That can be explained for this result: the lysine-containing peptides act as acyl acceptors, and they also can act as acyl acceptors. The superabundant MTGase could catalyze the reaction between collagen and collagen. So the overmuch MTGase makes side-effects on the reaction. Accordingly, the optimum mass ratio of MTGase to chitosan is about 0.1.

**Table 1**
Mositure-absorption and retention property of collagen–chitosan with different DS values.

| Sample | DS | $R_a$ (%) | | $R_h$ (%) |
|---|---|---|---|---|
| | | RH 81% | RH 43% | Silica gel |
| Chitosan | – | 33.6 | 9.7 | 46.1 |
| Collagen–chitosan | 0.259 | 34.8 | 16.5 | 49.2 |
| | 0.425 | 48.3 | 20.4 | 58.7 |
| | 0.660 | 56.9 | 23.7 | 72.9 |
| HA | – | 43.8 | 12.5 | 78.7 |

HA: hyaluronic acid; RH: relative humidity.

### 3.3. Water solubility of collagen–chitosan

Water solubility of collagen–chitosan is evaluated under different pH conditions. The transmittances of samples are more than 90%. Solutions of collagen–chitosan remain transparent over the entire pH range irrespective of DS values. Hence, the introduction of collagen to chitosan contributes to increase the hydrophilicity of chitosan. Thus, the collagen–chitosan is a water-soluble derivative.

### 3.4. Moisture absorption and retention properties

As shown in Table 1, the moisture-absorption and retention ability of collagen–chitosan depends on the DS values. At both 43% and 81% RH, the $R_a$ (%) of collagen–chitosan reveal better moisture-absorption ability compared with chitosan and hyaluronic acid (HA). And in the silica gel, collagen–chitosan with the DS 0.259, 0.425 and 0.660 shows fine moisture retention ability compared with chitosan. When the DS is 0.660, collagen–chitosan shows better moisture-retention ability. It demonstrates that the introduction of collagen to chitosan contributes to enhance both moisture-absorption and retention ability.

### 3.5. Antioxidant activities of collagen–chitosan

#### 3.5.1. The effect of DS value and concentration on $H_2O_2$ scavenging activity

Fig. 3 presents $H_2O_2$ scavenging activity of different DS and concentration of collagen–chitosan. Obviously, the $H_2O_2$ scavenging activity increases with the increase of concentration. And collagen–chitosan with the DS 0.568 shows better $H_2O_2$ scavenging activity. However, collagen–chitosan with the DS value at 0.259, 0.409 and 0.486 has unconspicuous effect on $H_2O_2$ scavenging activity. When the DS value is 0.568, the $H_2O_2$ scavenging activity of collagen–chitosan is 78.2–111.6% at 0.15–2.50 mg/ml. And Vc, as the positive control, is 77.6–117.5% at 0.15–2.5 mg/ml. It is reported that the antioxidative activity of collagen is inherent to their characteristic amino acid sequences. The collagen has the $H_2O_2$ scavenging activity due to the existence of Pro residues in the peptide sequence (Lin & Li, 2006).

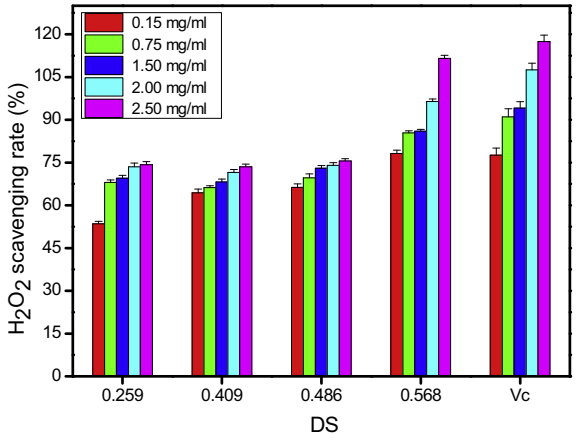


**Fig. 3.** $H_2O_2$ scavenging activity of collagen–chitosan as a function of DS value and concentration.

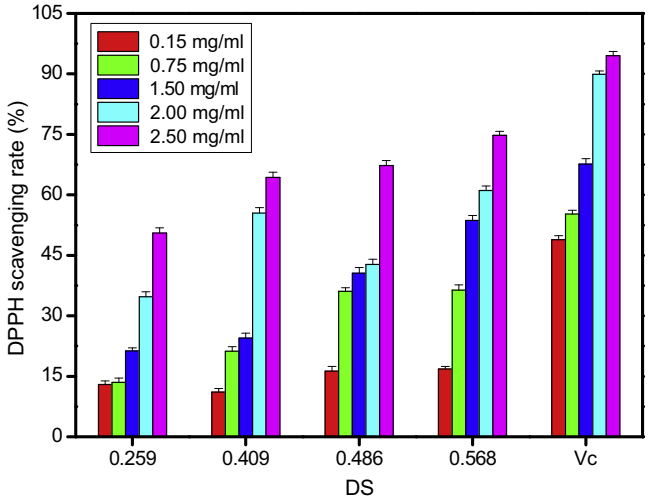


**Fig. 4.** DPPH scavenging activity of collagen–chitosan as a function of DS value and concentration.

#### 3.5.2. The effect of DS value and concentration on DPPH scavenging activity

As demonstrated in Fig. 4, collagen–chitosan exhibits strong DPPH radical scavenging activity. Obviously, with the increased concentration from 0.15 to 2.5 mg/ml, the DPPH scavenging activity slowly enhances at first stage, and then sharply increases at later stage. At 2.5 mg/ml, the DPPH scavenging activity increases with the increase of DS value. Among the levels used in the experiment, 2.5 mg/ml collagen–chitosan with DS 0.568 is the strongest one with a scavenging rate of 74.8%. That is about 79.1% of Vc at 2.5 mg/ml. It is indicated that His, Pro, Tyr and Trp are the most important residues in radical scavenging activity of collagen (Bamdad & Chen, 2013).

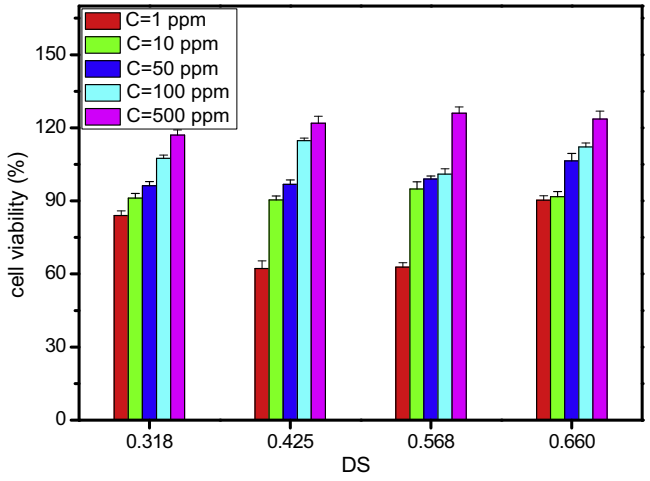


**Fig. 5.** The cell viability of collagen–chitosan as a function of DS value and concentration.

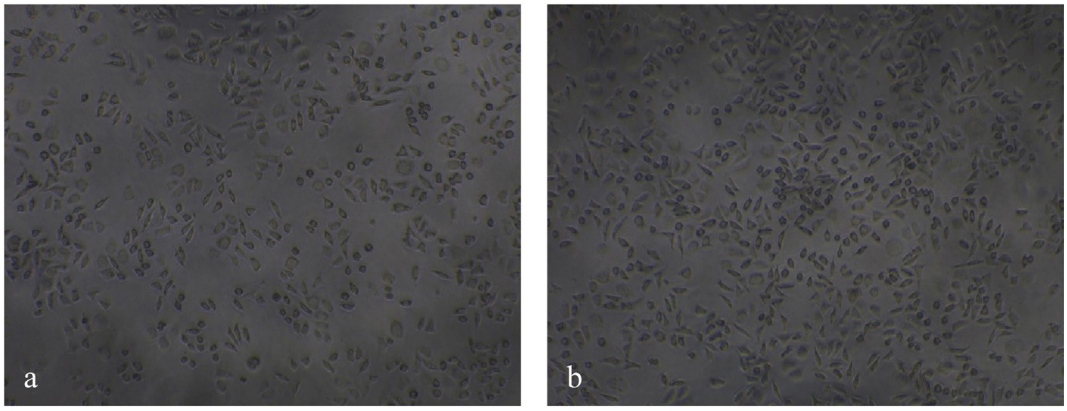


**Fig. 6.** Inverted microscope images of mouse fibroblast cell L929 at 37 °C for 22 h in the presence of 5% $CO_2$ in a cell culture incubator: (a) control; (b) collagen–chitosan (DS = 0.425, 100 μg/ml).

### 3.6. The effect of DS value and concentration on cell viability

Fig. 5 shows the effect of DS value and concentration on cell viability. It presents that the collagen–chitosan exhibits better cell viability with the DS of 0.660 between 0.05 and 0.5 mg/ml. At a relatively high concentration, the active ingredient of collagen–chitosan can enhance the survival rate of mouse fibroblast cells. And cell viabilities increases with the increase of concentration. However, it is still unclear the mechanism of cell viability for the collagen–chitosan. It is presumed that collagen could stimulate the synthesis of extracellular matrix component. Consequently it provides a suitable niche for mouse fibroblast cells adhesion, proliferation and differentiation. And further research needs to be carried out to confirm this hypothesis.

Fig. 6 shows inverted microscope images of L929 fibroblasts obtain after 22 h of incubation on blank (a) and collagen–chitosan (b). The cells with normal morphology, flat shape and irregular fibrous arrangement are observed in Fig. 6. Compared with the control group, the density and quantity of cells are increased. This result is in accord with the above conclusion as shown in Fig. 5.

## 4. Conclusion

In this work, MTGase was effective in catalyzing collagen chains onto the chitosan backone. Collagen–chitosan with DS value of 0.259–0.660 could be obtained by adjusting the reaction conditions. The biological properties of collagen–chitosan were evaluated including moisture absorption and retention properties, antioxidant activity and cell viability. And the DS value and concentration of collagen–chitosan had significant effect on antioxidant activity and cell viability. The results revealed that the enzyme-catalyzed grafting of collagen chains onto the chitosan backone contributed to enhance the moisture absorption and retention abilities, antioxidant activity and promote L929 cells growth efficiently. Therefore, collagen–chitosan could provide potentiality of reparative ability to skin in cosmetic, biomedical and pharmaceutical fields.

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