Influence of Blending Carboxymethylcellulose with Gelatin Scaffold on Mechanical Properties

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Abstract

Biopolymer scaffolds fabricated by freeze drying process have been widely used for tissue engineering applications. We took the interest in using gelatin scaffold blended with carboxymethylcellulose (CMC) to improve the mechanical properties of the scaffolds. The CMC solutions were blended with gelatin solutions of 5 ratios and fabricated to porous structure via freeze drying process. The thermal crosslinking technique was used to induce conjugation of free amide and carboxyl groups in protein structures of the scaffolds. The swelling and mechanical properties of the scaffolds were characterized. Adding of CMC in gelatin scaffolds effected in both physical and mechanical properties. The scaffold which used gelatin and CMC in the ratio of 80 and 20, respectively occurred in the highest level of water absorption with the ratio of 44.67 ± 3.86. The result from mechanical test showed that GC82T scaffold dramatically increased in compressive modulus of the scaffolds (0.70 ± 0.07 kPa) with significant different compared to pure gelatin scaffold. The high level in swelling ratio of the scaffold can help the scaffold to maintain a porous network during *in vitro* cell culture because they have to immerse in surrounding media. The high value in compressive modulus of the scaffold can also help the scaffold to provide 3D structure while implanting in the body. This results suggested that the blending gelatin and CMC scaffold in the ratio of 80:20 (gelatin:CMC) had tendency to improve in mechanical properties of the scaffolds for applying in tissue engineering applications.

***Keywords**:* carboxymethylcellulose; gelatin; scaffold; swelling; compressive modulus

1. Introduction

There have several biomaterials which fabricated into potential scaffolds for cell proliferation and differentiation. Among of these materials, gelatin which is a denatured structure of collagen has been used for medical application as a biomaterial and as an additive or gel capsules in drugs and also as a scaffold in tissue engineering [1,2]. However, gelatin has a very hydrophilic nature and relatively poor in mechanical properties which limited their potential applications [3].

Due to poor mechanical properties of gelatin, we took an interest in using carboxymethylcellulose (CMC) to blend with gelatin for improving the mechanical properties of the gelatin scaffolds. CMC is a derivative of cellulose by reacted with sodium hydroxide and chloroacetic acid. Due to its good viscosity building, high shear stability, biocompatibility, easily available and very cheap compared to other polysaccharides, It has widely used in various fields [4,5]. In medical applications, CMC was used as a hydrogel for wound dressing [6], a scaffold for various tissue engineering applications [7] and an injectable material for bone augmentation [8]. This study focused on the scaffolds made from gelatin blended with CMC in various ratios. Physical and mechanical properties of the scaffolds were characterized in comparison to the conventional gelatin scaffold.

2. Experimental

2.1 Materials

Type A Gelatin was purchased from BIO BASIC INC, Canada. It is a reagent grade and derived from porkskin with bloom number of 240-270 and pH 4.5-5.5 at 25°C. Its viscosity is 3.5-4.5 cps and has moisture less than 12.0%. Carboxymethylcellulose sodium salt (CMC) was purchased from Sigma-Aldrich, St. Louis, MO, USA. It has medium viscosity of 400-800 cps in a 2% aqueous solution at 25°C. We used a deionized water from our laboratory for preparing the gelatin and CMC solutions.

2.2 Preparation of gelatin/CMC scaffolds

Powders of Type A gelatin were immersed in deionized water at room temperature for 0.5 h before dissolved under agitation for 1 h at 50°C to obtain 0.8 wt% (w/w) gelatin solution. Then, CMC was dissolved in deionized water at 70°C for 1 h to form a 0.8 wt% (w/w) CMC solution. The gelatin solutions were blended with CMC solutions into five ratios. All of blended solutions were stirred for 1 h at 50°C, after that they were degassed by putting on the hot plate. Then, they were pipetted in a 24-well cell culture plate. Finally, they were fabricated into a porous structure by using a freeze drying process which freezed at -20 °C overnight and lyophilize at -50 °C for 24 h.

To improve the mechanical properties of the scaffolds, they were crosslinked by using thermal crosslinking which used the condition of 140°C for 48 h. The code of thermal crosslinked gelatin/CMC scaffolds in different ratios of gelatin and CMC were shown in Table. 1.

Table. 1 Thermal crosslinked gelatin/CMC scaffolds

Blending composition of gelatin/CMC	Thermal crosslinked gelatin/CMC scaffolds
100/0	G100T
90/10	GC91T
80/20	GC82T
70/30	GC73T
60/40	GC64T

2.3 Swelling properties of the scaffolds

The scaffolds were weighed and placed in phosphate buffered saline (PBS, pH 7.4) at 37 °C for 3 h. Excess PBS solution in scaffolds were removed by blotting them on lint-freed papers. Each scaffold was weighed again and the swelling ratio of each scaffold was calculated from the equation 1 [9]:

$$\text{swelling ratio} = \frac{W_t^s - W_o^s}{W_o^s} \qquad (1)$$

where: W_t^s is the weight of wet scaffolds

W_o^s is the weight of dry scaffolds.

The values were expressed as the mean ± standard deviation (number of each scaffold sample (n) = 5)

2.4 Evaluation on mechanical properties of the scaffolds

A Universal Testing Machine (UTM, Instron No. 5566, USA) was used to determine compressive modulus of the scaffolds at a constant deformation rate of 0.5 mm/min in the dry state at 25 °C [10]. The slope of the linear region in the stress-strain curve gave the stiffness or compressive modulus values [2]. The values were expressed as the mean ± standard deviation (n=5).

2.5 Statistical analysis

All experiments were repeated five times. The swelling ratio and compressive modulus of the scaffolds were plotted in the same graph to show the distribution of the data and significant differences between measured groups. The significant differences between two groups were evaluated using a student t-test with 95% confidence interval. The differences were considered to be statistically significant when $p<0.05$.

3. Results and Discussions

Every gelatin/CMC scaffolds possessed an interconnected porous structure with approximately 4.7 mm average in height and 13.8 mm average in diameter. The example of gelatin/CMC scaffolds in condition of GC73T was shown in Fig. 1. Gelatin-CMC scaffolds were tested for swelling properties which were weighed before and after immersed in PBS solution and calculated for the swelling ratio by using the Eq. (1). The example of GC73T scaffold after immersed in PBS solution at 37 °C for 3 h was shown in Fig. 2.

In order to evaluate the mechanical properties of the different types of scaffolds,

Fig. 1 Photograph of thermal crosslinked gelatin/CMC scaffolds (GC73T)

Fig. 2 Wet thermal crosslinked gelatin/CMC scaffold (GC73T) after immersed in PBS solution at 37 °C for 3 h

Universal Testing Machine was used to measure the compressive modulus of the scaffolds. The example of the gelatin scaffold compressed by UTM was shown in Fig. 3. The results from both of swelling test and compressive modulus were plotted in the same graph which was shown in Fig. 4. All mean values of swelling ratio and compressive modulus of gelatin/CMC scaffolds were represented by round dot line and solid line, respectively. The results showed that adding of CMC in gelatin scaffolds effected in swelling properties. The scaffold which used gelatin 80 and CMC 20 in ratio showed the highest level of water absorption with the ratio of 44.67 ± 3.86.

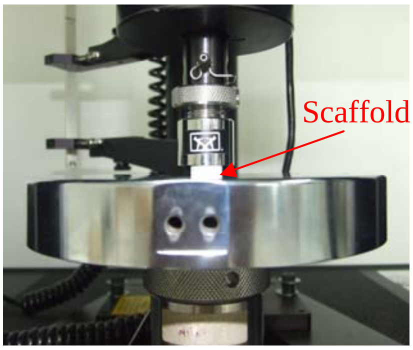

Fig. 3 Thermal crosslinked gelatin/CMC scaffold (GC73T) compressed by UTM

However, there were some ratios of gelatin/CMC blended scaffold showed in decreasing in swelling ratio with significant different when compared with pure gelatin scaffold (G100T) which its swelling ratio was 22.27 ± 3.95.

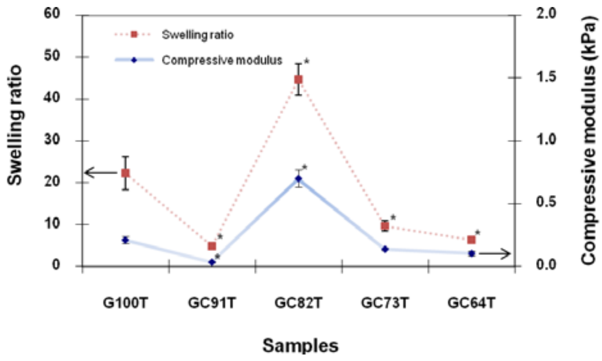

Fig. 4 Swelling ratio (round dot line) and compressive modulus (solid line) of thermal-crosslinked gelatin/CMC scaffolds

(significant different p<0.05 relative to G100T)*

In this experiment, we would like to compare the results between swelling test that was the new result and compressive modulus that we have done before [11]. The results from compressive test showed that GC82T scaffold dramatically increased with significant different in compressive modulus compared to pure gelatin scaffold. The compressive modulus of pure gelatin scaffold was 0.21 ± 0.03 kPa and the compressive modulus of GC82T was 0.70 ± 0.07 kPa.

However, there were some ratios of gelatin/CMC blended scaffold showed decreasing with significant different in compressive modulus when compared to pure gelatin scaffold. The data distributions of all scaffolds from swelling test and compressive modulus were in the same trend. Compressive modulus which represents the mechanical strength of the scaffolds has a crucial influence on swelling ratio which shows the water absorption of each scaffold. The results of swelling ratio and compressive modulus were in the same trend because the lower compressive modulus or the weak in mechanical strength should provide the less in swelling ratio. The scaffold GC82T showed in the highest level both swelling ratio and compressive modulus. The other ratios of gelatin blended CMC scaffolds which were GC91T, GC73T and GC64T showed decreasing with significant different of both swelling ratio and compressive modulus when compared to pure gelatin scaffold.

The reason why the highest in both compressive modulus and swelling ratio occurred in only GC82T that because the image from Scanning Electron Microscopy (SEM) [11] of GC82T appeared in homogenous porous structure and membrane-like structure while others showed non-homogeneous in pore shape and some area was torn especially in GC91T. Therefore, the best pattern of the GC82T structure can lead to have the best in swelling ratio and compressive modulus while others were lower especially in GC91T.

The benefit of high level in swelling ratio of the scaffold is to provide enough nutrients to fibroblast cells which cultured inside the scaffold. The high value in compressive modulus of the scaffold can help the scaffold to maintain a porous 3D structure while implanting in the patient.

4. Conclusions

The effects of blending CMC in gelatin scaffold were characterized in both swelling ratio and compressive modulus. Addition of CMC in the scaffolds effected both physical and mechanical properties. The blended scaffold which made from gelatin and CMC in the ratio of 80:20 (gelatin:CMC) showed the highest level of the swelling ratio and also in the compressive modulus. Whereas, decreasing in swelling ratio and compressive modulus of the scaffolds when compared to pure gelatin scaffold were observed in the other ratios of gelatin blended with CMC which were GC91T, GC73T and GC64T. This results suggested that using CMC to blend with biopolymer gelatin scaffold in the condition of gelatin 80 and CMC 20 is the best condition that can be used to test in the future experiments for applying in tissue engineering applications.

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6. References

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