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Evaluation of Gelatin/Carboxymethylcellulose Scaffolds Using Mooney-Rivlin Model

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Abstract

Scaffolds based on various ratios of gelatin blended with carboxymethylcellulose (CMC) were studied. The scaffolds were fabricated to porous structure via freeze drying process and crosslinked to induce conjugation of free amide and carboxyl groups in protein structures by using thermal crosslinking techniques. The mechanical properties of the scaffolds were characterized both experimental procedure and modeling. In order to evaluate the modeling, the stress-strain behavior of the scaffolds by fitting the data to a Mooney-Rivlin model was decribed. We utilized the Mooney-Rivlin constitutive relationship for soft networks which typically express nonlinear behavior of stress-strain curve from compression test. Results showed that the data distribution of both model and experiment are in the same trend. The models which evaluated CMC blended gelatin scaffold in the ratio of 80 and 20 of gelatin and CMC occurred in the highest average in shear modulus which was 18.12 kPa, compared to G100T, G91T, G73T and G64T scaffolds. Gelatin scaffold blending with 10, 30 and 40% of CMC showed dramatically decreased in the shear modulus which were 7.70, 3.10 and 1.53 kPa, respectively, compared to pure gelatin scaffold with significant difference. These results showed the possibility of using CMC as a low cost material to combine with biopolymers for using in tissue engineering applications.

Keywords: Gelatin, Carboxymethylcellulose, Shear modulus, Hyperelastic material, Mooney-Rivlin model

1. Introduction

Loss of skin in patients can result from multiple causes of disease or injury such as ulcer, burn and trauma. Due to lost of surface area, skin replacement marketplaces have to produce and develop their wound closure which meet many requirements of native skin for patients [1]. Medical science has thus turned to tissue engineering and materials science and engineering to design proper scaffolds for transplantation and reconstruction of tissues and organs [2]. Functionally, the skin has two layers which consist of epidermal and dermal layer. The epidermal layer or epidermis is a regenerative tissue which can recovers its structure completely at the site of the defect while the dermal layer or dermis is a nonregenerative tissue which the wound edges contract and close with simultaneous formation of scar tissue at the defected site [3]. To overcome the nonregenerative tissue in the dermis function, many researchers have developed artificial extracellular matrices or scaffolds for supporting the three-dimensional tissue formation at the wound site [4-6].

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The typical biomaterial used for fabrication of the scaffold is collagen which is a natural polymer that is widely used in many tissue repair and regeneration [7]. The basic collagen molecule contains three polypeptide-chains which consist of more than 1000 amino acids of each chain [8]. Collagen is an excellent biocompatibility because of low toxicity and poor immunogenic reactions [9-10]. However, there are some disadvantages of collagen such as a high cost of preparation of pure type I collagen, more hydrophilic compared with synthetic polymers and difficult to handling [11]. To overcome this, the new fabricated scaffolds should have sufficient strength to provide a good handling and an ability to maintain their stability of the 3D structure and pore size upon the contractile force which cause by the growing cells on the scaffold surface, the compressive modulus and the tensile force from surrounding tissues. The compressive modulus is the capacity of a material or structure to withstand loads tending to reduce size. The shear modulus is used to measure the stiffness of materials which can describe the response of materials to shear stress [7].

To overcome the high cost of collagen, we took an interest in using gelatin which is a denatured structure of collagen and the price of gelatin is cheap and easily available. Scaffold made from gelatin has shown to be positively interacted with cells which have a research approval of in vitro biocompatibility test of gelatin with fibroblast cells. The cells showed a good affinity and proliferation on the gelatin scaffolds after 14 days of culturing without any signs of biodegradation [12]. The second biological material used to blend with gelatin scaffold and can improve a strength of scaffold structure is carboxymethylcellulose (CMC). CMC is a derivative of cellulose by reacted with sodium hydroxide and chloroacetic acid. The properties of CMC are a good in viscosity building, flocculation and a high shear stability. CMC is easily available and very cheap compared to other polysaccharides [13].

The behavior of rubber-like is modeled in the framework of hyperelasticity. Numerous constitutive equations are available in the literature and have recently been compared and used in many works [14-16]. The scaffolds typically have nonlinear stress-strain responses due to the elastomeric behavior. However, the identification of material parameters which govern the constitutive equation is difficult. In this research, one homogeneous test is considered to identify constitutive parameter, namely uniaxial compressive test. It consists of performing several homogeneous tests which generate one type of strain state as uniaxial compression. For this purpose, the sample geometry and loadings condition are defined beforehand by numerical investigation. Finally the constitutive is defined using a curve-fitting method.

Therefore, the objectives of this research are to investigate gelatin/CMC scaffold by identifying the constitutive parameter using a curve-fitting method from the homogeneous test as uniaxial compressive with different ratios of CMC blended with gelatin. We focus on the Mooney-Rivlin [17-18] which is a simple one of hyperelastic material models for describing scaffold's constitutive behavior.

2. Methods

2.1 Materials and preparation of gelatin/CMC scaffolds

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

For the preparation of the gelatin/CMC scaffolds briefly, gelatin powder was immersed in deionized water at room temperature for 0.5 hour before dissolved under agitation for 1 h at 50^{o}C to obtain 0.8 wt% (w/w) gelatin solution. Then, CMC powder was dissolved in deionized water at 70^{o}C for 1 hour to form a 0.8 wt% (w/w) CMC solution. The gelatin solution was blended with the

CMC solution in various ratios as showed in Table 1. All of blended solutions were stirred for 1 hour at 50°C and then degassed on the hotplate. After that it was pipetted into 24-well cell culture plate. The gelatin/CMC solutions were fabricated into porous structure by freeze drying process which freezed at -20°C overnight and lyophilize at -50°C for 24 h. Finally, the scaffolds were crosslinked by using thermal crosslinking technique which used the condition of 140°C for 48 h.

Table 1. Blending Composition of Gelatin/CMC

Samples	Blending Composition	
	Gelatin	***CMC***
G100T	100	0
G91T	90	10
G82T	80	20
G73T	70	30
G64T	60	40

2.2 Curve fitting method

The data distribution from stress-strain relations was fit by hyperelastic model which was constitutive law of hyperelastic material using Mooney-Rivlin potential function [19]. NonlinearLeastSquares was used as the alternative method. The equation used to determine the parameter G was shown as follows

$$T = G\left((\varepsilon + 1) - \frac{1}{(\varepsilon + 1)^2}\right) \tag{1}$$

Where T is engineering stress, G is shear modulus and ε is strain.

2.3 Mechanical properties of material

The nonlinear stress-strain relation of scaffolds typically was known as nonlinear deformation response. The hyperelastic materials have a very small compressibility which is referred to incompressibility. The constitutive law for an isotropic hyperelastic material is usually defined by a relation relating the strain energy potential function (W). The strain energy potential function is a scalar function of the strain or deformation tensors which its derivative respects to a strain component that determines the stress component. Their relationship can be written as follows

$$\sigma_i = 2\left[\left(\lambda_i^2 - \lambda_j^2\right)\frac{\partial W}{\partial I_1} - \left(\frac{1}{\lambda_i^2} - \frac{1}{\lambda_j^2}\right)\frac{\partial W}{\partial I_2}\right] \tag{2}$$

Where σ_i is the Cauchy stress tensor on 1-direction (i), W is the strain energy function, λ_i is the principle stretch on 1-direction (i), λ_j is the principle stretch on 2-direction (j) and I is the principle invariant.

Generalized Mooney model (Adapted from Mooney, 1940) which its form likes nonlinear has been used to evaluate various large deformation of hyperelastic materials. Therefore this model is chosen to characterize the nonlinear mechanical responses (stress-strain relation) of gelatin/CMC scaffold. The specific form of generalized Mooney constitutive relationship is the strain energy potential function (W) which is depends on two invariants of the deformation tensor (I_1,I_2) by two constants (C_1 and C_2) which can be written in case of incompressibility as follow [20].

$$W = C_1(I_1 - 3) + (I_2 - 3) \tag{3}$$

$$C_1 = \frac{G_1}{2} \tag{4}$$

$$C_2 = \frac{G_2}{2} \tag{5}$$

Where G_1 and G_2 are material properties. For small deformation in case of rubber elasticity, the initial shear modulus (G) which is one of several quantities for measuring the stiffness of materials. The shear modulus of the solid is

$$G = G_1 + G_2 \tag{6}$$

From the simple extension, the engineering stress in simple extension of an isotropic incompressible hyperelastic material can be written by

$$T_{11} = 2\left(\lambda - \frac{1}{\lambda^2}\right)\left(\frac{\partial W}{\partial I_1} + \frac{\partial W}{\partial I_2}\right) \tag{7}$$

Where T_{11} is engineering stress, W is strain energy potential function, I_1 and I_2 are invariants of the deformation tensor, λ is equal to the extension ratio which is related to the strain (ε) by the following expression

$$\lambda = \varepsilon + 1 \tag{8}$$

The invariants I_i are given by

$$I_1 = \lambda_1^2 + \frac{1}{\lambda_1^2} + 1 \tag{9}$$

Where λ_1 is extension ratio of the material.

$$I_2 = I_1 \tag{10}$$

$$I_3 = 1 \tag{11}$$

Therefore the expression of engineering stress (T_{11}) with the form of Generalized Mooney model can be written as

$$T_{11} = G_1\left(\lambda - \frac{1}{\lambda^2}\right) + G_2\left(1 - \frac{1}{\lambda^3}\right) \tag{12}$$

Where T_{11} is engineering stress, G_1 and G_2 are initial shear modulus of the material and λ is extension ratio.

2.4 Determination of material parameters

For the simple material model which contains only two parameters, material parameters can be estimated by fitting to result of a compressive test. The Non-linear-Least-Squared method is chosen as the alternative method and the equation used to determine the parameter G was shown as Follows [17]

$$G = \frac{E}{2(1+\nu)} = 2(C_1 + C_2) \tag{13}$$

Where G is initial shear modulus, E is Young's modulus for infinitesimal deformation, ν is poisson's ratio and C_1 and C_2 are material constants.

2.5 Geometry and loading condition

All of gelatin/CMC scaffolds were approximately 4.7 mm in height and 13.8 mm in diameter as shown in Figure 1. For the statistical analysis, all experiments were repeated into five times. The compressive modulus and shear modulus of the scaffolds were plotted in the same graph to show the data distribution and significant differences between different ratios of gelatin blended with CMC scaffolds and pure gelatin scaffold. The significant differences between two groups were evaluated using a student t-test with 95% confidence interval. The differences were considered to be a statistically significant when $p<0.05$.

To evaluate the homogeneous compressive response of scaffolds, we used a Universal Testing Machine (UTM, Instron No. 5566, USA). The loading condition which applied to the machine was a constant deformation rate of 0.5 mm/min in the dry state at 25ºC [18]. The values were evaluated from initial compressive stress-strain curve which determine the slope from 10% to 30% strain of the scaffolds. The values were expressed as mean±standard deviation (n=5) and the raw data of compressive stress-strain of each scaffold was keep to evaluate shear modulus by used the Mooney-Rivlin model. The shear moduli of all different scaffolds were identified by fitting the stress-strain data by a Mooney-Rivlin model which described above. All data points were expressed as mean±standard deviation (n=5). The results of mechanical test from both experiment and model were compared in the same graph which was described in the results and discussion.

Figure 1. Photograph of 0.8% (w/w) gelatin/CMC scaffold which used 30% of CMC

3. Results and discussion

3.1 Compressive modulus of the scaffolds

The gelatin/CMC scaffolds were compressed by the UTM with two flat plates to analyze the mechanical properties of the scaffolds. The example of pure gelatin scaffold compressed by UTM was shown in Figure 2. Force versus displacement was converted into engineering stress and strain by using of the initial dimensions of the scaffolds.

The compressive moduli of the scaffolds were plotted in the same graph of shear modulus as shown in Figure 3. All mean values of shear modulus and compressive modulus of all gelatin/CMC scaffolds were represented by round dot line and solid line, respectively. The results from compressive modulus showed that gelatin scaffold with 20% CMC dramatically increased in compressive modulus with significant different compared to pure gelatin scaffold. The compressive modulus of pure gelatin scaffold was 0.21 ± 0.03 kPa and the compressive modulus of 20% CMC scaffold was 0.70 ± 0.07 kPa [21] as shown in Table 2. However, there were some ratios of gelatin/CMC blended scaffold showed decreasing in compressive modulus with significant different compared to pure gelatin scaffold.

Figure 2. Pure gelatin scaffold (0% CMC) compressed by UTM

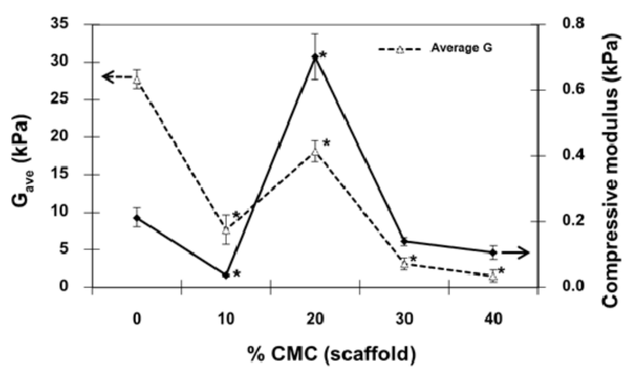

Figure 3. Shear modulus (kPa) using Mooney-Rivlin model of 15% strain and compressive modulus (kPa) of 0.8% (w/w) gelatin/CMC scaffold (n=5)

Table 2. Compressive modulus of 0.8% (wt/wt)

Samples	Average Compressive Modulus (kPa) ± SD
G100T	0.21 ± 0.03
G91T	0.04 ± 0.00
G82T	0.70 ± 0.07
G73T	0.14 ± 0.01
G64T	0.11 ± 0.02

3.2 Identification of constitutive parameter

The shear modulus of the scaffolds was expressed by fitting data of stress and strain to the Mooney-Rivlin model. We utilized the Mooney-Rivlin constitutive relationship for soft networks which typically express nonlinear behavior of stress-strain curve from compression test [17,22]. The example of scaffold which fit its stress-strain data to the Mooney-Rivlin model was pure gelatin scaffold (0% CMC) as shown in Figure 4. Stress curve of the scaffold was nonlinear represented by red line and the Mooney-Rivlin model could fit the curve with 10% strain represented by black line.

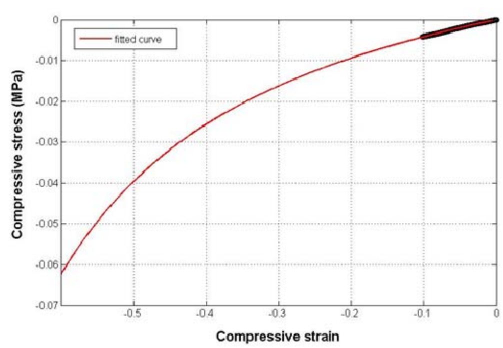

Figure 4. Stress versus strain curve in compression of pure gelatin scaffold (0% CMC). Stress curve was nonlinear (red line) and fit curve with Mooney-Rivlin model of 10% strain (black line).

All of the scaffolds showed different in shear modulus because it depended on CMC concentration in the gelatin scaffold (Figure 3). Using gelatin blended with 20% of CMC occurred in the highest value of average shear modulus which was 18.12 ± 1.38 kPa compared to G100T, G91T, G73T and G64T scaffolds. The shear modulus of the scaffolds decreased with significant different when using 10%, 30% and 40% of CMC blended with gelatin scaffolds compared to pure gelatin scaffold (0% CMC) which was 27.7 ± 1.24 kPa. The shear modulus of gelatin scaffold with 10%, 30% and 40% of CMC were 7.7 ± 2.00 kPa, 3.1 ± 0.8 kPa and 1.53 ± 0.78 kPa, respectively as shown in Table 3.

Table 3. Shear modulus of 0.8% (wt/wt) gelatin/CMC scaffold using Mooney-Rivlin model of 15% strain

Samples	Average Shear Modulus (kPa) ± SD	R^2
G100T	27.70 ± 1.24	0.9231
G91T	7.70 ± 2.00	0.6352
G82T	18.12 ± 1.38	0.6043
G73T	3.10 ± 0.80	1.000
G64T	1.53 ± 0.78	0.9349

3.3 Comparison of experimental test and model

The results of both compressive modulus from the experimental test and shear modulus from fitting the stress-strain data to the Mooney-Rivlin model were ploted in the same graph. The data distribution of both model (shear modulus) and experiment (compressive modulus) are in the same trend. The scaffold of 20% CMC showed both shear modulus and compressive modulus in high level. The other concentrations of CMC added into gelatin scaffolds (10%, 30% and 40% of CMC) showed decreasing in both shear modulus and compressive modulus compared to pure gelatin scaffold.

There have some advantages of high value in shear modulus and compressive modulus which express the mechanical strength of the scaffold. The high value in shear modulus and compressive modulus can help the scaffold to maintain a 3D porous structure when immersed in media. It is benefit for implanting the scaffold in the patient or culturing fibroblast cell in the scaffold. The strength of porous structure can help cell to receive enough nutrients to growth and differentiation.

4. Conclusion

The Mooney-Rivlin model was used to characterize mechanical properties of gelatin/CMC scaffolds. The CMC blended gelatin scaffold of ratio 80/20 (gelatin/ CMC) occurred the highest average of shear modulus which was 18.12 kPa. This was due to the arrangement of porous structure from SEM image showed better membrane-like structure compared to other ratios of gelatin/CMC scaffolds [21]. Gelatin scaffold blended with 10, 30 and 40% of CMC showed dramatically decreased in the shear modulus compared to pure gelatin scaffold with significant difference. This finding can be helped in reconstruction of skin tissue because the skin substitutes normally required a proper strength and stability when implanted in patients. The data distributions between shear modulus from model and compressive modulus from experiment were in the same trend. The novelty in this work showed that Mooney-Rivlin model have the potential to evaluate mechanical properties of biopolymers scaffold which can be used in tissue engineering applications.

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