Mechanical Properties Analysis of Scaffold Material Using Nonlinear Least Squares Fitting by Hyperelastic Model

Fasai Wiwatwongwana and Nattawit Promma

Abstract—**Nowadays, the number of chronic wounds is on the rise. Wounds can caused by many problems such as disease, burn, ulceration, trauma and accident. Tissue engineering aims to produce porous scaffold biomaterials to regenerate damaged tissues to growth of new tissue. This research, gelatin was blended with Carboxymethylcellulose (CMC) in various conditions and fabricated to porous structure by using freeze-drying method. All scaffolds were strengthen their structure by dehydrothermal crosslinking. Normally, the scaffold behavior is likely to be a foam-like hyperelastic material. Therefore, this research was selected Blatz-Ko model to describe the material behavior that acts as a foam rubber. This model could apply with both cases of compressible and incompressible material. The mechanical characterization of the scaffold was investigated by compressive test using universal testing machine (UTM). The experimental data obtained from the UTM was used to plot the stress-strain curve. The initial shear modulus of the material was identified by function derived from Blatz-Ko hyperelastic model using non-linear curve fitting method. The result revealed that Blatz-Ko model could fit curve at approximately 7% strain which was suitable for infinitesimal strain theory. The dehydrothermal treated scaffold with 90:10 gelatin/CMC ratio showed the highest shear modulus of 10.47±1.21 kPa. The structural collapse occurred in 60:40 gelatin/CMC scaffold. The physical characterization was done by using scanning electron microscopy (SEM) to investigate surface morphology and pore size of scaffolds. The results showed the appropriate pore size of the scaffold with average pore size of 117 μm to 197 μm. The 90:10 gelatin/CMC scaffold showed the biggest pore size.**

Index Terms—**Hyperelastic model, Blatz-Ko, mechanical properties, scaffold, gelatin, carboxymethylcellulose.**

I. Introduction

The designing of artificial extracellular matrices or scaffolds which uses material and fabrication technologies is very important for support three-dimensional tissue formation. The adult doses not regenerate organs such as skin that has been lost or removed either due to accident or deliberate excision. To design the structure of artificial skin by tissue engineering using various materials which can simulate the extracellular matrices for the skin tissues that is known as scaffolds or templates. It has to provide proper physical, mechanical and biological properties for cell proliferation, differentiation and skin tissue formation [1], [2]. There are various types of scaffold design such as fibers, sponges, freeze-dried materials and rapidly prototyped structures. The important properties for scaffold design structure are porosity, pore network, pore size, biocompatibility and mechanical properties. The mechanical strength and elasticity of materials are a major impact on creating a scaffold which can help to maintain its structure during implantation and provide 3D structure for nutrient diffusion and new tissue formation [3], [4]. Therefore, in order to overcome the disadvantages of high price materials and scaffold fabrication process, the purpose of this research is aim to design the affordable scaffold in local price with good physical and mechanical properties.

The mechanical characteristic of scaffold has to be analyzed to verify its behavior. Hyperelastic material is a constitutive model of stress-strain behavior of a material which is subjected to large deformation. It is used to describe material act as a rubber-like or foam-like material [5], [6]. The scaffold has to require stability and relatively affordable during implantation. It has to subject both axial and shear force. Therefore, Blatz-Ko model which suitable for foam-like material is selected in this research. The identified parameters are young's modulus and shear modulus [7], [8]. There have a previous study of surface wrinkling which generalized by Blatz–Ko model. The wrinking of a thin film boned to a soft compressible substrate was analyzed. The results revealed that Blatz–Ko model could investigate the effects of various material compressibility on various types of wrinkle formation in a class of non-linearly elastic materials [9].

The material used for scaffold fabrication in this research is gelatin which derived from collagen and has biocompatibility properties. There have a previous research studied on pore size in a 3D bioprinted gelatin scaffold on fibroblast proliferation. The pore size controlled 3D gelatin scaffolds were fabricated by 3D bioprinting with a low temperature processing step and a crosslinking process. The results showed that human dermal fibroblasts proliferation on the 3D gelatin scaffolds prepared with pores more than 580 μm exhibited higher growth rates compared to the scaffold prepared with pores 435 μm, after 14 days of culture [10].

Therefore, in order to overcome low mechanical strength of gelatin, this research selected carboxymethylcellulose

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(CMC) as a second material to blend with gelatin scaffold. CMC is a derivative of cellulose by reacted with sodium hydroxide and chloroacetic acid. It is good in viscosity building, flocculation and high shear stability. Normally, CMC is available, easily purchased and it has low price compared to other polysaccharides [11], [12]. There have previous study that CMC improved in mechanical strength and biocompatibility of scaffold structure. For example, there have a research of PVP-CMC-$CaCO_3$ hydrogel scaffold for bone tissue engineering. The PVP-CMC-$CaCO_3$ hydrogel scaffold improved structural and functional properties. Cell proliferation and cell viability were examined until 7 days using permanent cell lines MG63 (human osteosarcoma), L929 (murine fibroblasts) and cell cultures from mouse bone explants (CC-MBE). The results confirmed that hydrogel scaffolds exhibited a good biocompatibility. The scaffolds could be used as an implant for bone tissue regeneration and drug delivery purposes [13]. Moreover, there have a study of physical characteristics of composite bone scaffold fabricated from oil palm empty fruit bunch – carboxymethyl cellulose (CMC), chitosan (CS), and hydroxylapatite (HA). The result showed that the highly porosity provided higher value in compressive strength. CMC was found to be a suitable material as a reinforcement to cope with chitosan and HA/nHA produced better composite which could apply in biomaterials for orthopaedic application as bone scaffold [14]. Finally, there have a previous study of mechanical and biological properties of porous scaffolds of chitosan (CS) and carboxymethyl cellulose (CMC) reinforced with whisker-like biphasic and triphasic calcium phosphate fibers which fabricated by freeze drying method. The results revealed that addition of CMC to CS led to a significant improvement in the mechanical properties (up to 150%). The composite scaffolds of CS and CMC reinforced with 50 wt% triphasic fibers improved in terms of mechanical and biological properties with showed compressive strength and modulus of 150 kPa and 3.08 MPa, respectively, which was up to 300% greater than pure CS scaffolds. This composite scaffolds were suitable to be used in bone tissue engineering application [15].

II. Material Fabrication

The fabrication procedures of gelatin/CMC scaffold were the same as previous research [16]. 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 25ºC. Its viscosity was 3.5-4.5 cps and moisture less than 12.0%. CMC was purchased from Sigma-Aldrich, St. Louis, MO, USA. It was medium viscosity with 400-800 cps in a 2% aqueous solution at 25ºC. The gelatin and CMC solution were prepared using deionized water. Briefly, the gelatin solution was prepared by mixing gelatin powder to deionized water (DI water, 0.8 wt.%) then leaved it at room temperature for 1 hour before stirred it at 50 ℃ for 1 hour. CMC solution was done by mixing CMC and DI water together and stirred at 70 ℃ for 30 minutes. The blending conditions were made in five ratios of gelatin and CMC which were 100:0, 90:10, 80:20, 70:30 and 60:40, respectively and then stirred it at 50 ℃ for 15 minutes. The solution was pipetted into 24-well culture plate with volume 1 ml per well and freezed them at -20 ℃ for 24 hours. The scaffold was placed into a Lyophilizer (Freeze-Dry Machine) at -50 ℃ for 24 hours to obtain porous 3D structure. All scaffolds were stored in dry condition before further treatment. Dehydrothermal treatment (DHT) was selected in this research. It was performed by putting scaffolds into a vacuum oven at 140 ℃ for 48 hours to strengthen the scaffold structure.

III. Material Parameter Identification

A. Physical Properties Identification

The morphology of all scaffold conditions was analyzed by Scanning Electron Microscopy (SEM, JEOL: JSM-5910 LV SEM). All scaffolds were investigated by using 60x magnifications and 15 kV voltage acceleration. The obtained SEM images were used to determine the pore size by randomly selected for the pore size investigation of each condition.

B. Mechanical Properties Identification

The uniaxial compressive test for each scaffold condition was performed by using universal testing machine (UTM) with compression rate of 0.1 mm/minute in dry condition at 25 ℃ to collect load and displacement data from the experiment which provided stress-strain relation [17]. It was used to prove that at the same testing condition, the Blatz-Ko model could fit better than the Neo-Hookean model. The amount of samples used for the testing for each ratio of scaffold was 5 (n=5). The initial shear modulus of the material was identified by function which derived from Blatz-Ko hyperelastic model by using non-linear curve fitting method. Overall, there would be 25 scaffolds from heat treatment for UTM testing. It was divided into 5 mixtures which were pure gelatin, 90:10, 80:20, 70:30 and 60:40 of gelatin:CMC, respectively.

In continuum mechanics, large deformation theory deals with material deformations with both rotations and strains. It can be applied for the hyperelastic material behavior and also suitable for soft tissue description [18]. To specify the hyperelastic material model, it needs to select constitutive model and finds material parameters. The second Piola-Kirchhoff stress tensor, in case of hyperelasticity, can be expressed to determine components of the Cauchy stress tensor in terms of the principle invariants of the left Cauchy-Green deformation tensor. The Cauchy stress components can be expressed in (1) [19], [20].

$$\boldsymbol{\sigma} = -p\boldsymbol{I} + 2\frac{\partial \boldsymbol{W}}{\partial I1}\boldsymbol{B} - 2\frac{\partial \boldsymbol{W}}{\partial I2}\boldsymbol{B}^{-1} \tag{1}$$

where $\boldsymbol{W}$ represents the strain energy function per unit initial volume, $\boldsymbol{\sigma}$ represents the Cauchy stress tensor, $\boldsymbol{B}$ represents the left Cauchy-Green deformation tensor and p represents pressure value.

In order to avoid the pressure term, generally, the Cauchy stress is expressed in both i and j direction by difference between $\sigma_i - \sigma_j$ in case of uniaxial compressive test and giving σ_j to be zero. In the uniaxial extension or compressive case, the response occurs along the lateral, i, direction and the gradient deformation can be written in form of principle stretch (λ). From (1), writing Cauchy stress equation in Cartesian coordinate system and subtracting each other in order to obtain the Cauchy stress equation in uniaxial case ((2)). Engineering stress (T) can be expressed by dividing (2) by λ as shown in (3).

$$\sigma_i = 2\left(\lambda_1^2 - \frac{1}{\lambda_i}\right)\left(\frac{\partial \boldsymbol{W}}{\partial I_1} + \frac{1}{\lambda_i}\frac{\partial \boldsymbol{W}}{\partial I_2}\right) \quad (2)$$

$$T_i = 2\left(\lambda_i - \frac{1}{\lambda_i^2}\right)\left(\frac{\partial \boldsymbol{W}}{\partial I_1} + \frac{1}{\lambda_i}\frac{\partial \boldsymbol{W}}{\partial I_2}\right) \quad (3)$$

where the λ_i is the principal in-plane stretch ratios and has the value of $\varepsilon_i + 1$ (ε_i is an engineering strain). The term I_i is the invariant that can be calculated and written in case of incompressibility as shown in (4).

$$I_1 = \lambda^2 + \frac{2}{\lambda},\ I_2 = \frac{1}{\lambda^2} + 2\lambda,\ I_3 = 1 \quad (4)$$

This step consists of expressing the stress component as function of the measure strain component by introducing the constitutive equation. Blatz-Ko model was considered in this research and it described the material behavior that acted as foam rubber and could apply to both cases of compressible and incompressible material [21, 22]. Using Blatz-Ko model as incompressible material, after the derivation, it was apparently showed the equation of Neo-Hookean model [17]. However, incompressible case, the strain energy equation presents Poisson ratio value which is 0.25 (v = 0.25) and can be expressed as shown in (5).

$$W = \frac{\mu}{2}\left(\frac{I_2}{I_3} + 2I_3^{\frac{1}{2}} - 5\right) \quad (5)$$

where μ is initial shear modulus. Then the engineering stress equation in term of Blatz-Ko hyperelastic model can be written as shown in (6).

$$T = \mu\left(1 - \frac{1}{\lambda^3}\right) \quad (6)$$

The problem occurs this step is to identify the parameter that governs the Blatz-Ko law, namely initial shear modulus in (6). From the experimental data of stress-strain relation is obtained, nonlinear curve fitting using the function derived from Blatz-Ko hyperelastic model is represented as the calculation method. In this research, nonlinear least-squared was chosen. The initial shear modulus was obtained earlier from curve fitting method; therefore, Young's modulus (E) could be also obtained from (7) [20].

$$\mu = \frac{E}{2(1+v)} \quad (7)$$

IV. Result

A. Mechanical Characterization Experimental Result

The five ratios of gelatin/CMC scaffolds, 100:0, 90:10, 70:30, 80:20 and 60:40, were represented by the labels D100, D91, D82, D73 and D64, respectively. The Curve fitting method was being used to determine the parameter of each scaffold condition by using Blatz-Ko model. Fig. 1 showed the examples of stress-strain curve and curve fitting of gelatin/CMC scaffold at ratios 80:20 with 5 repetitions.

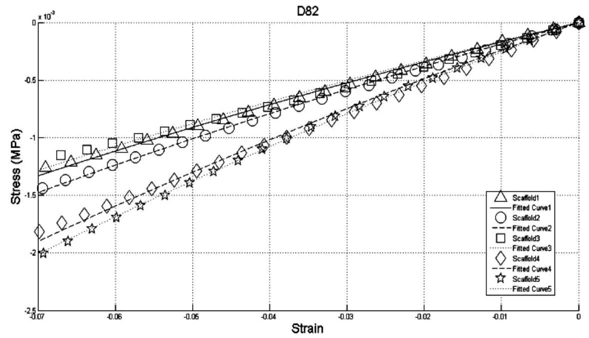

Fig. 1. Curve Fitting of Gelatin/CMC (D82) Scaffolds.

The result revealed that Blatz-Ko model could fit the curve at 7% strain of each scaffold which represented by different kinds of markers. The curve was fitted and shown as a line. The average values of shear modulus from fitting results and young's modulus from experiment were shown in table 1 where the average r square of each case was more than 0.99. The gelatin/CMC scaffold in condition of D91 showed the highest value of young's modulus which was 26.18 kPa. Whereas, the D64 scaffold showed the weakest in structure with occurred the lowest value of young's modulus which was 14.11 kPa.

TABLE I: Curve Fitting Results

Scaffolds	Average Shear Modulus (kPa)	SD	Average Young's Modulus (kPa)
D100	6.97	1.92	17.44
D91	10.47	1.21	26.18
D82	6.58	1.23	16.44
D73	7.20	1.01	18.01
D64	5.64	1.35	14.11

The average values of initial shear modulus of each scaffold condition were plotted as shown in Fig. 2. From this figure, it was found that D91 scaffold occurred the highest value of initial shear modulus which was higher than pure gelatin scaffold (D100) with significant difference. Whereas, increasing CMC in condition of D82, D73 and D64, the shear modulus was decreased, especially the lowest shear modulus was found in the scaffold D64. The result of average shear modulus of all scaffold conditions was consistency with the result of average young's modulus.

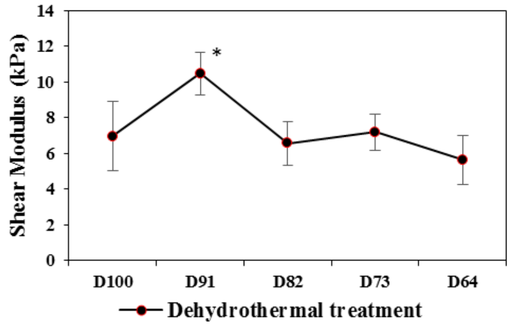

Fig. 2. Summary of Initial Shear Modulus of gelatin/CMC Scaffolds (n=5) (** significant different $p<0.05$ relative to D100*).

B. Physical Characterization Experimental Result Dehydrothermal Treatment Scaffolds

The freeze dried gelatin/CMC scaffolds were crosslinked to strengthen their structure by heated in a vacuum oven at 140 ℃ for 48 hours. After SEM testing, the morphology of cross-sectional structure of each scaffold condition was shown in Fig. 3. The SEM images showed homogeneous porous structure with interconnected pore, except D100 scaffold (Fig. 3a.) which expressed its structure as non-homogeneous porous structure. The scaffold should have interconnected porous structure to provide cell fibroblasts growth and differentiation for skin regeneration.

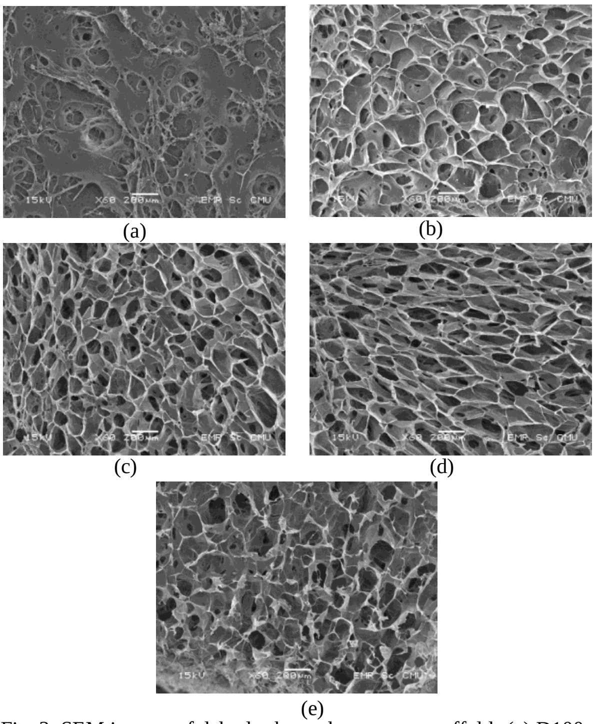

Fig. 3. SEM images of dehydrothermal treatment scaffolds (a) D100 (b) D91 (c) D82 (d) D73 and (e) D64.

The pore size of the scaffold should match the size of cell fibroblasts to growth and create a new tissue. After randomly calculated the pore size of each scaffold condition from SEM images, the average pore size of all scaffolds was shown in Table II. The pore size distribution of all scaffold conditions was approximately between 117 and 197 µm which was in the appropriate value for skin tissue engineering scaffold which the pore size should be between 100 and 200 µm.

TABLE II: AVERAGE PORE SIZE OF SCAFFOLDS

Scaffolds	Average Pore Size (µm)	SD
D100	168.51	45.96
D91	197.87	38.30
D82	117.87	23.83
D73	138.72	33.19
D64	179.15	28.09

V. DISCUSSION

This research showed that the Blatz-Ko model could fit the curve at 7% strain of all scaffold conditions according to the description of this model which was suitable in infinitesimal strain situation. All scaffold condition revealed interconnected and homogeneous porous structure except D100 which considered to be non-homogeneous structure. The results of average shear modulus and young's modulus showed that scaffold D91 occurred the highest values which were 10.47 kPa and 26.18 kPa, respectively which its shear modulus was higher than pure gelatin scaffold with significant difference. The scaffold structure collapsed in D64 scaffold with showed the lowest value of shear modulus and young's modulus. The result of D64 scaffold was consistency with its SEM image which showed thin-walled porous of all structure that seemed to be weak in structure. The average pore size of all scaffold conditions was from 117-197 µm. It revealed that pore size of D91 was the biggest pore which was approximately 197.87 µm. From the results, it could be summarized that big pore size of scaffold provided good mechanical strength as shown in D91. For further research, other models, such as Ogden could be used in case of foam-like material. In addition, the porosity and other crosslinking techniques such as chemical treatment or time and temperature condition of dehydrothermal treatment could be used for investigation. The biodegradability experiment should be performed to show the biological properties of the scaffold which should match the time of skin regeneration.

VI. CONCLUSION

The result from SEM images showed that all scaffolds were three-dimensionally interconnected porous structure, except D100. For the mechanical modeling evaluation, all scaffolds were compressed to 70% deformation. The Blatz-Ko model was used to identify the initial shear modulus by using curve fitting method. The result showed that Blatz-Ko model could fit the curve at 7% strain of all scaffold conditions according to the characteristic of this model (infinitesimal strain). The D91 scaffold provided the maximum initial shear modulus which was 10.47 kPa. The highest value of young's modulus of scaffold also obtained from D91 scaffold which was 26.18 kPa. Whereas, D64 scaffold showed the lowest value of shear modulus and young's modulus. This could be summarized that the Blatz-Ko model could be used to predict the mechanical response of soft materials. The average pore size of all scaffolds was considered to be in an appropriate range of pore size for skin regeneration. The biggest pore size was found in D91 scaffold. From the results, it was implied that big pore size provided good in mechanical strength of scaffold structure. It could be summarized that too much of CMC content did not significantly effect on material strengthening, but only an appropriate amount of CMC and using dehydrothermal treatment could improve in mechanical strength of the scaffold.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Asst. Prof. Dr. Fasai Wiwatwongwana and Asst. Prof. Dr. Nattawit Promma designed the concept of the project, writing literature review, design of the research outline and design of research tools. They also made a decision on data analysis method, analysis of data, interpretation of data analysis, writing draft of the article (first draft), revising draft of the article (second draft) and final approval of the article (final draft).

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