Mechanical Properties Analysis of Gelatin/Carboxymethylcellulose Scaffolds

F. Wiwatwongwana and S. Chaijit

***Abstract*—Gelatin blended with carboxymethylcellulose (CMC) scaffold was fabricated via freeze drying method. The various gelatin and CMC ratios were 100/0, 90/10, 80/20, 70/30 and 60/40, respectively. The mechanical characterization of the scaffold was done by compressive test using universal testing machine. The obtained data was used to determine compressive modulus and shear modulus which was analyzed from neo-Hookean model. The deformed scaffold and total strain energy time response were analyzed using finite element model (FEM). The scaffold G73T showed the highest value both compressive modulus (0.53±0.11 kPa) and shear modulus (1.02±0.11 kPa). The results were consistency with FEM that G73T showed the highest range of equivalent elastic strain and the highest value of total strain energy-time response. The results could imply the best condition for scaffold fabrication from mechanical analysis which might suitable for tissue engineering applications.**

***Index Terms*—Compressive modulus, shear modulus, scaffold, gelatin, carboxymethylcellouse, finite element model.**

I. INTRODUCTION

Tissue engineering can be referred to biomaterials development which is combinding scaffold, cells and biologically active molecules into functional tissues. Skin loss can cause by many different causes such as burns, accidents, ulcers and diseases. The scaffold, skin replacement material which is a biocompatible and biodegradable material, has been widely used and recently available. The functions of the scaffold are to prevent infection, accelerate the wound healing and generate skin tissues [1], [2]. Moreover, it has to allow skin to reproduce in a suitable condition and heal the wound. However, the available scaffolds are expensive due to its components. Therefore, the cheaper scaffold which has the same functions is the propose of this research. The scaffold fabrication has to be designed for supporting the recovery mechanism of skin functions. The application of tissue engineering usage is also the main propose of the scaffold design. It can be used the derived naturally or synthetically materials in the compositions [3], [4].

The important functions of the scaffold are appropriate strength which can be supported the compressive and tensile strength during implantation, appropriate pore size which can allow fibroblast cells to migrate and growth in the scaffold and have function that is biocompatible with cells and have to be biodegradable. In terms of mechanical characteristic, the scaffold behavior have to be investigated before testing with fibroblast cells. The basic knowledge of the interactions between mechanical stimuli, cells and biomaterials is growing but the quantitative effect of mechanical stimuli on cells attached to biomaterials is still unknown. There also have many research studies to develop finite element models (FEM) of various scaffolds in order to calculate the mechanical behavior of the scaffold and also load transfer from the biomaterial structure to the biological entities [5]-[8]. For the structure of the scaffold, it shows a behavior of rubber-like material which can be modeled in the framework of hyperelasticity. There are many studies of numerous constitutive equations and have recently been compared and used in different applications [9]-[11]. The scaffold behavior normally has nonlinear stress-strain responses due to the elastomeric behavior. Because of the identification of material parameters which govern the constitutive equation is still difficult. Therefore, the simple one of hyperelastic material models for describing scaffold's constitutive behavior is neo-Hookean medel. It can be identified constitutive parameter of scaffold by using a curve-fitting method from the homogeneous test or uniaxial compressive test [12].

Biopolymer that is the most widely used in scaffold composition is gelatin which is positively interacted with cells. There have many research studies approved for *in vitro* biocompatible test for gelatin with fibroblast cells [13], [14]. The results of those research fields showed that gelatin scaffolds could be able to maintain cells with good affinity and proliferation after 14 days of culturing without any signs of biodegradation [15]. The second biomaterial which can be used to help for scaffold strengthening is carboxymethylcellulose (CMC). CMC, a derivative of cellulose, is obtained from the reaction of cellulose with sodium hydroxide and chloroacetic acid. The good mechanical properties of CMC are high viscosity and shear strength which can help for mechanical integrity of the scaffold. The price of CMC is very cheap and easily purchased compared to other polysaccharides. Various treatments such as dehydrothermal treatment and chemical treatment have been used for strengthening the scaffold structure [16]-[18]. Therefore, in this research, gelatin and CMC were chosen for scaffold fabrication. Moreover, the various conditions of gelatin blended with CMC were selected and dehydrothermal treatment was used for strengthening the scaffold structure. The mechanical

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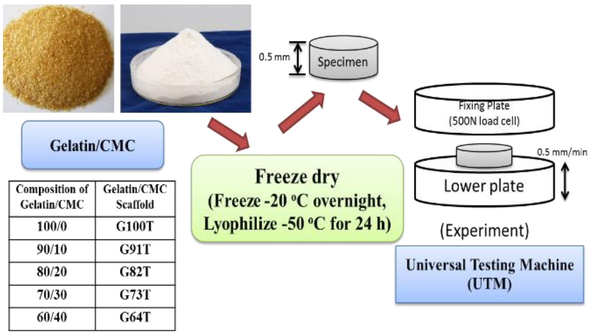
properties of various scaffold conditions were analyzed by experiment, FEM and neo-Hookean model.

II. MATERIAL FABRICATION

According to previous research, the conditions of dehydrothermal scaffold fabrication was the same [19]. The scaffold was made from gelatin blended with CMC which could help in scaffold strengthening. 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 °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 with 400-800 cps in a 2% aqueous solution at 25 °C. The gelatin/CMC solution was prepared by using deionized water (DI water) as a solvent. The scaffolds were made in five different gelatin/CMC ratios which were 100/0, 90/10, 80/20, 70/30 and 60/40, respectively. The gelatin/CMC scaffold fabrication was done by preparing gelatin solution by mixing gelatin powder with DI water at 0.8 wt% (w/w) then leaved it at room temperature for 1 hour before stirred it at 50°C for another 1 hour. CMC solution was prepared by mixing CMC with DI water at 0.8 wt% (w/w) and then stirred at 70 °C for 30 minutes. The blending gelatin/CMC ratios were 100/0, 90/10, 80/20, 70/30 and 60/40, respectively which stirred it at 50 °C for 15 minutes of each condition. Finally, the solutions were pipetted into 24-well culture plate with volume of 1 ml per well and freezed them for 24 hours at -20 °C. The scaffold was then placed in a Lyophilizer (Freeze-Dry Machine) at -50 °C for 24 hours and put all scaffolds in a humid controlled container. The schematic diagram of gelatin/CMC scaffold fabrication was shown in Fig. 1.



Composition of Gelatin/CMC	Gelatin/CMC Scaffold
100/0	G100T
90/10	G91T
80/20	G82T
70/30	G73T
60/40	G64T

Fig. 1. Schematic diagram of 0.8 wt% (w/w) gelatin/CMC scaffold preparation and experimental test.

III. MECHANICAL PROPERTIES IDENTIFICATION

A. *Geometry and Loading Condition*

The fabrication method of this scaffold normally used freeze-drying technique where porous structure was formed. The examples of gelatin/CMC scaffolds were shown in Fig. 2. The compressive testing was performed by using universal testing machine (UTM, Zwick/Roell Z1.0) to collect load-deformation data from experimental test to obtain stress-strain information. The compression rate was 0.5 mm/minute in dry condition at 25 °C [20]. The tested gelatin/CMC scaffolds were divided into 5 mixtures which were 100/0, 90/10, 80/20, 70/30 and 60/40, respectively. The compressive modulus was evaluated from initial compressive stress-strain curve which determined the slope from 15% to 25% strain of the scaffolds and expressed as mean ± standard deviation (*n*=5). The raw data of compressive stress-strain of each scaffold was used to evaluate initial shear modulus by using neo-Hookean model. The significant different of each blending composition was evaluated using a student t-test with 95% confidence interval. The differences were considered to be a statistically significant when p<0.05.

B. *Material Parameter Identification*

The shear modulus of each scaffold was determined from the expression of engineering stress (T_{11}) in the form of neo-Hookean strain energy potential as shown in (1) [20].

$$T_{11} = G\left(\lambda - \frac{1}{\lambda^2}\right) \tag{1}$$

where

G : is initial shear modulus (kPa)

T_{11} : is engineering stress (kPa)

λ : is principle stretch which was calculated from the measure of elongation *e* using equation:

$$\lambda = 1 + e$$

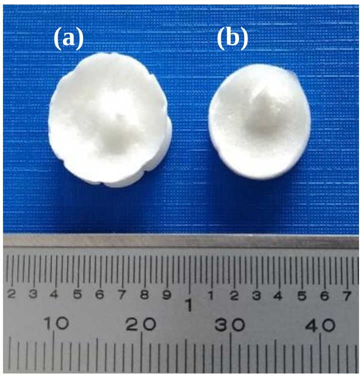


Fig. 2. 0.8 wt% (w/w) of gelatin/CMC scaffold (a) G100T and (b) G73T.

C. *Finite Element Modeling*

A non-linear elastic material law was used for the model and the finite element code MARC (MSC Software) was used to analyze equivalent elastic strain and total strain energy time response of the scaffolds. Typical models hold around 600,000 elements of dimensions of around 10-25 μm element size. The compressive modulus from experiment and shear modulus from neo-Hookean model of the scaffolds were used to analyze the mechanical properties by FEM. The deformed scaffold was used as 100 increments and time was used for 10 s. The equivalent elastic strain was obtained and the total strain energy time response was obtained from history plot of all increments. The principle strains and stresses as well as the von-Mises stress were calculated for all cylinders. The dilatation stress and octahedral shear strain were calculated since these parameters might be potential mechanical stimuli for tissue differentiation. The material flow of FE models was governed by hereditary integration for FEM Cauchy stress (or true stress) as shown in (2) [8]. The scaffold mesh from finite element was shown in Fig. 3.

$$\sigma = \int_0^t 2G(t-\tau)\frac{de}{d\tau}d\tau + I\int_0^t K(t-\tau)\frac{d\Delta}{d\tau}d\tau \qquad (2)$$

where

σ is Cauchy stress in FEM constitutional equation (kPa)
G is shear modulus in FEM function (kPa)
t is time in FEM constitutional equation (s)
τ is relaxation times (s)
I is unit tensor in FEM constitutional equation
e is strain in FEM constitutional equation
K is bulk modulus in FEM function (kPa)

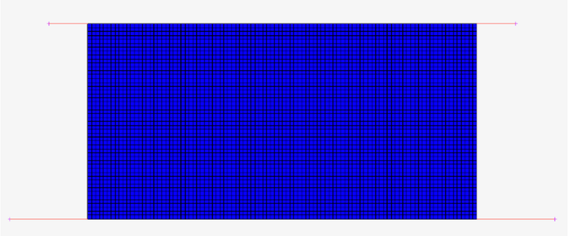


Fig. 3. The mesh was made of around 600,000 tetrahedral elements.

IV. Result

A. Compressive Modulus of the Scaffolds

The gelatin/CMC scaffolds were compressed by UTM with two flat plates to analyze the stress-strain relation of each condition of the scaffolds. The examples of G100 and G73 during compression test by UTM were shown in Fig. 4. Force versus displacement was converted into engineering stress and strain by using of the initial dimensions of each scaffold.

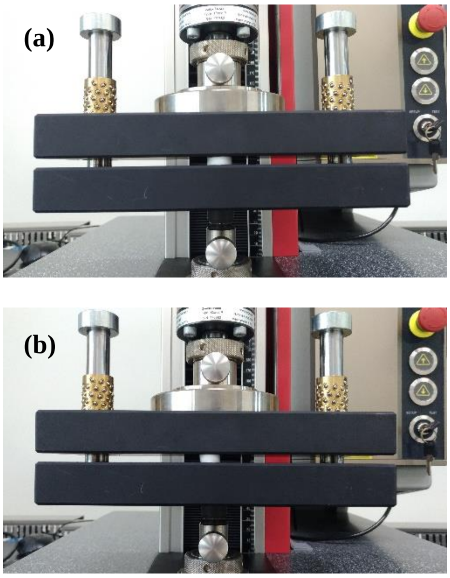


Fig. 4. The gelatin/CMC scaffolds during compression test (a) G100T and (b) G73T.

The average compressive modulus of all scaffold conditions which represented by circle marker of the scaffolds was plotted as shown in Fig. 5. The results showed that gelatin scaffold with 30% CMC (G73T) occurred the highest compressive modulus with significant different compared to pure gelatin scaffold (G100T). The compressive modulus of G73T was 0.53 ± 0.11 kPa and the compressive modulus of pure gelatin scaffold was 0.30 ± 0.05 kPa as shown in Table I. However, the compressive modulus of other compositions (G91T and G82T) of blending gelatin/CMC scaffolds showed the similar trend to pure gelatin scaffold with non-significant result. However, The G64T scaffold showed the lowest compressive modulus (0.12 ± 0.06 kPa) with significant different compared to G100T.

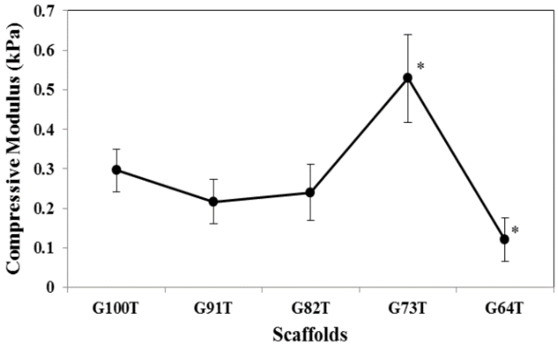


Fig. 5. Compressive modulus of 0.8 wt% (w/w) of gelatin/CMC scaffold (n=5) (* *significant different $p<0.05$ relative to G100T*).

TABLE I: Compressive Modulus of 0.8 wt% (w/w) of Gelatin/CMC Scaffold (N=5)

Scaffolds	Average Compressive Modulus (kPa)	SD
G100T	0.30	0.05
G91T	0.22	0.06
G82T	0.24	0.07
G73T	0.53	0.11
G64T	0.12	0.06

B. Shear Modulus of the Scaffolds

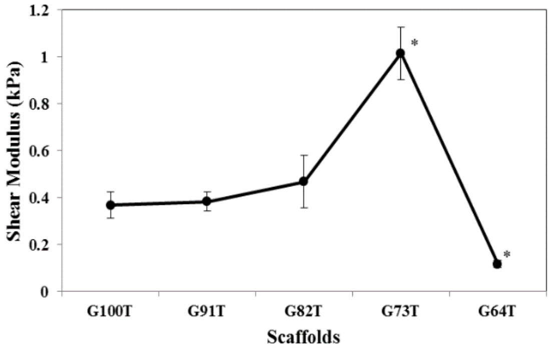


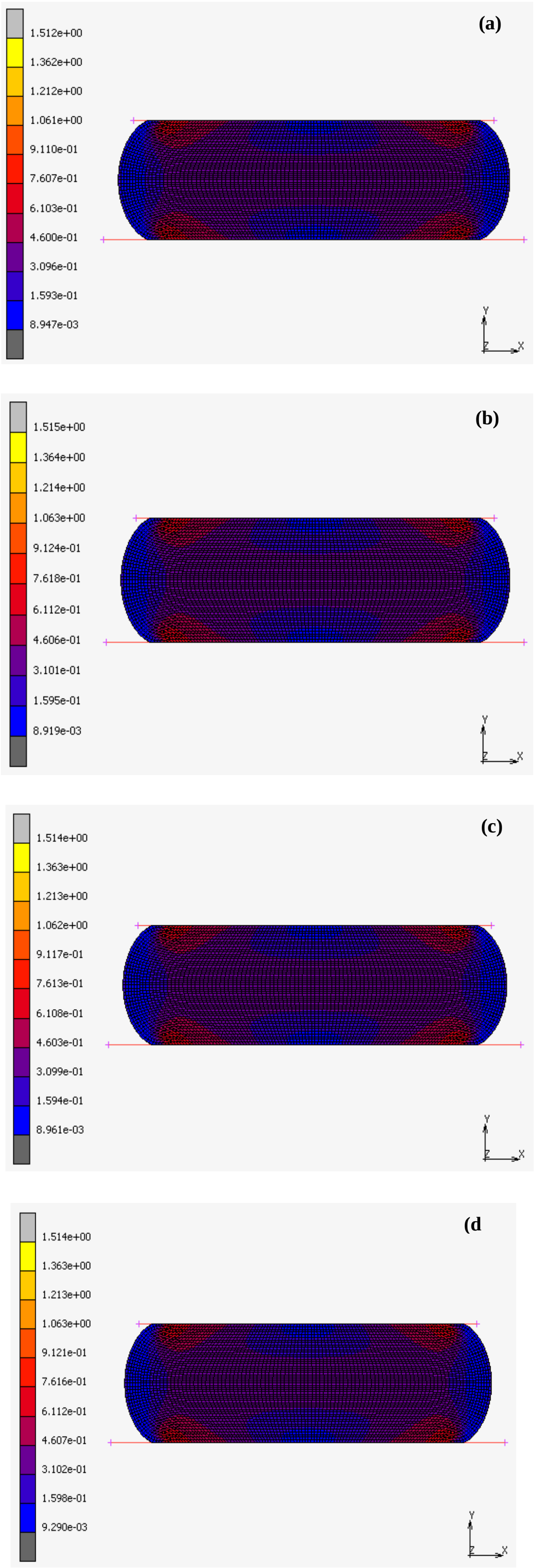
Fig. 6. Shear modulus of 0.8 wt% (w/w) gelatin/CMC scaffold from neo-Hookean model of 6% strain (n=5) (* *significant different $p<0.05$ relative to G100T*).

TABLE II: Shear Modulus of 0.8 wt% (w/w) Gelatin/CMC Scaffold From Neo-Hookean Model of 6% strain (N=5)

Scaffolds	Average Shear Modulus (kPa)	SD
G100T	0.37	0.06
G91T	0.38	0.04
G82T	0.47	0.11
G73T	1.02	0.11
G64T	0.12	0.02

The average shear modulus of all scaffold conditions which represented by circle marker was plotted as shown in Fig. 6. The results showed that gelatin scaffold with 30% CMC (G73T) occurred the highest shear modulus (1.02 ± 0.11 kPa) with significant different compared to pure gelatin scaffold (G100T). Whereas, the lowest shear modulus occurred in G64T which was 0.12 ± 0.02 kPa as shown in Table II. From the result of compressive modulus, all blending compositions of gelatin/CMC scaffolds showed the similar trend of shear modulus and compressive modulus.

C. Finite Element Model of the Scaffolds



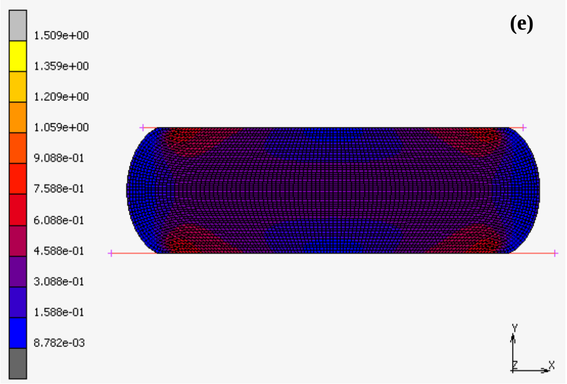


Fig. 7. The finite element modeling of 0.8 wt% (w/w) gelatin/CMC scaffold (a) G100T, (b) G91T, (c) G82T, (d) G73T, and (e) G64T.

After compressive test and neo-Hookean model analyzing, compressive modulus and shear modulus of each gelatin/CMC condition were analyzed the scaffold deformation behavior by using FEM. The equivalent elastic strain of deformed gelatin/CMC scaffold was shown in Fig. 7. The results shown in similar trend with deformable of 100 increments. The scaffold G73T shown the highest range of equivalent elastic strain of deformed material. Whereas, the scaffold G64T shown the lowest range of equivalent elastic strain.

The total strain energy-time response from FEM plot of all deformable of 100 increments from finite element analysis under uniaxial compression was shown in Fig. 8. The scaffold G73T which represented by circle green marker showed the highest of total strain energy compared to other scaffolds at the equivalent time. Whereas, G64T scaffold which represented by asterisk light purple marker showed the lowest of total strain energy at the equivalent time. The other scaffolds; G100T, G91T and G82T which represented by yellow, blue and red marker occurred the similar trend of total strain energy-time response.

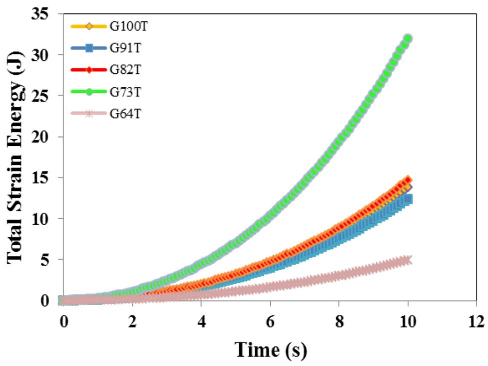


Fig. 8. Total strain energy-time response from finite element analysis under uniaxial compression of G100T, G91T, G82T, G73T and G64T.

V. DISCUSSION

According to the mechanical results of this research, it could be suggested that CMC could be used as a scaffold strengthening, especially for gelatin scaffold at a condition of gelatin/CMC of 70/30. This condition showed the highest value both compressive modulus and shear modulus which were 0.53 ± 0.11 kPa and 1.02 ± 0.11 kPa, respectively. Both compressive modulus and shear modulus of all gelatin/CMC conditions were in the same trend. However G61T showed the lowest compressive modulus and shear modulus which were 0.12 ± 0.06 kPa and 0.12 ± 0.02 kPa, respectively. The equivalent elastic strain of deformed gelatin/CMC scaffold from FEM results showed the deformation of all scaffolds with deformable of 100 increments. The G73T scaffold occurred the highest range of equivalent elastic strain (Fig. 7(d)) of scaffold deformation. It could be summarized that this scaffold condition could support loading better than other scaffolds. The FEM result was consistency with the results from compressive modulus and shear modulus. The total strain energy-time response from FEM plot of all 100 increments of deformed material was illustrated in Fig. 8. The scaffold G73T showed the highest value of total strain energy whereas G64T showed the lowest value at the equivalent time. The FEM plot result was in the similar trend of the result from experiment. It could be summarized that scaffold condition of G73T was the strongest structure and supported the most uniaxial compressive load. It might be the best condition for using in further experiment of tissue engineering application.

Further research can be focused on the other treatment methods for scaffold strengthening such as chemical treatment. The other scaffold fabrication technique such as salt-leaching technique, it can be compared with freeze dry technique. The above mentions may be allowed each type of scaffold to obtain maximum strength of material. In addition, a higher number of samples and repetition are encouraged in order to improve experimental accuracy. The porosity and biodegradability experiment are necessary to investigate. Moreover, sometime the scaffold may be used in wet condition during implantation, the mechanical properties in wet condition of the scaffolds can be identified to have the appropriate value.

VI. CONCLUSION

A skin replacement or scaffold is used to implant in patients after skin loss from various causes. Due to high price of skin replacement that currently available, this research was aim to fabricate the alternative scaffold as a new product to decrease cost of material fabrication. The scaffolds which made from gelatin blended with CMC as the scaffold strengthening were selected in this research. CMC solution was blended with gelatin solution and formed the scaffold at various gelatin/CMC ratio which were 100/0, 90/10, 80/20, 70/30 and 60/40, respectively. The mechanical characterization of all scaffold conditions was the investigation of compressive modulus by using Universal Testing Machine. The shear modulus of the scaffolds was evaluated from neo-Hookean model at 6% strain and all data from compressive modulus and shear modulus were used to analyze in finite element model. From the experimental test, all the scaffolds were compressed to 80% deformation. The results showed the maximum compressive modulus and shear modulus of scaffold which were from gelatin/CMC ratio at 70/30. However gelatin/CMC ratio at 60/40 showed the lowest compressive modulus and shear modulus. It was found that too much of CMC content decreased on material strengthening. Increasing of CMC content at appropriate condition could be improved in mechanical property of scaffold structure.

From finite element analysis of deformable material, G73T scaffold occurred in the highest range of equivalent elastic strain which could be implied that it could support loading better than other scaffolds. The result from finite element model was consistency with the results from compressive modulus and shear modulus. Moreover, the G73T scaffold occurred the highest value of total strain energy from FEM plot at the equivalent time compared to other scaffolds. On the other hands, G64T scaffold revealed the lowest value of total strain energy at the equivalent time. The other scaffolds (G100T, G91T and G82T) were in the same trend of total strain energy-time response. From all the results, it could be sumarized that the gelatin/CMC ratio at 70/30 might be useful for tissue engineering applications due to its good for supporting tension and compression. Thus, experiments, neo-Hookean model and FEM analysis could provide qualitative information regarding to mechanical properties of the scaffold and its deformation behavior. It could be used this information to predict the scaffold behavior and design an appropriate scaffold for their applications.

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