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Characterize of Composite Scaffold Using Gelatin-Carboxymethylcellulose from Waste Product for Wound Dressing by Salt Leaching Method

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The wound dressing can be used for burn patient or people who loss of skin. Blending gelatin with carboxymethylcellulose (CMC) can be used to produce skin graft for wound dressing application. Gelatin and CMC are waste from livestock production industry and agricultural waste, respectively. This study was emphasized on mechanical properties of composite material. Gelatin which was a biocompatibility material and CMC which was a scaffold strengthening and could improve in elasticity were selected for scaffold fabrication via salt leaching method. The blended scaffold was fabricated in various gelatin/CMC ratios which were 3/0, 2.7/0.3, 2.4/0.6, 2.1/0.9 and 1.8/1.2, respectively. The mechanical characterization of the scaffold was evaluated by compressive test using universal testing machine (UTM). The data obtained from the UTM was used to determine compressive modulus. The results showed the highest value of compressive modulus was obtained from gelatin/CMC at ratio of 1.8/1.2 with 15.16 ± 3.23 kPa. On the other hand, the compressive modulus from pure gelatin showed the lowest value which was 0.93 ± 0.26 kPa. Finite element models could predict the scaffold deformation with 1.8/1.2 scaffold showed the highest range of strain energy which could support loading more than other scaffolds. Therefore, the mechanical strength of 1.8/1.2 scaffold could be applied for wound dressing application. Moreover, this research needed to produce material from waste and added valuable for wound care applications.

1. Introduction

The development of biological skin substitutes in tissue engineering is fast growing up nowadays. It have to restore, maintain and improve biologically active molecules into tissue function when the disruption of the normal anatomic structure and function of the skin occurred. Normally, the skin wound or chronic wound can be caused by pressure ulcers, diabetic foot ulcers and venous leg ulcers, burns and diseases. The wound dressing or scaffold have to promote healing process which involves several phases including proliferation, maturation and tissue remodeling. It should be a biocompatible and biodegradable materials to prevent infection and inflammation and provide mechanical support for tissue regeneration (Ma 2006 and Talikowska et al. 2019). The scaffold conditions have to be designed for the recovery mechanism which depends on tissue engineering applications (Park 2002 and Hollinger 2012). To overcome the high price of wound dressing components, this research aims to fabricate the scaffold in lower price with suitable skin functions. There have various naturally derived polymeric materials such as collagen, gelatin, chitosan, fibrin, hyaluronic acid or synthetic polymers such as polycaprolactone (PCL), poly(glycolic acid) (PGA) and their copolymers and poly(lactic acid) (PLA) have been used to fabricate tissue scaffolds (Modaress et al. 2015 and Rad et al. 2019 and Romero et al. 2018 and Ferrari et al 2017). In addition, the mechanical strength and elastic properties are important functions of scaffolds. It have to support the different mechanical loading during implantation and tissue growth. It also should have appropriate pore size with high porosity, large surface area to volume ratio and interconnected porous structure. Finite element models can analyze the homogeneous deformations behavior of biological tissues such as hart wall, tendons, ligaments, articular cartilage and connective tissue or skin (Benítez 2017 and Groves et al. 2013). There have previous study of graphene oxide

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nanosheet (GONS) content effected on the linear and nonlinear mechanical properties of poly(acrylic acid) (PAA)/gelatin (Gel) hydrogels. The results shown the experimental data was well fitted with those predicted by the FE models. The Yeoh material model accurately defined the nonlinear behavior of hydrogels (Faghihi et al. 2014). The mechanical properties of electrospun gelatin meshes were studied. The result shown that the stiffness was shown to be largely unaffected by fiber diameter that was supported by FE models while the volume fraction of fibers was constant (Butcher et al. 2017). The viscoelastic properties of frozen-thawed agar/agar-gelatin gels based on binary-phase virtual structure have been studied. The results shown the prediction errors fell within 12.5% for NR analysis and 3.1% for FEM optimization (Kim et al. 2012).
Gelatin is the most widely used to fabricate the scaffold which is biocompatible interacted with fibroblast cell and good cell proliferation. There have previous study on a tri-polymer polycaprolactone (PCL)/gelatin/collagen type I composite nanofibrous scaffold. The result of MTT assay demonstrated the viability and high proliferation rate of L929 mouse fibroblast cells on the composite scaffold (Gautam et al. 2014). The gelatin scaffold fabricated by salt-leaching method showed that gelatin scaffold was biocompatible with cells and showed in good affinity and cell proliferation (Lee et al. 2003 and Lee et al. 2005 and Wiwatwongwana 2019). Carboxymethylcellulose (CMC), biocomplatible polymer, was chosen to blend with gelatin scaffold in this research. CMC is a available derivative of cellulose polysaccharide with low price. It is widely used in biology, medicine, nutrition, pharmaceutical and nanocarriers for delivery of anticancer therapeutic agents. It presents excellent properties such as biocompatibility to the skin, biodegradability, non-toxicity (Carvalho et al. 2018). CMC and polyvinyl alcohol (PVA) nanofibers tested with human fibroblast cells have been studied. The result shown the culturing cells on the nanofibers to assess the attachment, growth, and proliferation after 24 hours of culturing (Zulkifli et al. 2019). CMC is high in viscosity and shear strength which help to strengthen scaffold structure and dehydrothermal treatment has been used for strengthen scaffold structure (Capitani et al. 2000 and Biswal 2004). Salt leaching has been widely used for gelatin scaffold fabrication which easily forms into desired shape with a uniformly distributed which used excess NaCl crystals as a porogen to fabricate porous scaffolds and NaCl particles can be dissolved in water. The pore size can be adjusted independently depended on particles size (Lee et al. 2005 and Cho et al. 2014). Therefore, this research aimed to fabricate gelatin blend with CMC scaffold by salt leaching technique. The various conditions of gelatin blended with CMC were designed and dehydrothermal treatment was used for strengthening the scaffold structure. The mechanical properties of scaffold conditions were analyzed by compression test and evaluated the mechanical simulation by FEM.

2. Material Fabrication

The salt leaching scaffold was made from gelatin blended with CMC in various conditions. Type A gelatin was purchased from Fluka Analytical, Sigma-Aldrich, St. Louis, MO, USA. It was a reagent grade and derived from porcine skin with medium gel strength for microbiology. CMC was purchased from Sigma-Aldrich, St. Louis, MO, USA. It was from plant material with medium viscosity with 400-800 cps in a 2% aqueous solution at 25 ^{o}C. Sodium chloride (NaCl) 99% was purchased from Merck Co., Ltd. The gelatin/CMC solution was prepared by dissolving gelatin/CMC in DI water for five different ratios which were 3/0, 2.7/0.3, 2.4/0.6, 2.1/0.9 and 1.8/1.2 g, respectively. Then leaved it at room temperature for 1 hour before stirred it at 50°C for 1 hour until homogeneous. Leave it at room temperature for 2-3 mins before added 7 g of NaCl to each condition and stirred until it saturated to make a porous structure. The solutions were poured into casting teflon mold and leaved it dry in humid controlled container for 2 days to 1 week and rinse NaCl by DI water for 3 times and then leaved it dry again in humid controlled container for 2 days to 1 week. The obtained scaffold was heated at 140 ^{o}C for 48 h or dehydrothermal treatment to strengthen scaffold structure. Finally, keep all scaffolds in a humid controlled container and labeled it. The schematic diagram of gelatin/CMC scaffold fabrication by salt leaching method and experiment was shown in Figure 1.

3. Mechanical Properties Identification

3.1 Geometry and Loading Condition

The gelatin/CMC scaffold was fabricated by salt-leaching method which NaCl provided a porous structure. The examples of gelatin/CMC scaffolds of condition G2.7T and G2.4T were shown in Figure 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 (Wiwatwongwana et al. 2012). The tested gelatin/CMC scaffolds were divided into 5 mixtures which were 3/0, 2.7/0.3, 2.4/0.6, 2.1/0.9 and 1.8/1.2, 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 significant different of each condition was evaluated using a student t-test with 95% confidence interval. The differences were considered to be a statistically significant when $p<0.05$ compared to pure gelatin scaffold.

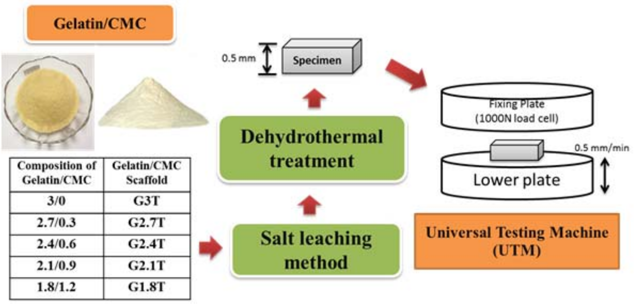

Figure 1: Schematic diagram of salt leaching gelatin/CMC scaffold preparation and experimental test.

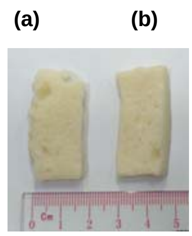

Figure 2: Salt leaching gelatin/CMC scaffold (a) G2.7T and (b) G2.4T, respectively.

3.2 Finite Element Modeling

Mechanical behavior of the gelatin/CMC scaffold from salt leaching method was evaluated by using FEM. The behavior of the scaffold was used a non-linear elastic material law for the finite element 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 as previous research (Wiwatwongwana 2019). The compressive modulus from experiment was used to analyze the mechanical properties of the scaffolds by FEM. The scaffold was deformed by using 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 Eq(1) (Kim et al. 2012). The example mesh of the scaffold from finite element was shown in Figure 3.

$$\sigma = \int_0^t 2G(t-\tau)\frac{de}{d\tau}d\tau + I\int_0^t K(t-\tau)\frac{d\Delta}{d\tau}d\tau \qquad (1)$$

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)

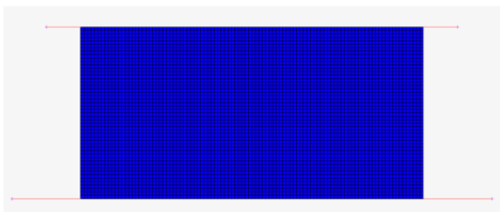

Figure 3: The mesh was made of around 600,000 tetrahedral elements.

4. Results and Discussions

4.1 Compressive Modulus of the Scaffolds

All scaffold conditions were compressed by UTM with two flat plates to analyze the stress-strain relation. Force versus displacement was converted into engineering stress and strain by using of the initial dimensions of each scaffold. The average compressive modulus of all scaffold conditions which represented by circle marker of the scaffolds was plotted as shown in Figure 4. The results showed that gelatin scaffold with 1.8 g of CMC (G1.8T) occurred the highest compressive modulus with significant different compared to pure gelatin scaffold (G3T). The compressive modulus of G1.8T was 15.16 ± 3.23 kPa and the compressive modulus of pure gelatin scaffold was 0.93 ± 0.26 kPa as shown in table 1. Increasing of CMC in the gelatin scaffold and using salt leaching method with dehydrothermal treatment provided increasing in compressive modulus of scaffold as shown in other compositions of blending gelatin/CMC scaffolds (G2.7T, G2.4T and G2.1T) with significant different compared to pure gelatin scaffold. It could be implied from the result of compressive modulus that CMC could be used as a scaffold strengthening with salt leaching technique, especially for gelatin scaffold at a condition of gelatin/CMC of 1.8/1.2. This condition showed the highest value of compressive modulus. Whereas G3T showed the lowest compressive modulus. The scaffold should have a strength structure for support of the surrounding tissues to prevent contraction and necrosis from stress during implantation and tissue regeneration. Compressive modulus is required to analyze for scaffold stiffness. CMC which improved the elasticity and strengthen structure for materials could enhance the modulus of gelatin scaffold fabricated using salt leaching method.

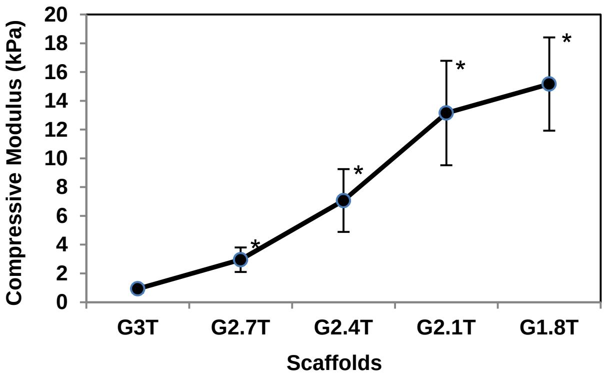

Figure 4: Compressive modulus of salt leaching gelatin/CMC scaffold (n=5) (significant different $p<0.05$ relative to G3T).*

Table 1: Compressive Modulus of salt leaching Gelatin/CMC scaffold (n=5)

Scaffold	Average Compressive Modulus (kPa)	SD
G3T	0.93	0.26
G2.7T	2.95	0.85
G2.4T	7.07	2.18
G2.1T	13.15	3.63
G1.8T	15.16	3.23

4.2 Finite Element Model of the Scaffolds

The biomechanical properties of scaffold should be analyzed for studied the tissue elastic behavior. The implant tissue was assumed to be elastic perfectly due to shear force and compressive force from surrounding tissue of wound. The compressive modulus was analyzed the scaffold deformation behavior by using FEM. The equivalent elastic strain of deformed various scaffold conditions was shown in Figure 5.

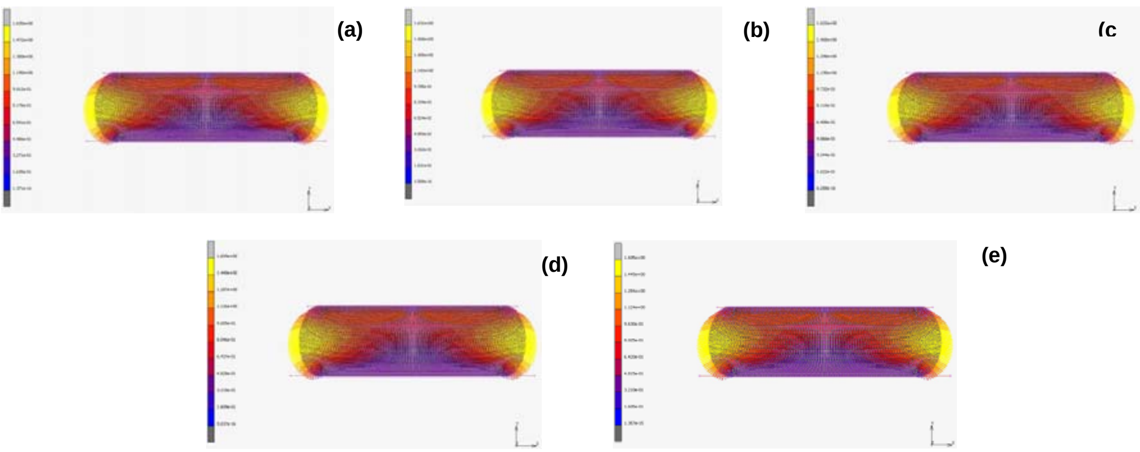

Figure 5: The finite element modeling of deformed salt leaching gelatin/CMC scaffold (a) G3T, (b) G2.7T, (c) G2.4T, (d) G2.1T and (e) G1.8T, respectively.

The results shown in similar trend with deformable of 100 increments with different colors of deformation. All scaffold conditions shown the similar range of equivalent elastic strain of deformed material. The distribution of the stress and strain of all scaffold conditions was similar due to their isotropic properties. The total strain energy-time response from FEM plot of all deformable of 100 increments under uniaxial compression was shown in Figure 6. The scaffold G1.8T which represented by circle blue marker showed the highest of total strain energy compared to other scaffolds at the equivalent time. Whereas G3T scaffold which represented by asterisk magenta marker showed the lowest of total strain energy at the equivalent time. The other scaffolds; G2.7T, G2.4T and G2.1T which represented by purple, orange and yellow marker, respectively occurred the increasing in total strain energy-time response when CMC increased in each condition. The FEM plot result was consistency with the result from experiment. It could be summarized that scaffold condition of G1.8T was the strongest structure and could support the most uniaxial compressive load. It might be the best condition for using in further experiment of tissue engineering application. FEM could be used for further biomechanical simulations of scaffold.

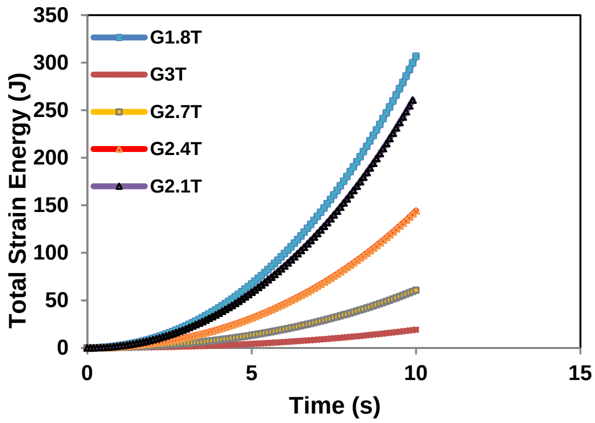

Figure 6: Total strain energy-time response from finite element analysis of G3T, G2.7T, G2.4T, G2.1T and G1.8T,respectively.

Further research can be focused on the other treatment methods for scaffold strengthening such as chemical treatment. The other scaffold fabrication technique such as rapid prototyping 3D printing can be used to compare with salt leaching technique. The other technique of salt leaching and particulate leaching fabrication should be investigated. The above mentions may be allowed each type of scaffold to obtain maximum strength of material. The other experiments such as porosity and biodegradability are necessary to investigate. In addition, scaffold may be used in wet condition during implantation, the mechanical properties in wet condition of the scaffolds should be analyzed to compare with dry condition for proper application.

5. Conclusion

Wound dressing or scaffold is used to help to promote skin regeneration in patients after skin loss from various causes. To overcome high price of ingredient in skin replacement which due to process of extraction. This research aimed to fabricate the alternative scaffold as a new product to reduce cost of material fabrication. The scaffolds which made from gelatin from porcine skin was blended with CMC from plant material which used as the scaffold strengthening at various gelatin/CMC ratio which were 3/0, 2.7/0.3, 2.4/0.6, 2.1/0.9 and 1.8/1.2, respectively. The results shown that the scaffold made from waste product both livestock waste and agricultural waste was successfully fabricated using salt leaching method with various conditions which helped reduce cost of the scaffold fabrication and was very environmentally friendly material. This was shown the improvement of waste product using in tissue engineering applications. The mechanical characterization of all scaffold conditions was the investigation of compressive modulus by using Universal Testing Machine. The data from compressive modulus was 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 of scaffold which were from gelatin/CMC ratio at 1.8/1.2. However, pure gelatin showed the lowest compressive modulus. It was found that increasing of CMC content and using dehydrothermal treatment increased in material strengthening. Increasing of CMC content in each condition improved in mechanical property. From finite element analysis of deformable material, the example of G2.4T and G1.8T scaffold shown in different colors of scaffold deformation from equivalent elastic strain. Moreover, G1.8T scaffold occurred the highest value of total strain energy from FEM plot at the equivalent time compared to other scaffolds. It could be summarized that this scaffold condition could support loading better than other scaffolds. On the other hands, G3T scaffold revealed the lowest value of total strain energy at the equivalent time. The other scaffolds (G2.7T, G2.4T and G2.1T) occurred increasing in total strain energy-time response when added CMC in each ratio. From all mechanical results, it could be summarized that the gelatin/CMC ratio at 1.8/1.2 might be useful for tissue engineering applications as a wound dressing due to its good for supporting compression. Thus, experiments 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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