Synthesis and Characterization Biocomposite Collagen-Chitosan- Glycerol as Scaffold for Gingival Recession Therapy

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Abstract

Gingivitis is considered high prevalence since nearly 75-90% of the world population suffers gingivitis in moderate category. The development of biomaterial in dentistry is divided into synthetic biomaterial and tissue engineering biomaterial includes scaffold engineering. Scaffold biomaterial engineering technique includes the development of tissue engineering and cell. The aim of this study is to determine the best compositions of collagen-chitosan scaffold by adding glycerol, marked by its mechanic characteristic. The synthesis process of the collagen-chitosan-glycerol scaffold is by freeze-dry method to form pores in the scaffold. The characterization of the synthesis was conducted through morphology test, tensile test, toxicity test, swelling test, and biodegradation test. Morphology test results in 50.89 - 143.5 μm pore size and thickness value of 412.0 - 514.9 mm which are appropriate for periodontal applications, especially gingivitis. The tensile test result is 0.46 MPa – 2.36 MPa and the highest value was seen on the variation of 0.46 with 2.36 MPa tensile strength value which is close to the value of UTS for periodontal. While the swelling test results in 1354.04 - 413.9%.

Based on the characterization results in this study, the collagen-chitosan scaffold with the addition of glycerol is a potent periodontal scaffold candidate, especially gingivitis.

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Introduction

Gingivitis or gum inflammation is a disease of the gingiva characterized by inflammation without the loss of gingiva attachment on the tooth[5]. Gingivitis is caused by the accumulation of plaque bacteria due to poor oral hygiene, calculus (tartar), mechanical irritation, and irregular tooth position as a contributing factor. A large quantity of plaque bacteria may hinder tissues connection and may trigger dental caries and periodontal disease. Clinical signs of gingivitis are reddish gingiva, swelling gingiva, bleeding and the presence of exudate. Generally, each individual experiences inflamed gums with various severity and exposures due to factors affecting the prevalence and severity of gingivitis which are age, oral hygiene (OHI), employment, education, geographical location, environmental pollution and dental care[11].

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In Indonesia, gingivitis patient numbers are increasing. Yet, the most common treatment is still surgery which can trigger trauma and required a fairly high cost. Thus, there is a need for new innovation namely implant material that can solve the problem. The scaffold is a tissue engineering that can be considered as a new alternative, since scaffold, as the product of tissue engineering, is deemed capable of helping the process of repairing the damaged tissues[7].

The scaffold is a porous biodegradable material with a three-dimensional structure. Scaffold works as a support for cell structures and becomes the extracellular matrix during regeneration process and natural tissues development. In tissue engineering application, an ideal scaffold must have the characteristic of homogeneous microstructure and high porosity. High porosity spaces accommodate cells and body fluids for transformation so that the scaffold contains lots of cells and nutrients to repair damaged tissues[7].

One material that can be used as an implant material for gingivitis case is collagen. Collagen plays a very important role at every stage of wound healing process. Collagen possesses many abilities such as homeostasis, interaction with platelets, interaction with fibronectin, increasing the fluid exudation, increasing cellular components, increasing the growth factors and encouraging the process of fibroplasia and sometimes on the epidermis proliferation[13]. However, the collagen scaffold often lost their shape and size due to rapid degradation when exposed to body fluids. In the selection of materials, collagen is synthesized with chitosan because chitosan is a material that has slower degradation rate compared to collagen so it is expected to improve the mechanical strength of the scaffold[7].

Chitosan is chosen to be applied on the scaffold for periodontal in order to increase the scaffold mechanical properties value. Chitosan has many advantages such as antibacterial, accelerate tissue regeneration and fibroblasts synthesis. Chitosan has also been widely used in tissue engineering[14,3]. In addition, chitosan also works as a bridge to improve the mechanical strength of the scaffold-based collagen derived from a number of amino groups in the molecule chain. Next, there is the need to add additives such as plasticizers to improve the mechanical strength. The plasticizer used in this research is glycerol. The material is used to make a scaffold by freeze-dry method.

Materials and methods

Tools and materials

The tools used include digital balance, glass beaker, magnetic bar, magnetic stirrer, freezer, lyophilizer, measuring cups, spatulas, pH meters, stopwatch, filter paper, and aluminum foil. Characterization tools include Tensile Strength (Autograph Imada HV-500NII), MTT Assay (Elisa reader), SEM (S50 inspect, FEI Corp., Japan), Freeze Drying (1-15 BETA OHRIST, Germany). The materials used in this study are collagen from Tissue Bank PAIR-Batan, chitosan from Biochitosan Indonesia, glycerol 2%, acetic acid, citric acid, phosphate buffered saline (PBS), simulated body fluid (SBF) and distilled water.

Synthesis of Collagen-Chitosan-Glycerol Scaffold

The making of scaffold begins by dissolving collagen and chitosan powders into the solvents which are acetic acid and citric acid. Then, they are mixed with a stirrer for 6 hours until it is homogeneous, which is marked by the absence of sediment in the solution when it is allowed to stand. The next process is synthesizing collagen with chitosan at a ratio of (v / v) 6: 4, 5: 5, 4: 6, 3: 7, and 2 ml of glycerol 2% is added to each variation and stirred until homogeneous. The solution is poured into a petri dish for lyophilization process to form pores with a thickness of 0.5 mm.

Morphology Test

The test carried out using scanning electron microscope (SEM), in order to reveal the morphology of the surface and cross-sectional samples of chitosan-collagen scaffold added with glycerol. SEM test shows the pore size and thickness formed on the scaffold. Before the samples are inserted into the scanning electron tool, the scaffold is cut to fit the holder and then the coating process with Au or Pd is conducted by sputtering coating method[1].

Mechanical Strength Test

The scaffold is cut in accordance to the standard test and both ends are attached to tensile test equipment in order to measure the mechanical strength of the collagen-chitosan scaffold added with glycerol[10]. The treatment results in a quantity that is used as a reference of the sample mechanical strength.

$$\sigma = \frac{F}{A}$$

In which: σ = Stress (N / m^2)
F = Load (N)
A = surface area (m^2)

Swelling Test

Swelling capability of the scaffold is determined by incubating or soaking it in phosphate buffered saline (PBS) at normal pH and at room temperature. Wet weight of the scaffold is calculated over several times and dried using a sponge filter paper to remove water adsorbed on the surface and then immediately weighed. The absorptions that occur in the scaffold can be calculated:

$$Esw\,(\%) = \frac{we - w0}{w0}\ x\ 100\,\%$$

Description: Esw = percentage of swelling scaffold
Wo = initial weight before the soaking
We = weight of the sample after the soaking

Results

Morphology Test Result

The thickness of all scaffolds ranged from 0.412 - 0.515 mm. The pore size of all scaffolds ranged between 102.4 - 143.5 µm.

Pull Test Results

Samples	UTS Value (Mpa)
A (Collagen-Chitosan-Glycerol) = 6: 4: 2	2.36
B (Collagen-Chitosan-Glycerol) = 5: 5: 2	0.80
C (Collagen-Chitosan-Glycerol) = 4: 6: 2	0.46
D (Collagen-Chitosan-Glycerol) = 3: 7: 2	0.67

Table 1. Pull Test Results.

Samples	Percentage of Swelling
A (Collagen-Chitosan-Glycerol) = 6: 4: 2	413,9 %
B (Collagen-Chitosan-Glycerol) = 5: 5: 2	801,91 %
C (Collagen-Chitosan-Glycerol) = 4: 6: 2	1021,78 %
D (Collagen-Chitosan-Glycerol) = 3: 7: 2	1354,04 %

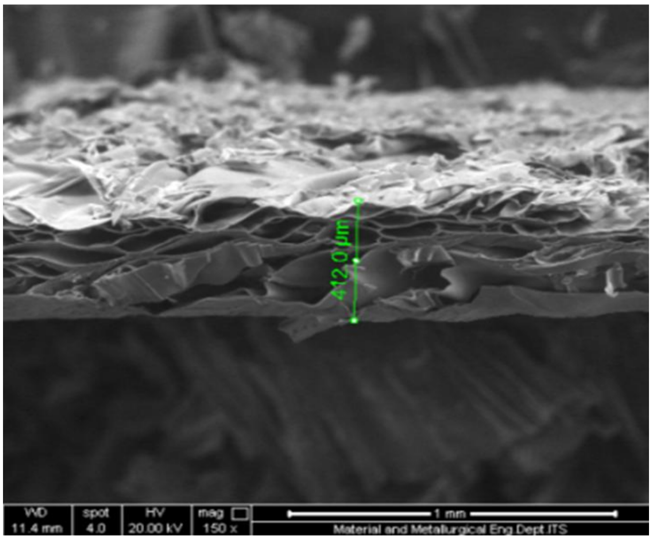
Table 2. Swelling Test Results.

Discussion

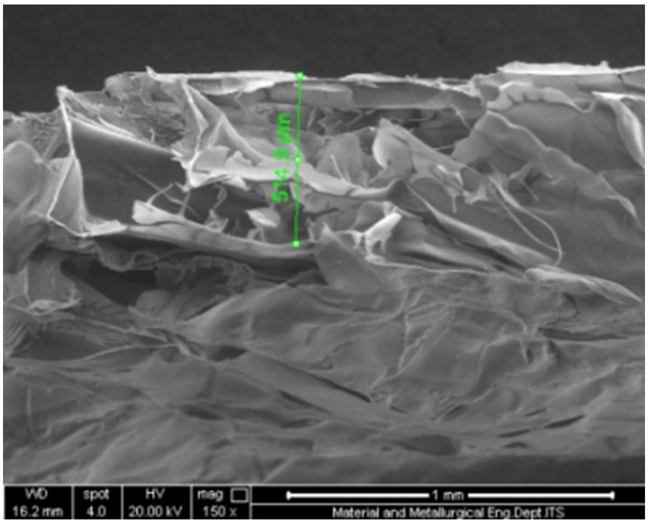
In this study, the samples were various collagen-chitosan compositions in the ratio of 6:4, 5:5, 4:6 and 3:7, and then each variation is added with 2 ml of glycerol.

Morphology Test

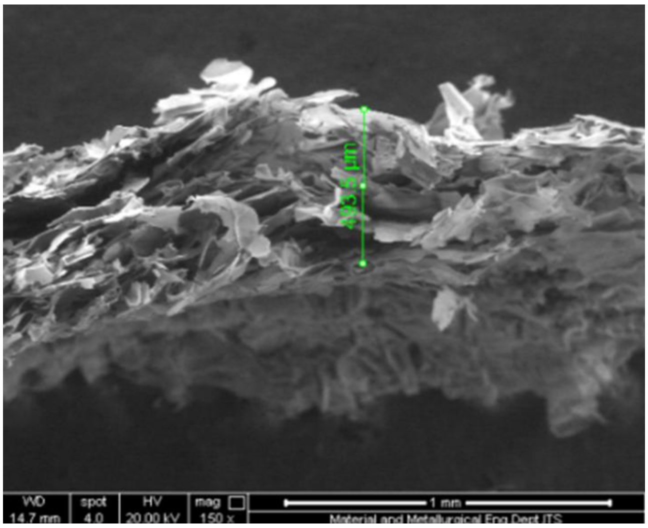
The first characterization was morphology test by scanning electron microscope (SEM) to reveal the surface structure of the samples. The magnification was at 300X, and the results were as follow :



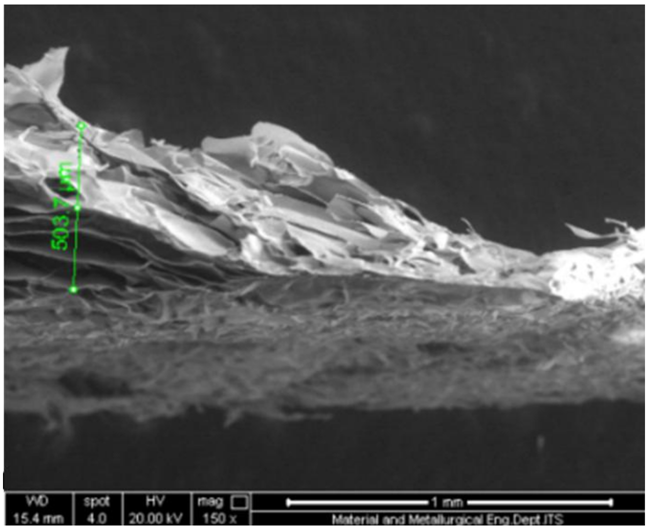
1.a



1.b



1.d



1.e

Figure 1. Observations with Vertical Cutting on the Scaffold For Thickness Measurement Results *(300X magnification): (a) 6: 4: 2; (b) 5: 5: 2; (c) 4: 6: 2; (d) 3: 7: 2 .*

The thickness of the scaffolds ranged from 0.412 to 0.515 mm, thus approaching the thickness mentioned by Peng et al (2006) that is 0.5 mm. The pore size obtained ranges between 102.4 to 143.5 μm which is suitable for a periodontal application. According to O'Brien et al (2005), the applications for periodontal cases needed pore size of 63-150 μm. Since smaller pore size may hinder cell growth in the scaffold, which later affects the wound healing process.

Pull Test

Next characterization is conducted mechanically, namely tensile test. This test is done to determine the elasticity of samples when given a certain pressure. According to Ralph in Rees, JS, and Jacobsen, P. H (1997), in the case of periodontal, UTS value that should be achieved is 2.4 Mpa. The results obtained for each sample namely sample A was 2.36 MPa, sample B was 0.80 MPa, sample C and D were 0.46 MPa and 0.67 MPa. The sample that reached the standard value for periodontal application is sample A that is equal to 2.36 MPa. This is because sample A has the largest composition of collagen compared to other samples. This is due to the fact that when collagen and chitosan are synthesized, they will form a complex bond that increases the value of the mechanical property[2].

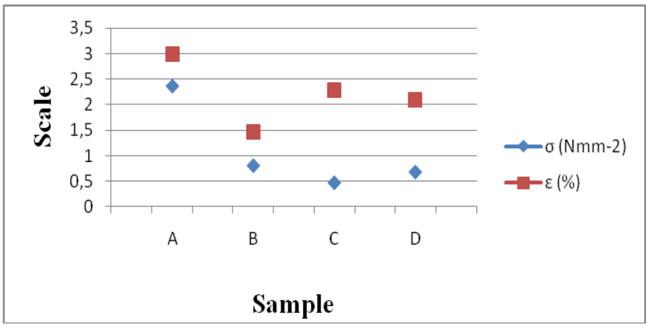


Figure 2. Characterization Results of Mechanical Test from Collagen-Chitosan-Glycerol Scaffold.

From the figure 3, all treated sample are swollen. The percentage of swelling in a sample 413,9 %; b sample 801,91 %, c sample 1021,78 % and d sample 1354,04 %. Based on figure 3, the percentage of swelling decreases as the amount of collagen in the scaffolds also decreased. The reason is that collagen that has many hydrophilic groups which have a tendency to chain the surrounding solution when soaked. This phenomenon is advantageous for collagen-chitosan composite which can improve scaffold hydrophilicity and swelling capacity. It is essential for tissue engineering scaffold to be able to store water, absorbing body fluids and transferring nutrients and metabolites into the cells embedded in the scaffold[4].

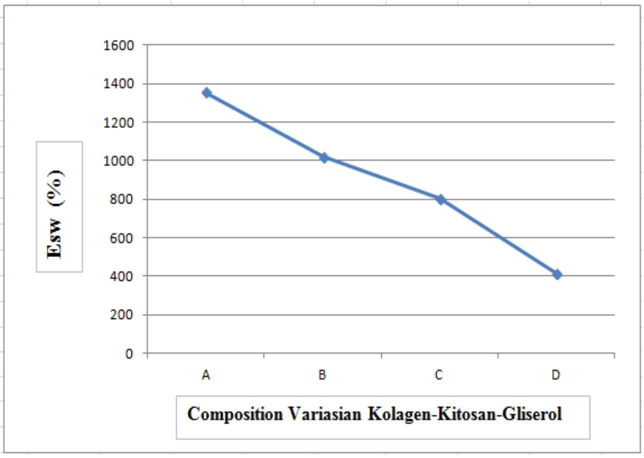


Figure 3. Swelling Percentage Graph of Collagen-Chitosan-Glycerol Scaffold.

Conclusions

1. On the morphology test pore size and thickness obtained in all samples were in accordance to the size of 0-2 mm thickness and pore size for periodontal applications which is 63-150 μm.
2. In the tensile test, samples can improve tensile strength along with a large amount of collagen composition (2.36 MPa) in the ratio of 6:4 and decreased on the composition ratio 5:5 , 4:6 and 3:7.
3. For the swelling test, the smaller amount of collagen in the composition, the smaller the percentage of swelling. Thus, the range of swelling percentage is 1354.0 to 4413.9%.

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Declaration of Interest

The author reports of conflict of interest.

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