Fabrication of Chitosan based-Scaffold as Potential Cornea Implant

Satria Rusdiputra¹, Arie Wibowo¹², Lia Amelia Tresna Wulan Asri¹, Bambang Sunendar Purwasasmita³*

¹Department of Materials Engineering, Faculty of Mechanical and Aerospace Engineering, Institut Teknologi Bandung
²Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung.
³Department of Engineering Physics, Faculty of Industrial Technology, Institut Teknologi Bandung

*Corresponding author: purwa@tf.itb.ac.id

Abstract. Corneal damage is the second cause of blindness in the world due to limitation of high quality corneal from donor. This condition promote rapid development of biodegradable and biocompatible scaffold for corneal tissue because it can reduce the patient's dependence on the cornea donor. The purpose of this research is to synthesize chitosan-based hydrogel scaffold with glutaraldehyde (GA) as crosslink agent and observe the influence of concentration chitosan and GA to transparency and mechanical properties of the scaffold. Scaffolds were prepared by solution casting combined with salt leaching method. Among all of chitosan-based scaffolds that have been prepared, sample with 6.7% of chitosan and 0.0% of GA possess the highest transparency (85%), while sample with 6.7% of chitosan and 0.3% of GA gave the highest Young’s modulus (3.09 MPa). However, sample with 6.7% of chitosan and 0.1% of GA might possess the highest potency as corneal tissue implant because of its transparency fulfill the minimum value of corneal tissue (80%) and its Young’s modulus (1.41 MPa) is higher than any chitosan-based scaffold with minimum transparency 80%.

Keywords: Chitosan, Cornea, Hydrogel, Scaffold, Tissue engineering

1. Introduction

Corneal is a transparent front part of the eyes with role as first protection of eyes and optical element that contribute to most of the eyes’s focusing power [1]. Corneal damage is the second cause of blindness in the world and current acceptable treatment to treat it is corneal transplantation from donor [1-4]. Nowadays, around 60,000 of corneal transplantation were performed every year globally, which is still too low compare to 10 million of blindness cases worldwide [2,3]. This problem occurred mainly because of shortage stock of corneal donor with high quality [4]. Therefore, development of synthetic scaffold biomaterials for corneal tissue application is needed.

For fruitfulness of corneal tissue engineering, scaffold for cornea tissue application should be non-toxic, possess good transparency, good biocompatibility, good permeability, suitable mechanical properties with corneal tissue, and promote cell growth [1-3]. Hydrogels-based scaffold is one of attractive materials for corneal tissue engineering.
application due to similarity of their properties with extracellular matrix (ECM) of cornea [3]. Hydrogels can be prepared by both of synthetic and natural based-polymers. One of natural based-polymers that widely explored is chitosan due to its advantages such as non-toxic, biodegradable, biocompatible, high antibacterial activities and high chelating abilities [5-8]. Nevertheless, chitosan-based scaffold suffer with their weakness such as low mechanicals properties ($\sigma_u$ compressive (2% w/v, dry) = 0.967 MPa) and low stability to maintain their shape in long term condition [9-12]. To overcome this challenge, cross linking agent such as glutaraldehyde are commonly used to increase mechanical properties and stability of scaffolds [13]. In this experiment, the influence of glutaraldehyde was used as cross linking agent in fabrication of chitosan-based scaffold and studying their optical and mechanical properties.

2. Materials and Methods

**Materials:** Chemicals that were used in this study are pro analyst (p.a) grade and purchased from Sigma Aldrich. Chitosan was purchased from CV. Biochitosan Indonesia.

**Preparation of Chitosan-based Scaffold:** Chitosan based-hydrogels was prepared by solution casting and particulate leaching method [14]. Briefly, chitosan and NaCl with various concentration were dissolved in acetic acid solution and mixed for 2 hours. Then, hydrogels was molded by pouring chitosan solution in glass substrate, followed by immersion in 2M NaOH solution for about three minutes. Obtained hydrogels was immersed in demineralized water for about 30 minutes to remove remaining NaOH and let the NaCl leach from scaffold. Excess amount of water in hydrogels was dried with tissue. Preparation parameter that were used in this experiment are concentration of chitosan, scaffold thickness and concentration of Glutaraldehyde (GA). Thus, samples code can be seen in table 1.

| Sample code | [chitosan] (%) | Sample thickness (mm) | [GA] (%) |
|-------------|----------------|-----------------------|----------|
| Ch15-1L-GA0.0% | 6.7 | 0.1 | 0.0 |
| Ch20-1L-GA0.0% | 5.0 | 0.1 | 0.0 |
| Ch15-2L-GA0.0% | 6.7 | 0.2 | 0.0 |
| Ch20-2L-GA0.0% | 5.0 | 0.2 | 0.0 |
| Ch25-2L-GA0.0% | 4.0 | 0.2 | 0.0 |
| Ch15-1L-GA0.1% | 6.7 | 0.1 | 0.1 |
| Ch15-1L-GA0.2% | 6.7 | 0.1 | 0.2 |
| Ch15-1L-GA0.3% | 6.7 | 0.1 | 0.3 |

**Characterization:** Functional group of chitosan were identified determined by Fourier Transform Infra-red (FTIR; Prestige 21 Shimadzu) method and their degree of deacetylation (DD) were determined by baseline method following Eq. 2.1 [15,16]:

$$DD[\%] = 100 - \left(\frac{A_{1655}}{A_{3450}} \times 115\right)$$  \hspace{1cm} (2.1)

Transparency of scaffolds were studied by UV-VIS Spectrophotometer (Agilent 8453 G1103A) method and mechanical properties of scaffolds were determined by Tensilon
3. Results and Discussion

3.1. FTIR result

Before used, it is important to ensure that DD of purchased chitosan are above 70%, to be classified as chitosan [17]. Thus, purchased chitosan was characterized using FTIR method and imaginary line on FTIR spectra of sample for calculation of DD of chitosan can be seen in Fig. 1. FTIR spectra showed that typical peaks of chitosan are observed in sample (~3400 cm\(^{-1}\) for O-H and/or N-H functional groups; 1656 cm\(^{-1}\) for amide I functional groups; 1600 cm\(^{-1}\) for amide II functional groups) [15,16]. Based on baseline method, calculated DD of sample was 76%. Since DD of sample is above 70%, it can be claimed that purchased chitosan is categorized as chitosan and can be used in this experiment.

![Figure 1. FTIR spectra of purchased chitosan](image)

3.2. UV-Vis results

Since transparency is important properties for synthetic corneal tissue, transparency of all of obtained chitosan scaffolds have been evaluated using UV-Vis spectroscopy method. UV-Vis results of chitosan scaffold can be seen in Fig. 2. UV-Vis results of samples with various chitosan concentration and without GA (Fig. 2a) revealed that obtained sample with 6.7% of chitosan and without GA showed the highest transparency, ± 85%, which is higher than minimum value for corneal tissue (80%) [3]. Thus, samples with chitosan 6.7% was cross-linked by GA as a function of concentration of GA (0.1; 0.2; and 0.3%) and their transparency were evaluated by UV-Vis spectroscopy method. UV-Vis results (Fig. 2b) showed that sample with cross-linked agent have lower transparency than sample without cross linking agent.
Figure 2. UV-Vis spectra of scaffold samples a) with various chitosan concentration and without GA, and b) with chitosan 6.7% and with various GA concentration.

Rolf Klein mentioned that deep of light penetration (denoted as a) and thickness of sample (z) can be used to predict interaction between materials with incident light, and can be calculated by equation:

\[ a = -\frac{z}{\ln T} \]  

(eq. 2.2)

If \( a >> z \) : Incident light will not be absorbed by materials

\( a \cong z \) : Incident light will be absorbed by bulk of materials

\( a << z \) : Incident light will be absorbed by surface of materials

Summary of deep of penetration light (a), thickness of sample (z) and materials characteristic is presented in table 1. As can be seen in Table 1, this rough estimation can be used to explain why sample with chitosan 6.7% without GA have the highest transparency while sample with chitosan 6.7% and GA 0.3% showed the lowest transparency.

Table 2. Summary of deep of penetration light (a), thickness of sample (z) and materials characteristic of all of samples

| No | Sample       | a (mm) | z (mm) | Characteristic | Transparency (%) |
|----|--------------|--------|--------|----------------|-----------------|
| 1  | Ch15-1L-GA0.0% | 0.519  | 0.116  | \( a >> z \)   | 85              |
| 2  | Ch20-1L-GA0.0% | 0.394  | 0.116  | \( a >> z \)   | 80              |
| 3  | Ch15-2L-GA0.0% | 0.403  | 0.194  | \( a >> z \)   | 70              |
| 4  | Ch20-2L-GA0.0% | 0.188  | 0.206  | \( a \cong z \) | 40              |
| 5  | Ch25-2L-GA0.0% | 0.229  | 0.198  | \( a \cong z \) | 50              |
| 6  | Ch15-1L-GA0.1% | 0.137  | 0.132  | \( a \cong z \) | 80              |
| 7  | Ch15-1L-GA0.2% | 0.168  | 0.114  | \( a \cong z \) | 60              |
| 8  | Ch15-1L-GA0.3% | 0.355  | 0.114  | \( a >> z \)   | 40              |

3.3. Mechanical Properties of Samples
Considering that mechanical properties is important properties for corneal tissue, mechanical properties of chitosan scaffold as function of GA concentration were performed and their results are presented in Fig. 3. Measurement of tensile strength (Fig. 3a) and elastic modulus/Young’s modulus (Fig. 3b) of samples showed that their strength and Young’s modulus are increased as concentration of GA increased. Meanwhile, measurement of elongation of samples (Fig. 3c) showed that elongation of samples decreased as concentration of GA increased. These results are reasonable since GA is cross linking agent that provide additional bond between polymer chain and 3D network in scaffold. Thus, scaffold become stronger and stiffer by addition of GA. Since corneal Young’s modulus that measured in vitro are varies from 0.1 to 57 MPa [17], all of chitosan-based scaffold that prepared with chitosan 6.7% (Young’s modulus were varied from 0.67 – 3.09 MPa) could be considered as acceptable materials for corneal tissue application. However, scaffold with higher Young’s modulus are more favorable for corneal tissue implant.

![Figure 3](image_url)

**Figure 3.** Mechanical properties of samples with chitosan 6.7% as a function of GA concentration: a) tensile strength of samples, b) elasticity modulus of samples, and c) elongation of samples

4. Conclusions

Chitosan based scaffold have been successfully prepared by SCPL method. Sample with chitosan 6.67% and GA 0.0% showed the highest transparency (± 85%). As concentration of GA increased, tensile strength and Young’s modulus of scaffold increased but elongation of scaffold tend to decreased. Considering that transparency of corneal tissue is higher than 80% and Young’s modulus that measured in vitro are varies from 0.1 to 57 MPa, sample that prepared with chitosan 6.67% and GA 0.1% showed the highest potency for corneal tissue implant with transparency 80% and its Young’s modulus (1.41 MPa) is higher than any chitosan-based scaffold with minimum transparency 80%.
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6. References

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