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THE EFFECT OF THE PERICARDIUM MEMBRANE ON ADSORPTION RATE OF BLOOD TYPE O ON CHITOSAN POWDER SIZE 150 -355 µm

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ABSTRACT

Tooth loss can have an impact on one's quality of life. One of the most common problems is alveolar bone loss induced by periodontal disease or surgery (trauma). A bone transplant is a material that may be used to repair bone loss. Chitosan has been demonstrated in studies to hasten wound healing. The pericardium membrane is an appropriate component for augmentation of the alveolar ridge deficiency. Barrier membranes have been designed and tested to keep epithelial and connective tissue cells from penetrating the void. To determine and explain the influence of the pericardium membrane on the adsorption rate of blood type O in chitosan powder sizes 150-355 µm. 14 chitosan samples measuring 150-355 m were separated into two groups: the control group, which included 7 samples of chitosan with pericardium membrane and 7 samples of chitosan without pericardium membrane, and the treatment group, which included 7 samples of chitosan without pericardium membrane. Immerse it in 75 mL of blood. For 10 minutes, measurements of group O blood adsorption rate were taken in each group. The results were statistically examined using the Mann-Whitney test ($p<0.05$). There was a statistically significant difference between the control and treatment groups. When the adsorption rate of blood type O in chitosan with and without membrane pericardium is compared, there are significant different. There is a difference in the rate of blood adsorption on 150-355 µm of chitosan with the pericardium membrane.

1. INTRODUCTION

Tooth loss can affect a person's quality of life. Loss of teeth means the loss of some orofacial structures, such as bone tissue, nerves, receptors, and muscles resulting in a decreased of most orofacial functions [1]. Patients who experience tooth loss will have difficulty in chewing. Moreover, this condition can also interfere with the patient's psychology because one of the functions of the teeth is also an aesthetic function [2]. Alveolar bone loss due to periodontal disease or surgery (trauma) is one of the most common complications. Alveolar bone damage can be prevented from getting worse with periodontal therapy so that regeneration occurs.

Periodontal regeneration involves repairing the bone, cementum, and periodontal fibers after the damage have occurred due to the periodontal disease process [3].

Bone graft is a substance that can replace the missing bone. Bone graft serves to stimulate the process of osteogenesis, osteoinduction, and osteoconduction. There are several types of bone grafts, namely autograft, allograft, and xenograft [4]. Autografts have limitations such as; donor site morbidity, limited bone that can be donated, and the possibility of inadequate resorption during the healing process. Allografts and xenografts also have several problems such as the risk of disease transmission, inflammation, immunogenicity, and loss of biological and mechanical properties [5]. The role of tissue engineering, namely alloplastic graft (synthetic bone graft) is needed to help regenerate damaged structures and tissues as an alternative to bone graft [6]. The use of bone graft is now widely accepted, because it is known that grafts have osteoconductive and osteoinductive properties [7].

Chitosan has many biological properties. Chitosan has been shown to accelerate wound healing by activating and modulating the function of inflammatory cells such as neutrophils, macrophages, fibroblasts, and endothelial cells as well as increasing the formation and regulation of granulation tissue [6]. Blood has an important role in the process of bone remodeling. Blood is a transport vehicle that contains platelets containing various kinds of growth factors. In the process of bone grafting, the space created by the graft is filled with blood clot. The clot will be absorbed and replaced with granulation tissue rich in new blood vessels filled with nutrients and mesenchymal stem cells, promoting osteoid formation. Blood mixed with chitosan will affect the activity of osteoblasts [8].

In blood type serology, type O blood has the most H antigens. Antigens on the surface of red blood cells will be recognized as foreign antigens when transfused to recipients who do not have identical antigens as the donor. The expression of a blood group antigen is controlled by genes. In the ABO and Lewis blood groups, the gene control is expressed by the enzyme responsible for the attached sugar/carbohydrate (Substance H) which will provide a specific antigen from the precursor substance. Type O blood has neither A nor B antigens but has both antibodies in the serum [9].

The pericardial membrane has good consistency, easy to manufacture, and can be processed to a thickness of 0.5mm [10- 13. One study found that bovine pericardium together with xenografts has been used in the augmentation of alveolar ridge defects [14]. The use of membranes as a barrier has been developed and tested to prevent epithelial cell and connective tissue cells from invading the deficient space [15]. Based on the background stated above, this study aims to examine the effect of the pericardial membrane on the rate of adsorption of type O blood on chitosan with a size of 150-355 μm.

2. Materials and Methods

This research consists of 3 stages of work. The first stage is making the samples by cutting the lower end of a glass pipette of approximately 3 cm to form a glass pipe. Samples using a pericardial membrane, the bottom of the pipe is covered with a pericardial membrane and in samples without the pericardial membrane, the bottom of the pipe is covered with gauze and each tied with a power O rubber, the chitosan was inserted into the pipe and vibrated.

The second stage is the preparation of the tool with a yellow background installed behind the pipe and the camera is placed parallel facing to the sample. The third stage is measuring at 0 seconds. The experiment begins when a series of pipes containing chitosan are inserted into a glass pot filled with blood, expressed as data 1 and until the 600th second, 21 data are obtained. The adsorption speed is calculated on each pipe by

dividing the volume/time.

3. Results

From the research that has been carried out, result of the mean and standard deviation of the calculation of the adsorption speed (ml/second) of chitosan size 150 – 355 µm with the pericardial membrane and without a pericardial membrane to blood group O with 30 second intervals as listed in Table 1.

Table 1. The average value and standard deviation of the adsorption speed (ml/second) of chitosan size 150 – 355 µm with a pericardial membrane and without a pericardial membrane on blood group O with an interval of 30 seconds.

Time	Chitosan without Pericardium Membrane			Chitosan with Pericardium Membrane		
	N	X	Std. D	N	X	Std. D
30 seconds	7	0.009172	0.004685	7	0.000002	0.000001
60 seconds	7	0.008430	0.003782	7	0.000002	0.000001
90 seconds	7	0.007915	0.003535	7	0.000002	0.000001
120 seconds	7	0.008030	0.003893	7	0.000002	0.000001
150 seconds	7	0.006630	0.002481	7	0.000002	0.000001
180 seconds	7	0.005387	0.002859	7	0.000002	0.000001
210 seconds	7	0.005115	0.002864	7	0.000003	0.000002
240 seconds	7	0.004972	0.002545	7	0.000002	0.000001
270 seconds	7	0.005601	0.002335	7	0.000002	0.000001
300 seconds	7	0.005544	0.002851	7	0.000002	0.000001
330 seconds	7	0.005544	0.003772	7	0.000002	0.000001
360 seconds	7	0.005087	0.002905	7	0.000003	0.000002
390 seconds	7	0.004187	0.001389	7	0.000002	0.000001
420 seconds	7	0.004658	0.004056	7	0.000002	0.000001
450 seconds	7	0.003630	0.003344	7	0.000002	0.000001

480 seconds	7	0.003315	0.002906	7	0.000002	0.000001
510 seconds	7	0.002344	0.002137	7	0.000002	0.000001
540 seconds	7	0.002030	0.001706	7	0.000002	0.000001
570 seconds	7	0.001087	0.000903	7	0.000002	0.000001
600 seconds	7	0.000772	0.000516	7	0.000002	0.000001

Description:
n: Number of sample
X: Average value
SD: Standard deviation

From Table 1, it was found that the average rate of adsorption of type O blood with the largest pericardial membrane occurred at 210 and 360 seconds, which was 0.000003 ml/second. For chitosan without pericardial membrane, the highest average rate of adsorption of group O blood occurred at the 30th second, namely 0.009172ml/second, with standard deviation values of 0.000001 with pericardial membrane and 0.009172 for without pericardial membrane, respectively. Both with the pericardial membrane and without the pericardial membrane, it was found that in the next second the rate of adsorption of type O blood was relatively decreasing. It can be seen the average value of the adsorption speed of type O blood, every 30 seconds for 10 minutes. At the 30th second, the highest average rate of adsorption of group O blood was seen in chitosan without a pericardial membrane. Data collection used a time interval of 30 seconds, starting at 30 seconds and then continued until the last data was taken at 600 seconds. There was an increase in blood in the tube containing chitosan without a pericardial membrane, whereas in the tube containing chitosan with a pericardial membrane, there was not much blood increase. The speed of blood adsorption by chitosan without a pericardial membrane formed a relatively stable regular pattern that decreased during the 30th to 600th seconds.

From table 2, the results of the Kolmogrov-Smirnov statistical test showed that the chitosan group with and without pericardial membrane at 30 seconds to 600 seconds had a significance value greater than 0.05 ($p > 0.05$). This shows that H0 is accepted so that the two treatment groups have a normal data distribution.

From table 3, the Mann-Whitney test results showed $p < 0.05$ at the 30th second to the 600th second, which means that there are significant differences between the treatment groups. From the test results, it was found that the rate of adsorption of group O blood on chitosan with a pericardial membrane and without a pericardial membrane showed a significant difference between the treatment groups.

Table 2. Kolmogrov Smirnov Test

	Kolmogorov-smirnov	
Times (second)	Chitosan without Pericardium Membrane (Sig.)	Chitosan with Pericardium membrane (Sig.)

30	0.200	0.012*
60	0.200	0.012*
90	0.200	0.013*
120	0.200	0.007*
150	0.200	0.007*
180	0.200	0.161
210	0.200	0.200
240	0.128	0.007*
270	0.185	0.013*
300	0.077	0.012*
330	0.200	0.161
360	0.200	0.200
390	0.050	0.012*
420	0.107	0.012*
450	0.079	0.161
480	0.177	0.007*
510	0.109	0.007*
540	0.200	0.012*
570	0.198	0.013*
600	0.198	0.012*

Information: *significant at p<0.05

Table 3. Mann-Whitney Test Result

Time (second)	Mann-Whitney (Sig.)*
30	0.001
60	0.002
90	0.001
120	0.001
150	0.001
180	0.002
210	0.002
240	0.001
270	0.001
300	0.001
330	0.002
360	0.002
390	0.002
420	0.001
450	0.002
480	0.001
510	0.001
540	0.001
570	0.001
600	0.001

Information: *significant at p<0.05

4. Discussion

Chitosan is easy to use as a material for tissue engineering. Chitosan has many biological properties and has been shown to accelerate wound healing [6]. The nature of chitosan is influenced by how this compound is processed [16]. Blood has an important role in the process of bone remodeling. In the process of bone grafting, the space created by the graft is filled with a blood clot. Blood clots have a role as a means for progenitor cells and blood vessels to reach the graft area to facilitate the formation of new blood vessels, as well as increase physical interlocking between the graft and the bone that will be repaired. Clot is absorbed and replaced with granulation tissue rich in new blood vessels filled with nutrients and mesenchymal stem cells, promoting osteoid formation. Blood mixed with chitosan will affect the activity of osteoblasts [8]. In Steigmann's study [14] the pericardial membrane was used in conjunction with xenografts for augmentation of alveolar ridge defects and demonstrated that the pericardial membrane is a suitable component for augmentation of alveolar ridge defects. The nature of the pericardial membrane is to inhibit bone regeneration through slow vascular penetration [17]. This study showed that in the 30th second the average speed of adsorption of type O blood by chitosan with gauze was faster than the average speed of chitosan with pericardial membrane. In the 30th second to the 600th second, there was a difference in speed where the speed of adsorption of type O blood by chitosan with gauze showed a faster speed than chitosan with pericardial membrane. The results of the Mann-Whitney statistical test in table 3 obtained $p < 0.05$ at the 30th second to the 600th second, which means that there are significant differences between the treatment groups. The significant difference between the treatment groups could be caused by various factors, for example, the occurrence of the blood clotting process, the porosity of the membrane so that the absorption that occurs in chitosan using a pericardial membrane is slower than chitosan without a pericardial membrane.

Important factors for the success of Guided Bone Regeneration (GBR) include factors such as occlusivity and stability of the membrane, size of the perforation, peripheral seal between the membrane and host bone, adequate blood supply, and access to bone-forming cells. Occlusivity is closely related to the porosity of the membrane, this factor has a major influence on the potential for cell invasion. The membrane pores facilitate oxygen, nutrients and bioactive substances for cell growth, which are important for bone and soft tissue regeneration [6].

Blood clotting changes the blood from a liquid to a semi-solid mass. In blood clotting, factor XII plays a role, where when factor XII comes into contact with a foreign object (in this study, glass blocks and glass pipes), so the blood clotting process (coagulation) will begin [19]. This study shows the pericardial membrane functions as a good barrier. In guided tissue regeneration (GTR) the membrane as a barrier serves to provide space for the defect so as to provide the opportunity for periodontal tissue to regenerate. In guided bone regeneration (GBR) the membrane is positioned to prevent fibroblastic cells from colonizing the intraosseous wound (with or without bone grafting) during healing, as the osseous cells migrate to fill the defect. So that bone regeneration can occur directly and its deposition can occur [15].

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