European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 05, 2020

THE ADSORPTION RATE OF BLOOD TYPE O TO CHITOSAN SIZE 150 – 355 µm WITH COLLAGENMEMBRANE

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ABSTRACT

Background: The tooth extraction process will always cause tissue damage, both hard tissue and soft tissue such as bleeding, pain, edema, and hard tissue such as loss of alveolar ridge volume after extraction. Substitutes for biological components have developed in increasing alveolar bone volume by using bone grafts. Chitosan can be used as an option. Addition of collagen membrane to post-extraction wounds can increase and protect the formation of initial blood clots to the root surface. Collagen membrane is a type of resorb able membrane that is often used. The function of this collagen membrane is increasing and maintaining blood clots and acting as scaffold for cell adhesion and growth. **Purpose**: To determine whether the use of collagen membranes does not slow down the chitosan adsorption speed of 150-355 µm in group O blood or not. **Methods:**There were chitosan samples measuring 150-355 µm were divided into two groups, namely control group, 7 samples for chitosan with gauze and 7 samples of the treatment group for chitosan with collagen membrane. Dip it in 75 ml of blood. Measurements of group O blood adsorption velocity in each group were seen for 10 minutes. **Result**: The result was analyzed statistically by using Mann-Whitney test with level of significance lower than 0.05 (Sig<0.05). There was significant difference between the control group and the treated group. **Conclusion**: There is a slowing of adsorption rate of blood type O on chitosan with membrane collagen.

***Keywords**: chitosan, adsorption speed, blood type O, collagen membrane.*

INTRODUCTION

Tooth extraction is a process of removing teeth from the alveolar process. Tooth extraction process will always cause tissue damage, both hard tissue and soft tissue such as bleeding, pain, and edema.[1]Preclinical and clinical studies have shown that loss of alveolar ridge volume after extraction is an irreversible process that involves reducing the dimensions of horizontal and vertical bones.[2] Atrophy in alveolar ridge has a considerable impact on tooth replacement therapy, especially when

implant-supported restorations are planned. Therefore, alveolar ridge preservation (ARP) has become a key component of dentistry today[3,4]

Bone graft is used in a variety of clinical settings to improve bone repair and regeneration[5]. Reconstruction of bone deficiency depends on certain mechanisms, which can be summarized into mechanisms of osteoconduction, osteoinduction, and osteogenesis.[6,7].

Chitosan is a natural biomaterial which has many biological activities. The importance of chitosan chemical composition has been explored in the field of tissue engineering[8]. Chitosan is obtained by converting chitin, while chitin can be obtained from fish scales. Chitosan is obtained by deacetylation of chitin with a high concentration alkaline solution.[9]Fish scales contain chitin in addition to some essential metals which are raw materials of chitosan. Chitosan can be obtained from chitin which is a substance forming fish scales.[10]Chitosan used in this study is in the form of powder with a size of 150-355 µm because the size has good wettability.[11]

Blood has an important role in the process of bone remodeling. Blood is a means of transportation containing platelets and various types of growth factors. Blood mixed with chitosan will affect the activity of osteoblasts. In the process of bone grafting, vascularization is very important. Blood clots have a role as a means for progenitor cells and blood vessels to reach the bone area to facilitate the formation of new blood vessels and increase physical interlocking between the graft and bone.[12]Blood type O does not have antigens A and B. The blood used in this study is blood type O because group O blood is the blood group most commonly found in humans around the world[13,14].

Collagen membrane is a type of resorbable membrane that is often used. The function of this collagen membrane is to increase and maintain blood clots and act as scaffold for adhesion and cell growth[15]. Utilization of resorbable collagen membranes is avoiding second surgery and reducing the number of side effects such as infections. Collagen membranes are also often combined with bone graft[16].

There have been no studies examining the speed of blood group O adsorption on chitosan size 150-355 µm with collagen membranes until now, therefore this study was conducted to determine the speed of blood group O adsorption on chitosan size 150-355 µm with collagen membranes. This study is expected that the use of collagen membranes does not slow down the chitosan adsorption speed of 150-355 µm in group O blood.This study aims to determine whether the use of collagen membranes does not slow down the chitosan adsorption speed of 150-355 µm in group O blood or not.

MATERIAL AND METHODS

This type of study was an experimental laboratory. The sample used was chitosan in the form of powder made from fish scales sized 150-355 μm which were inserted into the glass pipe with a volume size of 1 mL each of 0.7 grams. . The collagen membrane used by the MedPark Colla-DM® brand.This study used 7 samples for each training group, a control group consisting of 7 samples for control groups of blood type O with gauze and treatment groups consisting of 7 samples of blood type O with collagen membranes.The data obtained were analyzed by Kolmogorov-smirnov test and Mann-Whitney test.

Then the study sample was made byinserted chitosan being into each glass pipe which had a closed edge of 0.7 grams. All glass pipes are provided by chitosan in the support tool. The study sample that has been arranged was placed on a table with a flat surface.After that, blood type O was inserted into a glass box. The study sample was put in a glass box filled with blood. The blood adsorption speed was observed by reading the data every 30 seconds from the beginning to the 10th minute. This experiment was repeated by the same method and method on chitosan with collagen membrane. It was conducted with the speed of adsorption of blood group O on chitosan in the control group and the approval group.

RESULTS

The average adsorption speed (ml / sec) of chitosan sized of 150 - 355 μm with collagen membrane and gauze against group O blood at 30-second intervals for 10 minutes. It can be seen that the average group O blood adsorption rate is 30 seconds for 10 minutes. At the beginning of the study (30 seconds), the largest group O of blood group adsorption was seen on chitosan with gauze. Retrieval of data using a 30-second time interval, starting at 30 seconds and then continued until the last data is taken in seconds to 600. There is an increase in blood in the tube containing chitosan with gauze while the tube containing chitosan with collagen membrane does not appear to increase blood. The speed of blood adsorption by chitosan without collagen membrane is formed. A relatively stable regular pattern decreases over the 30^{th} second to 600^{th}second.

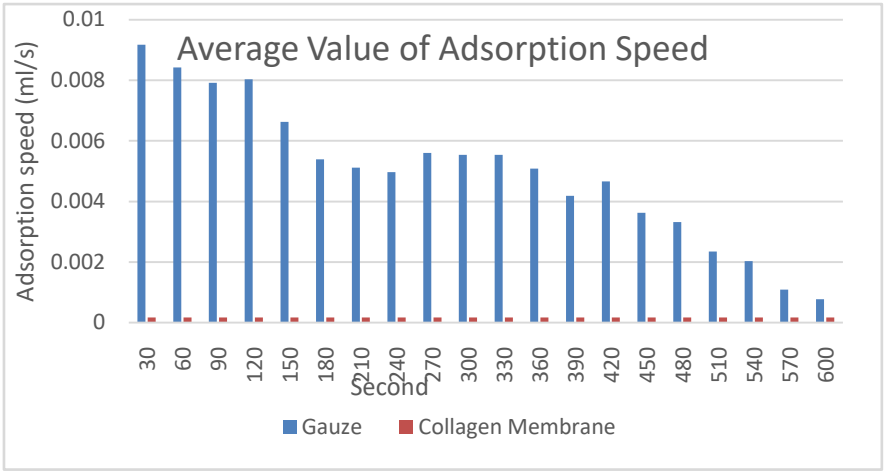

Figure 1. Graph of the average value of the group O blood adsorption speed against chitosan size 150-355 µm with collagen membrane and gauze.

Data normality test was examined by using the Kolmogrov-Smirnov test. The Kolmogrov-Smirnov statistical test results showed that the chitosan group with collagen and gauze membranes in the 30thto second seconds of 600 had a significance value greater than 0.05 (p> 0.05). This shows that H0 is accepted thus the two treatment groups have normal data distribution.

Furthermore, the Mann-Whitney test was conducted to determine whether there was a significant difference between the speed of blood group O adsorption on chitosan and the collagen membrane and gauze or not. Mann-Whitney test results obtained p <0.05 in the 30th second to the 600thminute which means that there were significant differences between the treatment groups. According to the results of the test, it was found that the blood group O adsorption speed of chitosan with collagen membrane and gauze showed significant differences between treatment groups.

DISCUSSION

Chitosan is a biomaterial that can be developed for bone tissue engineering in scaffold formation. Chitosan is produced through kirin derivative compounds with deacetylation process using high concentration NaOH. Linear polysaccharides are composed of ß- (l-4) -D-glucosamine and -N-acetyl-D-glucosamine (acetate)[17].

The size of chitosan used in this study is in accordance with the ideal pore size for bone growth i.e. 150 - 355 µm[18]. This size have a good wettability or attractiveness of an object's surface to liquid, increased surface area for bone remodeling by increasing activity osteoclasts which can

cause an increase in adsorption speed and osteogenic activity, and stimulated osteogenesis by increasing the number of pores and increasing protein in bone morphology[11].

The resorbable collagen membrane has been used in guided tissue regeneration and guided bone regeneration because of its biocompatibility and ability to improve wound healing, because of its bio-resorbability, no further surgery is needed. Collagen membranes have shown good regenerative results due to excellent cell affinity and biocompatibility for tissue regeneration[19].

In the process of bone grafting, vascularization is very important. 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 and increase physical interlocking between the graft and the part of the bone to be repaired. In the first 24 hours, the graft is filled with blood clot which will release growth factors and cytokines to attract neutrophils and macrophages. Furthermore, the clot will be absorbed and replaced with granulation tissue that is rich in young blood vessels containing nutrients and mesenchymal stem cells which can help the formation of osteoid. Furthermore, mineralization from osteoid will form bones.[12]The process of bone graft formation is influenced by the speed of blood adsorption by the bone graft, the speed of blood adsorption is directly proportional to the amount of substance absorbed. Because of that, blood adsorbed must be fast.

In the study by Iasella et al., 2003, it was shown that membrane use along with ridge augmentation procedures resulted in reduced thickness of soft tissue, thereby reducing the width of hard and soft tissue. Reduction in tissue thickness is most likely due to vascularity interference by the membrane and graft, because the membrane is between the flap and the surface of the bone, the vascular supply of the flap originates only from the flap base compared to the use of membranes, and vascular supply graft originating from the base of the underlying flap and bone[20]. This is in accordance with the results of study where there is a significant difference between the average velocities of chitosan adsorption in blood group O with the largest collagen membrane only 0.000003 ml / sec, whereas for chitosan with gauze, the average blood adsorption speed of group O is the highest which is 0.009172 ml / sec.

In a study by Taguchi et al., 2005 on collagen membranes, the newly formed alveolar ridge bone formation reached the same height as pre-existing bone; alkaline phosphatase-containing cells and osteocalcin and immune positive osteopontin bone matrix appear in the second week after implantation, showing osteoblastic differentiation in the porous layer of the membrane; collagen fibers produced by a membrane incorporated into the new bone matrix adjacent to the membrane;

and bone related membranes that are integrated with bone that extends from the oral cavity. In addition, they have found that the compact layer of the collagen membrane prevents the entry of unwanted connective tissue and can maintain sufficient space for osteogenic cells to produce bone in the oral cavity[21].

Collagen scaffold material has been designed to mimic one or more bone components, to facilitate blood vessel growth into the material, and to provide an ideal environment for bone formation. Scaffolds must have open pores, geometries that are fully interconnected in highly porous structures (enabling accurate cell growth and cell distribution throughout porous structures) and capable of supporting construct neovascularization of the surrounding tissue (extrinsic vowels in vivo). Pore size is a very important problem: if the scaffold pores are too small, cell occlusion pores can occur, prevent cellular penetration, extracellular matrix production, and neovascularization of the inner scaffold area. It is well received that for the purpose of bone tissue engineering, pore size must be in the range 200–900 μm[19].

When collagen membranes are used for guided bone regeneration, the porous and compact layers allow osteogenic cell migration and inhibit infiltration of connective tissue. Collagen fibers are the most abundant component in the bone matrix, can act as a reservoir of many local factors, and in the attachment of osteogenic cell matrix. Despite the absence of bone-specific proteins, collagen fibers from collagen membranes can function as scaffolds for osteogenic cells in bone defects and as a barrier against infiltration of surrounding connective tissue[19].

From the results of the study that has been conducted, it was found that the average velocity of blood group O adsorption with the largest collagen membrane occurred in seconds to 210 and 360, which is 0.000003 ml / second. While for chitosan with gauze, the average speed of blood group adsorption O was the largest in the 30th second, which was 0.009172 ml / sec, with a standard intersection value of 0.000001 with collagen membrane and 0.009172 for gauze. Both with collagen membrane and gauze were obtained at the next second the speed of adsorption of group O blood was relatively decreasing.

Based on the study, in the 30th second to the 600thsecond the average velocity of blood adsorption with the collagen membrane and without the collagen membrane showed a decrease in speed over time. This can be caused by several factors such as the blood clotting process, temperature, surface tension, and porosity. Possible differences in porosity and permeability of

collagen and gauze membranes affect the speed of adsorption, hence the adsorption speed of chitosan with collagen membrane is slower than chitosan without collagen membrane.

The existence of a blood clotting process, there is a change from liquid to semi-solid mass. The blood clotting factor that plays a role is factor XII, where when factor XII comes into contact with foreign matter (in this study, the glass beam and glass pipe) the blood coagulation process (coagulation) will begin[22].

Based on the results of the Mann-Whitney statistical test in Table 5.3, $p < 0.05$ in the 30th second to the 600thminute means that there are significant differences between treatment groups. Based on the results of the test, it was found that the blood group O adsorption speed of chitosan with collagen membrane and gauze showed significant differences between treatment groups. This can be caused by various factors, such as the occurrence of blood clotting processes, porosity of collagen membranes, thus the absorption that occurs in chitosan with collagen membrane is slower than chitosan without collagen membrane. From the results of the study that has been conducted, it can be concluded that there is a decrease in the speed of blood group O adsorption on chitosan size 150 - 355 μm with the addition of collagen membrane.

CONCLUSION

There is a slowing of absorption rate of blood type O on chitosan with membrane collagen.

CONFLICT OF INTEREST: There is no concflict of interest.

SOURCE OF FUNDING: This study is self-funded.

ETHICAL CLEARANCE: This study was approved by Ethical Commission of Health Research Faculty of Dental Medicine Universitas Airlangga.

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