Sys Rev Pharm 2021;12(1):1479-1486
A multifaceted review journal in the field of pharmacy

Potential Effect of Jellyfish *Aurelia aurita* Collagen Scaffold Induced Alveolar Bone Regeneration in Periodontal Disease

Ranny Rachmawati[1,2*], Mohammad Hidayat[3], Nur Permatasari[4], Sri Widyarti[5]

[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[2]Departement of Periodontics, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia
[3]Departement of Orthopaedics, Syaiful Anwar General Hospital, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[4]Departement of Oral Biology, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia
[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia

ABSTRACT

Periodontal disease is an inflammation disease that can cause alveolar bone resorption and make tooth mobility, eventually tooth loss. One of the important things in regeneration therapy of alveolar bone is the design and manufacture of a scaffold as osteoconductor. Collagen is an ideal choice to be used as scaffold because it has low immunity, good pores structure and permeability, biocompatible, and can be degraded. *Aurelia aurita* jellyfish is one of the potential marine animals for the development of collagen scaffold due to its high content of collagen. The aim of this research is to determine the potential of *A. aurita* jellyfish collagen scaffold as the new biomaterial in assisting the repair process of alveolar bone defect. Collagen was extracted from jellyfish by sonication, while scaffold was prepared by freeze-drying, and cross-linking with N-(3-dimethyl aminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC). This research was conducted on 10 male rats which is divided into two experimental groups. K+ is group that has alveolar bone defects and not given any treatment. KP is group that has alveolar bone defects and *A. aurita* jellyfish collagen scaffold is implanted. Then the calculation of osteoblast, osteoclast, STRO-1, and osteocalcin expression were conducted with immunohistochemistry. This resulted on significantly more osteoblast and less osteoclast cells on KP than on K+ group. The mean of STRO-1 and osteocalcin expressions was also significantly more on KP than on K+ group. Therefore, it can be concluded that the *A. aurita* jellyfish collagen scaffold is effective in helping regenerate alveolar bone.

Keywords: Collagen Scaffold, Jellyfish *Aurelia aurita*, Alveolar Bone Regeneration, Periodontal Disease

Correspondence:
Ranny Rachmawati
Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
Department of Periodontics, Faculty of Dentistry, Universitas, Brawijaya, Malang, Indonesia
Email: rannyperio.fk@ub.ac.id

INTRODUCTION

Periodontal disease is most commonly occurred disease in the community. Research of The World Oral Health Report on 2003 showed that periodontal disease ranked number 4 as the most expensive disease in terms of treatment cost. Survey conducted in USA showed that 75% of population once suffered periodontal disease. As survey from ministry of health, the prevalence of periodontal disease in Indonesia has reached 60%. Periodontal disease is an inflammation disease on tooth supporting tissue which covers gingiva, cementum, periodontal ligament, and alveolar bone (Laine & Crielaard, 2012). The etiology of periodontal disease might be caused by bacterial plaque or mechanical trauma. Both etiologies can cause alveolar bone resorption which can makes tooth mobility and tooth loss (Carranza, Camargo, & Takei, 2012; Michael G., Takei, R. Klokkevold, & Carranza, 2018). The cause of trauma on periodontal issue can be categorized into two main causes, unintentional trauma and intentional trauma. Unintentional trauma can happen due to fall incident, sport accident, traffic accident, improper usage of teeth such as pen biting and bottle opening. Intentional trauma can happen due to physical abuse on children and iatrogenic procedure done by the dentist (Auerkari *et al*., 2014; Farani & Nurunnisa, 2018).

To prevent tooth loss, regeneration therapy is needed to re-grow the damaged alveolar bone. Alveolar bone defect treatment aims to improve bone healing process and recover the function by tissue engineering effort which comprises three main components; natural bone growth, presence of cell and growth factor, and ideal scaffold (Peter *et al*., 2010). One of the important things in the development of artificial bone tissue is the design and the manufacture of a scaffold as osteoconductor which can speed up the alveolar bone regeneration process. Scaffold is a framework that acts as a microenvironment for stem cells that will carry out adhesion, proliferation, and differentiation which eventually produces the tissue we expect. Scaffold must also meet standard criteria such as being biocompatible, biodegradable, supporting cell adhesion, and having an extracellular matrix structure of alveolar bone so that the role of scaffold as an osteoconductor can be obtained (Horst *et al*., 2010). Scaffold material can be obtained from synthetic and natural sources. Collagen is an ideal choice of natural ingredients to be used as scaffold because it has low immunity factor, good pores structure, good permeability, is biocompatible with surrounding tissues, and can be degraded well (Addad *et al*., 2011; Dong & Lv, 2016).

So far, the source of collagen used for scaffold production comes from various organisms. Collagen from cows and pigs is a material that has long been used in the community as a scaffold, but the higher demand of collagen causes a shortage of collagen raw material originating from land animals, resulting in an increase in the price of raw materials in the market. This condition can be a major threat to the global needs. Religious constraints also limit Muslims, Hindus and Jews to using products made from pork and beef (Azizur Rahman, 2019; Gomez-Guillen *et al*., 2011; Silvipriya *et al*., 2015). Collagen sources that come from cows can cause a risk of infection such as bovine spongiform encephalopathy (Hoyer *et al*., 2014). As potential solution to reduce the demand for raw materials from land animals, it is possible to use other materials from the sea which are abundant, low price, and still underutilized (Hoyer *et al*., 2014; Khong *et al*., 2016). In this study, it underlies the selection of jellyfish as the basic material for scaffold development.

Based on statistical data in 2008, the number of jellyfish production in Indonesia reached 500,000 tons per year (Perikanan, 2008) and an increase in 2011 reaching 674,000 tons (Perikanan, 2011). The jellyfish production increase in Indonesia in 1998-2002 period by an average of 2.46% per year. Of all the jellyfish, *A.aurita* jellyfish is the most common in Indonesia and is a type of jellyfish that can be consumed. The content of amino acid in jellyfish is also very high, in fact, almost all of the human body's amino acid needs can be fulfilled by jellyfish, except for Methionine and Histidine which are a little less than the FAO / WHO provisions. Even the content of amino acid in jellyfish is higher than abalone, tuna, fresh goat's milk, fresh cow's milk and fresh crab (Kuvaini, 2012). In several previous research, it was known that the collagen contained in jellyfish has been used in various biomedical applications. Hoyer *et al* (2014) uses the whole body of *Rhopilema esculentum* jellyfish to extract collagen so that it can produce scaffold with the aim of regenerating cartilage (Hoyer *et al*., 2014). In the research of Nagai *et al*., (Nagai *et al*., 2000) collagen was extracted from jellyfish and then conducted cross-link with 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide hydrochloride (EDC) to be used as medical application . Besides having elastic mechanical properties, scaffold based on jellyfish collagen is characterized by the interconnection between the pores that allows mesenchymal stem cells to live and multiply, thus providing a good basis for osteogenic differentiation or osteogenesis (Hoyer *et al*., 2014).

In previous research, the scaffold was made using *R.esculentum* jellyfish. This type of jellyfish has a different composition from the *A.aurita* jellyfish. In *R.esculentum* jellyfish, 16 types of amino acids were found with the highest order of composition Glutamic acid (Glu), Lysine (Lys), Glycine (Gly), Aspartic Acid (Asp), Leucine (Leu), meanwhile in Indonesia, the highest number of jellyfish is *A.aurita (A. aurita)* which is found in almost all the waters of Kalimantan, Sumatra and Java. In *A.aurita* jellyfish, 15 types of amino acids were found with the highest order of composition Glycine (Gly), Glutamic acid (Glu), Phenylamine (Phe), Threonine (Thr), Tyrosine (Tyr), Lysine (Lys), Serin (Ser) and others. Of the entire *A.aurita* tissue, there are 57 mg protein/gram of dry weight. The largest composition in the collagen content is Glycine, and other amino acids, including proline and hydroxyproline (Leone *et al*., 2015).

Therefore, the aim of this research is to determine the potential of *A. aurita* jellyfish collagen scaffold as the new biomaterial in assisting the repair process of alveolar bone defects; by comparing osteoblasts and osteoclasts, and expression of STRO-1 and osteocalcin as biomarkers in alveolar bone regeneration.

MATERIAL AND METHODS

Scaffold Material

Dried jellyfish *A. aurita* was cut into small pieces then washed using sterile water three times. After that, the jellyfish pieces were washed using 0.1 M NaOH as much as 3 times, then added sterile aquabidest as solvent then blended until crushed. Sonication was done for 10 minutes and centrifuged at a speed of 10,000 rpm for 15 minutes at 25^{0}C. Pellet was taken and put in a petri dish. Freeze-drying was completed for 24 hours. Membrane collagen scaffolds were obtained after freeze-drying, then crosslinked in 1 wt.% solution of N-(3-dimethyl aminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) in 80% ethanol for 2 hours**.** Scaffold was carefully rinse in sterile water, in 1 wt.% glycine solution, and sterile water for last, and then freeze-drying.

Experimental Design in Animal Model

This experimental study was approved by the Ethic Commission of Universitas Brawijaya (Approval No.1052-KEP-UB). This research was conducted on 10 male Wistar rats aged ± 4 months weighing 250-300 grams from Animal House Universitas Brawijaya. The animals were randomly divided into two experimental groups. The first group (n = 5 animals in total) had alveolar bone defects and were not given any treatment (K+). In the second group (n = 5 animals in total), there were alveolar bone defects and *A.aurita* jellyfish collagen scaffold was implanted (KP). In the beginning, the ten rats were weighed and anesthetized with Ketamine HCl, then incised horizontally on the gingiva of the left mandible buccal area posterior with scalpel no.15 and ± 5 mm long. After that, the flap was opened using a small rasparatorium until the alveolar bone surface was visible and then irrigated with a sterile distilled water solution. After that, the alveolar bone defect is made with a deep basin 2 mm using a low speed round bur.

Collagen Scaffold Implantation

A. aurita jellyfish collagen scaffolds were weighed the same and cut according to the size of the defect and then implanted with surgical tweezers on the defect area. After fixation, the periodontal flap was returned back to its original position and sutured with nylon thread size 6.0 and then irrigated with povidone iodine to minimize infection. After 8 weeks, all rats were euthanized, and the left mandibular jaw was cut.

Histological Processing

Samples were fixated using 10% formalin for 24 hours and then decalcified using 14% EDTA, pH 7.4 for 30 days. Furthermore, dehydration was carried out using graded alcohol (30%, 50%, 70%, 80%, 96%, and absolute) for 60 minutes each. Clearing was then performed using xylene solution for 2 x 90 minutes, then embedding was performed using paraffin which was melted at 60^{o}C, then the paraffin was allowed to harden and let stand for 1 day. After 24 hours, the sample was cut with a longitudinal microtome, a thickness of 5 µm (for HE examination) and 3 µm (for immunohistochemistry) and then mounted on a slide with 5% gelatin.

Osteoblast and Osteoclast Staining with Hematoxylin Eosin Staining

The slides were deparaffinated by heating at 60^{o}C for 60 minutes then washed with Xylol solution for 2 x 10 minutes. After that, rehydration was performed using graded alcohol (absolute, 96%, 80%, 70%, 50%, 30%) for 5 minutes each. The slides were dripped with Hematoxylin for 10 minutes, then dehydrated with graded alcohol (30%, 50%) for 5 minutes each. After that, the slides were dripped with Eosin, dehydrated with graded alcohol, rinsed with sterile water, and dried. The slides were rinsed under flowing water and dried. The slides were dripped with emelian and covered with a coverslip. Slide observations were carried out using a light microscope (Nikon E100) with 1000x magnification for cell counting. Osteoblast and osteoclasts cells were counted in the alveolar bone on the defect area, performed 3 times and the mean was measured.

Immunohistochemistry Staining for STRO-1 and Osteocalcin Detection

The slides were deparaffinated by heating at 60^{o}C for 60 minutes then washed with Xylol solution for 2 x 10 minutes. After that, rehydration was conducted using

graded alcohol (absolute, 96%, 80%, 70%, 50%, 30%) for 5 minutes each. The slides then were fixated with absolute methanol for 5 minutes then washed with PBS pH 7.4 and then performed endogenous blocking using 4% H2O2 for 30 minutes. The slides were washed with PBS for 3 x 5 minutes. The slides were blocked using unspecific protein with 5% FBS containing 0.25% Triton X-100 then washed with PBS for 3 x 5 minutes. The slide was dripped with anti-STRO-1 mouse monoclonal and osteocalcin (Santa Cruz, USA) as the primary antibody (on different slides) and then dissolved in PBS buffer + 0.1% BSA, incubated for 2 hours at room temperature, washed with PBS 3 x 2 minutes, then put it in SA-HRP and incubated for 30 minutes at room temperature, and washed with PBS 3 x 2 minutes. The slides were rinsed and then dripped with DAB (DAB Chromogen : DAB Buffer = 1 : 40), incubated for 5 minutes at room temperature, then washed with distilled water for 3 x 2 minutes and dried. The slides were observed under a light microscope (Nikon E100) with 1000x magnification on the alveolar bone defect area. The expression of the two proteins was then counted with 3 times repetition and the mean was measured.

Statistical Analysis

The data normality was determined, and the results are expressed as mean ± SD for 5 (five) rats per each group. Statistical analysis was carried out using one way ANOVA and Post-hoc Tukey's to test the significant difference between groups using SPSS v.22 with statistical significance set at $p \leq 0.05$.

RESULT

Comparison of the Number of Osteoblasts and Osteoclasts with Harris-Hematoxylin Eosin Staining

The process of observing osteoblast cells was carried out using a Nikon E-100 microscope with a magnification of 400x and 1000x. Cell counts were performed in the 1000x field of view. Osteoblast cells appear to be plump-like, cuboidal, reddish purple in colour and located on the surface of the bone **(Figure 1, 2)**.

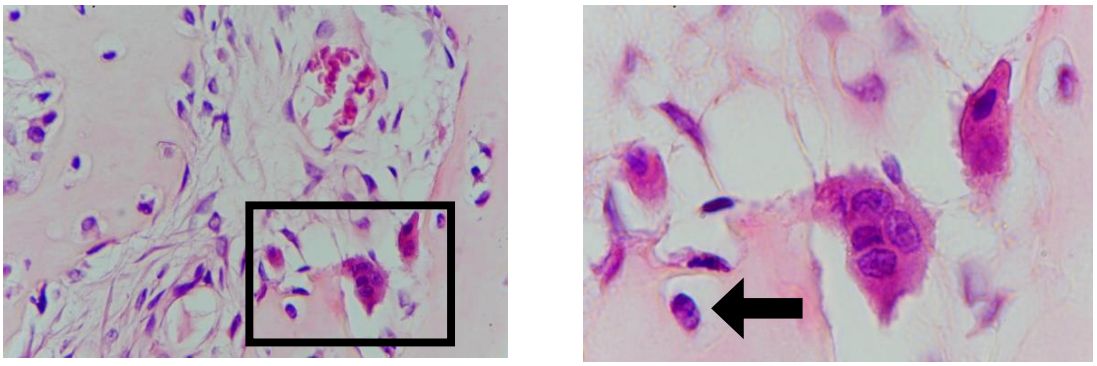

Figure 1: Osteoblast cells pointed with black arrow and HE is staining on K+ Group.
(A) 400x Magnification **(B)** 1000x Magnification

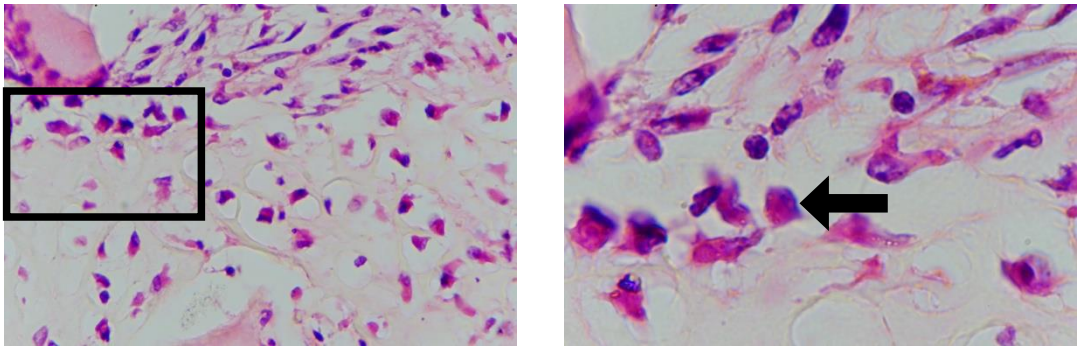

Figure 2: Osteoblast cells pointed with black arrow and HE is staining on KP Group.
(A) 400x Magnification **(B)** 1000x Magnification

Osteoclast cells were observed using a Nikon E-100 microscope with a magnification of 400x and 1000x. Cell counts were performed in the 1000x field of view. Osteoclast cells are shaped like giant cells with multinucleon which are on the side of the bone that undergoes resorption (lacuna howship), and the cytoplasm looks like a reddish-purple foam and is located on the surface of the bone **(Figure 3, 4)**.

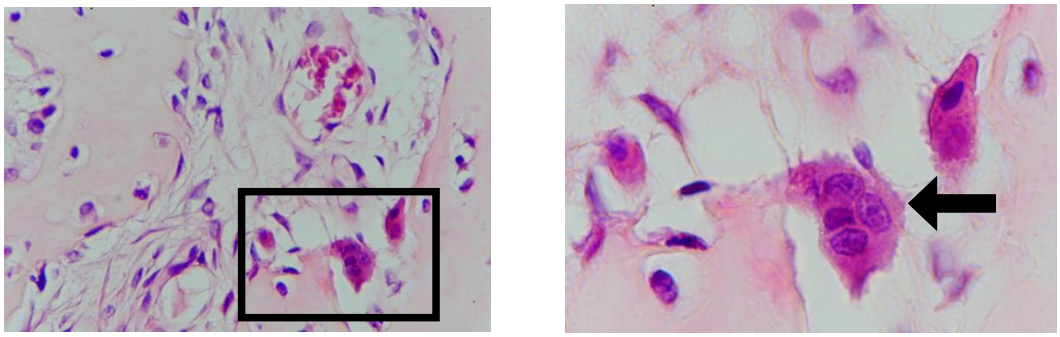

Figure 3: Osteoclast cells pointed with black arrow and HE is staining on K+ Group
(A) 400x Magnification **(B)** 1000x Magnification

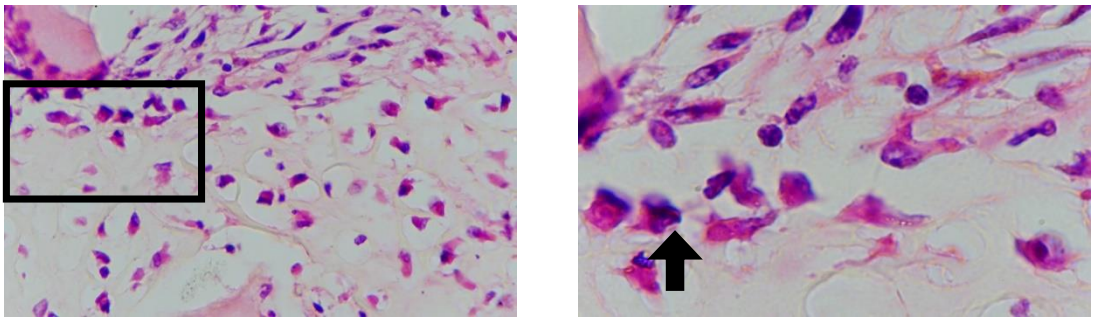

Figure 4: Osteoclast cells pointed with black arrow and HE is staining on KP Group.
(A) 400x Magnification **(B)** 1000x Magnification

Based on the results of statistical analysis using one-way ANOVA, it was found that the average number of osteoblasts showed a value of 6.0 (SD = 2.83) in the K+ group, and in the KP group showed a value of 14.00 (SD = 1.83). While the average number of osteoclasts based on ANOVA analysis, shows a value of 13.25 (SD = 2.50) in the K+ group, and the KP group shows a value of 6.75 (SD = 1.89) **(Figure 5).** When the comparison between the two groups was carried out using post hoc Tukey, it was found that the number of osteoblast and osteoclast cell comparisons in the K+ group was significantly different in the KP group ($p < 0.05$) **(Figure 5)**. These results indicate that the treatment group can play a role in improving the quality of alveolar bone with a significant difference in the number of osteoblasts and the number of osteoclasts compared to the K+ group (control). The mean number of osteoblasts from the treatment group was higher than that in the control group (K+). The mean number of osteoclasts was less than in the control group (K+). This condition, the number of osteoblasts that is greater than the number of osteoclasts, can help the regeneration process of alveolar bone.

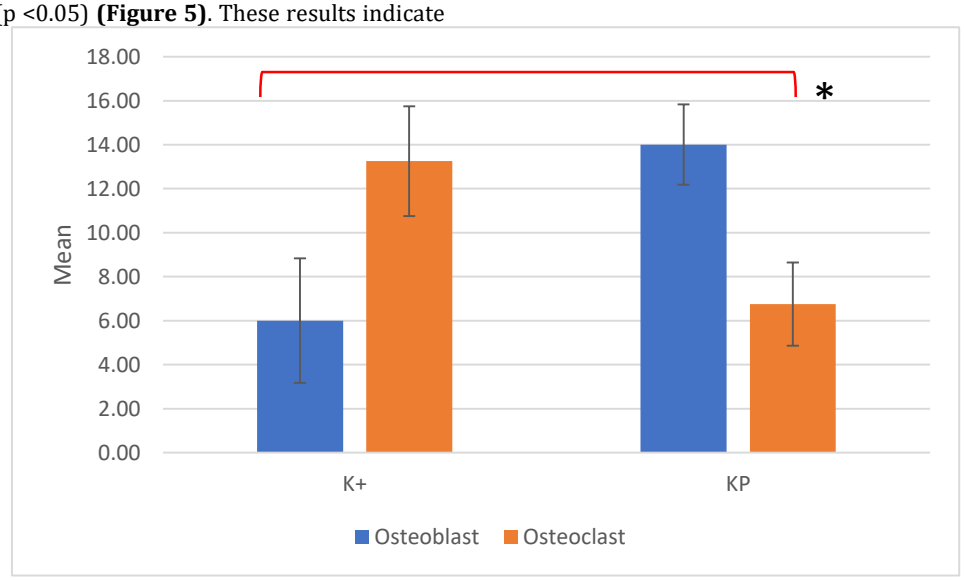

Figure 5: Histogram of the mean number of osteoblast and osteoclast cells in the two experimental groups. Results are expressed as mean ± SD. *Significant with $p < 0.05$.

STRO-1 Expression with Immunohistochemistry Method

STRO-1 observations were using a Nikon E-100 microscope with a magnification of 400x and 1000x in the alveolar bone defect area. The goal is to calculate the presence of mesenchymal stem cells in the scaffold. The protein expression was calculated at 1000x magnification in the selected field of view. STRO-1 expression is described with a brownish glow on the outside and around the cell **(Figure 6, 7).**

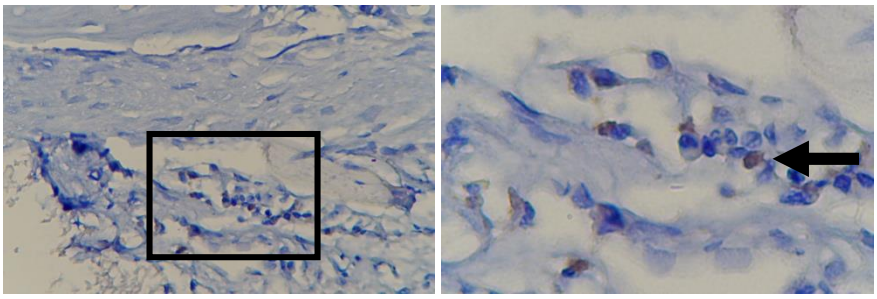

Figure 6: The STRO-1 expression pointed with the black arrow in the K+ group. **(A)** 400x magnification **(B)** 1000x magnification

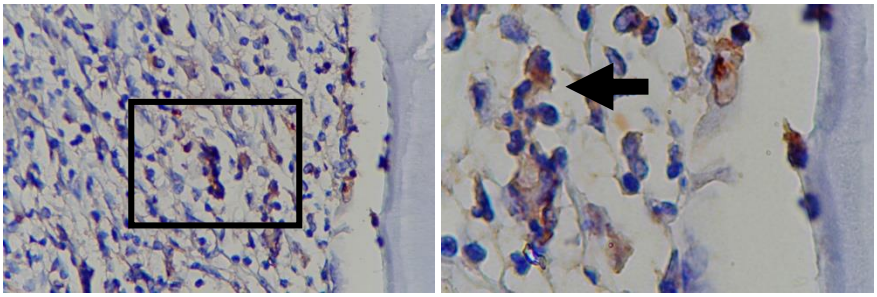

Figure 7: The STRO-1 expression pointed with the black arrow in the KP group. **(A)** 400x magnification **(B)** 1000x magnification

In the calculation of ANOVA statistical analysis and post hoc Tukey, the average result of STRO-1 expression in the K+ group was 2.00 (SD = 0.82) and the KP group was 7.75 (SD = 1.50). These results indicate that there is a significant difference in the K+ group to the KP group ($p < 0.05$) **(Figure 8)**. These results indicate that the KP group has the potential to regenerate alveolar bone with a significant difference in the number of STRO-1. The mean number of STRO-1 expression which was higher than that in the control group (K+) indicated that mesenchymal stem cells could proliferate and differentiate better in those two groups.

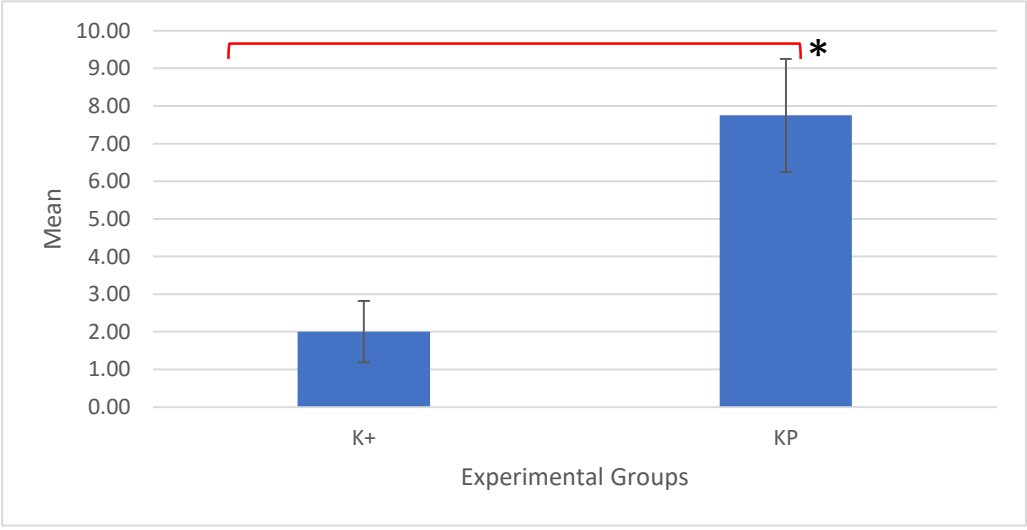

Figure 8: Histogram of the mean number of STRO-1 expression in the two experimental groups. Results are expressed as mean ± SD. *Significant with $p < 0.05$.

Osteocalcin Expression with Immunohistochemistry Method
The examination of osteocalcin expression with immunohistochemical procedures was using osteocalcin primary antibodies. The goal is to count the number of osteoblasts that express osteocalcin, hereinafter referred to as positive osteocalcin. Osteocalcin expression is the percentage of the number of osteoblast cells in the alveolar bone that expresses osteocalcin as a secretory protein that is outside and around the cell. The slides were observed under a Nikon E-100 microscope with 400x and 1000x magnifications on the alveolar bone defect area. The protein expression was calculated using a 1000x magnification microscope in the selected field of view. Positive osteocalcin expression is characterized by dark brown staining outside and around the cell, cytoplasm, dark purple, brown staining core **(Figure 9, 10).**

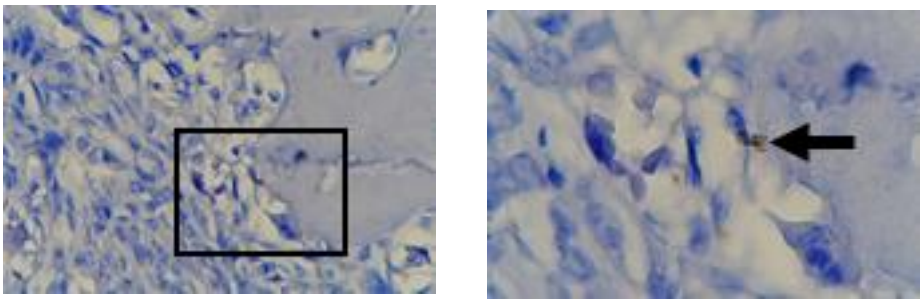

Figure 9: The Osteocalcin expression pointed with the black arrow in the K+ group. **(A)** 400x magnification **(B)** 1000x magnification

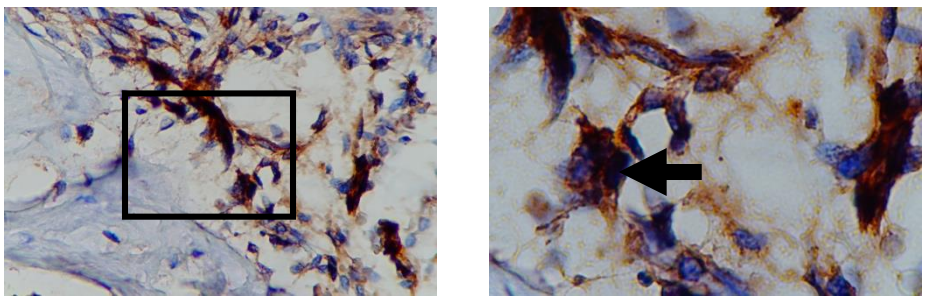

Figure 10: The Osteocalcin expression pointed with the black arrow in the KP group. **(A)** 400x magnification **(B)** 1000x magnification

Based on statistical analysis using ANOVA, the average results of positive osteocalcin expression found in each group of experimental animals. The group that showed a significant difference was in the K+ group with a mean of 4.50 (SD = 1.29) to the KP group with a mean of 10.75 (SD = 1.71) ($p < 0.05$) **(Figure 11)**. These results indicate that the KP group has the potential to regenerate alveolar bone with a significant difference in the amount of osteocalcin compared to the K+ group (control). The average number of osteocalcin expressions, that is higher than in the control group (K+), indicates that the quality of osteoblasts is better and can help in the formation of alveolar bone.

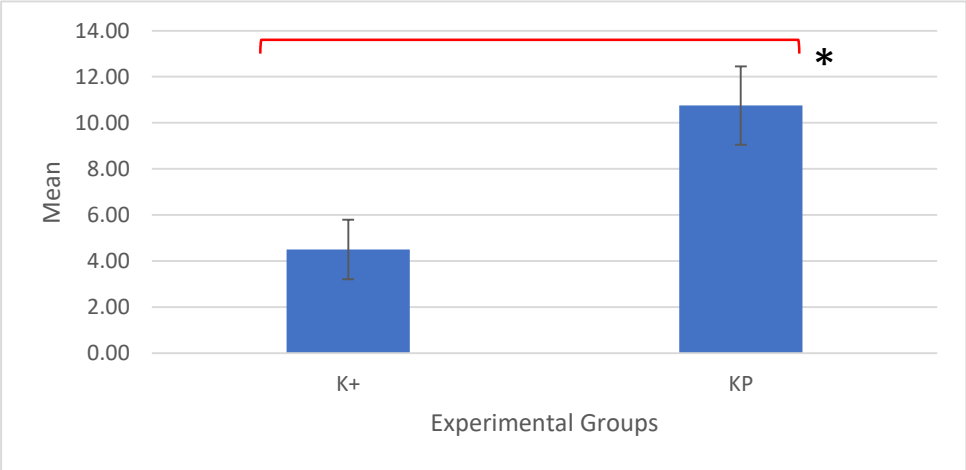

Figure 11: Histogram of the mean number of Osteocalcin expression in the two experimental groups. Results are expressed as mean ± SD. *Significant with $p < 0.05$.

DISCUSSION

The regeneration of periodontal tissues is a continuous physiological process. Regeneration also takes place during the development of destructive periodontal disease. Most gingival and periodontal diseases are chronic inflammatory diseases, meaning that they are a healing process. Since regeneration is a part of healing, when gingival and periodontal disease develops in the form of inflammation, regeneration takes place as well. However, because bacteria and bacterial products play a role in the disease process, and the resulting inflammatory exudate injures regenerating cells and tissues, the healing process during the disease does not end completely (Zeichner-David, 2006). The goal of periodontal treatment is to eliminate pathological conditions, create stable conditions in order to increase regeneration of periodontal tissues (alveolar bone, cementum and periodontal ligaments) (Izumi *et al*., 2011). Biomaterial is an important component in tissue engineering to assist the regeneration of periodontal tissues. Scaffold is one of the components in tissue engineering which has three main functions, which are having a form for construction in order to facilitate tissue regeneration, as a carrier of both molecular and mechanical signals to cells, and to optimize cell function in the tissue. This biomaterial can come not only from nature such as plants, animals and humans, but also be synthetic materials (Goldstein *et al*., 1999). In this research, a source of collagen from *A. aurita* jellyfish was used to be used as scaffold. The type of *A. aurita* jellyfish was chosen because until now there has never been any research using this type to be used as a scaffold, so it is necessary to know whether the *A. aurita* type has the potential to become a scaffold or not. In addition, *A. aurita* jellyfish are the source of jellyfish with the most production in Indonesia and have not been maximized for their utilization. Although collagen as the base material for scaffold has excellent biological properties, it also has disadvantages, which are poor mechanical properties and structural stability. To overcome these, it is recommended to use chemicals as crosslinkers to obtain intermolecular that can modify the properties of collagen scaffold (Dong & Lv, 2016; Velasco *et al*., 2015). This research used N-(3-dimethylaminopropyl)-N0-ethyl-carbodiimide hydrochloride (EDC) as a crosslinker to increase structural stability, strengthen mechanical properties, and prolong solubility time (Park *et al*., 2002). In the process of forming alveolar bone, it is inseparable from the role of osteoblast cells as bone-forming cells and osteoclast cells as bone resorption cells and together with nerve cells and blood vessel cells to form a unit known as Bone Multicellular Units (BMU). The function of this BMU in adult bone growth is to help the bone remodeling process (Borciani *et al*., 2020). The aim of this research was to see the ratio of the average number of osteoblasts and osteoclasts using HE is staining from 2 treatment groups, which are K+ (group with alveolar bone defect without treatment) and KP (group with alveolar bone defects treated with *A. aurita* jellyfish collagen scaffold treatment). The results in both groups showed that the average number of osteoblasts was higher than the number of osteoclasts **(Figure 5)**. The condition of more osteoblast cells than osteoclasts will help to accelerate the bone formation process (Borciani *et al*., 2020). The height and density of alveolar bone in normal circumstances have a balance between the size of the bone formation and resorption and is influenced by systemic and local factors. When the amount of resorption is greater than the amount of bone formation, the alveolar bone height and density may decrease (Michael G. *et al*., 2018). From the results of statistical analysis showed a significant difference between the treatment group (KP) and the control group (K+) on the mean number of osteoblasts and osteoclasts. This shows that *A. aurita* jellyfish collagen scaffold can potentially help the regeneration process of alveolar bone. The integrity of bone microstructure is largely determined by the balance of the bone remodeling process which includes the formation and resorption processes of the bone. These two processes are influenced by the balance of osteoblast and osteoclast metabolism (Praba, 2015). This research also observed the STRO-1 expression through immunohistochemical methods, which is the marker for the presence of mesenchymal stem cells and is able to isolate mesenchymal stem cells which have high stemness characteristics and have the potential to differentiate into osteoblasts. The average number of STRO-1 expressions in this group of mice indicated that the KP group was more numerous than the K+ group and the difference was significant ($p < 0.05$) **(Figure 8)**. This result supports previous studies which stated that collagen as a natural material has the advantages of better biocompatible and bioactive properties than synthetic materials (hydroxyapatite) (Dong & Lv, 2016; Hoyer *et al*., 2014; Khong *et al*., 2016; Velasco *et al*., 2015), thus making mesenchymal stem cells easier to infiltrate and proliferate because they obtain a supportive microenvironment for cell growth. Osteocalcin expression used in this research aims to determine the quality of osteoblasts because osteocalcin is a specific protein that is only produced by osteoblasts. Osteocalcin examination is often used as an initial biomarker in studies aimed at bone formation and can be used to assess the effectiveness of research results. The results of the osteocalcin examination are quite accurate and stable in assessing the bone formation process (Zoch *et al*., 2016). The results of the mean amount of osteocalcin in this research indicated that the KP group expressed more osteocalcin than the K+ group and showed a significant difference ($p < 0.05$) **(Figure 11)**. This data describes that when osteoblasts can express osteocalcin, it means they have good cell quality and can play a role in the bone mineralization process (Lian & Gundberg, 1988). Osteocalcin can only be detected in the consolidation phase where minerals have begun to form in the bone callus. Osteocalcin is also a non-collagen protein that is most abundant in bones and plays an important role in the mineralization process and the process of calcium ion homeostasis (Wolf, 1996; Zoch *et al*., 2016).

CONCLUSION

Based on this research, it can be concluded that the *A. aurita* jellyfish collagen scaffold is effective in helping regenerate alveolar bone. This is based on the presence of a higher number of osteoblasts than osteoclasts, the expression of STRO-1 and osteocalcin which is significantly different compared to the control group with a higher mean number.

ACKNOWLEDGMENT

The authors wish to thank the Faculty of Dentistry, Universitas Brawijaya for financial support. Special thanks to Rizka Pantris from Biomolecular Laboratory for their enlightening, encouraging discussions, and support. We also thank Yuanita Lely from Faculty of Dentistry, Universitas Brawijaya for the discussion and support the statistical analysis.

REFERENCES

1. Addad, S., Exposito, J. Y., Faye, C., Ricard-Blum, S., & Lethias, C. (2011). Isolation, characterization and biological evaluation of jellyfish collagen for use in biomedical applications. *Marine Drugs*.
2. Auerkari, E. I., Suryandari, D. A., Umami, S. S., Kusdhany, L. S., Siregar, T. W. A., Rahardjo, T. B. W., Hogervorst, E. (2014). Gene promoter polymorphism of RUNX2 and risk of osteoporosis in postmenopausal Indonesian women . *SAGE Open Medicine*. https://doi.org/10.1177/2050312114531571
3. Azizur Rahman, M. (2019). Collagen of extracellular matrix from marine invertebrates and its medical applications. *Marine Drugs*. https://doi.org/10.3390/md17020118
4. Borciani, G., Montalbano, G., Baldini, N., Cerqueni, G., Vitale-Brovarone, C., & Ciapetti, G. (2020). Co–culture systems of osteoblasts and osteoclasts: Simulating in vitro bone remodeling in regenerative approaches. *Acta Biomaterialia*. https://doi.org/10.1016/j.actbio.2020.03.043
5. Carranza, F. A., Camargo, P. M., & Takei, H. H. (2012). Bone Loss and Patterns of Bone Destruction. In *Carranza's Clinical Periodontology*. https://doi.org/10.1016/b978-1-4377-0416-7.00014-7
6. Dong, C., & Lv, Y. (2016). Application of collagen scaffold in tissue engineering: Recent advances and new perspectives. *Polymers*. https://doi.org/10.3390/polym8020042
7. Farani, W., & Nurunnisa, W. (2018). Distribution of Frequency of Permanent Tooth Fractures in Dental Hospital and Mouth. Universitas Muhammadiyah Yogyakarta. *Insisiva Dental Journal: Majalah Kedokteran Gigi Insisiva*. https://doi.org/10.18196/di.7193
8. Goldstein, A. S., Zhu, G., Morris, G. E., Meszlenyi, R. K., & Mikos, A. G. (1999). Effect of osteoblastic culture conditions on the structure of poly(DL-lactic-co-glycolic acid) foam scaffolds. *Tissue Engineering*. https://doi.org/10.1089/ten.1999.5.421
9. Gomez-Guillen, M. C., Gimenez, B., Lopez-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*. https://doi.org/10.1016/j.foodhyd.2011.02.007
10. Horst, M., Madduri, S., Gobet, R., Sulser, T., Hall, H., & Eberli, D. (2010). Scaffold Characteristics for functional hollow organ regeneration. *Materials*. https://doi.org/10.3390/ma3010241
11. Hoyer, B., Bernhardt, A., Lode, A., Heinemann, S., Sewing, J., Klinger, M., Gelinsky, M. (2014). Jellyfish collagen scaffolds for cartilage tissue engineering. *Acta Biomaterialia*. https://doi.org/10.1016/j.actbio.2013.10.022
12. Izumi, Y., Aoki, A., Yamada, Y., Kobayashi, H., Iwata, T., Akizuki, T., Ishikawa, I. (2011). Current and future periodontal tissue engineering. *Periodontology 2000*.
13. Khong, N. M. H., Yusoff, F. M., Jamilah, B., Basri, M., Maznah, I., Chan, K. W., & Nishikawa, J. (2016). Nutritional composition and total collagen content of three commercially important edible jellyfish. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2015.09.094
14. Kuvaini, A. (2012). Management and Utilization of Jellyfish Amino Acids for Human Health as the Implementation of the Nagoya Protocol. *Jurnal Citra Widya Edukasi*.
15. Laine, M. ., & Crielaard, W. (2012). Functional Foods/Ingredients and Periodontal Diseases. *Eur J Nutr 51, 27-30*. https://doi.org/https://doi.org/10.1007/s00394-012-0325-5
16. Leone, A., Lecci, R. M., Durante, M., Meli, F., & Piraino, S. (2015). The bright side of gelatinous blooms: Nutraceutical value and antioxidant properties of three Mediterranean jellyfish (Scyphozoa). *Marine Drugs*. https://doi.org/10.3390/md13084654
17. Lian, J. B., & Gundberg, C. M. (1988). Osteocalcin. Biochemical considerations and clinical applications. *Clinical Orthopaedics and Related Research*. https://doi.org/10.1097/00003086-198801000-00036
18. Michael G., N., Takei, H., R. Klokkevold, P., & Carranza, F. (2018). Newman and Carranza's Clinical Periodontology 13th ed. *Clinical Periodontology*.
19. Nagai, T., Worawattanamateekul, W., Suzuki, N., Nakamura, T., Ito, T., Fujiki, K., … Yano, T. (2000). Isolation and characterization of collagen from rhizostomous jellyfish (Rhopilema asamushi). *Food Chemistry*. https://doi.org/10.1016/S0308-8146(00)00081-9
20. Park, S. N., Park, J. C., Kim, H. O., Song, M. J., & Suh, H. (2002). Characterization of porous collagen/hyaluronic acid scaffold modified by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide cross-linking. *Biomaterials*. https://doi.org/10.1016/S0142-9612(01)00235-6
21. Perikanan, K. K. dan. (2008). *Government Agency Performance Accountability Report.* Jakarta.
22. Perikanan, K. K. dan. (2011). *Government Agency Performance Accountability Report*. Jakarta.
23. Peter, M., Binulal, N. S., Nair, S. V., Selvamurugan, N., Tamura, H., & Jayakumar, R. (2010). Novel biodegradable chitosan-gelatin/nano-bioactive glass ceramic composite scaffolds for alveolar bone tissue engineering. *Chemical Engineering Journal*. https://doi.org/10.1016/j.cej.2010.02.003
24. Praba, F. W. (2015). Effect of topical application of human lactoferin on the bone defect healing process: study in Wistar Rats with histomorphometric analysis. *Jurnal Kedokteran Gigi Universitas Gajah Mada*.
25. Silvipriya, K. S., Krishna Kumar, K., Bhat, A. R., Dinesh Kumar, B., John, A., & Lakshmanan, P. (2015). Collagen: Animal sources and biomedical application. *Journal of Applied Pharmaceutical Science*. https://doi.org/10.7324/JAPS.2015.50322
26. Velasco, M. A., Narváez-Tovar, C. A., & Garzón-Alvarado, D. A. (2015). Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. *BioMed Research International*. https://doi.org/10.1155/2015/729076
27. Wolf, G. (1996). Function of the bone protein osteocalcin: Definitive evidence. *Nutrition Reviews*. https://doi.org/10.1111/j.1753-4887.1996.tb03798.x
28. Zeichner-David, M. (2006). Regeneration of periodontal tissues: Cementogenesis revisited. *Periodontology 2000*. https://doi.org/10.1111/j.1600-0757.2006.00162.x
29. Zoch, M. L., Clemens, T. L., & Riddle, R. C. (2016). New insights into the biology of osteocalcin. *Bone*. https://doi.org/10.1016/j.bone.2015.05.046