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Original article

Evaluation of osteogenic properties after application of hydroxyapatite-based shells of *Portunus pelagicus*

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ABSTRACT

Background: *After tooth extraction, the socket leaves a defect on the alveolar bone. The administration of shell crab-derived hydroxyapatite maintains bone dimensions that are important for achieving successful prosthodontic treatment. Purpose: The aim of the study was to determine the osteogenic properties, such as the number of osteoclasts, osteoblasts and osteocytes, after the application of hydroxyapatite-based shell crab in the post-extraction sockets of Wistar rats.* ***Methods:*** *There were two groups: the control group (K) and the treatment group (T). Wistar rats were randomly divided into control and treatment groups. After tooth extraction, hydroxyapatite gel derived from Portunus pelagicus shells was applied to the tooth sockets of Wistar rats. Observations and calculations of osteoclasts, osteoblasts and osteocytes were carried out on the 14th and 28th days under a light microscope with 400 times magnification. Statistical analysis was performed using one-way ANOVA.* ***Results:*** *There was a significant difference ($p<0.05$) between the K14 and P14 groups, K28 and P28 groups, K14 and K28 groups, and P14 and P28 groups. The results indicated that there were significant differences between groups of variables.* ***Conclusion:*** *The application of shell crab-derived hydroxyapatite (Portunus pelagicus) was able to decrease the number of osteoclasts and increase the number of osteoblasts and osteocytes.*

Keywords: *hydroxyapatite; Portunus pelagicus; osteoblasts; osteoclasts; osteocytes*

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INTRODUCTION

Tooth extraction is the process of removing the tooth from the alveolar bone when teeth are unable to withstand further treatment.[1] The post-extraction socket healing process leaves an alveolar defect. Along with the growth of bone in post-extraction sockets, there is also a process of resorption on the alveolar ridge.[2] There is a decrease in the buccolingual dimensions, as well as a decrease in the apicoronal dimensions of the alveolar ridge, as is often found after tooth extraction.[3] Reduction of the alveolar ridge can interfere with prosthodontic treatment. Resorption can lead to a loss of aesthetic and function, which can be harmful when paired with dental implants, especially in the anterior maxilla.[4]

Bone resorption after tooth extraction would cause difficulties for implant placement. This can be overcome by preserving the socket. There are several methods that can be used to minimise the occurrence of bone resorption. Among them is the use of demineralised freeze-dried bone allograft (DFDBA), Bioglass and hydroxyapatite (HA), which has been used in the form of both a resorbable and non-resorbable membrane.[5]

Calcium phosphate bioceramics, such as HA, are popular materials for bone reconstruction.[6] HA bioceramic materials form up to 70% of bone structure. HA can be produced synthetically from chemical reagents or can be synthesised from natural resources through hydrothermal transformation and the high-temperature calcination of bones.[7] Raw HA biomaterials are easily available and are abundant in Indonesia. Among the abundant raw materials is shell crab, which is one of Indonesia's export commodities. Indonesia exports 604,215–625,000 tons of crab without shells per year.[8] Flower crabs (*Portunus pelagicus*) has been a mainstay of Indonesia's export commodities to various countries around the world.[8] Crab shells contain calcium

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carbonate ($CaCO_3$), which can be processed further into HA [$CA_5(PO_4)_3(OH)$]. HA's structure is identical to that of human bone, which renders it a potential source of synthetic bone for bone grafts. In the field of dentistry, bone grafts are used to increase the alveolar ridge height, remodel the jawbone, transfer tissue free of microvascular problems and re-establish the alveolar crest.[8]

HA has osteoconductive properties and can stimulate mesenchymal cells to proliferate and differentiate in the bone regeneration process. Porous HA forms a strong bond between the bones, accelerating the process of vascularisation. The porosity of the bone graft increases the osteoconductive properties and the colonisation of osteoblasts, facilitates the penetration of osteoblast cells and provides a medium for osteoblasts to attach to.[9] Osteoblasts and osteocytes secrete osteoprotegerin (OPG), which acts as a binder for the RANKL receptor and decreases the differentiation of osteoclasts.[10] OPG has been shown to function as an inhibiting factor for osteoclastogenesis in vivo and in vitro.[11] This study aimed to determine the effect of HA crab shell on the number of osteoclasts, osteoblasts and osteocytes in the tooth sockets of Wistar rats.

MATERIALS AND METHODS

This study was approved by the Institutional Health Research Ethical Clearance Commission with certificate number 177/HRECC.FODM/VII/2018. An HA powder was made from crab shell by soaking the shell in water (ratio 3:20) for 15 minutes. The powder was immersed in chlorine and dissolved in water (10 ml of chlorine was used for 20 litres of water). It was soaked for 5 minutes. The calcination process was carried out by heating the material in a furnace at an initial temperature of ± 50°C, which slowly increased by 5°C/minute until the temperature reached 1000°C. It was maintained at this temperature for two hours. The HA powder was made into a gel by adding carrageenan powder and water with a ratio of 6:3:2, then mixed and heated slowly at 70°C for 10 minutes to form a gel compound.

The experimental subjects were 36 Wistar rats divided into four subgroups. Each group consisted of nine Wistar rats: control group at 14 days (K14), control group at 28 days (K28), treatment group at 14 days (P14) and treatment group at 28 days (P28). The rats were sedated with 10% ether. Each had its mandibula left incisor extracted using sterile forceps. For the treatment groups, HA gel was applied to the sockets, which were then sutured with silk thread 3/0. Meanwhile, the sockets of the control groups were sutured without the application of HA gel.

All the Wistar rats were sacrificed on the 14th and 28th days. Rats were euthanised using ketamine at a lethal dose (66–88 mg/kg of body weight). The mandible was cut and immersed in a 10% formaldehyde solution for at least 24 hours. Decalcification was performed using ethylenediaminetetraacetic acid. The tissue then underwent dehydration and was stored at 60°C for some time before being submerged in liquid paraffin. The paraffin blocks containing tissue were then cut using a microtome machine (3–4μm).

The slices of tissue were then inserted into a water bath (30–40°C). The tissue pieces were carefully attached to a glass object and 1–2 drops of albumin were added to the tops of the tissue pieces. After that, the glass object was heated with a hot plate at a temperature of 30–40°C. Haematoxylin and eosin staining was then used to measure the number of osteoclasts, osteoblasts and osteocytes. This observation was carried out under a light microscope with 400 times magnification. Statistical analysis was performed using one-way ANOVA with a p value of <0.05.

RESULTS

The measurements of the osteoblasts, osteoclasts and osteocytes on the 14th and 28th days from all groups can be seen in Figure 1. Histological imaging of the osteoblasts, osteoclasts and osteocytes can be seen in Figures 2, 3 and 4. The data was analysed using the Kolmogorov-Smirnov test and Levene's test, and the results showed that the data were normally distributed ($p>0.05$) and homogenous ($p>0.05$).

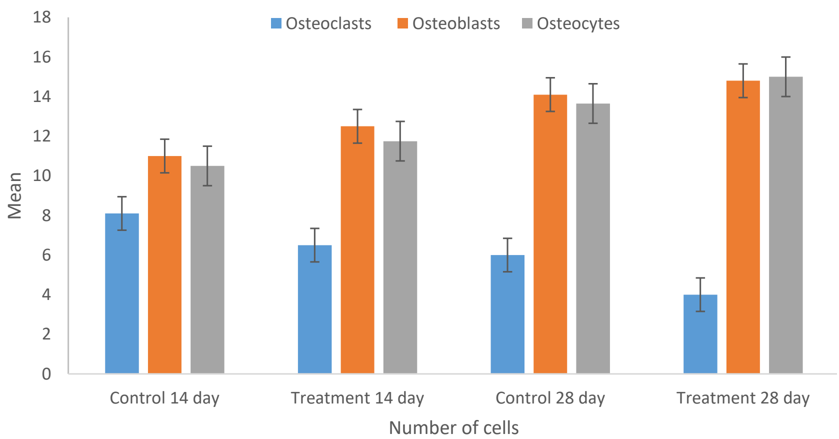

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts and osteocytes from the control and treatment groups.

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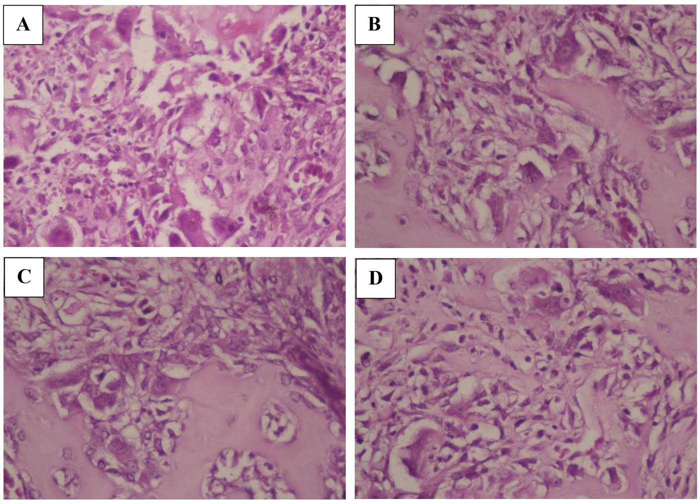

Figure 2. A histological view of the osteoclasts on the 14th (A) and 28th (B) days of the control group and the 14th (C) and 28th (D) days of the treatment group.

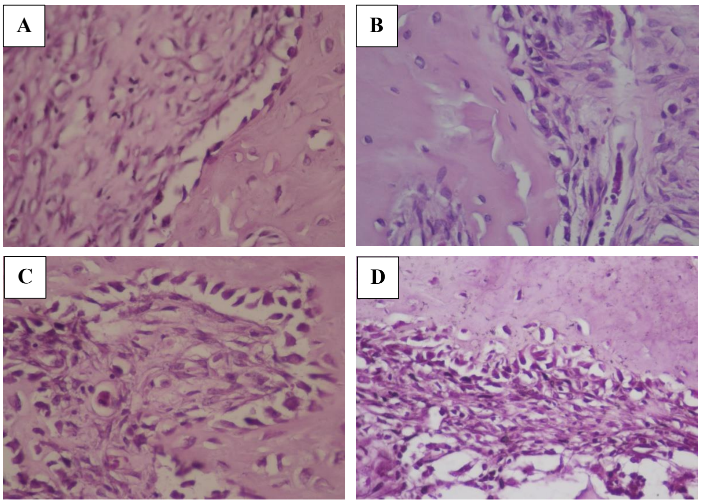

Figure 3. A histological view of the osteoblasts on the 14th (A) and 28th (B) days of the control group and the 14th (C) and 28th (D) days of the treatment group.

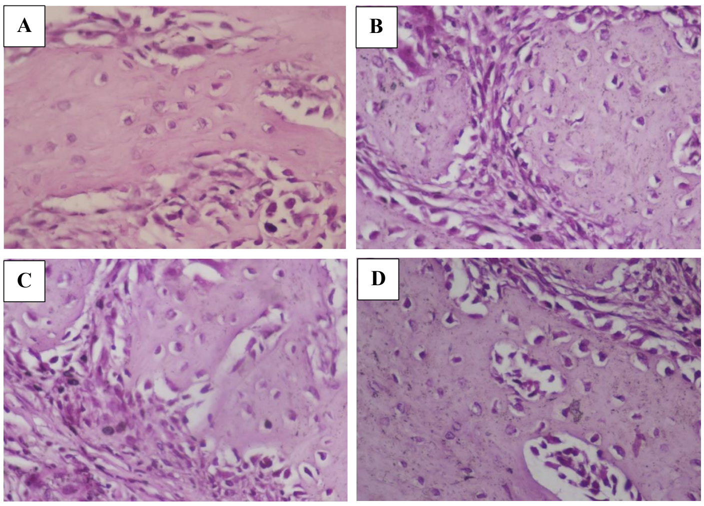

Figure 4. A histological view of the osteocytes on the 14th (A) and 28th (B) days of the control group and the 14th (C) and 28th (D) days of the treatment group.

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Table 1. This data represents the p value between each group of all-day treatment. All groups showed significant differences ($p<0.05$)

	K14	K28	P14	P28
K14		0.044	0.000	0.000
K28			0.024	0.000
P14				0.024
P28				

After the homogeneity test was carried out, a significance test was conducted using the one-way ANOVA test. The results showed that there were significant differences between groups of variables ($p<0.05$). A post hoc Tukey test was also conducted to determine the significance of the number of osteoclasts, osteoblasts and osteocytes in each study group. The significant differences between each group can be seen in Table 1.

DISCUSSION

Tooth extraction is the most common procedure in the field of dentistry. The response to the body's normal healing process after tooth extraction often causes significant bone resorption.[12] After tooth extraction, the alveolar bone is gradually absorbed by the body. Then, a remodelling process occurs, which results in a decrease in the dimensions of the alveolar bone. The vertical plane decreases and tends to be more palatal than its original position.[13]

The bone remodelling process consists of several phases, beginning with the activation phase. The activation phase involves the recruitment and activation of osteoclast monocyte-macrophage precursors from the circulation, resulting in the interaction of osteoclast precursor cells and osteoblasts. Then, during the resorption phase, osteoclasts begin to dissolve the mineral matrix and decompose the osteoid matrix.[12] The resorption phase is dominated by osteoclasts. Next comes the recovery phase, in which the transition from bone resorption to bone formation occurs. Bone absorbed in the resorption phase contains various mononuclear cells, including monocytes, osteocytes released from the bone matrix and preosteoblasts, which function to begin the process of new bone formation. In the formation phase, osteoblast cells are released on the surface to begin bone formation.[13] The process is completed by the mineralisation phase, which begins 30 days after osteoid deposition.[14]

To maximise bone regeneration after tooth extraction and minimise the occurrence of bone resorption, the socket is filled with bone graft material. When filling the socket, actions that could cause trauma to the bone should be avoided, thereby reducing the occurrence of buccal, lingual and ridge alveolar resorption.[12]

Calcium phosphate bioceramics, such as HA, are popular materials for bone reconstruction. Bioceramic HA material forms up to 70% of the bone structure. HA is effectively used to replace part or all of the bone tissue. It can be used as a bone filling material. HA can produce a physicochemical interaction between ceramics and bone tissue, thus encouraging the binding and growth of new tissue.[15]

The HA in this study was made from crab shell, which was first made into a HA powder using a furnace, then converted into a crab shell-based HA gel. The crab shell-based HA gel used in this study contained 87.11% HA.

The results showed a significant difference in the number of osteoclasts on the 14th and 28th days. This was because, on the 14th day, the resorption phase was dominated by osteoclasts. Osteoclasts need 2–4 weeks for the remodelling cycle to complete bone resorption. Meanwhile, on the 28th day, there was a decrease in the number of osteoclasts due to the commencement of the initial stage of the recovery phase. It was found that the number of osteoclasts on day 14 was higher than the number of osteoclasts on day 28 in both the control groups and the treatment groups. The results also showed that there was a decrease in the number of osteoclasts in the treatment group when compared with the number of osteoclasts in the control group on the 14th and 28th days. This indicates that the administration of crab shell-based HA can reduce the number of osteoclasts in sockets after extraction.

The number of osteoblasts in P14 and P28 was higher than the number of osteoblasts in K14 and K28. No significant differences were found between P14 and P28. This is because, on the 28th day, an insignificant number of osteoblasts were formed due to the continuation of osteoblast cells in the maturation phase forming osteocytes for apoptosis.[16]

Figure 1 also shows that there were significant differences between K14 and P14 and K28 and P28. This is because HA gel can trigger osteocytes to differentiate, so there is an increase in the number of osteocytes. However, the differences were only significant between the K14 and P14; the differences between K28 and P28 were not significant. This was due to osteocyte apoptosis occurring after a period of 10 to 14 days. Osteocyte apoptosis plays a key role in activating the bone remodelling mechanism.[17] Although the P28 showed the highest number of osteocytes, maximum cell growth actually occurred before the 28th day. Thus, the number of osteocytes did not increase much between days 14 and 28. This is because crab shell-based HA has osteoconductive and osteoinductive properties, facilitating the growth of new bone tissue in the gap between mineral particles in HA. Adding crab shell-based HA particles can significantly reduce the number of osteoclasts. The formation of an apatite layer on the surface of a biomaterial has the ability to bind living bones. The potential for the osteoinductive properties of HA has been confirmed in previous studies. Furthermore, the administration of HA is found to deposit a higher number of collagen fibres around the HA particles.[18]

HA can bind to bone tissue and provide a specific biological response that can stimulate osteoblast cells to form

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new bone tissue and help the bone regeneration process.[18] Combined with osteoconduction, it can increase osteoblast attachment. The activation of osteoblasts and osteocytes can produce OPG.[19] OPG is one of the main factors in regulating osteoclast differentiation. OPG is found to inhibit the spontaneous induction of bone absorption.[20] OPG is a feed receptor for RANKL and competes with RANK to bind RANKL. As a result, OPG can be an effective inhibitor for osteoclast cell maturation and osteoclast cell activation. When the bone resorption phase by osteoclasts is complete, the resorbed bone cavity contains various mononuclear cells, including monocytes, osteocytes released from the bone matrix and preosteoblasts, which function to initiate new bone formation. In conclusion, the administration of HA-based shell crab to Wistar rats after tooth extraction can reduce the number of osteoclasts and increase the number of osteoblasts and osteocytes.

REFERENCES

1. Pedersen GW. Buku ajar praktis bedah mulut. 4th ed. Jakarta: EGC; 2013. p. 36.
2. Alani AFI. Multiple techniques have been proposed to preserve alveolar bone after tooth loss. Ann Med Health Sci Res. 2018; 8(1): 65–8.
3. Hansson S, Halldin A. Alveolar ridge resorption after tooth extraction: a consequence of a fundamental principle of bone physiology. J Dent Biomech. 2012; 3: 1–8.
4. van Heerden P. Treatment concepts for socket grafting. Int Dent – African Ed. 2012; 2(1): 70–4.
5. Kim Y-K, Yun P-Y, Um I-W, Lee H-J, Yi Y-J, Bae J-H, Lee J. Alveolar ridge preservation of an extraction socket using autogenous tooth bone graft material for implant site development: prospective case series. J Adv Prosthodont. 2014; 6(6): 521–7.
6. Ari MDA, Yuliati A, Rahayu RP, Saraswati D. The differences scaffold composition in pore size and hydrophobicity properties as bone regeneration biomaterial. J Int Dent Med Res. 2018; 11(1): 318–22.
7. Komur B, Altun E, Aydogdu MO, Bilgiç D, Gokce H, Ekren N, Salman S, Inan AT, Oktar FN, Gunduz O. Hydroxyapatite synthesis from fish bones: Atlantic salmon (Salmon salar). Acta Phys Pol A. 2017; 131(3): 400–2.
8. Raya I, Mayasari E, Yahya A, Syahrul M, Latunra AI. Shynthesis and characterizations of calcium hydroxyapatite derived from crabs shells (Portunus pelagicus) and its potency in safeguard against to dental demineralizations. Int J Biomater. 2015; 2015: 469176.
9. Ardhiyanto HB. Stimulasi osteoblas oleh hidroksiapatit sebagai material bone graft pada proses penyembuhan tulang. Stomatognatic (J K G Unej). 2012; 9(3): 162–4.
10. Smith SY, Varela A, Samadfam R. Bone Toxicology. New York: Spinger; 2017. p. 27–93.
11. Jana S, Shah R, Thomas R, Kumar ABT, Mehta DS. Techniques for preservation of post-extraction alveolar bone loss: A literature review. J Adv Med Med Res. 2021; 33(10): 33–42.
12. Udeabor S, Halwani M, Alqahtani S, Alshaiki S, Alqahtani A, Alqahtani S. Effects of altitude and relative hypoxia on post-extraction socket wound healing: A clinical pilot study. Int J Trop Dis Heal. 2017; 25(3): 1–7.
13. Pagni G, Pellegrini G, Giannobile W V., Rasperini G. Postextraction alveolar ridge preservation: biological basis and treatments. Int J Dent. 2012; 2012: 1–13.
14. Fogelman I, Gnanasegaran G, Van der Wall H. Radionuclide and hybrid bone imaging. Heidelberg: Springer; 2012. p. 44–6.
15. Anchana devi C, Perumal P. Synthesis & application of hydroxyapatite bioceramics from different marine sources. J Res Environ Earth Sci. 2016; 2(11): 7–15.
16. Bellido T. Osteocyte-driven bone remodeling. Calcif Tissue Int. 2014; 94(1): 25–34.
17. Cardoso L, Herman BC, Verborgt O, Laudier D, Majeska RJ, Schaffler MB. Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. J bone Miner Res. 2009; 24(4): 597–605.
18. Sotto-Maior BS, Senna PM, Aarestrup BJ V, Ribeiro RA, Assis NM de SP, Cury AADB. Effect of bovine hydroxyapatite on early stages of bone formation. Rev Odonto Ciência. 2011; 26(3): 198–292.
19. Supangat D, Cahyaningrum SE. Synthesis and characterization of crab shell hydroxyapatite (Scylla serrata) by wet application method. UNESA J Chem. 2017; 6(3): 143–9.
20. Bonucci E, Ballanti P. Osteoporosis-bone remodeling and animal models. Toxicol Pathol. 2014; 42(6): 957–69.

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Evaluation of osteogenic properties after application of hydroxyapatite- based shells of Portunus pelagicus Michael Josef Kridanto Kamadjaja, Alya Nisrina Sajidah Gatia, Agtadilla Novitananda, Lintang Maudina, Harry Laksono, Agus Dahlan, Bambang Agustono Satmoko Tumali and Muhammad Dimas Aditya Ari ABSTRACT Background: After tooth extraction, the socket will leave a defect on the alveolar bone. The administration of shell crab - derived hydroxyapatite will maintaining bone dimensions that is very important to achieve successful prosthodontic treatment. Purpose: The study was aimed to determine the osteogenic properties such as the number of osteoclasts, osteoblasts and osteocytes after application of hydroxyapatite-based shell crab in post extraction sockets of Wistar rat. Methods: There were 2 groups, control group (K) and treatment group (T). Wistar rats were randomly divided into control and treatment group. After tooth extraction, tooth socket of Wistar rats was given hydroxyapatite gel derived from Portunus pelagicus shell. Observation and calculation of osteoclasts, osteoblasts and osteocytes were carried out on the 14th and 28th day under light microscope with 400 times magnification. Statistical analysis was performed using one way ANOVA. Results: There was a significant difference ($p<0.05$) beetween K14 and P14 group, K28 and P28 group, K14 and K28 group, P14 and P28 group. This results indicated that there were significant differences between groups of variables. This results indicated that there were significant differences between groups of variables. Conclusion: Application of shell crab-derived hydroxyapatite (Portunus pelagicus) was able to decrease the number of osteoclasts and increase of the number of osteoblast and osteocyte. Keywords: hydroxyapatite; Portunus pelagicus; osteoblasts; osteoclasts; osteocytes INTRODUCTION Tooth extraction is a process of removing the tooth from the alveolar bone where the teeth are already unable to do the treatment again.1 Post-extraction socket healing process will leave an alveolar defect. Along with the growth of bone in post-extraction sockets, there is also the process of resorption on the alveolar ridge.2 There is a decrease in buccolingual dimension as well as a decrease in alveolar ridge apicoronal dimensions, it is often found after tooth extraction.3 Reduction of alveolar ridge can interfere prosthodontic treatment. Resorption can lead to a loss of aesthetic and functional, which can harm when paired dental implants, especially in the anterior maxilla.4 Bone resorption after tooth extraction would be difficult for implant placement. This can be overcome by preserve the socket. There are several methods that can be done to minimize the occurrence of bone resorption. Among them is the use of demineralized freeze-dried bone allograft (DFDBA), bioglass and hydroxyapatite which has been used both in the form of a resorbable membrane or nonresorbable.5 Calcium phosphate bioceramics such as hydroxyapatite (HA) is a very popular material for bone reconstruction.6 HA bioceramics material forms up to 70% of bone structure. HA can be produced synthetically from chemicals reagents or can be synthesized from natural resources through a hydrothermal transformation, and high-temperature calcination of bones.7 Raw material of hydroxyapatite biomaterials is very easily available and abundant in Indonesia. Among the abundant raw material is shell crab, which is part of Indonesia's export commodities. Crab by Indonesian export commodities of 604215-625000 tons / year without a shell.7 Crab (Portunus pelagicus) has been the mainstay of Indonesia's export commodities to various countries in the world.8 Crab shells containing calcium carbonate ($CaCO_3$) can be processed further into hydroxyapatite [$CA_5(PO_4)_3(OH)$].8 HA structure is identical to that of human bone which renders a potential source of synthetic bone for bone grafts. In the field of dentistry, bone graft is used to increase alveolar ridge height, remodel the jawbone, transfer tissue free of microvascular problems, and reestablish alveolar crest.8 Hydroxyapatite have an osteoconductive properties and able to stimulate mesenchymal cells to proliferate and differentiate in the bone regeneration process. Porous hydroxyapatite to form a bond between the bones strong and accelerating the process of vascularization. The porosity of the bone graft will increase the osteoconductive properties, the colonization of osteoblasts, and facilitate the penetration of osteoblast cells as well as a medium for osteoblasts to attach.10 Osteoblasts and osteocytes secrete osteoprotegerin (OPG), which acts as a binder and the RANKL receptor decrease the differentiation of osteoclasts.11 OPG has been shown to function as an inhibiting factor for osteoclastogenesis in vivo and in vitro.12 This study aimed to determine the effect of hydroxyapatite crab shell towards the number of osteoclasts, osteoblasts and osteocytes in tooth socket of Wistar rats. MATERIALS AND METHODS A hydroxyapatite powder from the crab shell (Portunus pelagicus) was made by means of a crab shell Portunus pelagicus soaked in water (ratio 3:20) for 15 minutes. The powder immersed with chlorine is dissolved into water (10 ml of chlorine is used for 20 liters of water). Soaking was done for 5 minutes. The calcination process was carried out by heating using a furnace with an initial temperature of ± 50°C and slowly increasing it with an increase in temperature of 5°C / minute until the temperature reaches 1000°C and maintained for 2 hours. The hydroxyapatite powder from the crab shell (Portunus pelagicus) was made into a gel by adding carrageenan powder and water with a ratio of 6:3:2 then mixed by heating slowly at 70°C for 10 minutes to form a gel compound. The experimental animals were 36 Wistar rats divided into 4 subgroups, each group consisted of 9 Wistar rats: control group 14 days (K14), control group 28 days (K28), treatment group 14 days (P14), and treatment group 28 days (P28). Rats were sedated with 10% ether in order to reach sedation phase. Their mandibula left incisor was extracted using sterile forceps. For treatment group, HA gel was applied to the socket then sutured with silk thread 3/0. Meanwhile, control group was directly sutured without application of HA gel. All Wistar rats were sacrificed on the 14th and 28th day. Rats were euthanized using ketamine at lethal dose (66-88 mg/kg body weight). The mandible was cut and immersed into 10% formaldehyde solution for at least 24 hours. Decalcification was done using ethylene diamine tetraacetic acid (EDTA). The tissue then was underwent dehydration and stored at 60°C for a while before poured with liquid paraffin. Paraffin blocks containing tissue then were cut using microtome machine (3-4μm). Slice of tissues was then inserted into the waterbath (30-40°C). The tissue pieces were carefully attached to the glass object and added 1-2 drops of albumin on top of the tissue pieces. After that, the glass object was attached to the tissue and heated with hot plate at a temperature of 30-40°C. Hematoxilin eosin (HE) was then performed to measure the number of osteoclasts, osteoblasts and osteocytes. This observation were carried out under light microscopic with 400 times magnification. Statistical analysis was performed using one way ANOVA with p value < 0.05. RESULTS The result of osteoblasts, osteoclasts, and osteocytes measurement from 14th day and 28th day from all groups can be seen in Figure 1. Histological imaging of osteoblast, osteoclast and osteocyte can be seen in Figure 2, 3 and 4. The data were analyzed using Kolmogorov-Smirnov and Levene test, the results showed that the data were normally distributed (p> 0.05) and homogenous (p> 0.05). After the homogeneity test was carried out, a significance test was conducted using one- way ANOVA test. The results showed that there were significant differences between groups of variables (p <0.05). Post Hoc Tukey test was also conducted to see the significance of the number of osteoclasts, osteoblasts, and osteocytes between the study groups. The results found that there was a significant difference between K14 and P14 group , K28 and P28 group, K14 and K28 group, P14 and P28 group. Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes from control and treatment groups. Figure 2. A histological view of osteoclast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group. Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group. Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group. DISCUSSION Tooth extraction is the most common procedure in the field of dentistry. The response to normal healing by the body after tooth extraction often causes significant bone resorption.13 After tooth extraction, the alveolar bone will be gradually absorbed by the body. Then a remodeling process will occur which results in a decrease in the dimensions of the alveolar bone. A decrease in the vertical plane and tends to be more palatal than its original position.14 The bone remodeling process consists of several phases, namely the activation phase, in the activation phase involves the recruitment and activation of osteoclast monocyte-macrophage precursors from the circulation, resulting in interaction of osteoclast precursor cells and osteoblasts. Then the resorption phase, osteoclasts begin to dissolve the mineral matrix and decompose the osteoid matrix.13 The resorption phase is dominated by osteoclasts. Furthermore, the recovery phase, the phase of the transition from bone resorption to bone formation, bone cavity absorbed in the resorption phase contains various mononuclear cells, including monocytes, osteocytes released from the bone matrix, and preosteoblasts, which function to start new bone formation. phase of formation, there is the release of osteoblast cells on the surface function to start bone formation.14 Finished by the mineralization phase, this phase begins 30 days after osteoid deposition.15 To maximize bone regeneration after the tooth extraction action and minimize the occurrence of bone resorption, after tooth extraction the socket is filled with bone graft material. When filling the bone graft, avoid actions that can cause trauma to the bone, thereby reducing the occurrence of buccal, lingual, and ridge alveolar resorption.13 Calcium phosphate bioceramics, such as hydroxyapatite (HA) are very popular ingredients for bone reconstruction. The bioceramics HA material forms up to 70% bone structure.6 Hydroxyapatite is effectively used to replace part or all parts of bone tissue. Can be used as bone filling material. Hydroxyapatite can produce a physicochemical interaction between ceramics and bone tissue, thus encouraging the binding and growth of new tissue.16 Hydroxyapatite in this study was made from crab shell (Portunus pelagicus) which was made into hydroxyapatite powder first by means of the furnace and then converted into a crab shell-based hydroxyapatite gel (Portunus pelagicus). The crab shell-based hydroxyapatite gel (Portunus pelagicus) used in this study contained 87.11% hydroxyapatite. The results showed a significant difference between the number of osteoclasts on the 14th day and the number of osteoclasts on the 28th day, this was because on the 14th day the resorption phase was dominated by osteoclasts. Osteoclasts need 2-4 weeks for the remodeling cycle to do bone resorption.19 Whereas on the 28th day there was a decrease in the number of osteoclasts, this was due to starting to enter the initial phase of the recovery phase. So it was found that the number of osteoclasts on day 14 was higher when compared with the number of osteoclasts on day 28 in the control group and in the treatment group. The results also showed that there was a decrease in the number of osteoclasts in the treatment group when compared with the number of osteoclasts in the control group on the 14th day and 28th day. Indicates that the administration of crab shell-based hydroxyapatite (Portunus pelagicus) can reduce the number of osteoclasts in sockets after extraction. In the 14th day treatment group and 28th day treatment group, the number of osteoblast cells was higher when compared to the 14th day control group and the 28th day control group. In the treatment group on the 14th day with the 28th day treatment group no significant differences were found. This happens because on that day there are not many osteoblasts formed due to the continuation of osteoblast cells in the mature phase to form osteocytes or apoptosis.19 In Figure 1 it also shows that there are significant results in the 14th day control group with the 14th day treatment group and in the 28th day control group with the 28th day treatment group because hydroxyapatite gel can trigger osteocytes to differentiate so there is an increase in the number of osteocytes. However, in the treatment group the 14th day was only significant with the 14th day control group, not significant with the 28th day control group and 28th day treatment this was due to osteocytes apoptosis after a period of 10 to 14 days. Osteocyte apoptosis plays a key role in activating the bone remodeling mechanism.20 Although in the 28th day treatment group showed the highest number of osteocytes, it turned out that maximum cell growth was before the 28th day. So that the number of osteocytes does not increase so much in the span of between 14 and 28 days. This is because crab shell-based hydroxyapatite (Portunus pelagicus) has osteocondutive and osteoinductive properties, facilitating the growth of new bone tissue in the gap between mineral particles in hydroxyapatite. The osteoconductive and osteoinductive properties of crab shell-based hydroxyapatite (Portunus pelgicus) could facilitate the growth of new bone tissue Adding crab shell based hydroxyapatite particles (Portunus pelagicus) can significantly reduce the number of osteoclasts. The formation of an apatite layer on the surface of a biomaterial has the ability to bind living bones. The potential for osteoinductive properties possessed by hydroxyapatite has been confirmed in previous studies. And the administration of hydroxyapatite is found to deposit higher collagen fibers surrounding the hydroxyapatite particles.21 Hydroxyapatite can bind to bone tissue and provide a specific biological response that can stimulate osteoblast cells to form new bone tissue so that it can help the bone regeneration process.21 With the presence of osteoconduction, it can increase osteoblast attachment. Activation of osteoblasts and osteocytes can produce osteoprotegerin (OPG).22 One of the first factors that regulates osteoclast differentiation is OPG. OPG is found to inhibit spontaneous and induction of bone absorption.23 OPG as a feed receptor for RANKL and competes with RANK to bind RANKL. As a result, OPG can be an effective inhibitor for osteoclast cell maturation and osteoclast10 cell activation. When the bone resorption phase by osteoclasts is complete, the resorbed bone cavity contains various mononuclear cells, including monocytes, osteocytes released from the bone matrix, and preosteoblasts, which function to initiate new bone formation.20 In conclusion, administration of hydroxyapatite-based shell crab (Portunus pelagicus) Wistar rats after tooth extraction can reduce the number of osteoclasts and increase the number of osteoblasts and osteocytes.