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Judul Artikel : **(C17)** Evaluation of osteogenic properties after application of hydroxyapatite-based shells of Portunus pelagicus

Jurnal : Dental Journal (Majalah Kedokteran Gigi)

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No	Perihal	FEBRUARI Tanggal	Halaman
1	Bukti submit dan artikel yang disubmit	21 April 2021	1-2
2	Bukti Revisi 1	24 Mei 2021	3-47
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Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes

Authors: Michael Josef Kridanto Kamadjaja[1], Alya Nisrina Sajidah Gatia[2], Agtadilla Novitananda[2], Lintang Maudina[2], Harry Laksono[1], Agus Dahlan[1], Bambang Agustono Satmoko Tumali[1], Muhammad Dimas Aditya Ari[1]

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Dr. Michael Josef Kridanto Kamadjaja, drg.,M.Kes., Sp.Pros.
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Kami beritahukan bahwa naskah sejawat dengan judul:

Provision of Hydroxyapatite-based Shells of Portunus pelagicus Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes

Authors: Michael Josef Kridanto Kamadjaja, Alya Nisrina Sajidah Gatia, Agtadilla Novitananda, Lintang Maudina, Harry Laksono

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Atas perhatiannya, kami ucapkan terima kasih.

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Judul Naskah: **Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes**

Tanggal Kirim : 29 April 2021 Tanggal Kembali ke Redaksi : 5 Mei 2021

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Penyunting Ahli,

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Research Report

Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes

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ABSTRACT

Background: *After tooth extraction on the socket will leave a defect of the alveolar bone in the form of a decrease in the dimensions of the alveolar ridge, thus maintaining bone dimensions is very important to get successful prosthodontic treatment.* ***Purpose:*** *To determine the number of osteoclasts, osteoblasts, and osteocyte after the administration of shell crab-derived hydroxyapatite Wistar rat after extraction of tooth.* ***Methods.*** *Treatment by giving hydroxyapatite gel shell crab Portunus pelagicus from species to socket after extracting tooth Wistar rats osteoclasts the which will be observed on the 14th and 28th days.* ***Results:*** *There was a decrease in the number of osteoclasts, increase of the number of osteoblast and osteocyte in the treatment group compared with the number of the cells in the control group on the 14th day and 28th day.* ***Conclusion:*** *Shell crab-derived hydroxyapatite (Portunus pelagicus) after extraction of a tooth can Wistar rats 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 to form 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].

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 based on the number of osteoclasts, osteoblasts and osteocytes in after tooth extraction sockets 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 H_2O_2:Water (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 ± 50ºC and slowly increasing it with an increase in temperature of 5ºC / minute until the temperature reaches 1000ºC and maintained for 2 hours.

Second, the hydroxyapatite powder from the crab shell (Portunus pelaagicus) was made into a gel by adding carrageenan powder and water with a ratio of 6:3:2 then mixed bt heating slowly at 70 ° C for 10 minutes to form a gel compound.

The experimental animals were 36 wistar rats then divided into 4 groups, each group consisted of 9 wistar rats consisting of the control group for 14 days (K14), the control group for 28 days (K28), the treatment group for 14 days (P14) and the treatment group for 28 days (P28). Revocation of the lower left incisor. A crab-based hydroxapatite gel (*Portunus*

pelagicus) was applied to the socket after extraction of the treatment group while the control group was given nothing. Giving the gel was done by injection syringe technique as deep as 3mm until the gel filled the post extraction socket. Then the socket was sutures with a non-absorble.

Specimens collection were perfomed on day 14 and day 28, all of the subjects were sacrificed. Then the rat mandible was cut, the mandible was inserted into a fixation solution with 10% formalin solution. Soaking at least 1 x 24 hours with a volume of 10 times the size of the specimen. Decalcification was done by using Ethylene Diamine Tetraacetic Acid (EDTA). Next cut to a tissue thickness of 0.3 - 0.5 mm and arranged into a tissue cassette. Then put it into the machine automatic processor, the network undergoes a process of dehydration. Followed by removing air from the network using a vacuum machine for 30 minutes. Tissue cassettes were removed and stored at a temperature of 60 ° C for a while before printing is done with liquid paraffin. Paraffin blocks containing tissue, then cut using a microtome machine (3-4µm).

One piece of tissue taken was then inserted into the waterbath (30-40ºC). The tissue pieces were carefully attached to the glass object. Drops 1-2 drops of albumin on top of the tissue pieces. After that, the glass object that has been attached to the tissue is heated with a hot plate at a temperature of 30-40ºC. Then staining of Hematoxilin Eosin (HE) was performed. After being entangled, cover with a glass cover carefully so there are no bubbles. Observation and calculation of osteoclasts, osteoblasts and osteocytes were carried out using microscopic magnification 400 times. Statistical analysis was performed using one way ANOVA.

RESULTS

The observations were obtained from the results of the number of osteoclasts, osteoblasts and osteocytes on day 14 and day 28 after extraction of mandibular left incisors after the administration of crab shell based hydroxyapatite gel in 36 wistar rats, the study sample consisted of 9 control groups 14th day, 9 control group 28th day, 9 treatment groups for 14 days, and 9 treatment groups for 28 days. In the dental socket control group, no treatment was given, only sewing was done after extraction. In the socket treatment group filled with hydroxyapatite gel.

The tooth extraction socket on the 14th and 28th day was prepared with Hematoxilin Eosin (HE) staining. Then calculating the number of osteoclasts, osteoblasts and osteocytes in preparations using a microscope. Cell count results on preparations can be seen in table 1.

Data from the research results can be seen using the bar diagram in Figure 1. On histopathological observations osteoblast, osteoclast and osteocyte were obtained in the microscope visual field as follows in figure 2, 3 and 4. Data analysis in this study started with the normality test of each data using the Kolmogorov-Smirnov test to see whether the data generated is normally distributed ($p > 0.05$). Next, a homogeneity test was carried out by using Levene Test to test the similarity of (homogeneous) variants of several samples. In the Levene statistical test this study shows data $p > 0.05$ which means that the data in this study are homogeneous.

After the homogeneity test was carried out, a significance test was conducted using One Way Anova to see the differences between groups of variables. On One Way Anova Test a value of 0.00 was produced ($p < 0.05$). This shows that there are significant differences between groups of variables. After that, the Post Hoc Tukey Test was conducted to see the significance of the number of osteoclasts, osteoblasts, and osteocytes between the study groups. A value is considered to have a significant difference if $p > 0.05$. In the analysis of data this study found a significant difference in the K14 group to the P14 group, the K28 group to the P28 group, the K14 group to the K28 group and the P14 group to the P28 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. 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. 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 structure6. Hydroxyapatite is a bioceramics that has good bioactive and stability properties. 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.

This study uses wistar rats (*Rattus norvegicus*) because wistar rats are the most commonly used animal models in medical and scientific research[17]. Wistar rats were used as male sex because it was feared that using female wistar rats could be influenced by the hormonal cycle of female rats there by reducing the homogeneity of the population used at the time of the study and could affect the effects of the treatment carried out in the study[18].

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 needed about 2-4 weeks during the remodeling cycle. to do bone resorption. 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. 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[15]. In this study showed that administration of hydroxyapatite-based shell crab

(Portunus pelagicus) Wistar rats after tooth extraction can reduce the number of osteoclasts as well as increase the number of osteoblasts and osteocytes.

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Type the authorities or persons which contribute to funding or assistance in your research if any.

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Table 1. Results of reading the number of osteoclasts, osteoblasts, and osteocytes

No	Group Reseearch	N	Ostoclast		Osteoblast		Osteocyte	
			Mean	SD	Mean	SD	Mean	SD
1	K14	9	8,00	0,78	11,11	1,54	10,50	1,08
2	K28	9	6,00	0,88	12,44	1,59	11,80	1,05
3	P14	9	6,40	1,00	14,22	1,92	13,50	0,92
4	P28	9	4,10	1,05	14,77	2,49	15,10	1,26

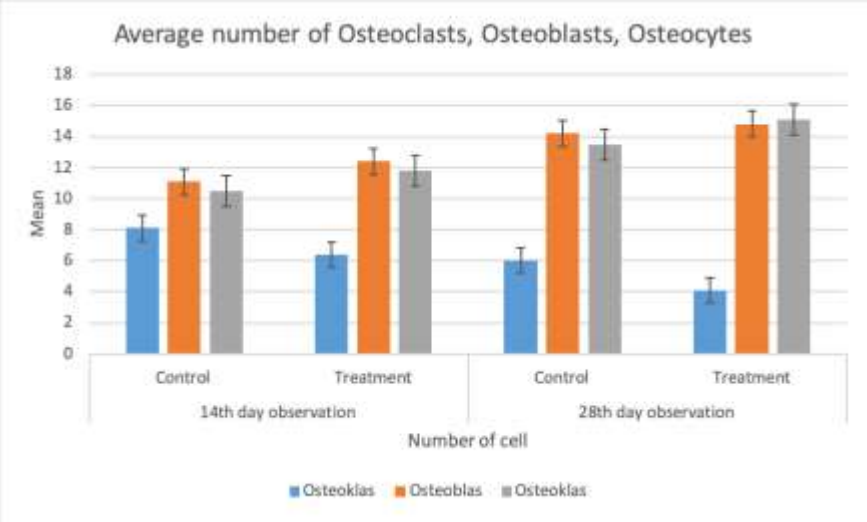
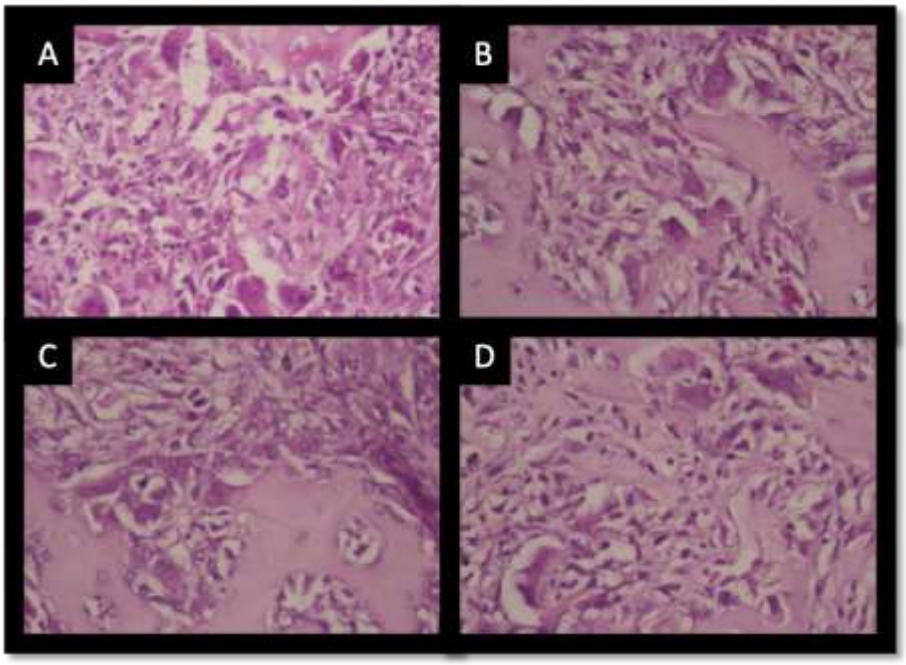


Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes



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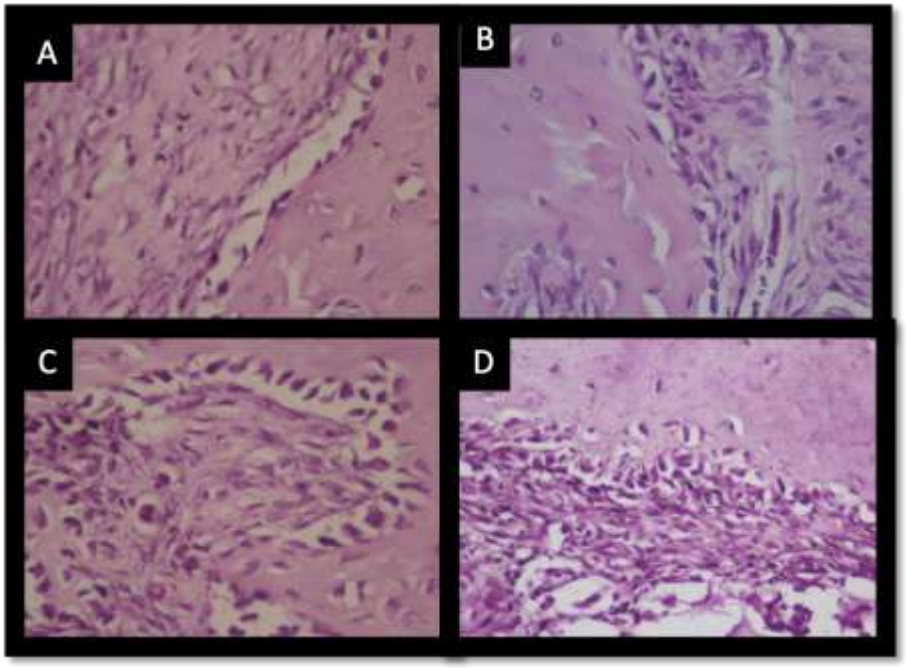

Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

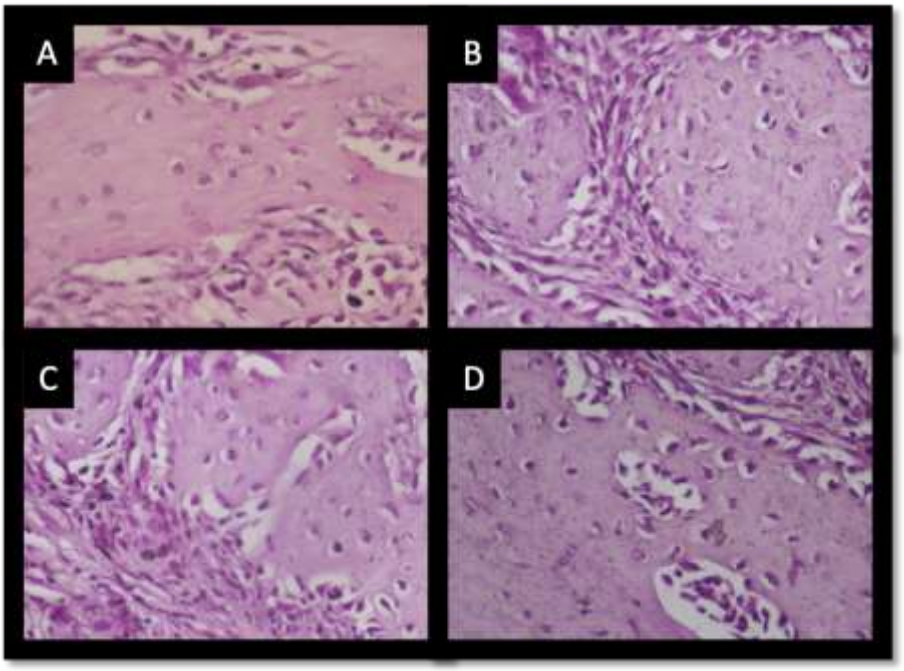

Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

FORMAT PENILAIAN NASKAH DENTAL JOURNAL HASIL PENELITIAN (untuk Penyunting Ahli)

Judul Naskah: **Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes**

Tanggal Kirim : 29 April 2021 Tanggal Kembali ke Redaksi : .....

HAL YANG DISUNTING	YA*	TIDAK*
1. Apakah naskah ini pernah dimuat pada media lain ? Keterangan: tidak pernah		v
2. Apakah judul tepat, singkat, jelas, dan menggambarkan kontribusi pengembangan keilmuan ? (maksimal 10 kata, melingkupi variabel yang diteliti) Keterangan: judul selain lebih dari 10 kata, sebaiknya diubah agar lebih mudah dimengerti dan lebih menarik.		
3. Apakah pada naskah hasil penelitian :		
a) Pendahuluan mencakup latar belakang secara jelas ? Keterangan: Sudah cukup jelas		
b) Tujuan cukup jelas ? Keterangan: Sudah cukup jelas		
c) Metode dan rancangan penelitian sesuai dengan tujuan penelitian ? Keterangan: Sudah sesuai		
d) Prosedur penelitian diuraikan secara tepat dan rinci, sehingga menjamin validitas internal/ eksternal Keterangan: Sudah di uraikan secara terperinci		
e) Hasil penelitian dapat menjawab *research question* ? Keterangan: Hasil penelitian sudah menjawab research question		
f) - Pembahasan tidak mengulang hasil ? - Selaras dengan lingkup penelitian dan dibandingkan dengan hasil penelitian sejenis ?		

- Menerangkan makna hasil penelitian dalam menjawab permasalahan ? Keterangan: pembahasan sudah baik, tidak mengulang hasil.		
g) Acuan selaras dengan materi penelitian dan menggunakan literatur 10 tahun terakhir? Keterangan: 6 dari 23 literatur masih menggunakan literatur yang lebihdari 10 tahun		
h) Kesimpulan sesuai dengan judul, permasalahan? - Hasil penelitian memberi kontribusi untuk pengembangan Ilmu kedokteran gigi ? - Melakukan sintesis berdasar hasil penelitian sejenis yang mendahului Keterangan: penelitian ini memberi kontribusi pengembangan ilmu pengetahuan		
i) Pustaka perlu ditambahi/ dikurangi**)? Keterangan: Pustaka perlu ditambhakan, agar dapat mengganti literatur yang telah lebih dari 10 tahun		
4. Apakah ada bagian yang perlu ditambahi/ diringkas**) ? Keterangan: - Mohon dilakukan perbaikan table/ gambar. - Mohon dilihat kembali pengulangan-pengulangan kalimat atau penjelasan yang sama pada setiap babnya		

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Research Report

Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes

ABSTRACT

Background: *After tooth extraction on the socket will leave a defect of the alveolar bone in the form of a decrease in the dimensions of the alveolar ridge, thus maintaining bone dimensions is very important to get successful prosthodontic treatment.* ***Purpose:*** *To determine the number of osteoclasts, osteoblasts, and osteocyte after the administration of shell crab-derived hydroxyapatite Wistar rat after extraction of tooth.* ***Methods.*** *Treatment by giving hydroxyapatite gel shell crab Portunus pelagicus from species to socket after extracting tooth Wistar rats osteoclasts the which will be observed on the 14th and 28th days.* ***Results:*** *There was a decrease in the number of osteoclasts, increase of the number of osteoblast and osteocyte in the treatment group compared with the number of the cells in the control group on the 14th day and 28th day.* ***Conclusion:*** *Shell crab-derived hydroxyapatite (Portunus pelagicus) after extraction of a tooth can Wistar rats 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 to form 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].

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 based on the number of osteoclasts, osteoblasts and osteocytes in after tooth extraction sockets 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 H_2O_2:Water (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 ± 50ºC and slowly increasing it with an increase in temperature of 5ºC / minute until the temperature reaches 1000ºC and maintained for 2 hours.

Second, the hydroxyapatite powder from the crab shell (Portunus pela~~-~~gicus) was made into a gel by adding carrageenan powder and water with a ratio of 6:3:2 then mixed bt heating slowly at 70 ° C for 10 minutes to form a gel compound.

The experimental animals were 36 wistar rats then divided into 4 groups, each group consisted of 9 wistar rats consisting of the control group for 14 days (K14), the control group for 28 days (K28), the treatment group for 14 days (P14) and the treatment group for 28 days

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(P28). Revocation of the lower left incisor. A crab-based hydroxapatite gel (*Portunus pelagicus*) was applied to the socket after extraction of the treatment group while the control group was given nothing. Giving the gel was done by injection syringe technique as deep as 3mm until the gel filled the post extraction socket. Then the socket was sutures with a non-absorble.

Specimens collection were ~~perfomed~~performed on day 14 and day 28, all of the subjects were sacrificed. Then the rat mandible was cut, the mandible was inserted into a fixation solution with 10% formalin solution. Soaking at least 1 x 24 hours with a volume of 10 times the size of the specimen. Decalcification was done by using Ethylene Diamine Tetraacetic Acid (EDTA). Next cut to a tissue thickness of 0.3 - 0.5 mm and arranged into a tissue cassette. Then put it into the machine automatic processor, the network undergoes a process of dehydration. Followed by removing air from the network using a vacuum machine for 30 minutes. Tissue cassettes were removed and stored at a temperature of 60 ° C for a while before printing is done with liquid paraffin. Paraffin blocks containing tissue, then cut using a microtome machine (3-4µm).

One piece of tissue taken was then inserted into the waterbath (30-40ºC). The tissue pieces were carefully attached to the glass object. Drops 1-2 drops of albumin on top of the tissue pieces. After that, the glass object that has been attached to the tissue is heated with a hot plate at a temperature of 30-40ºC. Then staining of Hematoxilin Eosin (HE) was performed. After being entangled, cover with a glass cover carefully so there are no bubbles. Observation and calculation of osteoclasts, osteoblasts and osteocytes were carried out using microscopic magnification 400 times. Statistical analysis was performed using one way ANOVA.

RESULTS

~~The observations were obtained from the results of the number of osteoclasts, osteoblasts and osteocytes on day 14 and day 28 after extraction of mandibular left incisors after the administration of crab shell based hydroxyapatite gel in 36 wistar rats, the study sample consisted of 9 control groups 14th day, 9 control group 28th day, 9 treatment groups for 14 days, and 9 treatment groups for 28 days. In the dental socket control group, no treatment was given, only sewing was done after extraction. In the socket treatment group filled with hydroxyapatite gel.~~

~~The tooth extraction socket on the 14th and 28th day was prepared with Hematoxilin Eosin (HE) staining. Then calculating the number of osteoclasts, osteoblasts and osteocytes in preparations using a microscope.~~ Cell count results on preparations can be seen in table 1.

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Data from the research results can be seen using the bar diagram in Figure 1. On histopathological observations osteoblast, osteoclast and osteocyte were obtained in the microscope visual field as follows in figure 2, 3 and 4. Data analysis in this study started with the normality test of each data using the Kolmogorov-Smirnov test to see whether the data generated is normally distributed (p> 0.05). Next, a homogeneity test was carried out by using Levene Test to test the similarity of (homogeneous) variants of several samples. In the Levene statistical test this study shows data p> 0.05 which means that the data in this study are homogeneous.

After the homogeneity test was carried out, a significance test was conducted using One Way Anova to see the differences between groups of variables. On One Way Anova Test a value of 0.00 was produced (p <0.05). This shows that there are significant differences between groups of variables. After that, the Post Hoc Tukey Test was conducted to see the significance of the number of osteoclasts, osteoblasts, and osteocytes between the study groups. A value is considered to have a significant difference if p> 0.05. In the analysis of data this study found a significant difference in the K14 group to the P14 group, the K28 group to the P28 group, the K14 group to the K28 group and the P14 group to the P28 group.

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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. 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. 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 structure6.~~ Hydroxyapatite is a bioceramics that has good bioactive and stability properties. 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].

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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.

This study uses wistar rats (*Rattus norvegicus*) because wistar rats are the most commonly used animal models in medical and scientific research[17]. Wistar rats were used as male sex because it was feared that using female wistar rats could be influenced by the hormonal cycle of female rats there by reducing the homogeneity of the population used at the time of the study and could affect the effects of the treatment carried out in the study[18].

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 needed about 2-4 weeks during the remodeling cycle~~.~~ to do bone resorption. 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.

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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. 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[15]. In this study showed that administration of hydroxyapatite-based shell crab (Portunus pelagicus) Wistar rats after tooth extraction can

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reduce the number of osteoclasts as well as increase the number of osteoblasts and osteocytes.

ACKNOWLEDGEMENT

Type the authorities or persons which contribute to funding or assistance in your research if any.

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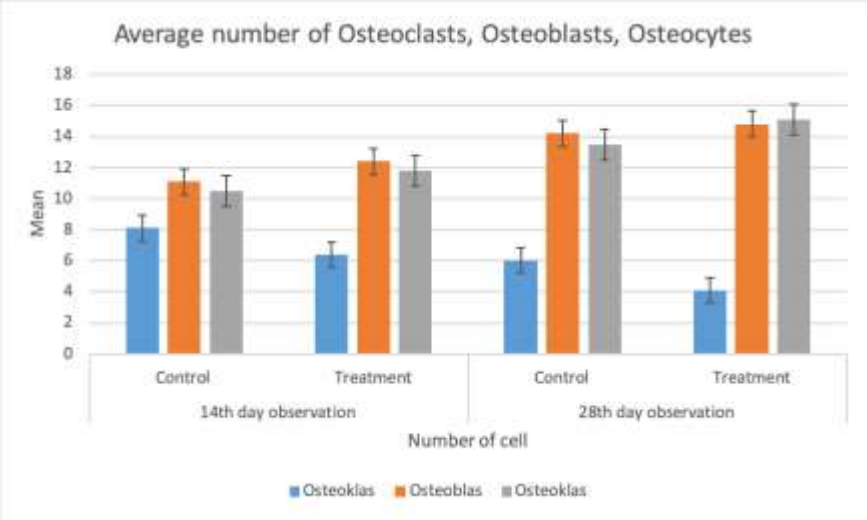

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes

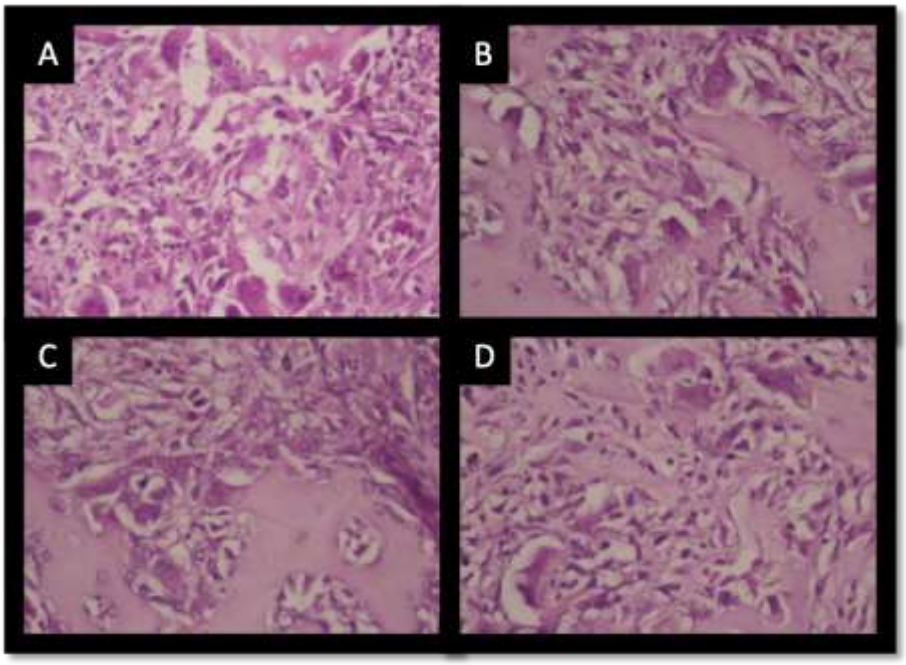

Figure 2. A histological view of osteoclast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

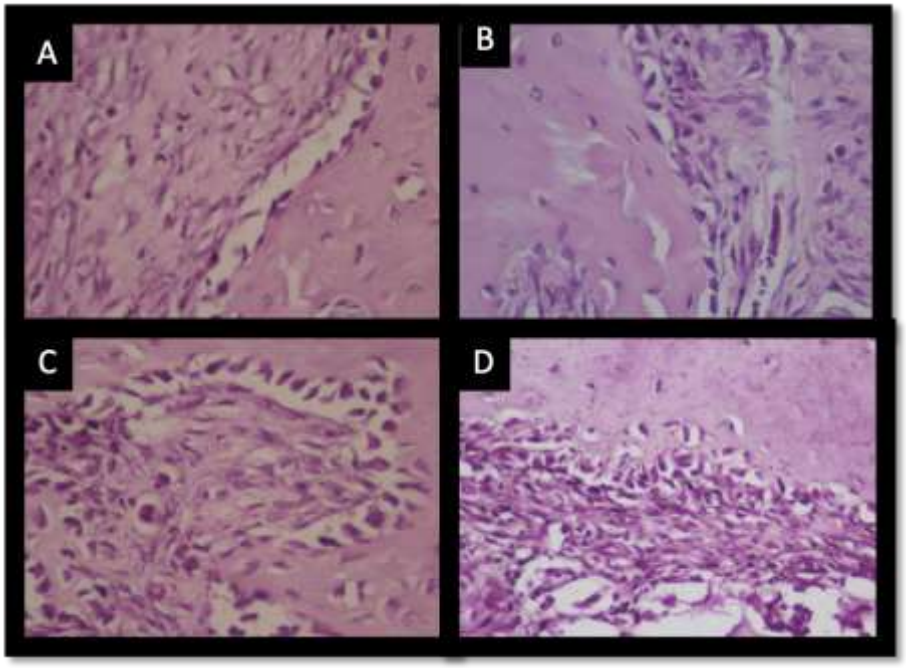

Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

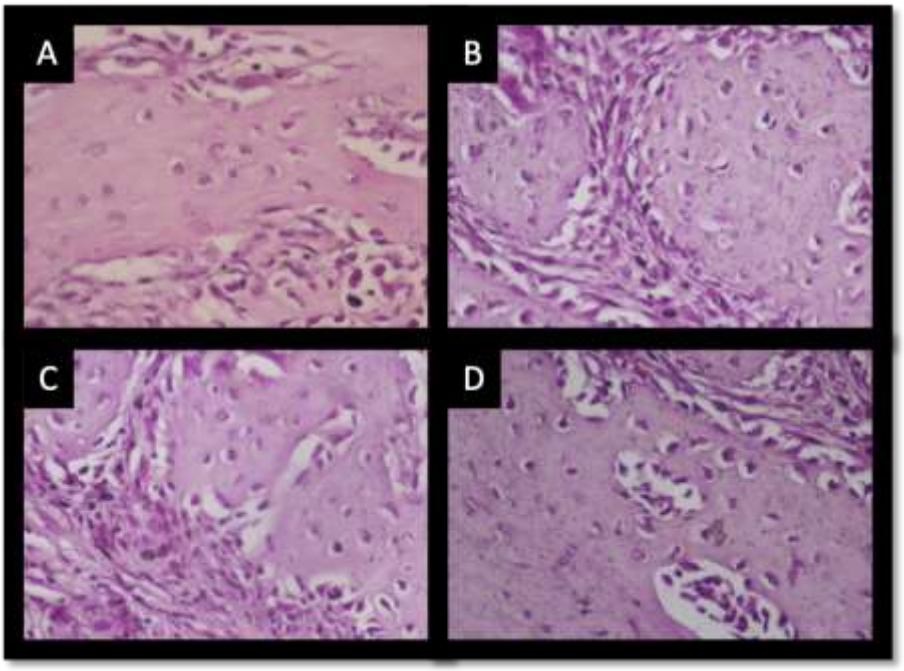

Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

DAFTAR TILIK *MANAGING EDITOR*

Judul Naskah: **Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes**

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HAL YANG DISUNTING	KETERANGAN*)
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Apakah panjang naskah cukup memadai? ▪ (10-12 halaman, 1,5 spasi, dengan ukuran kertas HVS A4, *times new roman* ukuran font 12)	Ya/ Tidak
▪ Bagian-bagian isi naskah proporsional (Pembahasan lebih panjang dari Pendahuluan)	Ya/ Tidak
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Surabaya, 28-04-2021

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Research Report

Provision of Hydroxyapatite-based Shells of *Portunus pelagicus* Post Tooth Extraction Wistar Rats to Osteoclasts, Osteoblasts and Osteocytes

ABSTRACT

Background: *After tooth extraction on the socket will leave a defect of the alveolar bone in the form of a decrease in the dimensions of the alveolar ridge, thus maintaining bone dimensions is very important to get successful prosthodontic treatment.* ***Purpose:*** *To determine the number of osteoclasts, osteoblasts, and osteocyte after the administration of shell crab-derived hydroxyapatite Wistar rat after extraction of tooth.* ***Methods.*** *Treatment by giving hydroxyapatite gel shell crab Portunus pelagicus from species to socket after extracting tooth Wistar rats osteoclasts the which will be observed on the 14th and 28th days.* ***Results:*** *There was a decrease in the number of osteoclasts, increase of the number of osteoblast and osteocyte in the treatment group compared with the number of the cells in the control group on the 14th day and 28th day.* ***Conclusion:*** *Shell crab-derived hydroxyapatite (Portunus pelagicus) after extraction of a tooth can Wistar rats decrease the number of osteoclasts and increase of the number of osteoblast and osteocyte.*

Keywords: *Hydroxyapatite; Portunus pelagicus; Osteoblasts; Osteoclasts; Osteocytes*

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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 to form up to 70% of bone

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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].

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 based on the number of osteoclasts, osteoblasts and osteocytes **in after** tooth extraction sockets 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 H_2O_2:Water (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 ± 50ºC and slowly increasing it with an increase in temperature of 5ºC / minute until the temperature reaches 1000ºC and maintained for 2 hours.

Second, the hydroxyapatite powder from the crab shell (Portunus pelaagicus) was made into a gel by adding carrageenan powder and water with a ratio of 6:3:2 then mixed bt heating slowly at 70 ° C for 10 minutes to form a gel compound.

The experimental animals were 36 wistar rats then divided into 4 groups, each group consisted of 9 wistar rats consisting of the control group for 14 days (K14), the control group for 28 days (K28), the treatment group for 14 days (P14) and the treatment group for 28 days

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(P28). Revocation of the lower left incisor. A crab-based hydroxapatite gel (*Portunus pelagicus*) was applied to the socket after extraction of the treatment group while the control group was given nothing. Giving the gel was done by injection syringe technique as deep as 3mm until the gel filled the post extraction socket. Then the socket was sutures with a non-absorble.

Specimens collection were perfomed on day 14 and day 28, all of the subjects were sacrificed. Then the rat mandible was cut, the mandible was inserted into a fixation solution with 10% formalin solution. Soaking at least 1 x 24 hours with a volume of 10 times the size of the specimen. Decalcification was done by using Ethylene Diamine Tetraacetic Acid (EDTA). Next cut to a tissue thickness of 0.3 - 0.5 mm and arranged into a tissue cassette. Then put it into the machine automatic processor, the network undergoes a process of dehydration. Followed by removing air from the network using a vacuum machine for 30 minutes. Tissue cassettes were removed and stored at a temperature of 60 ° C for a while before printing is done with liquid paraffin. Paraffin blocks containing tissue, then cut using a microtome machine (3-4µm).

One piece of tissue taken was then inserted into the waterbath (30-40ºC). The tissue pieces were carefully attached to the glass object. Drops 1-2 drops of albumin on top of the tissue pieces. After that, the glass object that has been attached to the tissue is heated with a hot plate at a temperature of 30-40ºC. Then staining of Hematoxilin Eosin (HE) was performed. After being entangled, cover with a glass cover carefully so there are no bubbles. Observation and calculation of osteoclasts, osteoblasts and osteocytes were carried out using microscopic magnification 400 times. Statistical analysis was performed using one way ANOVA.

RESULTS

The observations were obtained from the results of the number of osteoclasts, osteoblasts and osteocytes on day 14 and day 28 after extraction of mandibular left incisors after the administration of crab shell based hydroxyapatite gel in 36 wistar rats, the study sample consisted of 9 control groups 14th day, 9 control group 28th day, 9 treatment groups for 14 days, and 9 treatment groups for 28 days. In the dental socket control group, no treatment was given, only sewing was done after extraction. In the socket treatment group filled with hydroxyapatite gel.

The tooth extraction socket on the 14th and 28th day was prepared with Hematoxilin Eosin (HE) staining. Then calculating the number of osteoclasts, osteoblasts and osteocytes in preparations using a microscope. Cell count results on preparations can be seen in table 1.

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Data from the research results can be seen using the bar diagram in Figure 1. On histopathological observations osteoblast, osteoclast and osteocyte were obtained in the microscope visual field as follows in figure 2, 3 and 4. Data analysis in this study started with the normality test of each data using the Kolmogorov-Smirnov test to see whether the data generated is normally distributed (p> 0.05). Next, a homogeneity test was carried out by using Levene Test to test the similarity of (homogeneous) variants of several samples. In the Levene statistical test this study shows data p> 0.05 which means that the data in this study are homogeneous.

After the homogeneity test was carried out, a significance test was conducted using One Way Anova to see the differences between groups of variables. On One Way Anova Test a value of 0.00 was produced (p <0.05). This shows that there are significant differences between groups of variables. After that, the Post Hoc Tukey Test was conducted to see the significance of the number of osteoclasts, osteoblasts, and osteocytes between the study groups. A value is considered to have a significant difference if p> 0.05. In the analysis of data this study found a significant difference in the K14 group to the P14 group, the K28 group to the P28 group, the K14 group to the K28 group and the P14 group to the P28 group.

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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. 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. 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 structure6. Hydroxyapatite is a bioceramics that has good bioactive and stability properties. 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.

This study uses wistar rats (*Rattus norvegicus*) because wistar rats are the most commonly used animal models in medical and scientific research[17]. Wistar rats were used as male sex because it was feared that using female wistar rats could be influenced by the hormonal cycle of female rats there by reducing the homogeneity of the population used at the time of the study and could affect the effects of the treatment carried out in the study[18].

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 needed about 2-4 weeks during the remodeling cycle. to do bone resorption. 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

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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. 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[15]. In this study showed that administration of hydroxyapatite-based shell crab (Portunus pelagicus) Wistar rats after tooth extraction can

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reduce the number of osteoclasts as well as increase the number of osteoblasts and osteocytes.

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ACKNOWLEDGEMENT

Type the authorities or persons which contribute to funding or assistance in your research if any.

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Table 1. Results of reading the number of osteoclasts, osteoblasts, and osteocytes

No	Group Reseearch	N	Ostoclast		Osteoblast		Osteocyte	
			Mean	SD	Mean	SD	Mean	SD
1	K14	9	8,00	0,78	11,11	1,54	10,50	1,08
2	K28	9	6,00	0,88	12,44	1,59	11,80	1,05
3	P14	9	6,40	1,00	14,22	1,92	13,50	0,92
4	P28	9	4,10	1,05	14,77	2,49	15,10	1,26

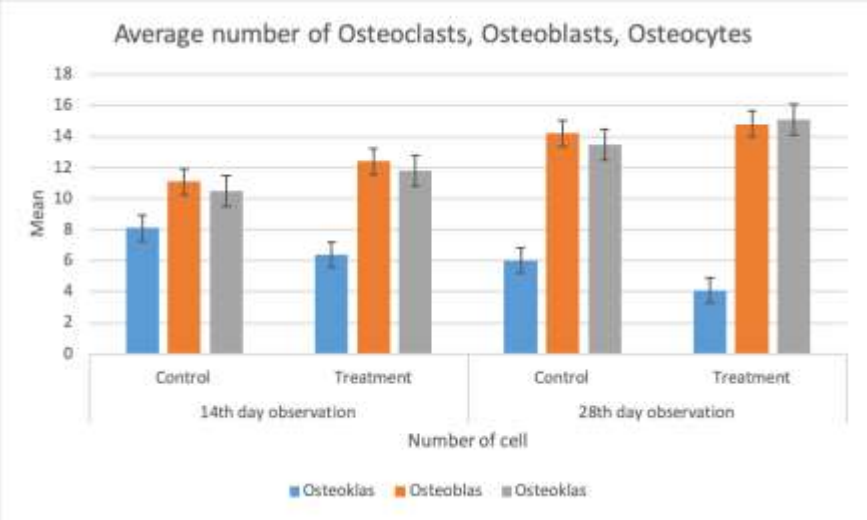

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes

Commented [HP30]: TABLE 1 dan FIGURE 1 isinya sama. HARAp dipilih salah satu saja (bisa pakai Figure 1 saja!!)

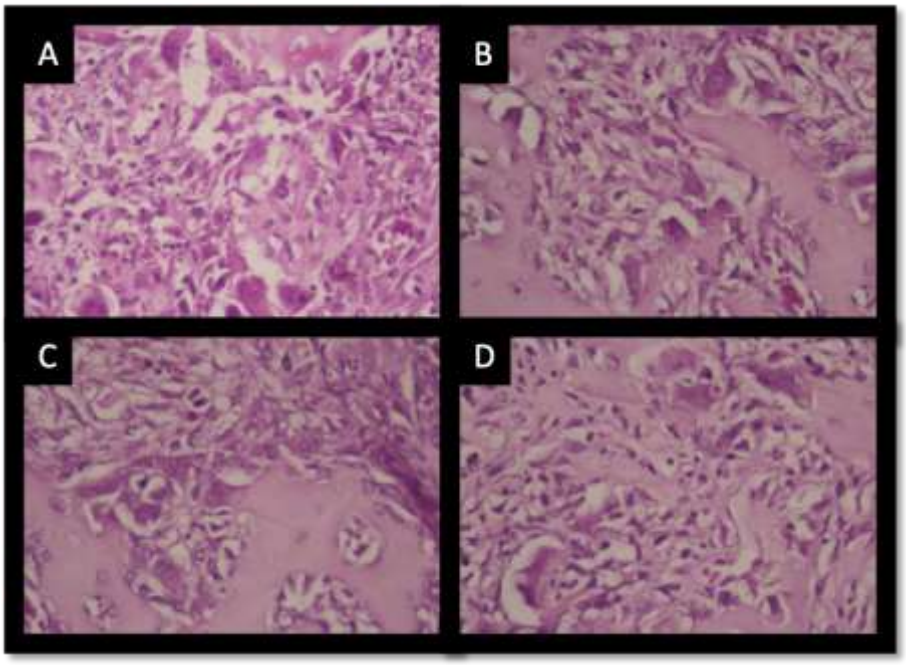

Figure 2. A histological view of osteoclast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

Commented [HP31]: Harap ditunjukkan yang mana gambaran osteoclast, tunjukkan dengan tanda panah.

Apa yang membedakan antara kontrol dan treatmen group? Apa yang membedakaan antara day 14 dan day 28????

Bgt juga utk Figure 3 dan 4!!

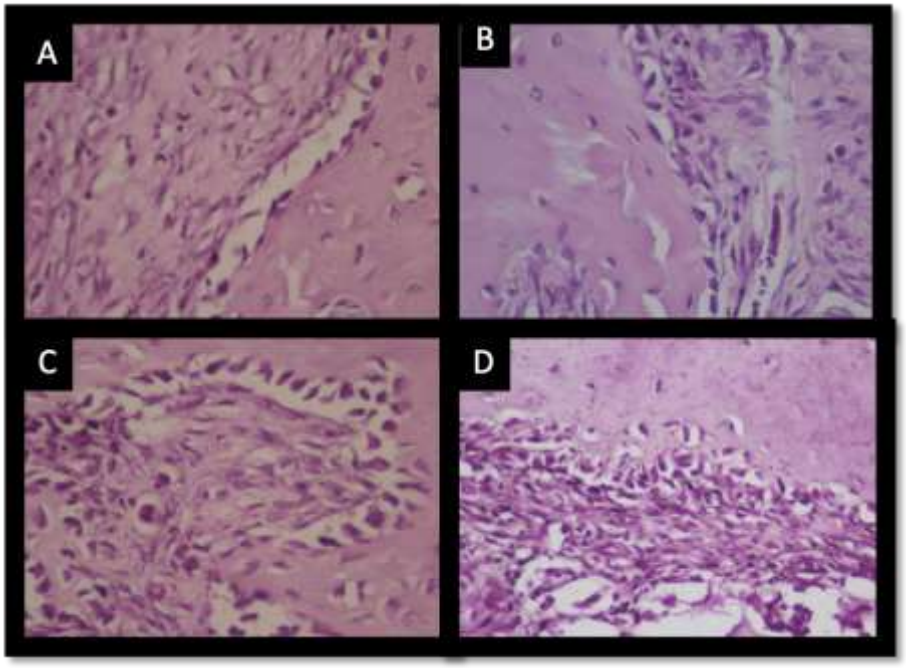

Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

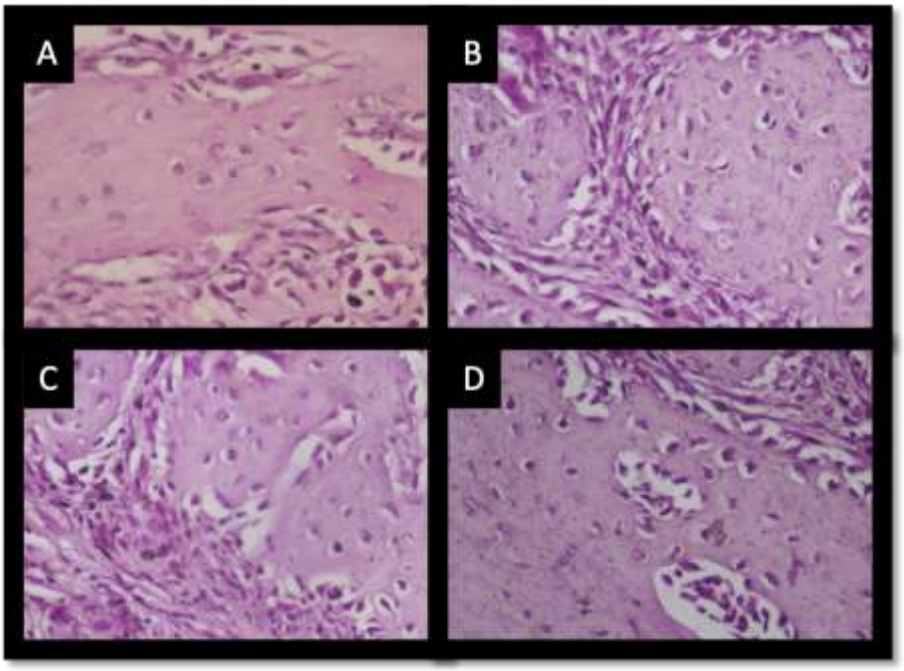

Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

Pemberitahuan revisi ke-2

Dari: Dental Journal (Majalah Kedokteran Gigi) (dental_journal@fkg.unair.ac.id)

Kepada: michael-j-k-k@fkg.unair.ac.id; josef_310563@yahoo.com

Tanggal: Sabtu, 17 Juli 2021 pukul 11.51 WIB

Kepada Dr. Michael Josef K. Kamadjaja, drg

Berikut terlampir review ke-2 dari reviewer, mohon merevisi sesuai komentar dan memberi highlight warna pada perubahan yg dilakukan.
Revisi mohon dikirim kembali paling lambat tanggal 23 Juli 2021.
Terimakasih.

Salam,
Muhammad Dimas Aditya Ari

review 2_Evaluation of osteogenic properties after application of Hydroxyapatite-based shells of Portunus pelagicus.docx
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Research Report

Evaluation of osteogenic properties after application of Hydroxyapatite-based shells of Portunus Pelagicus on animal models

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 aimed to determine the number of osteoclasts, osteoblasts, and osteocyte after the application of shell crab-derived hydroxyapatite Wistar rat on tooth socket post extraction.* ***Methods.*** *There was 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 between 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.* ***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

Preparation of hydroxyapatite powder

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 ± 50ºC and slowly increasing it with an increase in temperature of 5ºC / minute until the temperature reaches 1000ºC 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.

Tooth Extraction and application of HA gel

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.

Tissue preparation

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 hour. 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 (30-40ºC). 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 30-40ºC. 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.

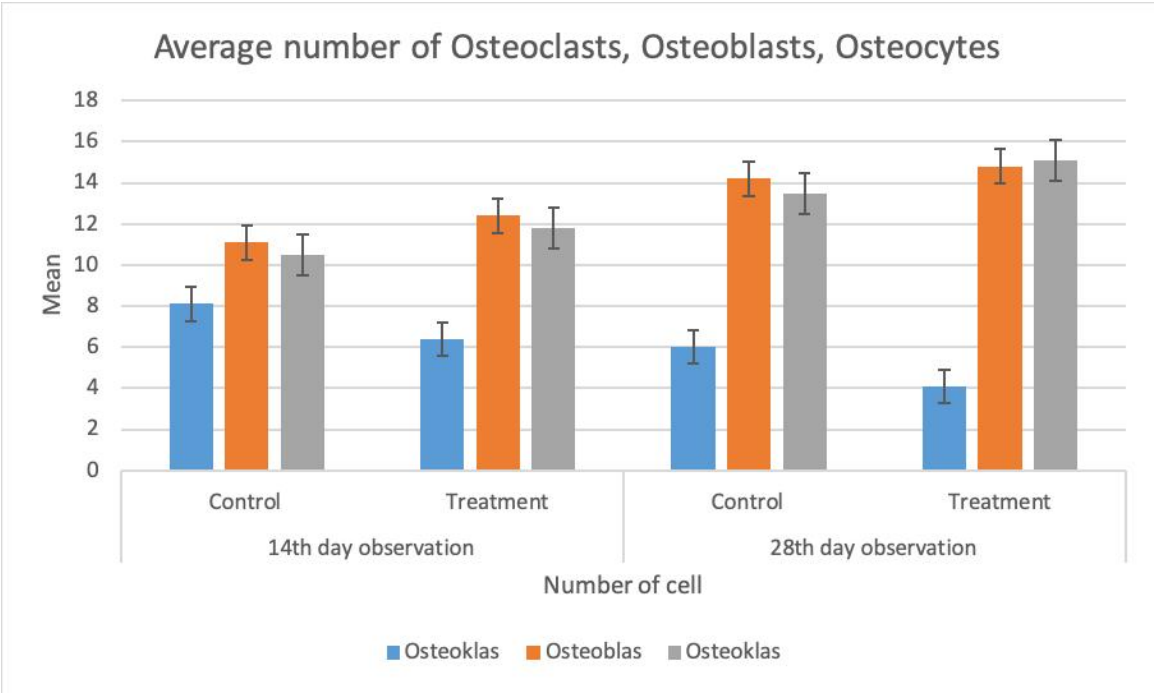

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes from control and treatment groups.

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.

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 osteoclast[10] 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].

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.

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ACKNOWLEDGEMENT

There was no funding and materials support in this study

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Table 1. Results of reading the number of osteoclasts, osteoblasts, and osteocytes

No	Group Reseearch	N	Ostoclast		Osteoblast		Osteocyte	
			Mean	SD	Mean	SD	Mean	SD
1	K14	9	8,00	0,78	11,11	1,54	10,50	1,08
2	K28	9	6,00	0,88	12,44	1,59	11,80	1,05
3	P14	9	6,40	1,00	14,22	1,92	13,50	0,92
4	P28	9	4,10	1,05	14,77	2,49	15,10	1,26

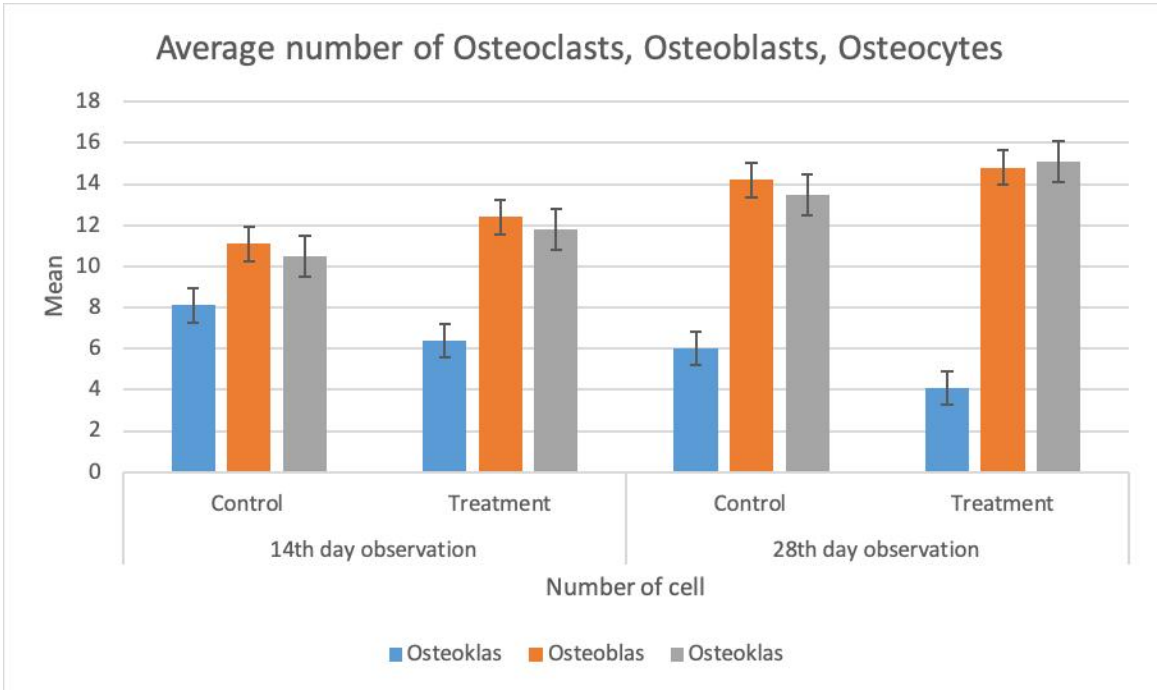

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes

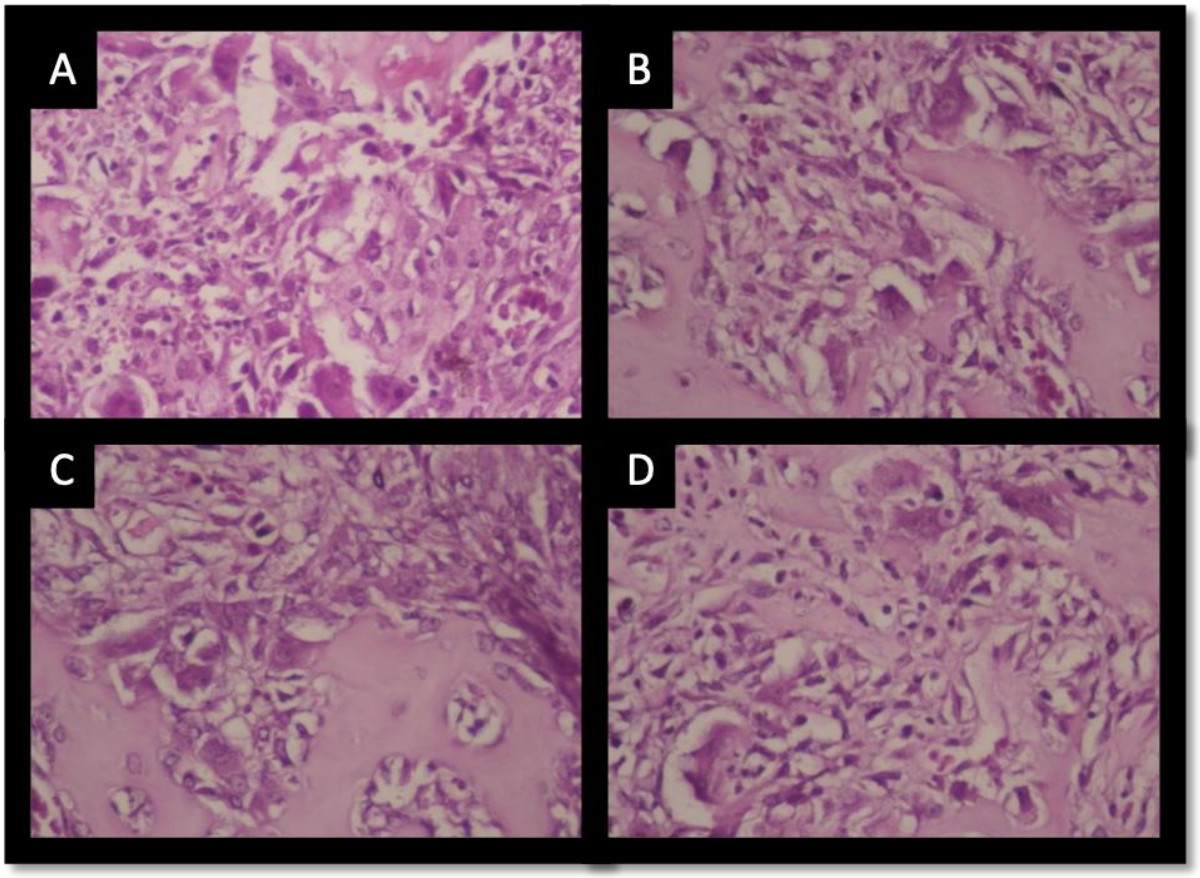

Figure 2. A histological view of osteoclast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

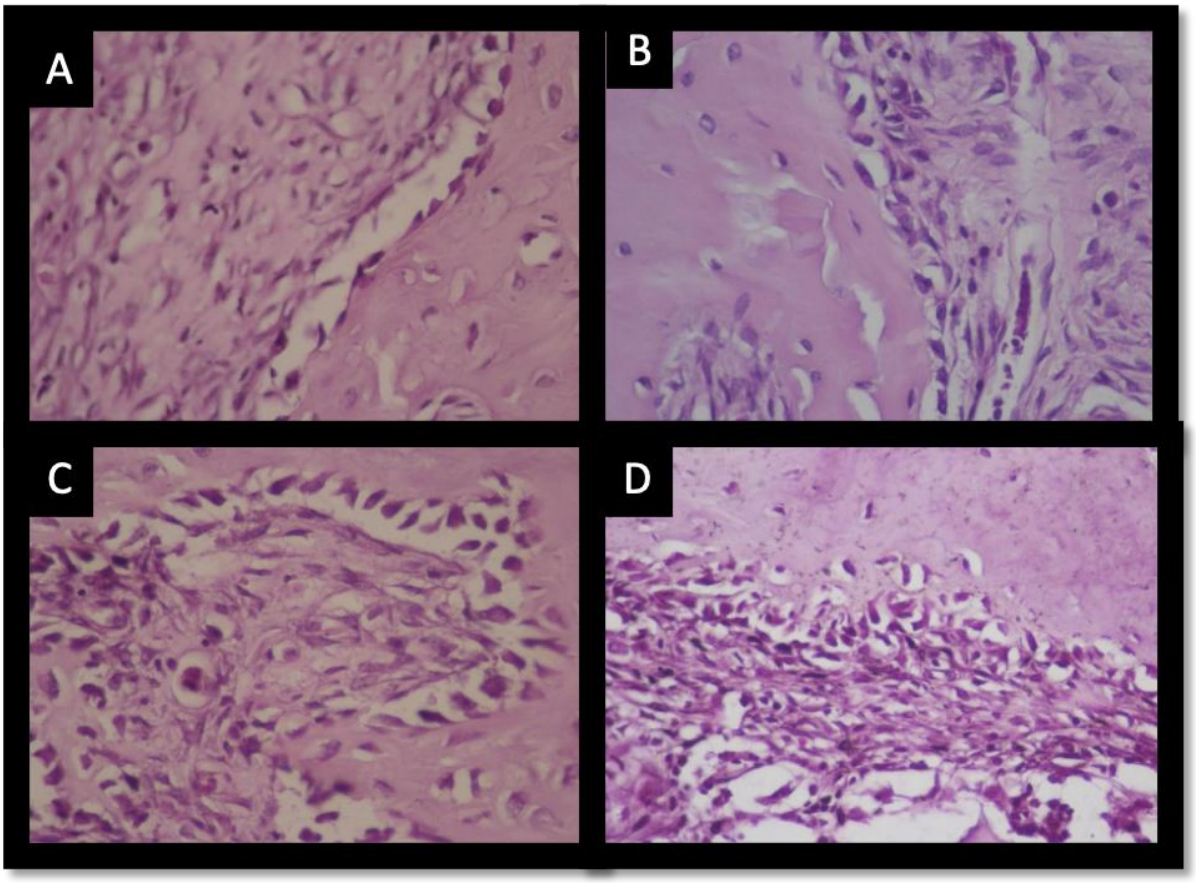

Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

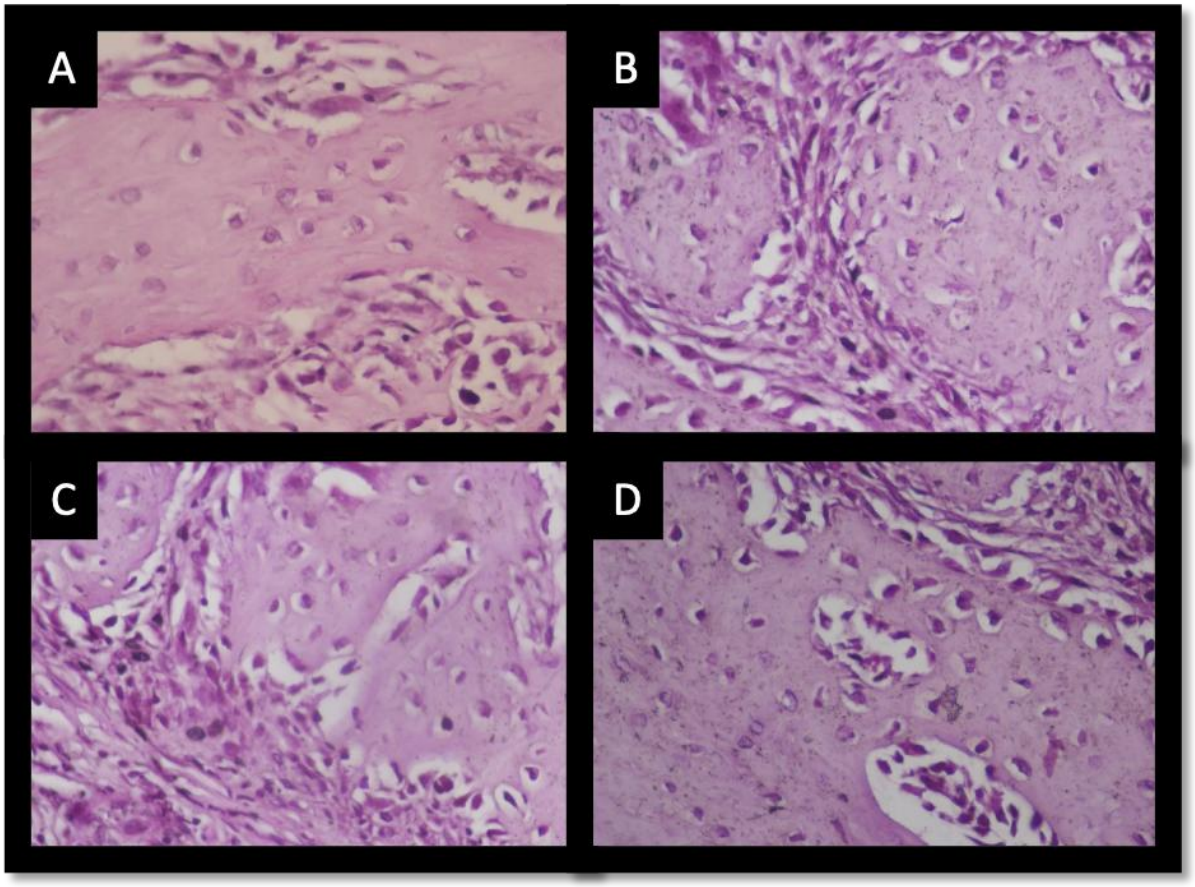

Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

Pemberitahuan revisi ke-3

Dari: Dental Journal (Majalah Kedokteran Gigi) (dental_journal@fkg.unair.ac.id)

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Tanggal: Senin, 19 Juli 2021 pukul 16.22 WIB

Kepada Dr. Michael Josef K. Kamadjaja, drg

Berikut terlampir review ke-3 dari reviewer, mohon merevisi sesuai komentar dan memberi highlight warna pada perubahan yg dilakukan.
Revisi mohon memakai file terlampir dan dikirim kembali paling lambat tanggal 23 Juli 2021.
Terimakasih.

Salam,
Muhammad Dimas Aditya Ari

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Review 3_Evaluation of osteogenic properties after application of Hydroxyapatite-based shells of Portunus pelagicus.docx
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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
Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya – Indonesia

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*

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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.

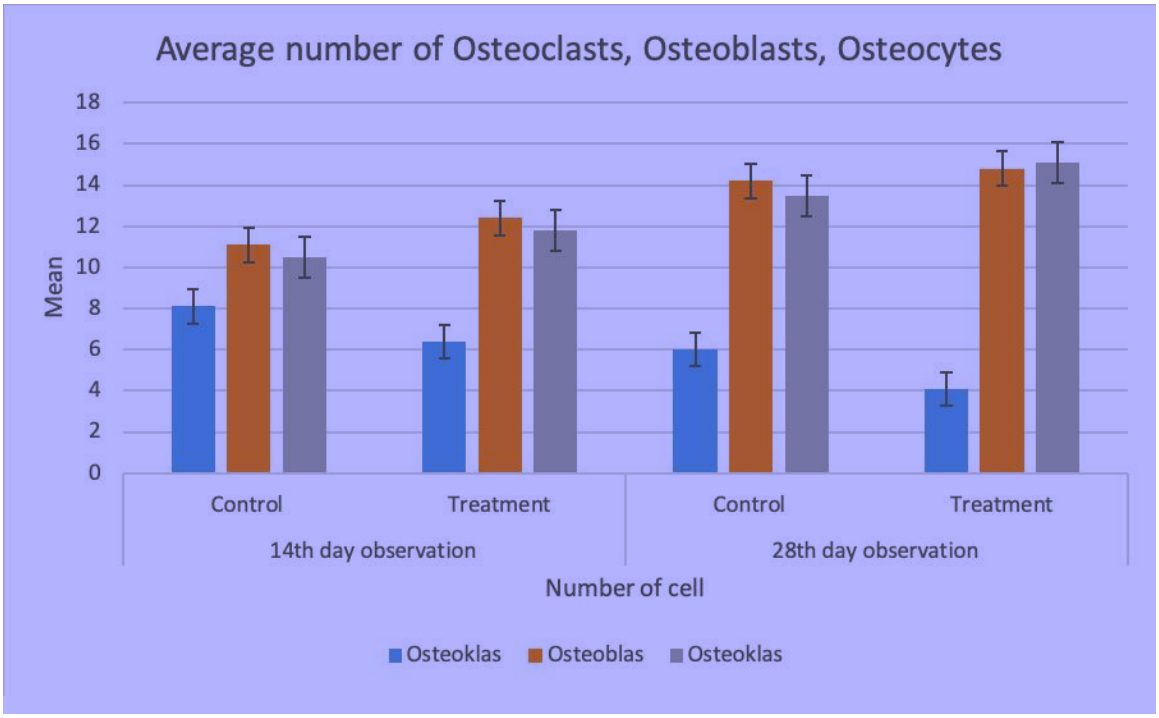

Figure 1. Diagram of the mean number of osteoclasts, osteoblasts, and osteocytes from control and treatment groups.

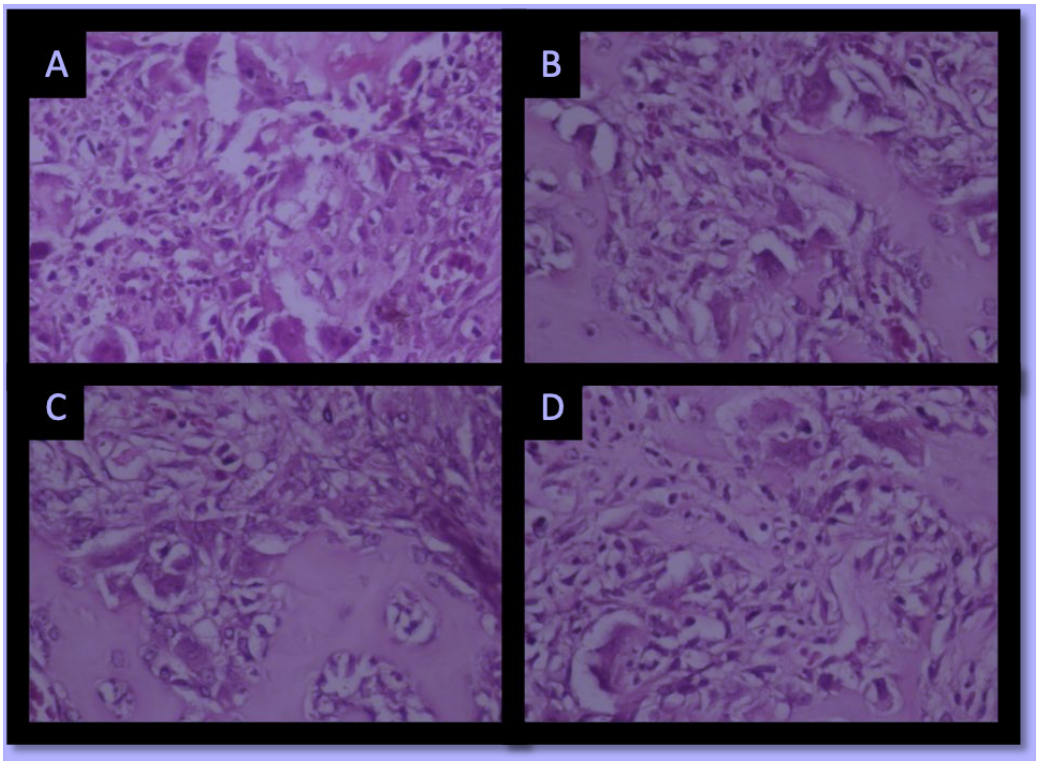

Figure 2. A histological view of osteoclast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

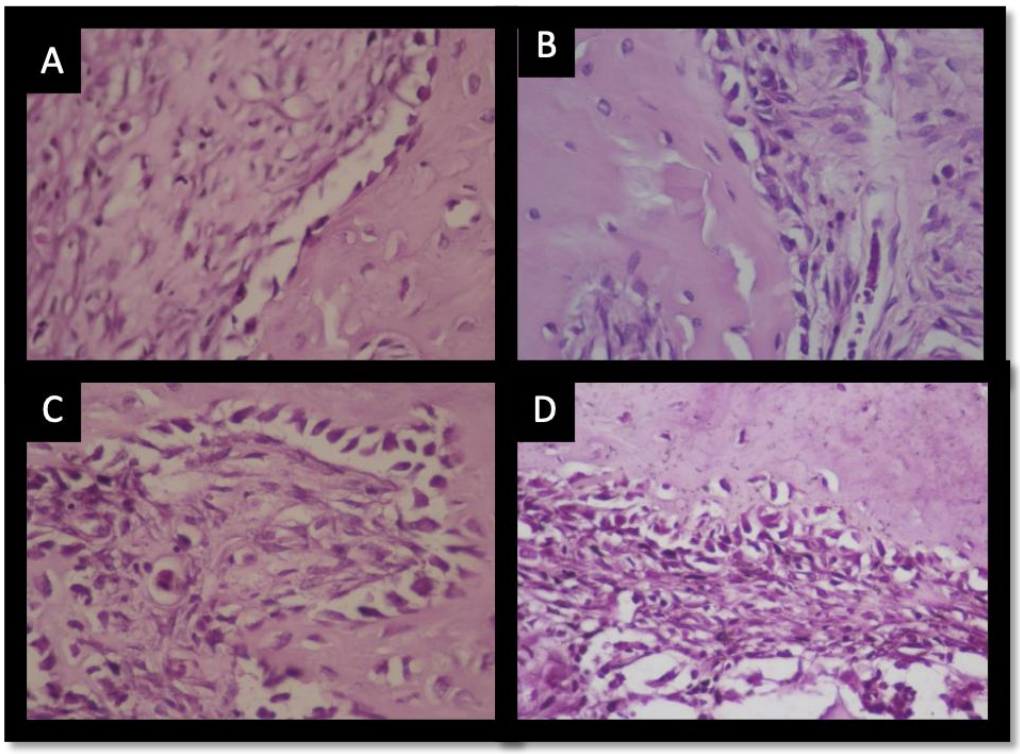

Figure 3. A histological view of osteoblast on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

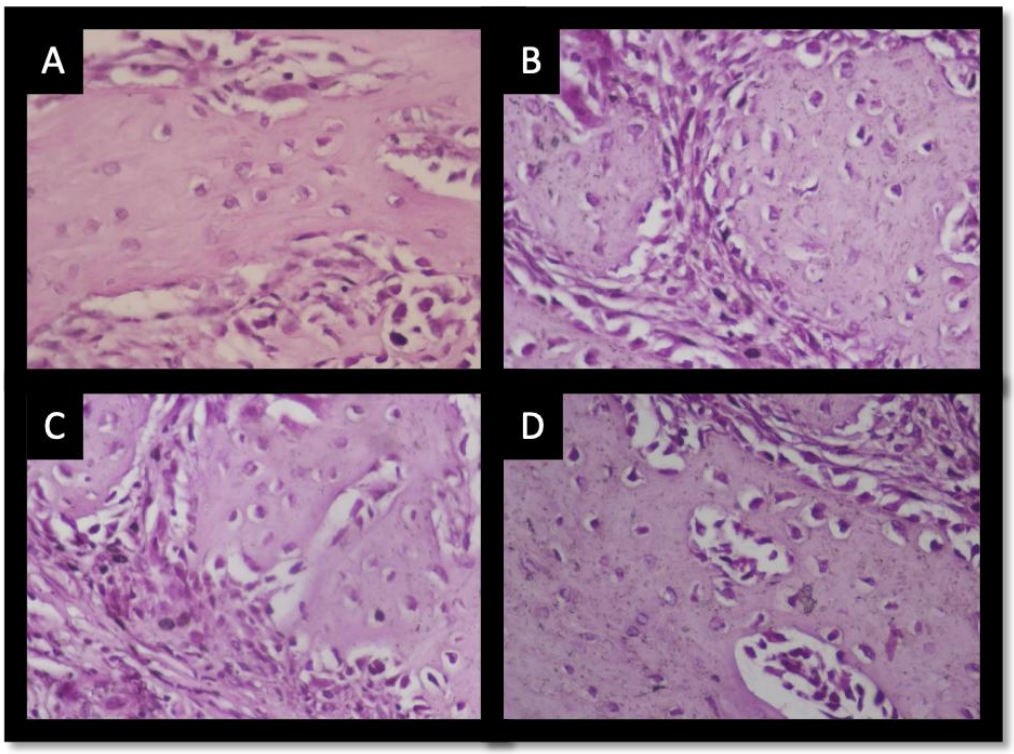

Figure 4. A histological view of osteocyte on 14th(A) and 28th(B) days of control group and 14th(C) and 28th(B) days treatment group.

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 osteoclast[10] 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.

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Kepada: josef_310563@yahoo.com

Tanggal: Rabu, 21 Juli 2021 pukul 08.21 WIB

Michael Josef Kridanto Kamadjaja:

Thank you for submitting the manuscript, "Evaluation of osteogenic properties after application of hydroxyapatite-based shells of Portunus pelagicus" to Dental Journal (Majalah Kedokteran Gigi). With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Acceptance letter

Dari: Dental Journal (Majalah Kedokteran Gigi) (dental_journal@fkg.unair.ac.id)

Kepada: michael-j-k-k@fkg.unair.ac.id; josef_310563@yahoo.com

Tanggal: Selasa, 27 Juli 2021 pukul 15.30 WIB

Dear Dr. Michael Josef Kridanto Kamadjaja,

We have reached a decision regarding your submission to Dental Journal (Majalah Kedokteran Gigi),
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Authors: 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

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Best Regards,

Muhammad Dimas Aditya Ari

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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 ($CaCO3$) can be processed further into hydroxyapatite [$CA5(PO4)3(OH)$].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

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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.

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