Original Article

Utilization Effectivity Of Pearl Oyster Shell (Pinctada Maxima) As Industrial Waste In Pangkep To Osteogenesis Bone Graft To Cavia Porcellus Femur

M.Hendra Chandha[1], Surijana Mappangara[2], Harun Achmad[3], Sri Oktawati[2], Muthmainnah Buddin[4], DanielTetan-El[4], Firman Salam[4], Handayani Halik[5]

[1]Department of Oromaxilofacial Surgery, Faculty of Dentistry, Hasanuddin UniversityMakassar, Indonesia
[2]Department of Periodontology, Faculty of Dentistry, Hasanuddin University Makassar,Indonesia;
[3]Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar,Indonesia;
[4]Periodontology Specialist Educational Program, Faculty of Dentistry, HasanuddinUniversity, Makassar, Indonesia
[5]Hasanuddin medical research center, faculty of Medical, Hasanuddin University, Makassar,Indonesia;
*Corresponding Author: Sri Oktawati; periounhas_sri@yahoo.com Institutional email address: fdhu@unhas.ac.id; web page: http://dent.unhas.ac.id/
DOI: 10.47750/pnr.2023.14.S02.29

Abstract

Background and objective: Pinctada Maxima which is one of the nacres some of the structures of marine biota whose contents can be used to build tooth structures are damaged due to disease. The aim of this study is to see the effectiveness of pearl shells on osteogenesis of bone graft in periodontal tissue and to determine the effectiveness of pearl shells on periodontal tissue through the formation of osteoblasts so that later it can be considered as an ideal alternative bone graft. **Methods**: Cavia Porcellus were divided into 3 groups of 30: Bone graft using Hydroxyapatite Pinctada maxima, Bovine Xenograft as positive control and placebo as negative control, then sacrificed on 14th and 21st days. The Real Time-Polymerase Chain Reaction (RT-PCR) analysis was performed to see the expression of osteocalcin (OCN) and Scan electron microscope (SEM) examination to see the formation of osteoblasts **Results**: Data were analyzed with Independent T test and processed in SPSS 24.0. It was shown that the osteoblast increases in the Batan and Placebo groups from day 14 to day 21, in contrast to the Hydroxyapatite Pinctada Maxima test group with SEM analysis. For the OCN increase in all the Batan, Placebo and HydroxyapatitePinctada Maxima groups and the most significant increase was in the Hydroxyapatite PinctadaMaxima Test group although it was not significant with the RT-PCR analysis. **Coclusion:** Hidroxyapatite pinctada maxima has quite good potential as a bone graft.

Keywords: Osteocalcin, Osteoblast, Pinctada Maxima, Real Time-Polymerase Chain Reaction (RT-PCR), Scan electron microscope (SEM).

INTRODUCTION

Indonesia is one of the world's biodiversity hotspots which has a wide variety of marinesbiota diversity. Medical researchers began to turn their attention to the ocean because they believed the earth's oceans could provide better treatment than chemicals. Some marine biota structures can be used to build tooth structures (bone, dentin, pulp, and periodontal ligament) that are damaged or lost due to disease.[1–3]

Based on the RISKESDAS of the Ministry of Health in 2018, there are 57.6% of the Indonesian population who experience oral health problems, one of which is infection in the periodontal tissue. Severe periodontal disease is characterized by tissue resorption of both alveolar bone and cementum which can result in tooth loss. Spontaneous regeneration does notalways occur, so regenerative therapy is needed that can accelerate healing and

new bone formation. Tissue engineering is a biomedical technology developed to help regenerate limbs that cannot be repaired by the tissue itself.[4–6]

One type of tissue engineering for periodontal regeneration is the application of bone graft. Bone grafts are used to reconstruct intraosseous defects formed by periodontal disease. Bone graft can help bone regeneration through three methods, namely osteoinductive, osteoconductive, and osteogenesis. Broadly speaking, there are four types of bone grafts, namely autograft, allograft, xenograft and alloplastic synthetic material. Autograft is still the main choice in restoring bone defects but it is still very limited so that a replacement bone graftmaterial is needed that can help bone regeneration.[7–10]

To accomplish normal physiological bone remodeling, the proper coupling of bone formation and bone resorption requires direct communication among different bone cells. Cells of the osteoblast lineage (osteoblasts, osteocytes, and bone-lining cells) and bone-resorbing cells (osteoclasts), together with their precursor cells, are organized in specialized units called bone multicellular units.[11,12] The remodeling cycle consists of three consecutive phases: resorption, reversal, and formation. Resorption begins with the migration of partially differentiated mononuclear preosteoclasts to the bone surface where they form multinucleated osteoclasts.[13–15]

In bone damage caused by periodontal disease, the application of graft material is provento be better than the open flap debridement method only. The goal of bone grafting on the periodontal tissue is to reduce pocket depth, improve clinical attachment, fill bone in the defectarea and regenerate new bone, cementum and periodontal ligament so that they can properly support the teeth. Bone substitute is a natural or synthetic material, often containing only a mineralized bone matrix with no viable cells, that is able to achieve the same purpose [8,16–18]

The discovery of dental implants in the skull of the Mayan tribesman was the beginningof a number of studies on clam shells. "Nacre" commonly called "mother of pearl" is part of the clam shell which has the main content of $Ca(CO3)^2$. Nacre is able to facilitate the proliferation of osteoblasts, accelerate the production of extracellular matrix, and mineralization.[19,20] Nacre contains inorganic and organic materials that have a basic structure similar to bone. Research on nacre material as bone graft has been carried out in many countriesusing different species. Pinctada maxima is a type of shellfish that has been cultivated in the Pangkep Islands, South Sulawesi. This species has also been widely studied in several countriesas bone graft material, but there are still limited studies using this species in Indonesia.[2,21–23]

According to the description above, the aim of this study is to see the effectiveness of pearl shells on osteogenesis of bone graft in periodontal tissue and to determine the effectiveness of pearl shells on periodontal tissue through the formation of osteoblasts so that later it can be considered as an ideal alternative bone graft.

METHODS

This study was according to the ARRIVE guidelines for animal pre-clinical research.

Animals

This was experimental laboratory research conducted on guinea pigs (Cavia cobaya). Male Cavia cobaya weighing 250–300 g and aged 2–3 months were utilized. All experimentalprotocols were approved by the Health Research Ethical Committee 0064/PL.09/KEPK FKG-RSGM UNHAS/2022 Before treatment, Cavia cobaya were adapted to a 12-h light/12-h dark cycle and given free access to tap water and standard food for a week. Unhealthy Cavia porcellus were excluded if they lose more than 10% of their body weight after a week of adaption.

Preparation of Hydroxyapatite Bone Graft from Pinctada Maxima

Preparation of pearl oyster shell powder (Pinctada Maxima) were done using the precipitation method. This is done first by cleaning the shell. The shells are brushed clean, thendried using sunlight. The shells were broken into smaller sizes and roasted for 2 hours. The results of the sample were mashed using a mortar so that pearl oyster shell powder was obtained. The powder was tested using AAS to obtain the CaO content. Pearl oyster shell powder was synthesized using H3PO4 compound at a temperature of 100^0C. The solution wasallowed to sit for 24 hours to obtain a hydroxyapatite precipitate. This precipitate was calcinedat a temperature of 800^0C. The synthesis results were characterized using FTIR.

Experimental Procedures

Following the adaptation period, male Cavia cobaya were randomly assigned to one ofthree groups (each with ten Cavia porcellus): (1) Bone graft using nacres from Pinctada maxima, (2) Bovine Xenograft as positive control and (3) placebo as negative control. The guinea pigs were anesthetized using ketamine (20 mg/kg) and Xylazine 10 mg/kg. Furthermore, each guinea pig went through surgery on one of the femurs, the surgical area wasmade with a horizontal incision with a scalpel no. 15 C to reduce bleeding and a full thicknessflap was made. A cavity was made in the femur with a diameter of 3 mm and a depth of 3 mmwith a round bur, and then irrigated using metronidazole infusion. The hole in the femur was given a placebo in the negative control group, pearl oyster shell hydroxyapatite powder in thetreatment group, and BATAN hydroxyapatite material in the positive control group. Suturing was done using absorbable suture (vycril 5.0) on muscle and silk on skin, all suturing were done using Interrupted Suturing technique, then closed with hypafix. Antibiotics and analgesicswere then given. On 14th and 21st day the Cavia Porcellus were sacrificed using ether. The femur was removed and sent Microstructure Laboratory, Faculty of Engineering, Indonesian Muslim University for Scan electron microscope (SEM) analysis to measure the osteoblast cellformation and the other sample was sent to Hasanuddin University HUM_RC Laboratory for RT-PCR analysis to identify of osteocalcin expression which is a marker of osteogenesis. Thedata were analyzed Independent T test data were processed in SPSS 24.0 and displayed in tableand graphs.

RESULT

This research was conducted from March to September 2022 and has been approved by the Ethics Commission Number: 0064/PL.09/KEPK FKG-RSGM UNHAS/2022. The research data were then analyzed with the help of the IBM SPSS Statistic version 21 data analysis program. Shapiro-Wilk test used to determine the normality of the data and Levene's test for the homogeneity of the data. The results of the normality test using Shapiro-Wilk showed thatthe data were normally distributed with a mean value of p> 0.05. The results of the data homogeneity was tested using Levene test, and was found that the variance of the data on day14 and 21 between groups was not homogeneous. The next test is the t-pari test to compare between the groups on day 14 and day 21 formation of osteoblasts the expression of osteocalcin(OCN) and in the femur of Cavia Porcellus

- **Formation of osteoblasts in the femur of Cavia Porcellus with Scan ElectronMicroscope (SEM) examination**

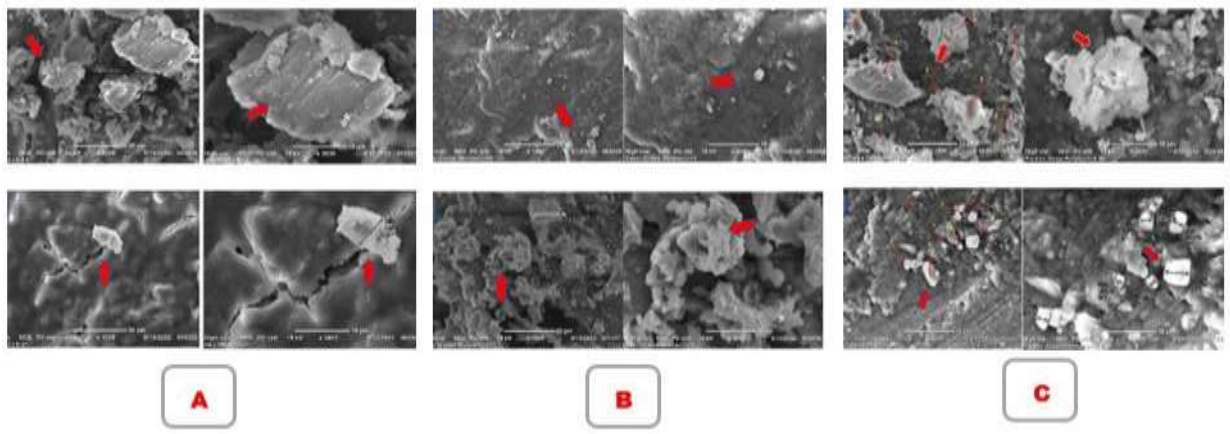


Figure 1. Osteoblast expression on days 14 (picture A) and 21 (picture B)
using a Scan Electron Microscope Scan Electron Microscope (SEM) magnification 1500x and 3000x.

(A) Hydroxyapatite Pinctada Maxima, (B) Batan, (C) Placebo sample marked with red arrows

The additional liquid granules of Hydroxyapatite Pinctada Maxima (A) H-21 amounted to1 grain with a particle size of 7.26 m and there were several cracks on the surface area of the bone scraping, this amount was less than that of Hydroxyapatite Pinctada Maxima (A) H-14, which amounted to 12 grains with an average size of 13.31 m particles. This shows that the length of time/day does not affect the number of grains (particles) on the surface area of the bone scraping for the Hydroxyapatite Pinctada Maxima (A) sample. Additional fluid granules Batan (B) H-21 amounted to 16 granules with an average particle size of 7.03 m on the surfacearea of bone scraping, this amount was more than Batan (B) H-14(picture A), which was 7 granules. This shows that the length of time/day affects the number of grains (particles) on thesurface area of the bone scraping for the Batan (B) sample Additional liquid granules on Placebo (C) H-21 amounted to 9 granules with an average particle size of 2.79 m on the surfacearea of bone scraping, this amount was less than Placebo (C) H-14 which amounted to 11 granules with an average particle size 12.33 m. This shows that the length of time/day does notaffect the number of grains (particles) on the surface area of the bone scraping for the Placebo
(C) sample

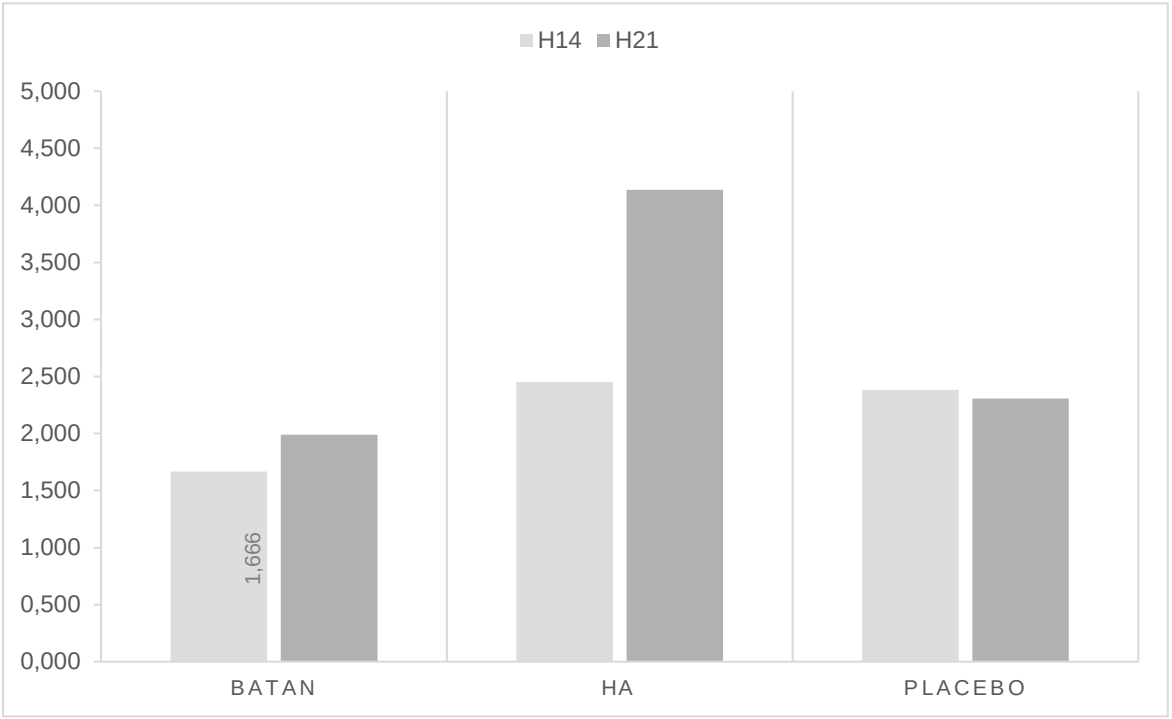
- **Expression of osteocalcin (OCN) The Real Time-Polymerase Chain Reaction (RT-PCR)**

This analysis was performed to see the expression of osteocalcin (OCN) as a biomarkerof osteogenesis in the femur of Cavia Porcellus

Tabel 1. The mean number of OCN expressions in the study group, on RT-PCR observations of the femur defects of guinea pig cavia porcellus on day 14 and day 21

Observation (days)	Variable	(Mean ± SD)
14	Batan	(1.666 + 2.653)
	Placebo	(2.381 + 1.48)
	Hydroxiapatite Pinctada maxima	(2.451 + 2.512)
21	Batan	(1.989 + 2.141)
	Placebo	(2.307 + 1.278)
	Hydroxiapatite Pinctada maxima	(4.135 + 2.77)

Based on the description in table 1, the average OCN expression on day 14 in the HA group had the highest average value (2,451 + 2,512) comparison between study groups showed the Batan group had the lowest average value (1,666 + 2,653). On day 21 the HA group had the highest average score (4.135 + 2.77) comparison between study groups showed the Batan grouphad the lowest average score (1.989 + 2.141)



Grafik 1. The mean number of OCN expressions in the study group, on RT-PCR observations of the femur defects of guinea pig cavia porcellus on day 14 and day 21

This graph shows that OCN expression has increased from day 14 to 21 in the hydroxyapatiteand Batan samples, while showing decrease in the placebo sample. The Hydroxyapatite test sample showed the highest increase, being 2.451 and 4.135 on the 14th and 21st day respectively.

Table 2. Test of different mean OCN levels between study groups, on RT-PCR observations of guinea pig cavia porcellus femur defects on day 14 and 21

Observation (days)	Inter-group variabes	p-value*
14	Batan – Hydroxiapatite Pinctada Maxima	0.644
	Batan - Placebo	0.613
	Hydroxiapatite Pinctada Maxima - Placebo	0.959
21	Batan – Hydroxiapatite Pinctada Maxima	0.208
	Batan - Placebo	0.783
	Hydroxiapatite Pinctada Maxima - Placebo	0.217

Based on the 2-variable difference test table, RT-PCR Osteocalsin observations in the femoraldefects of guinea pig Cavia porcellus. In all study groups on day 14 and day 21, there was no significant p*value> 0.05, which meant that there was no significant difference between the 3observational variables.

DISCUSSION

Hydroxyapatite (HA) as a bone graft has a chemical composition that is almost similarto real bone. HA has both osteoconductive and osteoinductive properties. HA has the characteristics of being biocompatible and bioresorbable. The use of bone grafts to replace lostor damaged bone structures due to defects after trauma or periodontal disease is expected to improve the wound healing process and induce new bone formation. [15,24–26]

Several researchers have conducted material tests in the processing stage of oyster shells so that the processed product still contains high amounts of calcium. The material processing technique that the researchers used in this study was precipitation. From the research that has been done, the mineral composition of shells from 3 different sources is the same for all samples. Calcium carbonate and carbon combined comprise more than 98.7% of the total mineral content.[27–30]

The main factor in bone destruction in periodontal disease is the interaction of bacteriawith the host. Plaque bacterial products cause differentiation of bone progenitor cells into osteoclasts and stimulate gingival cells to release mediators that have the same effect.[31–33] Lipopolysaccharide and other bacterial toxins play a role in immune cells and osteoblasts present in the gingival tissue which secrete IL-1 α, IL-1β, IL-6, prostaglandin E2 and Tumor Necrosis Factor (TNF)- α. These factors regulate osteoclast formation and activity.[5,34]

Osteocalcin (OCN) is the most abundant non-collagenous protein in bone secreted solely by osteoblasts. Most OCN secreted by osteoblasts is incorporated into the organic matrix that will later ossify into bone, however, a small fraction is secreted into the circulation. For this reason, OCN is widely considered a bone formation marker and OCN concentration correlates with direct measurement of bone formation.[35–37] In its carboxylate form it binds calcium directly and thus concentrates in bone. Osteocalcin is used as a marker of the mature osteoblast, also known as a marker ofmatrix mineralization [15,34,38]

In this study, two tests were carried out using Scanning electron microscope (SEM) examination to see the formation of osteoblasts and the Real Time-Polymerase Chain Reaction(RT-PCR) analysis was performed to see the expression of osteocalcin (OCN) as a biomarkerof osteogenesis and Scan electron microscope (SEM) examination to see the formation of osteoblasts in the femur of Cavia Porcellus.

New bone is subsequently maintained through bone remodelling, regulated by continuous cycles of bone resorption and formation. Increases in serum osteocalcin levels are associated with rapid bone loss. In osteoporosis, there is a deficiency of the calcium level and since osteocalcin is known as a calcium-dependent biomarker and has a strong affinity with hydroxyapatite responsible for bone mineralization. Osteoporosis leads to decreased hydroxyapatite crystal formation, and hence, results in an increase in serum osteocalcin levels.[39,40]

From the results of observations of the three treatments, osteoblast expression on drugsand placebo on H-14 was more than H-21, in contrast to Hydroxyapatite Pinctada Maxima onday 14 which was less than H-21 at 1500X magnification, as a previous study by Ismardianitaet al (2017) [41] by using animals sample which proved the increase of osteoblast cell number by day 14. It is also supported by a research by Salim et al (2015) which says that the process of resorption and bone formation in marmots takes about 2-4 weeks [42]

The results of the study by RT-PCT examination showed changes in increased expression level from day 14 to day 21 and Hydroxyapatite Pinctada Maxima showed the highest increase of 2.451 on day 14 and 4.135 on day 21. Osteocalcin is the most abundant noncollagenous protein that constitutes 1–2% of the total matrix proteins. Osteocalcin is a vitamin K-dependent protein and exclusively secreted by osteoblasts. Such research the first helix of OC islocated in the middle region and is resistant to fragmentation because of the structure of the molecules and protease inaccessibility All fragments of OC found in vivo are acidic forms withno amidated fragments reported to date.[42,43]

RT-PCT examination showed increased expression levels from day 14 to 21 and HA Pinctada Maxima

showing the highest increase of 2,451 and 4,135 respectively. Research on the first helix of OC is located in the middle region and is resistant to fragmentation due to structural molecules and protease inaccessibility. All fragments of OC found in-vivo are acidicin forms and there was no amidated fragments found. [44–46]

CONCLUSION

Based on the test results for the content and characteristics of bone graft containing pearl oyster shells (Pinctada maxima), this material contains a high enough element ofHydroxyapatite so that this material has quite good potential as a bone graft.

Scanning Electron Microscope examination in the Batan and Placebo groups in day 14to 21 showed a significant increase of osteoblasts, in contrast to Pinctada Maxima Hydroxyapatite group. Osteocalcin test using RT-PCR examination showed increase in all groups, however the most significant was found in the Pinctada Maxima group, however this was found to be statistically insignificant.

Content and characteristics of bone graft containing pearl oyster shell (Pinctada Maxima) were found to contain high element of Hydroxyapatite therefore making it a suitablecandidate for bone graft.

BIBILIOGRAPHY

1. Suprianto K, Nilam C, Khairiyah N, Amelia R, Siti Rahmadita dan, Periodontologi D, et al. Hidroksiapatit dari cangkang telur sebagai bone graft yang potensial dalam terapi periodontal. Clinical Dental Journal) UGM. 2019;5(3).
2. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone grafts and substitutes in dentistry: A review of current trends and developments. Vol. 26, Molecules. MDPI AG; 2021.
3. Poernomo H. Teknik Bone Tissue Engineering untuk Regenerasi Jaringan Periodontal dan Estetik Pada Edentulous Ridge. Interdental Jurnal Kedokteran Gigi (IJKG). 2019 Dec 27;15(2):56–9.
4. Jitendra Kumar VJ, LastName SK, LastName HP. Journey of Bone Graft Materials in Periodontal Therapy: A Chronological Review. Journal of Dental and Allied Sciences [Internet]. 2016 [cited 2023 Jan 7];5(1):30–4. Available from: https://www.jdas.in/temp/JDentAlliedSci5130-4253161_114851.pdf
5. Bowo H, Ardhiyanto. Peran Hidroksiaoatit Sebagai Bone Graft dalam Proses Penyembuhan Tulang. Stomatognatic. 2011;8(2):118–21.
6. Matuda Y, Okamura T, Tabata H. Periodontal Regeneration Using Cultured Coral Scaffolds in Class II Furcation Defects in Dogs. Journal of Hard TIussue Bilogy [Internet]. 2019 [cited 2023 Jan 7];28(4):329–34. Available from: https://www.jstage.jst.go.jp/article/jhtb/28/4/28_329/_pdf/-char/ja
7. Balaji V, Manikandan D, Ramsundar A. Bone grafts in periodontics. Matrix Science Medica [Internet]. 2020 [cited 2023 Jan 8];4(3):57. Available from: https://www.researchgate.net/publication/342914238_Bone_grafts_in_periodontics
8. Alakpa VE, BUrgess KE v., Chung P, Riehle MO, Gedegaard N. Nacre Topography Produces Higher Crystallinity in Bone than Chemically Induced Osteogenesis. Acs Publication [Internet]. 2017 [cited 2023 Jan 8];11:6717–27. Available from: https://pubs.acs.org/doi/pdf/10.1021/acsnano.7b01044?src=getftr
9. Siddiqui JA, Partridge NC. Physiological Bone Remodeling: Systemic Regulation and Growth Factor Involvement. Physiology [Internet]. 2016 May 1 [cited 2023 Jan 8];31(3):233. Available from: /pmc/articles/PMC6734079/
10. Chaparro O, Linero I, Chaparro O, Linero I. Regenerative Medicine: A New Paradigm in Bone Regeneration. Advanced Techniques in Bone Regeneration [Internet]. 2016 Aug 31 [cited 2023 Jan 8]; Available from: https://www.intechopen.com/state.item.id
11. Mohamed AM. An overview of bone cells and their regulating factors of differentiation. Malays J Med Sci. 2008 Jan;15(1):4–12.
12. Florencio-Silva R, Sasso GR da S, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int. 2015;2015:421746.
13. Siddiqui JA, Partridge NC. Physiological bone remodeling: Systemic regulation and growth factor involvement. Vol. 31, Physiology. American Physiological Society; 2016. p. 233–45.
14. Hadjidakis DJ, Androulakis II. Bone remodeling. In: Annals of the New York Academy of Sciences. Blackwell Publishing Inc.; 2006. p. 385–96.
15. Shankar Ram V, Parthiban, Sudhakar U, Mithradas N, Prabhakar R. Bonebiomarkers in periodontal disease: A review article. Vol. 9, Journal of Clinical and Diagnostic Research. Journal of Clinical and Diagnostic Research; 2015. p. ZE07-ZE10.
16. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL. Regeneration of periodontal tissue: bone replacement grafts. Dent Clin North Am. 2010 Jan;54(1):55–71.
17. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. Molecules. 2021 May 18;26(10).
18. Gharpure AS, Bhatavadekar NB. Clinical Efficacy of Tooth-Bone Graft. Implant Dent. 2018 Feb;27(1):119–34.
19. Dahlan K. Potensi Kerang Ranga sebagai Sumber Kalsium dalam Sintesis Biomaterial Substitusi Tulang. Prosiding Semirata FMIPA Universitas Lampung. 2013;147–51.
20. Rahayu S, Kurniawidi DW, Gani A. Pemanfaatan Limbah Cangkang Kerang Mutiara (Pinctada Maxima) Sebagai Sumber Hidroksiapatit. Jurnal Pendidikan Fisika dan Teknologi. 2018 Nov 8;4(2):226–31.
21. Aghaloo TL, Tencati E, Hadaya D. Biomimetic Enhancement of Bone Graft Reconstruction. Oral Maxillofac Surg Clin North Am. 2019 May;31(2):193–205.
22. Brundavanam RK, Fawcett D, Poinern GEJ. Synthesis of a bone like composite material derived from waste pearl oyster shells for potential

bone tissue bioengineering applications. Int J Res Med Sci. 2017 May 27;5(6):2454.
23. Ni M, Ratner BD. Nacre surface transformation to hydroxyapatite in a phosphate buffer solution. Biomaterials. 2003 Oct;24(23):4323–31.
24. Akbar F, Kusumaningrum R, Jamil MS, Noviyanto A, Widayatno WB, Wismogroho AS, et al. Sintesis Ca2P2O7 dari Limbah Kerang sebagai Bahan Baku Limbah Cangkang Kerang dengan Metode Solvothermal. Jurnal Fisika dan Aplikasinya. 2019 Oct 14;15(3):110.
25. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: Classic options, novel strategies, and future directions. Vol. 9, Journal of Orthopaedic Surgery and Research. BioMed Central Ltd.; 2014.
26. Kapasa E, Giannoudis P, Jia X, Hatton P, Yang X. The Effect of RANKL/OPG Balance on Reducing Implant Complications. J Funct Biomater. 2017 Sep 22;8(4):42.
27. Rahayu S, Kurniawidi DW, Gani A. Pemanfaatan Limbah Cangkang Kerang Mutiara (Pinctada Maxima) Sebagai Sumber Hidroksiapatit. Jurnal Pendidikan Fisika dan Teknologi. 2018 Nov 8;4(2):226–31.
28. H. Silva T, Mesquita-Guimarães J, Henriques B, Silva FS, Fredel MC. The Potential Use of Oyster Shell Waste in New Value-Added By-Product. Resources. 2019 Jan 5;8(1):13.
29. Jung S, Heo NS, Kim EJ, Oh SY, Lee HU, Kim IT, et al. Feasibility test of waste oyster shell powder for water treatment. Process Safety and Environmental Protection. 2016 Jul;102:129–39.
30. Yoon GL, Kim BT, Kim BO, Han SH. Chemical–mechanical characteristics of crushed oyster-shell. Waste Management. 2003 Jan;23(9):825–34.
31. Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun. 1996 Jul;64(7):2371–80.
32. Osta B, Benedetti G, Miossec P. Classical and Paradoxical Effects of TNF-α on Bone Homeostasis. Front Immunol. 2014;5:48.
33. Zhao B. TNF and Bone Remodeling. Curr Osteoporos Rep. 2017 Jun;15(3):126–34.
34. Gani A, Yulianty R, Supiaty S, Rusdy M, Dwipa Asri G, Eka Satya D, et al. Effectiveness of Combination of Chitosan Gel and Hydroxyapatite from Crabs Shells (Portunus pelagicus) Waste as Bonegraft on Periodontal Network Regeneration through IL-1 and BMP-2 Analysis. Int J Biomater. 2022;2022:1817236.
35. Moser SC, van der Eerden BCJ. Osteocalcin-A Versatile Bone-Derived Hormone. Front Endocrinol (Lausanne). 2018;9:794.
36. Zoch ML, Clemens TL, Riddle RC. New insights into the biology of osteocalcin. Bone. 2016 Jan;82:42–9.
37. Ettinger S. Osteoporosis and Fracture Risk. Nutritional Pathophysiology of Obesity and its Comorbidities. 2017;209–34.
38. Glenske K, Donkiewicz P, Köwitsch A, Milosevic-Oljaca N, Rider P, Rofall S, et al. Molecular Sciences Applications of Metals for Bone Regeneration. Int J Mol Sci [Internet]. 2018 [cited 2023 Jan 8];19:1–32. Available from: www.mdpi.com/journal/ijms
39. Kungvarnchaikul I. MMP12-1 Gene Expression Analysis of Osteopontin and Osteocalcin in Maxillary Sinus Floor Augmentation Using Xenograft. NGRC [Internet]. 2021 [cited 2023 Jan 8];22:627–33. Available from: http://www.ncbi.nlm.nih.gov/tools/primer-blast
40. Halai M, Ker A, Meek RD, Nadeem D, Sjostrom T, Su B, et al. Scanning electron microscopical observation of an osteoblast/osteoclast co-culture on micropatterned orthopaedic ceramics. J Tissue Eng. 2014;5:2
41. Ismardianita E, Nasrul E, Yanwirasti Y, Hemiawati M. The effect of ethanol extract of myrmecodia pendens to tgf-β1 expression and osteoblast cells after tooth extraction (the experimental research on cavia cobaya). Journal of Dentomaxillofacial Science. 2017 Dec 1;2(3):150.
42. Agustina N, Dwipura Hasbullah I, Uli Arta Panjaitan F, Hasan Aman G. The Effect of Hydoxyapatite Xenograft of Haruan Fish (Channa Striata) Bone on The Number Of Osteoblast And Osteoclast (In Vivo Study On Mandibular Bone of Male Guinea Pigs). Dentino : Jurnal Kedokteran Gigi [Internet]. 2018 Sep 28 [cited 2023 Jan 8];3(2):116–21. Available from: https://ppjp.ulm.ac.id/journal/index.php/dentino/article/view/5364
43. Bahrambeigi V, Salehi R, Hashemibeni B, Esfandiari E. Transcriptomic comparison of osteopontin, osteocalcin and core binding factor 1 genes between human adipose derived differentiated osteoblasts and native osteoblasts. Adv Biomed Res. 2012;1:8.
44. Rahayu S, Kurniawidi DW, Gani A. Pemanfaatan Limbah Cangkang Kerang Mutiara (Pinctada Maxima) Sebagai Sumber Hidroksiapatit. Jurnal Pendidikan Fisika dan Teknologi. 2018 Nov 8;4(2):226–31.
45. Burkhardt JK, Halama D, Frerich B, Gaunitz F. Real-time RT-PCR discriminating mRNA encoding osteocalcin from unspecific targets. Anal Bioanal Chem. 2009 Feb;393(4):1351–5.
46. Okamura K, Inagaki Y, Matsui TK, Matsubayashi M, Komeda T, Ogawa M, et al. RT-qPCR analyses on the osteogenic differentiation from human iPS cells: an investigation of reference genes. Scientific Reports [Internet]. 2020 Jul 16 [cited 2023 Jan 8];10(1):1–11. Available from: https://www.nature.com/articles/s41598-020-68752-2