Stimulation of Osteoblast and Osteocalcin in the Bone Regeneration By Giving Bonegraft Golden Sea Cucumber

Mardiana Adam[1], Harun Achmad[2*], Mansjur Nasir[3], Sri Wahyu Putri[4], Azizah Azizah[4], Dian Eka Satya[4]

1. Periodontology Department, Faculty of Dentistry Hasanuddin University, Makassar 90245 Indonesia.
2. Pediatric Dentistry Department, Faculty of Dentistry Hasanuddin University, Makassar 90245 Indonesia.
3. Orthodontic Dentistry Department, Faculty of Dentistry Hasanuddin University, Makassar 90245 Indonesia.
4. Periodontology Educational Program of Dentistry Faculty Hasanuddin University, Makassar 90245 Indonesia.

Abstract

Regeneration therapy in periodontal defects and requires a material that is biocompatible, especially in the form of bone graft material, to reduce pocket depth, restore attachment, fill bone defects, and regenerate new bones, cementum, and periodontal ligaments. Golden sea cucumber (Stichopus hermanni) contains various bioactive components that are beneficial for human health.

Objectives is to analyze the process of bone regeneration after the administration of bone graft Golden Sea cucumber through an increase in the number of Osteoblasts and Osteocalcin. This study used raw material in Golden Sea cucumber. After going through the process of deproteinization and maceration, performed XRD and XRF test analysis. The study was an experimental laboratory with the subject being a male guinea pig. The number of samples in this study was thirty guinea pigs. After 14 and 21 days, guinea pig sacrificed for the histological examination and immuno-histochemistry.

Expression of Osteoblasts and Osteocalcin in the Golden Sea cucumber group significantly differ from the positive control group.

Bone graft material from golden sea cucumber has the bone regeneration mechanism as bone graft material, so golden sea cucumber can be used as an alternative material for bone regeneration.

Experimental article (J Int Dent Med Res 2022; 15(1): 140-147)

Keywords: Bone regeneration, golden sea cucumber, stichopus hermanii.

Received date: 12 January 2022 **Accept date:** 13 February 2022

Introduction

Bones can regenerate their cells as part of the process of responding to trauma or infection. Similarly, in the alveolar bone, the presence of an infection that occurs in the alveolar bone will cause severe bone loss if not treated.[1]

Periodontitis is one of the most common diseases of the oral cavity in humans, characterized by damage to supporting tissues of teeth such as alveolar bone, ligament periodontal (PDL) and cementum. Its pathogenesis involves complex interactions between host immune responses to localized microbial colonies in the periodontal section and modifying host factors, including smoking, genes, and systemic disorders such as diabetes, cardiovascular disease, and rheumatoid arthritis.[2,3,4] There are 57.6% of Indonesia's population experiencing oral health problems, one of which is an infection of periodontal tissue, according to the health ministry's RISKESDAS in 2018.[5]

Regeneration therapy in periodontal defects requires *biocompatible* material, especially in the form of Bone Grafting (BG), to lower pocket depth, restore attachment, fill bone defects, and regenerate new bone, cementum, and periodontal ligaments. In periodontal surgery, wound healing patterns consist of primary healing and secondary healing. Successful healing of periodontal wounds after treatment can support optimal periodontal tissue regeneration.[6,7,8]

Indonesia is a country that has high

Corresponding author:
Prof. Dr. Muh. Harun Achmad, drg., M.Kes., Sp.KGA (K)
Pediatric Dentistry Department, Faculty of Dentistry
Hasanuddin University, Makassar 90245 Indonesia.
E-mail: harunachmader@gmail.com

potential marine resources because most of its territory is in the form of waters. One of them is The Golden Sea cucumber *(Stichopus Armani*), better known as gamat, a group of *echinoderms* that have long been used as nutritional ingredients and traditional medicine in Asian countries. Sea cucumber contains active ingredients that have therapeutic properties, antioxidants and can accelerate wound healing.[9,10] Research in Golden Sea cucumber as a drug in dentistry has only developed in the last decade. It is expected that its bioactive compounds can provide new hope for treating various diseases, including as an adjunct therapy in dentistry.[11,12,13,14]

Regenerative Treatment of Periodontal Tissue

Creating a regeneration process from tissue defects is challenging because it requires enough power to maintain its mechanical strength and architectural properties. Using cost-effective and straightforward techniques requires optimal tissue and surfaces for cell attachment, migration, proliferation, and differentiation. Bone regeneration is the process involving many cellular and molecular events similar to bone formation during embryogenesis. Many growth factors released include Transforming Growth Factor-ß (TGF-ß), Bone Morphogenetic Protein (BMPs), Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF),and Platelet-Derived Growth Factor (PDGF).[15,16,17,18]

Regenerative treatment of periodontal tissue should reduce probing depth, clinical attachment strengthening, and radiographic replenishment of bones and defect closure through periodontal regeneration (i.e., formation of root cementum, periodontal ligament, and new alveolar bone). Bone graft materials used in regenerative treatments can come from the same species (Allograft), other species (autografts), synthesis materials, hydroxyapatite (Alloplastic), and from the other species, not human (Xenograft).[19,20,21]

Regeneration in periodontal effect can occur when the remaining bone walls are still preserved and can provide blood supply and mechanical support for the placement of bone fillers at resort sites. Treatment of periodontal tissue bone defects can be an autologous bone graft, allogenic bone graft, and substitute regeneration technique.[22,23,24]

Alveolar bone regeneration follows a series of events, among others:

- Hemostasis and the formation of blood coagulum
- Inflammatory phase
- Angiogenesis: the growth of new cell cells and capillaries

Mesenchymal cell activity, temporary non-mineralization matrix deposition followed by interactive processes involving mineralization, differentiation of bone-forming cells and eventually the formation of new bone o the role of growth and differentiation factors o the process of woven and lamellar bone formation.

Remodeling newly formed bones: The incorporation of osteoclasts and osteoblasts that continues throughout life.[25,26]

The bone graft material is widely used in periodontal surgery for periodontal bone regeneration and is the standard gold for periodontal treatment. Compared to *open-flap debridement* procedures, *bone-replacement grafts* generally increase bone levels and clinical attachment levels and reduce probing depth. Bone grafts play a role in the correction of periodontal bone defects through the process of osteoinduction and osteoconduction. Occoinuctive materials can induce bone formation by activating undifferentiated mesenchymal cells to form osteoblast cells, while osteoinductive materials act as material, new bone formation scaffolding.[27,28,29,30]

Biomarkers of bone remodeling can consist of two types, markers of bone resorption or osteoclasts and markers of bone formation, i.e. osteocalcin produced by osteoblasts and are non-collagen proteins. The process of resorption and bone formation is used as a marker of bone regeneration.[31,32,33,34]

Golden Sea cucumber (*Stichopus hermanni*)

Golden sea cucumber (*Stichopus hermanni)* is one type of marine biota that benefits from regenerating cells, which is often used to heal various diseases. In addition, sea cucumber contains 86.8% protein (functional as a building block that plays a role in the process of growth and maintenance of body tissues), minerals (one of the minerals essential for bone and tooth formation is calcium Ca), mucopolysaccharides, glycosaminoglycans (GAGs), antiseptics, flavonoids, droitin, saponins, hyaluronic acid, omega 3, 6, and 9 as well as

amino acids 21. Phosphorus and calcium contained in Golden Sea cucumber can increase osteoblast and osteoclasts resorption. In addition, the content in sea cucumbers that calcium ions will be used as bone raw materials in osteocytes and ultimately play a role in forming new bone. Calcium metabolism has a dominant role in the process of bone formation.[35,36,37,38,39]

The purpose of this study was to analyze the process of bone regeneration after the bone graft of golden sea cucumber through increasing the number of Osteoblasts and Osteocalcin, as well as produce a natural material that had a bone regeneration effect that can accelerate healing so, it expected to be used as a periodontal tissue regeneration material that can be widely used in the Dentistry.

Materials and methods

This research was a *true experimental* with *post-test* design −*only control group design.* The research was conducted at the Biology Laboratory of Makassar State University to make bone graft of Golden Sea cucumber preparations consisting of two methods, namely: *deproteinized* and maceration and mixed into one until a bone graft preparation is formed from Golden Sea cucumber. After that, XRF analysis tested to see the compounds in the bone graft content of Golden Sea cucumber through X-rays and XRD analysis to see the crystallization structure phase through the peak of crystal diffraction. Pet Shop Lacoste for animal maintenance trials, Laboratory of Anatomical Pathology Faculty of Medicine Hasanuddin University to manufacture osteocalcin and Osteoblast slides, and Laboratories biochemist - Biomolecular Faculty of Medicine, University of Brawijaya Malang for slide reading Osteocalcin and Osteoblast.

After adaption process during seven days, thirty-male guinea pigs were randomly selected and divided into three groups (ten each group), namely group A received (golden sea cucumber's bone graft), group B received (*bovine bone xenograft* (BATAN)) and group C without any treatment. On the eighth day, weight loss was carried out on each group of guinea pigs. Then the manufacture of bone defects in the femur. After 14 days and 21 days, five guinea pig from each group were sacrificing for the next test. This research was approved by the ethical committee of the Faculty of Dentistry, Hasanuddin University.

Making bone-graft Golden Sea cucumber:

De-proteinezed Method:

Preparation of the sample is done by washing sea cucumber from the remaining dirt left behind, then manually drying with the sun directly until dry. After that, it is recommended to dry using an oven.

Sea cucumber is cut into small shapes, and she has it blended to smooth with a size of ± 60 Mesh.

Make bone-graft preparations through the deproteinized method by adding Sodium Hydroxide (NaOH) 3.5% with a ratio of 1:10 (b/vol) to the mashed powder Golden Sea cucumber. After that, heated with an electric stove at a temperature of 65°C while stirring with *a magnetic stirrer* for 2 hours. (Amri, Ilza, & Sumarto, 2018)

Lastly, it should be cooled at room temperature for easy filtration process to obtain the precipitate and drying process in the oven with a temperature of 65°C

The packaging on a tightly sealed sterile bottle

Method of Maceration:

Preparation of the sample is done by washing golden sea cucumber from the remaining dirt left behind, then manually drying with the sun directly until dry. After that, it is recommended to dry using an oven.

Golden sea cucumber is cut into small shapes, and she has it blended to smooth with a size of ± 60 Mesh.

The method of maceration by soaking Golden Sea cucumber powder in NaOH solution for 3 x 24 hours. After that, it is filtered with filter paper. Then evaporated in a water bath to get deposits of extract Golden Sea cucumber.

Treatment of experimental animals:

Guinea pigs were adapted for seven days, on the eighth day, weight scales were carried out on each group of guinea pigs.

Guinea pigs are anesthetized using ketamine (20mg/kg).

Furthermore, the surgical stage was performed on one guinea pig femur, done inside horizontal with scalpel no. 15.

Made a 3 mm profound defect on the femur bone using round bur.

The application of bone graft Golden Sea cucumber was in the treatment group.

Application of bovine bone graft in the positive control group, and saline solution was in the negative control group.

f. Before the wound was closed, antibiotics were given first, then suture using absorbable vycril 5.0.

Results

The normality and homogeneity of the result data was tested using the *Shapiro-Wills* test. The ANOVA one way test is carried out to determine the influence of each test group on the expression of Osteocalcin and Osteoblast cells in the formation of male guinea pigs. The results of ANOVA's one-way analysis showed significant differences between groups (p<0.001).

Table 1 shows the lowest values on group K- on days 14 and 21, namely 5.20 and 8.20, while the highest values are in group K + on day 14, which is 8.40 and group P on day 21, which is 12.60, in addition, the second highest is in group P on day 14, which is 8.20 and group K + on day 21, that is 12.00.

Group	Day	OCN			Value P
		Sample	Mean	SD	
P	14	5	8.20	1.30	0.011*
K+		5	8.40	1.82	
K-		5	5.20	1.48	
P	21	5	12.60	2.07	0.013*
K+		5	12.00	2.35	
K-		5	8.20	1.92	

Table 1. The lowest values on group.

Group	Day	Osteoblast			Value P
		Sample	Mean	SD	
P	14	5	10.00	1.58	0.012*
K+		5	10.20	1.92	
K-		5	6.40	2.07	
P	21	5	13.00	2.24	0.016*
K+		5	12.80	2.28	
K-		5	9.00	1.58	

Table 2. Demonstrate the lowest value is in group K- on.

In table 2 demonstrate the lowest value is in group K- on days 14 and 21, namely 6.40 and 9.00, while the highest value is in group K + on day 14, which is 10.20 and group P on day 21, which is 13.00, in addition to the second highest in group P on the 14th day, that is 10.00 and group K+ on the 21st day, which is 12.80 (Fig1,2).

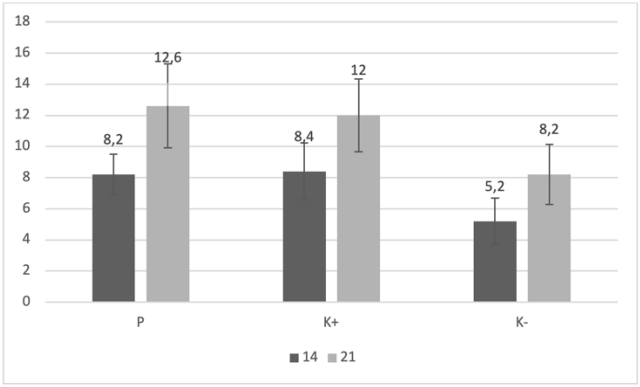

Figure 1. Demonstrate value in group.

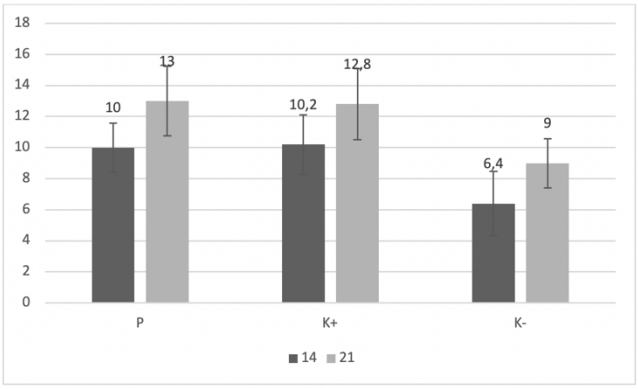

Figure 2. Demonstrate value in group.

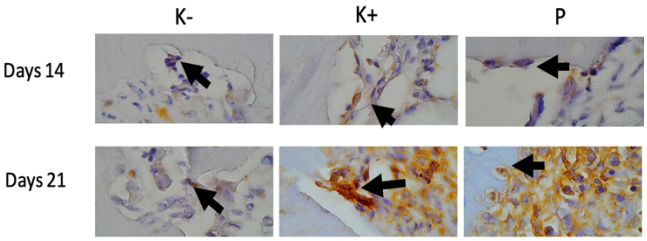

Figure 3. Observations using a light microscope at a magnification of 1000x.

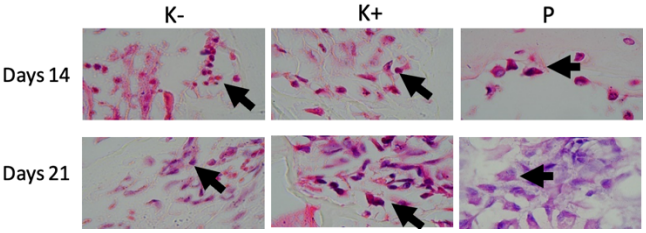

Figure 4. Observations using a light microscope at a magnification of 1000x.

Osteocalcin and Osteoblast cell expression data were obtained from observations on guinea pig bone defects of each group on days 14 and 21. Each tissue sample is made by slice preparations with a thickness of 4 um, then the immunohistochemical examination of

Osteocalcin in Osteoblast cells and histological examination of Osteoblast cells. The expression Osteocalcin indicates a positive reaction to the brown coloring of the cytoplasm, which indicates the reaction of osteocalcin and anti-osteocalcin. Observations using a light microscope at a magnification of 1000x shown in figures 3 & 4.

Discussion

To help the regeneration process in a defect in the bone, the one of strategy is to provide a substitution material in the form of bone graft that has osteogenic, osteoinductive and /or osteoconductive properties. This bone graft material should ideally be biocompatible, easy to shape, well-integrated with the original bone, have good mechanical properties with an ideal replacement rate, and be predictable with a reasonable patient acceptance rate.[41,42,30] In this study, we used materials derived from Golden Sea cucumbers processed into bone grafts. Researchers chosed to use maceration and deproteinization techniques based on previous studies. Isolation and characterization of cellular and extracellular components of a material play a role in the development of molecular markers thought to reflect bone formation or bone resorption, thus aiding in diagnostic and therapeutic assessment.[31,43,44] Research aims to analyze the process of bone regeneration after bone-graft Golden sea cucumber through improving the number of osteoblasts and the expression of Osteocalcin. The results of this study are expected to produce a natural ingredient that has a bone regeneration effect that can accelerate healing so that it can be used as a regeneration material—periodontal tissue.

Several studies on natural ingredients, whether combined with other ingredients or not having good results, when used as bone grafts for bone regeneration, include the research conducted by Ashrin MN (2021) which combined Anadara granosa and gelatin with various concentrations for bone regeneration. The result of this research is that there is a significant difference in the compressive and tensile values of Anadara granosa's scaffold, with the highest value found in the gelatin concentration of 20%. Meanwhile, for the pore size and hydrophobicity of the scaffold which matched bone regeneration biomaterials, the scaffold with the lowest ratio was obtained (ari et al, 2018). The combination of the use of bone grafts and non-absorbable membranes has also been shown to accelerate bone regeneration, where the function of the membrane here is to prevent bacteria from entering the socket or wound.[45,46,47]

Bargowo L (2020) in his research extracted collagen from gouramy fish scales to see its effectiveness in bone regeneration. The result of this research is that there is an increase in osteogenesis and angiogenesis in the process of bone regeneration. In line with the research of Pratiwi et al (2017) that chicken shank collagen scaffold can increase VEGF expression at week 2 during bone regeneration. Another study by Utari kresnadi (2020) which combined propolis extract and bovine bonegraft in tooth extraction sockets, found that TGF-ß expression increased in osteoblast cell formation and reduced IL-1ß expression and decreased the number of osteoclasts in alveolar bone regeneration. The same thing happened to the addition of Platelet Rich Plasma on implant placement can increase bone regeneration on the surface of the implant after 2 weeks of implantation. Nguyen et al (2021) also demonstrated a marked increase in contrast density was observed after 2, 4, 6, and 8 weeks, in which the radiolucent areas were gradually replaced with increased contrast density in the extracted rabbit socket.[48,49,50,51,52]

This study showed that by giving bone graft material Golden Sea cucumber to defects in the bone femur guinea pigs, the number of Osteoblast and expression of Osteocalcin increased significantly. Significant compared to defects that are not treated. Goldis rich in growth factors so that it can repair damaged cells. Protein contained up to 82%, 80% of which is collagen. High collagen content in Golden Sea cucumber plays a role in cell regeneration. Collagen stability will improve inflammation recovery and increase the number of fibroblasts and osteoblasts. It has a beneficial effect on osteogenesis.[53,54,55,42] Osteocalcin, a bone calcium-binding protein, a large part synthesized by osteoblasts, odontoblasts, and condrosits Hypertrophic, plays a role in the resorption and mineralization of bones, and is a specific marker of the bone formation process. Bone biomarkers play an essential role in the diagnosis, monitoring, and discovery of drugs. The ability to monitor health status, disease onset & development and treatment outcomes through noninvasive means is a desirable goal in the promotion and delivery

of health care.[31,56,57,19]

Our results show the excellent effectiveness of bone graft materials because, in addition to organic materials, Golden Sea cucumber also contains an-organic material that plays an anti-organic material. Inflammation plays a role in bone mineralization that can increase bone synthesis for the formation of new bone. In line with research from Safina.et al (2016) stating that the material Golden Sea cucumber emphasis effectively increases the number of Osteoblasts. Golden sea cucumber also contains cell growth factor (GCF), the one of its components is VEGF which plays a role in inducing neovascularization (angiogenesis) and osteoblast differentiation for bone formation in vivo. This is also in line with research conducted by Arundina Ira et al (2016) which stated that the administration of Gold Sea Cucumbers on MSCs can increase the proliferative ability of MSCs and can also increase the ability of MSCs to differentiate into osteoblasts.[39,12,58]

Our results are also in line with previous research by Rima Parwati et al. (2019) stating that the collagen content of golden sea cucumber can help increase the amount of Osteoblast in the bone regeneration process. The study showed that hyaluronic acid contained in golden sea cucumbers can increase TGF-β resulting in cell migration due to the release of various growth factors. This activation, in turn, triggers the proliferation and differentiation of osteoprogenitor cells into osteoblast cells that play an essential role in the formation of the bone matrix.[59,39,60,61]

Based on the results of observations in this study, it is known that the expression of Osteocalcin and the number of Osteoblasts increased in the entire research group. Significant improvement results are seen as the healing process occurs. The osteogenic effect exerted by the bone-graft material of golden sea cucumber is very effective compared with the positive control group and negative control. Lining with Wozney (2002), saying various bone graft materials are used to improve bone ability and bone healing, its relative success and depending on the many factors. More research is needed to augment the osteoinduction effect of golden sea cucumber material in stimulating the bone healing.[62]

Conclusions

The organic and an-organic ingredients of Golden Sea cucumber (*Stichopus hermanni*) extracted and put together into bone grafts can regenerate bone tissue. This can be seen from the accreation of the Osteoblasts and the expression of Osteocalcin in the defect of bone femur guinea pig.

Acknowledgments

Thanks to lectures and staff members of Department of Periodontology, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia for their technical assistance.

Declaration of Interest

The authors report no conflict of interest.

References

1. Hlaing TT, Compston JE. Biochemical markers of bone turnover – uses and limitations Bone formation. 2014;51(2):189–202.
2. Shue li. periodontal Regeneration: Promising and Challenging for Periodontal Complex Regeneration. Wuhan Union Hospital, China; 2017.
3. Slots J. Primer on etiology and treatment of progressive / severe periodontitis : A systemic health perspective. Periodontol 2000. 2020;83:272–6.
4. Hienz SA, Paliwal S, Ivanovski S, Cells B, Homeostasis B. Mechanisms of bone resorption in periodontitis. J Immnology Res. 2015;1–10.
5. tim kementerian kesehatan RI. Laporan Nasional Riskesdas 2018. 2019.
6. Tarnow D. Clinical Periodontology and Implant Dentistry, 5th edition. Vol. 18, Implant Dentistry. 2009. 101 p.
7. Newman MG, H.Tahei H, Klokkevold PR, Carranza FA. Newman and Carranza's Clinical Periodontology , Thirteenth Edition. Elsevier. 2019;1(1):944.
8. Cho YD, Kim KH, Lee YM, Ku Y, Seol YJ. Periodontal wound healing and tissue regeneration: A narrative review. Pharmaceuticals. 2021;14(5):1–17.
9. Motahereh Ghadiri, Soodabeh Kazemi, Behrooz Heidari MR. Bioactivity Of Aqueous And Organic extracts of sea cucumber holothuria lueucospilota (Brandt 1835) on pathogenic candida and streptococci. Aquat Res. 2018;10:32.
10. Ratih pangestuti Z arifin. Medicinal and health benefit effects of functional sea cucumbers. J Tradit Complement Med. 2018;8:341–2.
11. Oedjoe marcelien dj ratoe, Eoh crisca b. Keanekaragaman Timun Laut (Echinodermata: Holothuroidea) di Perairan Sabu Raijua, Pulau Sabu, Nusa Tenggara Timur. J Ilmu Dan Teknol Kelaut Trop. 2015;7(1):309–20.
12. Mulawarmanti D. Biota Laut Sebagai Alternative Bahan Obat (Pemanfaatan Teripang Emas Sebagai Terapi Ajuvan Di Kedokteran Gigi). Pros Semin [Internet]. 2019;1–10. Available from: http://prosidingseminakel.hangtuah.ac.id/index.php/ps/article/view/256
13. Kustiariyah. Teripang sebagai Sumber Pangan dan Bioaktif. J Pengolah Has Perikan Indones. 2010;10(1):1–8.
14. Damaiyanti D, Hangtuah U, Emas T. KARAKTERISASI ESKTRAK AIR TERIPANG EMAS (Stichopus hermanii). 2018;(November).

15. L. Larsson, A.M.Decker, L.nibali, S.P. Pilipchunk, T. Berglundh and WVG. Regenerative Medicine for Periodontal and Peri-implant Diseases. Crit Rev oral Biol Med. 2015;1–12.
16. Dimitriou R, Jones E, McGonagle D, Giannoudis P V. Bone regeneration: Current concepts and future directions. BMC Med [Internet]. 2011;9(1):66. Available from: http://www.biomedcentral.com/1741-7015/9/66
17. Yiting lou, Huiming Wang, Guanchen Ye, Yongzheng li, Chao liu MY and BY. Periosteal Tissue Engineering: Current development and Perspectives. Adv Healthc Mater. 2021;2–5.
18. Rima Parwati Sari, Sri agus Sudjarwo, Retno Pudji Rahayu, Widyasari Prananingrum, Syamsulina Revianti HK and AFB. The effects of Anadara granosa shell-Stichopus hermanni on bFGF expression and blood vessels counts In The Bone Defect Healing Process of Wistar rats. Dent hournal (Majalah Kedokt Gigi). 2017;50(4):194–8.
19. Eli halimah, Wiwik Rositawati IP. N-MID Osteocalcin (N-MID Oc) dan β-Crosslaps (β-CTx) sebagai penanda Biokimia Bone Turn Over pada wanita menopause. J Farm Klin Indones. 2016;5(1):67=74.
20. Kumar P, Vinitha B, Fathima G. Bone grafts in dentistry. J Pharm Bioallied Sci. 2013;5(SUPPL.1):125–7.
21. Jalaluddin M, Mahesh J, Mahesh R, Jayanti I, Faizuddin M, Kripal K, et al. Effectiveness of Platelet Rich Plasma and Bone Graft in the Treatment of Intrabony Defects: A Clinico-radiographic Study. Open Dent J. 2018;12(1):133–54.
22. Tonetti PC& MS. Clinical concepts for regenerative therapy in intrabony defects. Periodontol 2000. 2015;68:282–307.
23. William Anderson AP. Management of Intrabony Defects in Periodontal Disease. Dent Update. 2019;46:257–65.
24. Wu S, Xiao Z, Song J, Li M, Li W. Evaluation of BMP-2 enhances the osteoblast differentiation of human amnion mesenchymal stem cells seeded on nano-hydroxyapatite/ collagen/poly(L-lactide). Int J Mol Sci. 2018;19(8).
25. Giannobile W V, Berglundh T, Araujo BAM, Bartold PM, Bouchard P, Chapple I, et al. Biological factors involved in alveolar bone regeneration Consensus report of Working Group 1 of the 15 th European Workshop on Periodontology on Bone Regeneration. 2019;46(December 2018):6–11.
26. Baharara J, Amini E, Kerachian MA, Soltani M. The osteogenic differentiation stimulating activity of Sea cucumber methanolic crude extraction on rat bone marrow mesenchymal stem cells. Iran J Basic Med Sci. 2014;17(8):626–31.
27. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials. 2018;180:143–62.
28. Yuichi Izumi, Akira Aoki, Yoichi Yamada, Hiroaki Kobayashi, Takanori Iwata, Tatzuya Akizuki, Tomonari Suda, Sayaka Nakamura, Nawarat Wara-Aswapati MU& II. Current and future periodontal tissue engineering. Periodontol 2000. 2011;56:166–87.
29. Hughes FJ, Ghuman M, Talal A. Periodontal Regeneration: A Challenge for the Tissue Engineer. 2010;
30. Ansari M. Bone tissue regeneration, biology, strategy, and interface studies. Prog Biomater. 2019;8:223–37.
31. V. Shankar Ram, Parthiban, Uma Sudhakar, Nimisha Mithradas RP. Bone biomarkers in Periodontal Disease : A Review Article. J Clin Diagnostic Res. 2015;9:7–10.
32. Owen R, Reilly GC. In vitro models of bone remodelling and associated disorders. Front Bioeng Biotechnol. 2018;6.
33. Thahir H, Oktawati S, Gani A, Mappangara S, Cangara MH, Patimah, et al. The effectiveness bone graft of snakehead fish bones (Channa striata) in the gelatin form on the osteocalcin (ocn) expressions. Int J Pharm Res. 2020;12(2):4365–9.
34. Hadi A, Roelianto M, Subiyanto A, Yuanita T. Nuclear Factor of Activated T cells c -1 (NFATc-1) and Osteocalcin Expression in Periapical Bone Destruction Due to Enterococcus faecalis INDUCTION. Conserv Dent J. 2017;7(2):138–44.
35. Suryaningrum TD. teripang: Potensinya sebagai bahan NUTRACEUTICAL dan TEKNOLOGI PENGOLAHANNYA. Squalen Bull Mar Fish Postharvest Biotechnol. 2008;3(2):63.
36. Putri A, Prameswari N, Handayani B. Pengaruh Teripang Emas (Stichopus Hermanii) pada Remodeling Ekspansi Sutura Maksila Terhadap Lebar Palatal Menggunakan Analisis Sefalometri (Effect of Stichopus Hermanii to Palatal Width on Maxillary Suture Expansion using Cephalometric Analysis). :82–9.
37. Sandana IKI, Velisia J, Yunior A, Brahmanta A, Prameswari N. Potensi gel Stichopus hermanii dan Hyperbaric Oxygen Therapy untuk mempercepat perawatan ortodonti Potential of Stichopus hermanii gel and Hyperbaric Oxygen Therapy in accelerating orthodontic treatment . J Kedokt Gigi Univ Padjadjaran. 2017;29(3).
38. Wijaya S, Prameswari N, T ML. PENGARUH PEMBERIAN GEL TERIPANG EMAS TERHADAP REMODELING TULANG PERGERAKAN GIGI ORTODONTI (The Effect of Stichopus hermanii Gel on The Number of Osteoclast in the Pressure Area Bone Remodeling Ortodontic Tooth Movement). :2–6.
39. Majdina S, Mulawarmanti D, Rizka Y. Efektifitas Kombinasi Terapi Oksigen Hiperbarik dan Gel Teripang Emas (Stichopus hermanii) terhadap Peningkatan Jumlah Osteoblas pada Tikus Diabetes Melitus yang Diinduksi Bakteri Porphyromonas gingivalis. Denta. 2016;10(1):30.
40. Amri, Ilza M, Sumarto S. Characteristics of Chitosan Quality from Skin Sea Cucumber (Holothuria scabra) With Different Heating Time. Period J Terubuk Fish [Internet]. 2018;46(1):1–10. Available from: https://www.scribd.com/document/432903906/5912-12155-1-SM#download
41. Tri Cahyo Wahyudi, Irza Sukmana SS. Potensi Pengembangan Material Implan Tulang Hidroksiapatit Berbasis Bahan Alam Lokal. 2019.
42. Sheikh et al. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications; A Review. Biomed Res Int. 2017;21(9).
43. Adam M, Thahir H, Achmad H, Putri SW, Satya DE. The Potential of Golden Sea Cucumber (Stichopus hermanii) in the Regeneration of Periodontal Tissues : a Literature Review. 2021;25(6):4407–18.
44. Richard akin, Dahlia Herawati K murdiastuti. Pengaruh Penambahan Asam Hialuronat pada DFDBX Terhadap Keberhasilan Perawatan Kerusakan Intraboni. J Kedokt gigi. 2014;5(3):298–9.
45. Meinar Nur Ashrin, Widyasri Praningrum, Fitria Rahmitasari, Teofilus Timon Lirungan, Ryski Dea Citra Anindita RPS. Mechanical Evaluation of Anadara Granosa Scaffold with Various Gelatin Concentrations for Bone Regeneration. J Int Dent Med Res. 2021;14(2):514–8.
46. Ari MDA, Yuliati A, Rahayu RP, Saraswati D. The differences scaffold composition in pore size and hydrophobicity properties as bone regeneration biomaterial. J Int Dent Med Res. 2018;11(1):318–22.
47. Nilminie Rathnayake, Branko Trajkovski, Betul Rahman G-GZ. Clinical Applications and Outcomes of non-resorbable Polytetrafluoroethylene (PTFE) Membranes in Guided Bone Regeneration: Review. J Int Dent Med Res. 2019;12(4):1626–35.
48. Bargowo L, Airlangga U, Airlangga U, Brawijaya U. Vascular Endothelial Growth Factor and Bone Morphogenetic Protein Expression after Induced by Gurami Fish Scale Collagen in Bone Regeneration. J Int Dent Med Res. 2020;14(1):141–4.
49. Pratiwi AR, Yuliati A, Ariani MD. Vascular endothelial growth factor expression after induced by chicken shank collagen scaffold in bone regeneration. J Int Dent Med Res. 2017;10(2):333–7.
50. Kresnoadi U, Setio T, Prabowo Y, Airlangga U, Airlangga U. Expression of Interleukin-1β and TGF-B due to Induction with Natural Propolis Extract and Bovine Bone Graft Combination in Tooth Extraction Sockets Leading to Alveolar Bone Regeneration. J Int Dent Med Res. 2020;13(3):935–8.
51. Utama MD, Surya ES, Tetelepta R, Hasminar, Susaniawaty Y, Catarina Anita K, et al. The histomorphometric analysis of initial bone regeneration with platelet -rich- plasma post implantation. J Int Dent Med Res. 2019;12(1):42–8.
52. Nguyen TDT, Le MH, Tran TT, Bae TS, Nguyen-Thanh T.

Establishment and Characterization of Rabbit Model for Alveolar Bone Regeneration. J Int Dent Med Res. 2021;14(3):887–95.
53. Ira Arundina, Ketut Suardita, Hendrik Setiabudi MDA. Golden Sea Cucumbers (Stichopus Hermanii) as Growth Factors of Stem Cells. J Int Dent Med Res. 2016;9(3):242–8.
54. Noengki Prameswari ABR. The Effect of Stichopus hermanii to TLR-4 in Mediating Periodontal Ligament Remodeling During Orthodontic Relapse. Syst Rev Pharm. 2020;11(3):667–73.
55. Endang Wahyuningtyas, Ling Chuan Hsu, Wen Chien Lan, Shih Cheng Wen, Keng Liang Ou, Hsin Hua Chou MSH& ES. Application of a Promising Bone Graft Substitute in Bone Tissue Regeneration: Characterization, Biocompatibility, and In vivo Animal Study. Biomed Res Int. 2019;1–7.
56. J. E., Aubin EB. Osteoprotegerin and its Ligand : A New Paradigm for Regulation of Osteoclastogenesis and Bone Resorption. Osteoporos Int. 2000;11:905–13.
57. Rucci N. Molecular biology of bone remodelling IO RN IN RN. 2008;5(1):49–56.
58. Arundina I, Suardita K, Setiabudi H, Ariani MD. Golden sea cucumbers (Stichopus Hermanii) as growth factors of stem cells. J Int Dent Med Res. 2016;9(3):242–8.
59. Sari RP, Kurniawan H. Effectiveness of Anadara granosa shell-Stichopus hermanni granules at accelerating woven bone formation fourteen days after tooth extraction. Dent J (Majalah Kedokt Gigi). 2019;52(4):177.
60. Mills JC. Hyaluronan , inflammation , and breast. 2015;6(June):1–12.
61. Krejner-bienias A, Gauto AR, Grzela T. Hyaluronic acid i inflammation and tissue regeneration. 2016;(January 2019).
62. Wozney JM. Overview of Bone Morphogenetic Proteins. 2002;27(16):2–8.