31st IADR-SEA 28th SEAADE

31st IADR-SEA (International Association for Dental Research, South-East Asia Division)

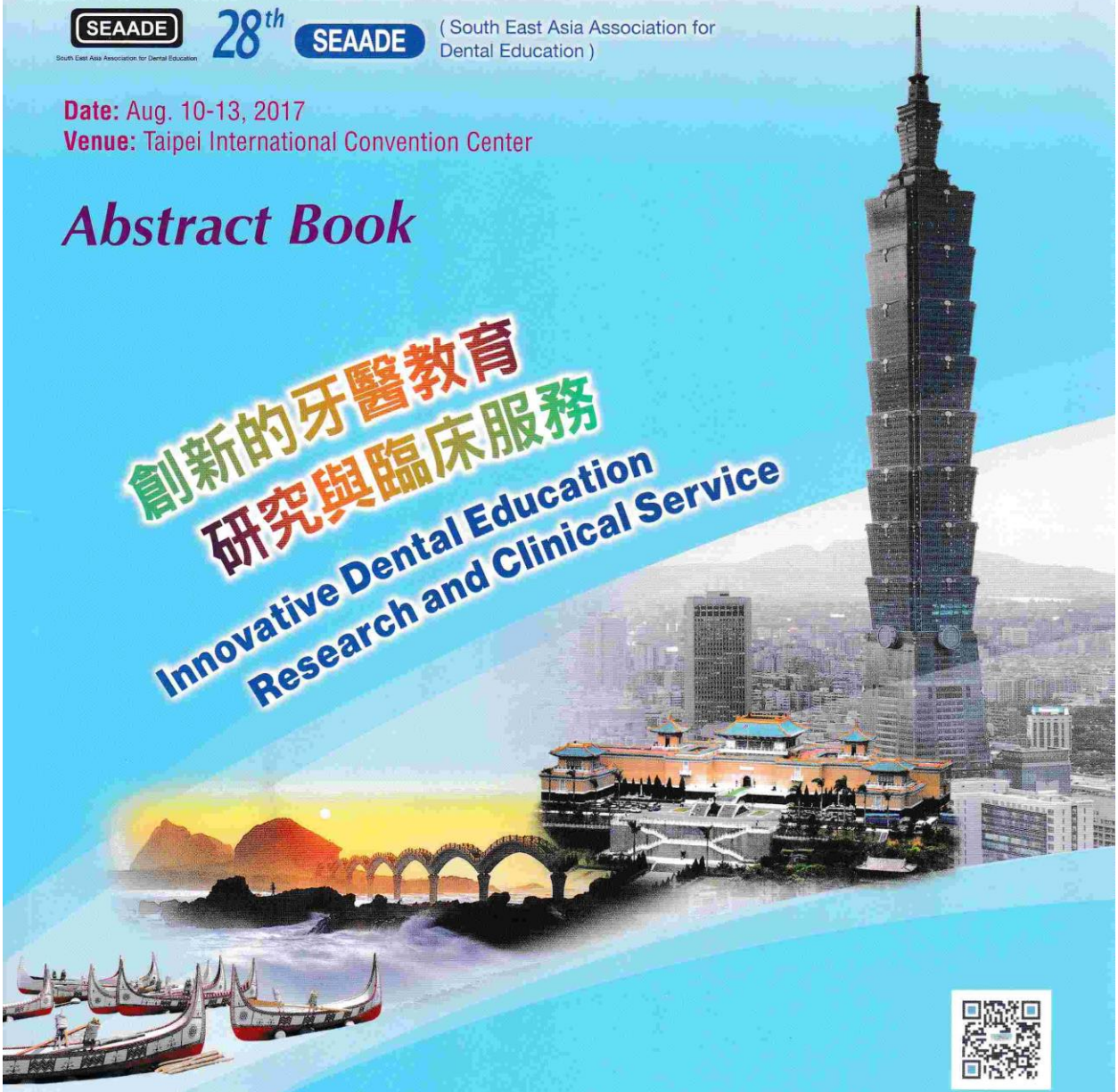
28th SEAADE (South East Asia Association for Dental Education)

Date: Aug. 10-13, 2017
Venue: Taipei International Convention Center

Abstract Book

創新的牙醫教育 研究與臨床服務

Innovative Dental Education Research and Clinical Service



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Message from the President of Chinese Taipei Association for Dental Sciences

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The Association for Oral Biotechnology and Medical Devices (TAPO)

Distinguished Guests, Ladies and Gentlemen:

On behalf of the Chinese Taipei Association for Dental Sciences (CTADS), I heartily welcome you to the 31st IADR-SEA and 28th SEAADE on August 10-13, 2017 in TICC Taipei, Taiwan. The CTADS is honored to have the privilege of hosting the Conference of IADR-SEA & SEAADE and promises to make it an exciting and memorable experience for all participants.

The theme is **"Innovative Dental Education, Research and Clinical Service"**. You will realize how the CTADS continues to organize the highest-quality dental scientific and educational meetings in Taiwan, also how important it is to the continuing education for innovation in dentistry, conducting researches and clinical practices. Our intent is not only to provide a platform for exchanging experiences and expertise in dentistry, but also for a channel to communicate with professionals from all around the world.

I am honored and delighted to welcome you to this beautiful Formosa Island. Taipei has everything you would look for in an international city, from its safe environment for visitors to its convenient transportation network and friendly people with hospitality.

I hope Taipei will become a city of acceptance and inclusiveness, bringing together innovation, sincerity, and thoughtfulness in a unique culture of happiness. I wish you all a wonderful time in the conference in Taiwan.

林俊彬

Chun Pin Lin

Chun-Pin Lin, DDS, MS, PhD, FICD

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The Effects of Blood Cockle's Shell and Golden Sea Cucumber on Osteoblast-Osteoclast in Vivo

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Objectives: The complication of tooth extraction, periodontal disease, pathological conditions can followed by the alveolar bone loss. Calcium carbonate derived from blood cockle shells has an osteoconductive properties. On the other hand, golden sea cucumber contains hyaluronic acid can stimulate the healing process by decreasing the pro-inflamatory cytokines. We have developed the sea cucumber and calcium carbonate from blood cockle shells for biomedical application. This study aimed to determined the effects of combination blood cockle shell and sea cucumber on osteoblast and osteoclast's number in vivo.

Methods: 25 male wistar rats were divided into 5 groups: Group 1= sham; Group 2= blood cockle's shell; Group 3= blood cockle's shell and sea cucumber 0.4%; Group 4= blood cockle's shell and sea cucumber 0.8%; Group 5= blood cockle's shell and sea cucumber 1.6%. The graft material were implanted into rat femur for 14 days. Thereafter, histological analysis were perfomed and the number of osteoclast and osteoblast were evaluated. The statistical anaysis done by Anova One-Way, LSD, $p<0.05$.

Results: The average number of osteoblast showed in Group 1 (9.7±3.6), Group 2 (20.7±0.5), Group 3 (32.46±0.557), Group 4 (40.73±0.894) and Group 5 (49.5±1.178). On the other hand, the average number of osteoclast showed in Group 1 (12.5±2.8), Group 2 (4.2±2.1), Group 3 (3.8±0.8), Group 4 (2.8±1.3) and Group 5 (2.0±0.9). In the comparison between the groups, there were significant differences for the number of osteoblast and osteoclast.

Conclusions: It was revealed from our study that the combination graft material that contain with blood cockle's shell and sea cucumber 1.6% has a good effect on the number of osteoblast and osteoclast in vivo.

The effects of blood cockle's shell and golden sea cucumber on osteoblast-osteoclast in vivo

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ABSTRACT

Objectives: The complication of tooth extraction, periodontal disease, pathological conditions can followed by the alveolar bone loss. Calcium carbonate derived from blood cockle shells has an osteoconductive properties. On the other hand, golden sea cucumber contains hyaluronic acid can stimulate the healing process by decreasing the pro-inflamatory cytokines. We have developed the sea cucumber and calcium carbonate from blood cockle shells for biomedical application. This study aimed to determined the effects of combination blood cockle shell and sea cucumber on osteoblast and osteoclast's number in vivo. **Methods:** 25 male wistar rats were divided into 5 groups: Group 1= sham; Group 2= blood cockle's shell; Group 3= blood cockle's shell and sea cucumber 0.4%; Group 4= blood cockle's shell and sea cucumber 0.8%; Group 5= blood cockle's shell and sea cucumber 1.6%. The graft material were implanted into rat femur for 14 days. Thereafter, histological analysis were perfomed and the number of osteoclast and osteoblast were evaluated. The statistical anaysis done by Anova One-Way, LSD, $p<0.05$. **Results:** The average number of osteoblast showed in Group 1 (9.7±3.6), Group 2 (20.7±0.5), Group 3 (32.46±0.557), Group 4 (40.73±0.894) and Group 5 (49.5±1.178). On the other hand, the average number of osteoclast showed in Group 1 (12.5±2.8), Group 2 (4.2±2.1), Group 3 (3.8±0.8), Group 4 (2.8±1.3) and Group 5 (2.0±0.9). In the comparison between the groups, there were significant differences for the number of osteoblast and osteoclast. **Conclusions:** It was revealed from our study that the combination graft material that contain with blood cockle's shell and sea cucumber 1.6% has a good effect on the number of osteoblast and osteoclast in vivo.

Keywords: blood cockle's shell, golden sea cucumber, osteoblast, osteoclast, in vivo.

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INTRODUCTION

The complication of tooth extraction, periodontal disease, pathological conditions can be followed by alveolar bone loss. The bone healing process phase consists of inflammatory, reparative, and remodeling phases.[1] Tooth removal causes the alveolar socket to be filled by a blood clot replaced by granulation tissue

within 1 week. The second week the sockets begin to be covered by blood vessels and young inflammatory cells.[2] The components of bone cells consist of four cell types, namely osteoprogenitor, osteoblasts, osteocytes and osteoclasts.[3]

Bone remodeling occurs due to osteoclast precursor cells differentiating into osteoclasts after receiving signals from osteoblasts. Increased osteoblasts will produce two ligands, the ostoprogenitor ligand (OPG) and the Receptor Activator of Nuclear Factor Ligand (RANKL) ligand as a receptor counterweight and compete with the Receptor Activator of Nuclear Factor (RANK).[4] Mature Osteoclasts synthesize proteolytic enzymes that digest collagen matrix.[5] Bone resorption is affected by osteoclast activating factors, ie prostaglandins, endotoxin bacteria, and activator complement products consisting of cytokines, Interleukin-1 (IL-1), Tumor Necrosis Factor α (TNF-α), Interleukin-6 (IL-6) and Interleukin-11 (IL-11) osteoclast cell formation process (osteoclastogenesis) osteoclast differentiation factor bonding occurs with the receptor. Increased osteoclastogenesis process results in bone resorption.[1] Day 14th, the transplant on the socket shows graft particles covered in connective tissue and coated by nucleated cells when the unsolved portion has already shown the formation of new bone plates to occupy a portion of the socket.[6]

Improvement of bone using bone graft material is expected to have better clinical improvement in bone compared with the usual surgical procedure without the addition of graft material. The bone graft material must have three basic functions, ie osteogenesis, osteokonduksi, and osteoinduction, must be bioactive and biocompatible to the body, have good mechanical properties, and are easily manipulated.[7] In this study using xenograft because of the large amount of bone obtained, osteoconduct, biocompatible in stabilizing blood clotting and revascularization resulting in osteoblast displacement.[8] This study uses ceramic material because it is able to be absorbed by the body and heal itself without the rest of foreign matter.[9]

Blood clams (Anadara granosa) are one of the most popular types of shellfish in Southeast Asia, have important economic value and have a fairly high protein composition.[10] Calcium carbonate in the blood shell plays a role in increasing the production of BMP (Bone Marrow Puncture) in the body. BMP turns the surrounding cell into an active osteoblast. The calcium carbonate content of the shells is biocompatible, osteoconductive and biodegradable.[11] The healing process due to bone damage can be maximized with other active ingredients ie sea cucumbers.[12] Sea cucumbers (Stichopus hermanni) can increase osteoblast activity due to the glycosaminoglycan (GAG) sulfate content comprising chondroitin sulfate 1,72%, dermatan sulfate 1,11%, 2% sulfuric sulfate and non-sulfated GAG of hyaluronic acid (AH) with the highest content of 75,7% .[13,14,15] Hialuronic acid (AH) is also able to influence velocity cell migration in wound healing, inflammation, angiogenesis, reepitalization and cell proliferation.[16]

Based on this, golden cockroaches and blood clams are known to help in the healing phase of bone, so researchers want to know the effectiveness of combination of blood clams with golden sea cucumber gel concentration 0.4%, 0.8%, 1.6% against the number of osteoclasts in the process bone healing. This study aimed at the combination of blood cockle shell and sea cucumber on osteoblast and osteoclast's number in vivo.

MATERIALS AND METHOD

This study was true eksperimental laboratories study. 25 male wistar rats were divided into 5 groups: Group 1 (G1) = sham; Group 2 (G2) = blood cockle's shell; Group 3 (G3) = blood cockle's shell and sea cucumber 0.4%; Group 4 (G4) = blood cockle's shell and sea cucumber 0.8%; Group 5 (G5) = blood cockle's shell and sea cucumber 1.6%. Each rat was anesthetized with 0.1 cc / 100 grBB ketamine

mixture and 0.01cc / 100 grBB xylazine, then the femur mice was shaved and isolated with 10% povidone iodine for 5 min, then femur defect was made.

The femur defect was filled with blood shell powder. The graft material were implanted into rat femur for 14 days. For preparing the blood shell powder, the shell of blood clams brushed and cleaned the outside and inside and then dried. After that, the shell of the blood shell was crushed and put into the crussible for the calcining process to form a blood shell powder.

The golden sea cucumber gel administrated topically to 0.01ml Wistar mice given once daily for 7 days on bone defects. For preparing the golden sea cucumber gel, golden sea cucumber was washed under running water and removed the contents of the stomach then dried with freeze dryer method and blended so that the powder is dissolved with 2% CMC Na to become golden cucumber gel by 0.4%, 0.8%, 1.6% .

Thereafter, histological analysis were perfomed and the number of osteoclast and osteoblast were evaluated. The statistical anaysis done by Anova One-Way, LSD, $p<0.05$.

RESULTS

Tabel 1. The mean and deviation standard of osteoblast on day 14th

Group	Mean ± Dev Std
G1	9.73 ± 3.57
G2	20.66 ± 0.527
G3	32.46 ± 0.557
G4	40.73 ± 0.894
G5	49.5 ± 1.178

Group 1 (G1) = sham; Group 2 (G2) = blood cockle's shell; Group 3 (G3) = blood cockle's shell and sea cucumber 0.4%; Group 4 (G4) = blood cockle's shell and sea cucumber 0.8%; Group 5 (G5) = blood cockle's shell and sea cucumber 1.6%.

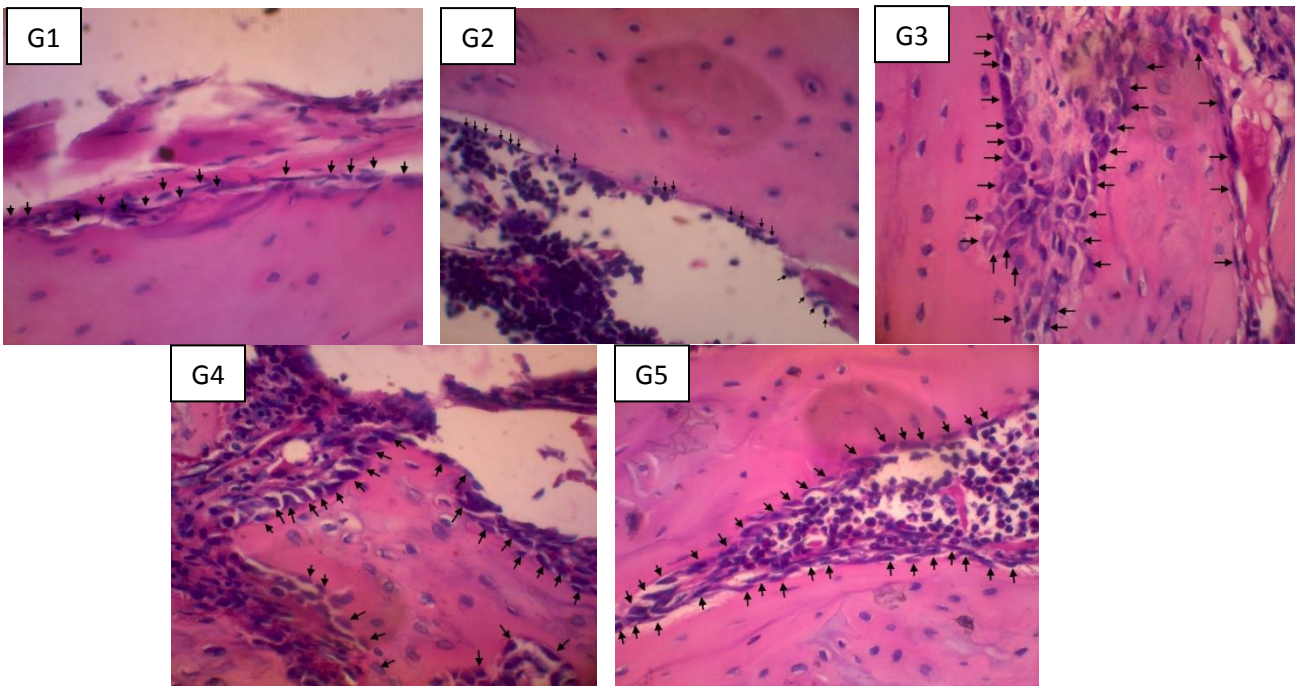

Figure 1. Histological images of osteoblast on the day 14th

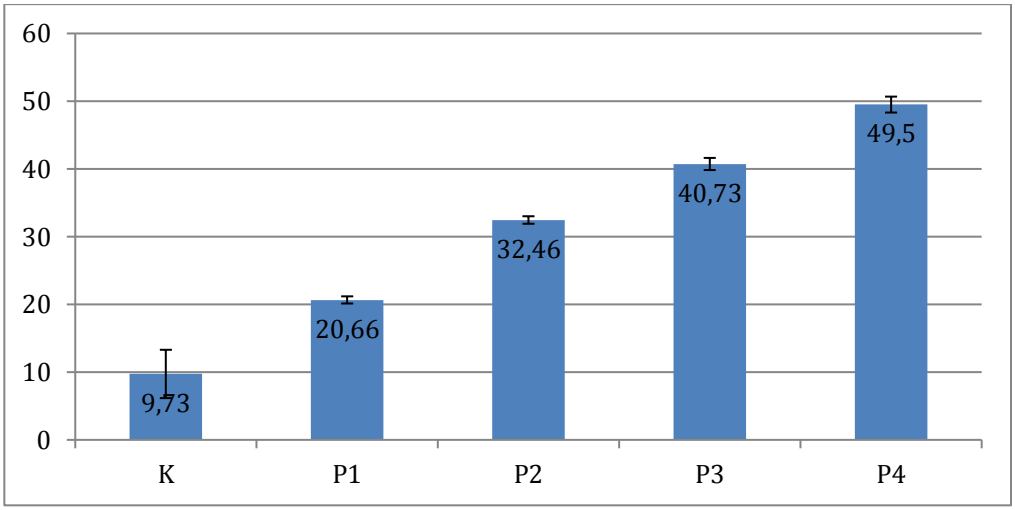

Figure 2. The number of osteoblast on the day 14th

Tabel 2. The mean and deviation standard of osteoclast on day 14th

Group	Mean ± Dev Std
G1	12.50 ± 2.881
G2	4.17 ±2.137
G3	3.83 ±0.753
G4	2.83 ±1,329
G5	2.00 ±0.894

Group 1 (G1) = sham; Group 2 (G2) = blood cockle's shell; Group 3 (G3) = blood cockle's shell and sea cucumber 0.4%; Group 4 (G4) = blood cockle's shell and sea cucumber 0.8%; Group 5 (G5) = blood cockle's shell and sea cucumber 1.6%.

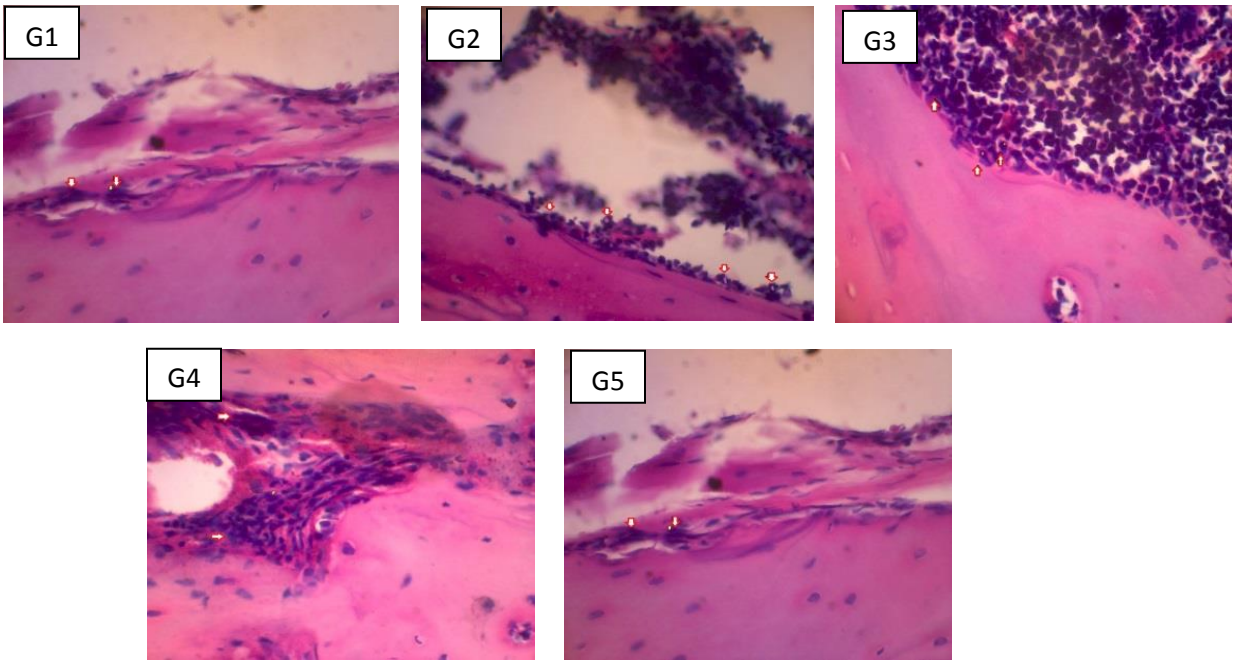

Figure 3. Histological images of osteoclast on the day 14th

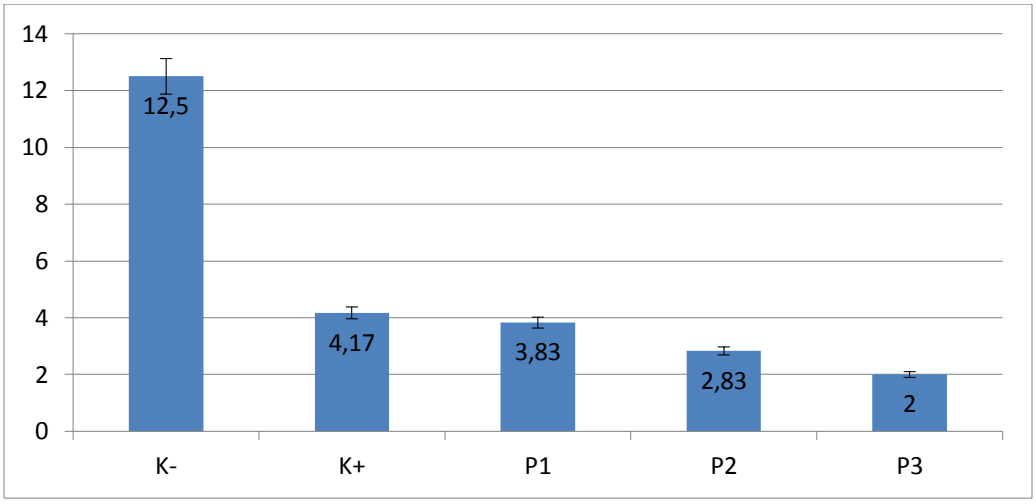

Figure 4. The number of osteoclast on the day 14th

The average number of osteoblast showed in Group 1 (9.7±3.6), Group 2 (20.7±0.5), Group 3 (32.46±0.557), Group 4 (40.73±0.894) and Group 5 (49.5±1.178). On the other hand, the average number of osteoclast showed in Group 1 (12.5±2.8), Group 2 (4.2±2.1), Group 3 (3.8±0.8), Group 4 (2.8±1.3) and Group 5 (2.0±0.9). In the comparison between the groups, there were significant differences for the number of osteoblast and osteoclast.

DISCUSSIONS

This study was conducted to determine the effectiveness of xenograft combination of shellfish shells (Anadara granosa) and sea cucumber (Stichopus hermanni) against the amount of osteoblasts and osteoclasts in bone healing. Osteoclasts and osteoblasts regulate a dynamic balance in the process of bone remodeling.[20] Bone remodeling imbalances due to osteoclast cells are more numerous than osteoblasts resulting in bone resorption processes. Bone resorption is influenced by active osteoclast, an activator complement product consisting of cytokines, IL-1, TNF-β, IL-6, and IL-11 (Ingle and Bakland, 2008). Cytokines, IL-1s, TNF-β, IL-6, and IL-11 affect RANK receptor work to bind to RANKL present in osteoblasts so that osteoclast activity increases and leads to bone resorption. [21,22]

Table 1, group G2 has an average yield of more osteoblasts than G1. In group G2 showed significant differences in the number of osteoblasts compared to the G3, G4 and G5 groups. In the G2 group only given bone graft shells of blood clams in the form of powder that is less than optimal in the process of osteogenesis, so less support in the healing process of bone. the membrane used to close the defect does not stick in place. In the G3 group, G4 and G5 use a gel preparation that has better adhesion than the powder. Good material attachment to the defect could enhance bone healing process. [20]

Figure 2 shows that there is a significant difference between increasing the number of osteoblasts in P2, P3 and P4 and clinically seen a good closed defect but not yet optimal. This suggests that the combination of the shells of clams (Anadara granosa) and the golden sea cucumber gel (Stichopus hermanni)) with a concentration of 0.4%, 0.8%, 1.6% can increase the number of osteoblasts in the bone healing process. The shells of oyster mushrooms have osteoconducting properties that function as scaffolds where new bone deposition in platelet formation process and golden cucumber have a content of AH (Hialuronic Acid) which can decrease inflammatory process by cooperating in fibrin formation. [20, 21] This

causes the platelet count to increase and the inflammatory process becomes decreased because the macrophages feed on the lesions arising from the inflammatory process so that the number of macrophages decreases. Additionally such combinations can lead to decreased proinflammation characterized by decreased inflammation and increased anti-inflammation characterized by increased healing processes. The healing process triggers the onset of GF in the form of FGF which can trigger the formation of more osteoblasts so that bone healing will occur faster. The treatment group (P4) shows the highest average osteoblast cell count among the P2 and P3 groups due to the different concentrations applied. The treatment group (P4) was given a 1.6% concentration which led to an increased platelet adhesion and aggregation process and increased scaffold as a new bone deposition site. The higher the concentration of golden sea cucumber given the adhesion and platelet aggregation also increased in the bone healing process.

Table 2, Group K is a group with normal bone healing, where the number of osteoclasts in this group shows a considerable amount compared to the other group. This can be seen statistically in the P1 group having a difference in the number of osteoclasts compared with group K. Significantly the defect appears slightly closed and begun to appear. Group P2, P3, P4 is a bone healing group that was given application of combination of shell powder and golden sea cucumber gel with concentration 0,4%, 0,8%, 1,6%. The addition of materials such as the golden cucumber rich in AH plays an important role in influencing the speed of cell migration in the process of wound closure, inflammation, angiogenesis, reepitalization and cell proliferation. This occurs because of the rapid adhesion and platelet aggregation that can affect the rate of cell migration.[18] In addition, AH can also decrease the inflammatory process by cooperating in fibrin formation.[23] In addition, the combination may lead to decreased proinflammation characterized by decreased inflammation and increased anti-inflammatory marked by increased healing process. The healing process will trigger the onset of GF in the form of FGF which can trigger the formation of more osteoblasts and can decrease the RANKL / OPG ratio so that bone healing will be faster. [22, 23, 24]

Statistically in groups P2, P3, P4 showed that there was a significant difference between the decrease in the number of osteoclasts between groups of one another. The treatment group (P4) showed the lowest average osteoclast cell count among the P2, P3 and P4 groups. In the treatment group (P4) was given a concentration (1.6%) where the highest concentration compared to the others led to the increasing adhesion and aggregation process performed by AH from Anadara granosa. Anadara granosa has osteoconducting properties that function as scaffolds where new bone deposition and golden cucumber have a content of AH (Hialuronic Acid) which can decrease inflammatory process by cooperating in fibrin formation and forming granulation tissue to cover the wound surface and start the remodeling phase. The remodeling phase is the final phase in wound healing.[3,25,26]

CONCLUSION

It was revealed from our study that the combination graft material that contain with blood cockle's shell and sea cucumber 1.6% has a good effect on the number of osteoblast and osteoclast in vivo.

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