ORIGINAL ARTICLE

The number of osteoblasts and osteoclasts in bone remodeling of bone defects caused by peri-implantitis using *Anadara granosa* bone graft: an experimental study

Vivin Ariestania[1*] Nike Hendrijantini[2] Chiquita Prahasanti[3] Hansen Kurniawan[4] Meinar Nur Ashrin[1] Chaterina Diyah Nanik[1] Anindita Apsari[1] Rizko Wira Arta[1] Rima Parwati Sari[5] Eddy Hermanto[6] Bunga Fauzia[7] Miwa Matsuyama[8]

[1]Departement of Prosthodontics, Faculty of Dentistry, Hang Tuah University, Surabaya
[2]Departement of Prosthodontics, Faculty of Dental Medicine, Airlangga University, Surabaya
[3]Department of Periodontics, Faculty of Dental Medicine, Airlangga University, Surabaya
[4]Department of Periodontics, Faculty of Dentistry, Hang Tuah University, Surabaya
[5]Department of Oral Biology, Faculty of Dentistry, Hang Tuah University, Surabaya
[6]Department of Oral Maxillofacial Surgery, Faculty of Dentistry, Hang Tuah University, Surabaya
[7]Department of Dental Materials, Faculty of Dentistry, Hang Tuah University, Surabaya
[8]Department of Oral Health Care and Rehabilitation, Institute of Biomedical Sciences, Tokushima University Graduate School,Japan

* Correspondence:
vivin.ariestania@hangtuah.ac.id

Received: 17 September 2025
Revised: 20 October 2025
Accepted: 22 November 2025
Published: 30 November 2025
DOI: 10.24198/pjd.vol37no3.64295

p-ISSN 1979-0201
e-ISSN 2549-6212

Citation:
Ariestania, V, Hendrijantini, N, Prahasanti, C, Kurniawani, H, Ashrin, MN, Nanik, CD, Apsari, A, Arta, RW, Sari, RP, Hermanto, E, Fauzia, B, Matsuyama, M. The number of osteoblasts and osteoclasts in bone remodeling of bone defects caused by peri-implantitis using Anadara granosa bone graft: an experimental study. Padjadjaran J. Dent, November. 2025; 37(3): 408-420.

ABSTRACT

Introduction: Peri-implantitis treatment aims to replace the damaged bone with new and healthy tissue during bone remodeling process. Bone grafts are materials used to stimulate the formation of new bone. Bone graft material derived from *Anadara granosa* (AG) can be synthesized into hydroxyapatite–tricalcium phosphate (HA-TCP) at a 70:30 ratio, which supports bone regeneration, as indicated by an increase in osteoblast numbers and decrease in osteoclast numbers. This study aims to analyze the effectiveness of *Anadara granosa* bone graft in the number of osteoblasts and osteoclasts during bone remodeling process. **Methods**: This study employed an experimental design with a post-test only control group. A total of 84 mice were divided into 12 groups (7 mice per group): negative control (K−), positive control (K+), and treatment group (P), each observed on days 14 and 28. Histological analysis was performed to count osteoblasts and osteoclasts. Data were analyzed using one-way ANOVA. **Results**: The number of osteoclasts was significantly reduced in the treatment groups (P14: 7.00 ± 1.528; P28: 6.57 ± 1.512) compared to the positive controls (K+14: 13.86 ± 2.410; K+28: 14.29 ± 1.496). On the contrary, the number of osteoblasts increased in the treatment groups (P14: 7.14 ± 1.676; P28: 8.57 ± 1.272) compared to the positive controls (K+14: 2.57 ± 1.512; K+28: 3.86 ± 1.574). Statistical analysis indicated that osteoblasts showed significant differences after AG treatment ($p<0.05$), and the ANOVA test showed significant differences in osteoclast number after AG treatment ($p<0.05$). **Conclusion**: The number of osteoblasts increases while the number of osteoclast reduces in bone remodelling of bone defect caused by peri-implantitis using AG bone graft.

KEYWORDS

Bone graft, *anadara granosa*, peri-implantitis, osteoblast, osteoclast

INTRODUCTION

A dental implant is a device surgically placed into the soft tissue or jawbone to serve as an artificial root, supporting a prosthetic tooth to replace one that has been lost. [1] Dental implant treatment patients are expected to manage risk factors effectively to achieve optimal treatment outcomes. The success rate for dental implants is currently high, approximately 97% for male and 98% for female. An implant higher success is at 53% for lower molar and 33% for upper premolar.[2] Risk factors for patients with dental implant treatment include systemic disease and periodontal disease.[3] One of the periodontal diseases that often occurs in patients with implants is peri-implantitis.

Peri-implantitis is a disease that causes inflammatory lesions in the soft tissue around dental implants, induced by microorganisms that are pathogenic to the periodontal.[4] Reports on the prevalence of peri-implantitis vary widely, ranging from 4% to 45%, highlighting its clinical significance.[2] The cause of peri-implantitis is generally the presence of bacteria caused by plaque buildup around the implant.

This study's microorganism associated with dental implant failure was gram-negative anaerobic bacteria, *Porphyromonas gingivalis* (Pg). Pg bacteria produce bacterial products or pathogens called *Lipopolysaccharide* (LPS), which are key in the development of periodontal disease. LPS can provide an early warning of bacterial infection and initiate an immune response; this molecule is produced from the extraction of bacterial membranes and vesicles by LPS Binding Protein (LBP). The inflammatory reaction around the implant that is stimulated by LPS can cause bone damage.[5]

Periodontal tissue experiences inflammation, characterized by the release of macrophages to produce bone resorption mediators. Macrophage 1 increases the secretion of pro-inflammatory cytokines (TNF-α and IL-1β), while macrophage 2 is responsible for the secretion of anti-inflammatory cytokines in the tissue regeneration process. Pro-inflammatory cytokines will increase osteoclastogenesis activity so that pre-osteoclasts will release *Receptor Activator of Nuclear factor Kappa-B* (RANK) and a mediator that can improve the regulation of *Receptor Activator of Nuclear factor-B Ligand* (RANKL), expressed by osteoblasts.[6]

The therapy that is usually used for peri-implantitis and is the protocol for peri-implantitis cases is conventional therapy. This therapy includes cleaning the area around the implant, debriding, and administering antibiotics. The disadvantage of traditional treatment is that it cannot benefit absorbed and inflamed bone. Therefore, additional therapy is needed to help stimulate and regenerate bone to heal optimally. Because of this, additional induction is necessary to stimulate osseointegration.[7]

One of the therapies currently used to treat peri-implantitis is bone grafting, which aims to help bone regeneration.[8] The bone graft material readily available now is xenograft as this material comes from natural ingredients and is available in large quantities. One example of xenograft material can come from marine biota. Some marine biota content can create soft and hard tissue structures such as bone, dentin, and periodontal ligament damaged or lost due to disease.[9]

This bone graft can be applied to bone defects damaged in the area around the implant. The bone graft will induce new bone formation and help wound healing by interacting with and increasing macrophage activity, which can increase the secretion of anti-inflammatory cytokines and growth factor.[10] Anti-inflammatory cytokines will increase growth factors to accelerate the bone regeneration process. Anti-inflammatory cytokines will also signal osteoprogenitors in the form of pre-osteoblasts and will stimulate Runt-Related Transcription Factor 2 (RUNX2) to induce the formation of mature osteoblasts. Thus, bone graft therapy can speed up the bone healing process.[11]

Biomaterials used in bone grafts have, in effect, lent themselves to enhanced bone regeneration. Isolation from a variety of sources of natural hydroxyapatite (HA) has been raised: fish bone and mollusk shells, for example. HA is one of the

most widely used types of apatite or calcium phosphate in the medical world because of its good compatibility and chemical and physical mineral content similar to human bones and teeth.[12]

HA sources are often found in beef bones, clam shells, and crab shells.[13] HA can be produced using various methods, including the hydrothermal method, continuous deposition, sol-gel method, solid-phase reaction and electrophoretic deposition.[14] Recently, there has been an increase in interest in creating high-performance hydroxyapatite powders by the natural resource synthesis of HAP. Nonetheless, research focusing on the simple and affordable production of Hap utilizing biowaste resources is still limited. Therefore, there is much promise for research on Hap synthesis from biowaste.

Previous research had been carried out using abalone shells containing hydroxyapatite, which are suitable as biomaterial candidates with the most optimum variation of settling time, namely 48 hours based on crystal size, particle size, degree of crystallinity, and polydispersity.[15] Dewi et al. (2024) found that nano-hydroxyapatite derived from snakehead fish bones exhibited antibacterial activity against *Streptococcus mutans*, supporting the prospect of natural sources of HA in dental materials.[16]

Hydroxyapatite can accelerate bone regeneration, resulting from a bone graft framework (scaffold) similar to bone structure. Tricalcium phosphate (TCP) has a higher absorption ability because TCP has biodegradable and osteoconductive features. TCP also has an increased ability for biodegradation and incorporation when combined with HA. The combination of HA and TCP is ideal to overcome the shortcomings of HA and TCP alone.[17] This combination is expected to help accelerate the wound-healing process.[18]

A natural material that can be used is shell. One type of this is blood cockles *Anadara granosa* (AG). Apart from being easy to find, blood cockles AG also contain hydroxyapatite. The mineral content in clam shells is 98.7% CaC, 0.05% Mg, 0.9% Na, 0.02% P, and 0.2% other contents. Blood cockles contain high amounts of calcium carbonate (CaC), which can function as a source of calcium so that it can be used for the synthesis of hydroxyapatite (HA) to be used as a bone repair biomaterial.[19] The HA-TCP scaffold has also been reported to have safe toxicity properties when used as therapy; this has been proven in research by Ariestania et al. (2022), which stated that the HA-TCP scaffold at a dose of 1000 µg to 5 µg can be used for therapy of bone defects without causing toxic effects on the human body.[17]

Based on research by Sari, the synthesis of HA is formed via hydrothermal for 12 hours, amounting to 72%; in TCP, it is formed by 21%, while the remaining 7% is a calcium carbonate compound to obtain a compound of HA-TCP combination. This combination of compounds is then processed into scaffold.[20] HA-TCP (72:21) is close to the ratio of HA-TCP, which is good as a graft material according to (Kim, et al., 2020) than HA-TCP (70:30, this combination has the same four properties as bone structure, namely physical, mechanical, and biological properties, so that it can provide good bone healing.[21] HA combined with TCP is an efficient choice because of its osteoinduction, osteoconduction, and resorbability characteristics.[17] Hydroxyapatite can stimulate the presence of macrophages with inflammatory cells, which can help strengthen the process of forming new blood vessels in the wound area.[18]

Osteoblasts play a role in regulating osteoclasts and bone matrix deposition. Osteoblasts can precipitate organic elements of the bone matrix and assist in synthesizing organic components (type I collagen, proteoglycans, and glycoproteins) from the bone matrix.[19]

Osteoclasts are large, multinucleated cells found along the surface of bones where bone resorption, remodeling, and repair occur. These osteoclasts are often found within a shallow depression in the bone that is resorbed or eroded enzymatically, called a Howship lacuna.[21] Osteoclast differentiation initially depends on signals via c-fms, the receptor for macrophage Colony-stimulating

factor (MCSF), on mononuclear precursor cells, which regulates RANK expression. RANKL regulates osteoclast formation and activation, where many hormones and cytokines produce effects. RANKL is expressed on osteoblast cells and binds to RANK receptors on osteoclast cells.[22]

Osteoclasts form the enzymes phosphohydrolase and tartrate-resistant acid phosphatase (TRAPase). The TRAPase enzyme is released into the lacunae at the site of bone resorption during bone disassembly and is thought to play a role in dissolving bone matrix minerals. During bone dismantling, osteoclasts also release collagen crosslinks resulting from protein matrix degradation (type 1 collagen), such as pyridinium crosslinks (free pyridinoline and deoxypyridinoline), cross-linked telopeptides (N-telopeptides and C-telopeptides), in addition to the TRAP enzyme and calcium, as marker substances: biochemical bone resorption or disassembly.[23]

According to Sari, 2021, pathological conditions in the control group (3 days and 7 days) after tooth extraction showed that IL-10 expression on macrophages had increased using AG bone graft. Mechanism of inflammation process initiated by macrophages (M2) was found and marked by the appearance of anti-inflammatory cytokines such as interleukin-10 (IL-10), interleukin-4 (IL-4), and interleukin-13 (IL-13). The presence of IL-10 which is an anti-inflammatory cytokine that modulates alveolar bone homeostasis increases osteoblast production.[24]

The novelty of this research lies in the AG bone graft which can accelerate bone remodelling in peri-implantitis related bone defect. The bone remodelling process is characterized by the formation of increased osteoblasts and a decrease in the number of osteoclasts. This study aims to analyze the effectiveness of Anadara granosa bone graft in the number of osteoblasts and osteoclasts during bone remodeling process.

METHODS

This type of research is an animal experimental study with a randomized, genuine experimental laboratory using a post-test-only control group design. The sample of this study was AG bone graft formed into a scaffold made by a hydrothermal process at a temperature of 200°C with a sintering time variation of 12 hours to produce HA synthesis of 72% and TCP 21% (72:21), then sterilized by gamma-ray irradiation with a dose of 25 Kgy with a temperature of 35-37°C at BATAN. The sample was divided into 12 groups, with each group consisting of 7, to be examined for osteoblasts and osteoclasts, with each negative control group (K-), positive control group (K+), and treatment group (P).

The materials needed in this study were a blood clam shell scaffold AG in the form of 2 microgram granules, 10% Ketamine, Xylazine, suture thread, and a needle, also PZ solution, Xylol solution, absolute Ethanol, 70% Ethanol, Meyer's Hematoxylin Eosin solution, Eosin solution, DPX adhesive fluid, P.Gingivalis LPS, Implant Tohoku®

The study population used experimental animals: adult male Rattus Norvegicus strain Wistar rats aged 20 weeks with a body weight of 325-350 grams that had been acclimatized for several weeks and obtained from the Experimental Animal Laboratory of the Institute of Tropical Disease (ITD) Airlangga University. The rats were given implants in the right femur region by drilling the femur, then given 10 microliters of LPS fluid in the post-drilling hole before the implant was inserted, after which stitching was performed to create a peri-implantitis condition in the rats. The rats were made in a peri-implantitis condition and waited for 30 days to develop inflammation.

Rats that had experienced inflammation (peri-implantitis) were re-incised to see the position of bone loss in the peri-implant area. AG scaffold, 2 micrograms in size, was applied to the bone defect of mice that experienced bone loss using

an excavator. The wound was closed again with stitches, and waited for 14 days and 28 days.

The rats were anesthetized with 1 cc of 10% ketamine added with 1 cc of Xylazine, and then injected intramuscularly into the semi-tendonous muscle as anesthesia induction in the gluteus. Then, the perfusion technique was performed using formalin Paraformaldehyde (PFA) fluid. After the rat died, an incision was made on the skin, and the soft tissue and muscles on the right femur were removed. The specimen examined was a piece of femur containing an implant, soaked in a 10% buffered formalin fixation solution for at least 8 hours before the decalcification process was carried out.

The research specimens were immersed in a 10% EDTA (Ethylene Diamine Tetra Acetic Acid) solution with 50 times the volume of the material being examined and then observed to ensure that air bubbles were coming out and the decalcification process was running well. The EDTA solution was replaced every three days, so the decalcification reaction was perfect. If there were no more air bubbles, then it was likely that the specimen had become soft (confirmed by needle puncture). After decalcification, the specimen was processed for histopathological and immunohistochemical examination.

The resulting specimens were processed for preparations, and HE staining was performed to count osteoblast and osteoclast using a light microscope at 1000x magnification in 20 fields of view. The research data were analyzed using a One-way ANOVA to detect differences in the numbers of osteoblasts and osteoclasts across three groups. Subsequently, post hoc LSD was conducted to determine the distinctions between the groups.

RESULTS

This research was carried out at the Experimental Animal Laboratory of the Institute of Tropical Disease (ITD) Airlangga University using bone graft from AG shell formed by a scaffold of 1.5 micrograms as the treatment group, placing implants in rat femurs as a control and installing implants accompanied by administration of LPS as a negative control for the number of osteoblasts and osteoclasts on the 14th and 28th days.

The data scale used in this research was the ratio data scale. Data analysis was conducted on the number of osteoclasts using the Shapiro-Wilk normality test, as the sample size was less than 50. In this research, the number of groups was more than 2, and there were no pairs, so a parametric test was carried out. If the data distribution was normal and homogeneous, a one-way ANOVA test was conducted. The mean and standard deviation of each group, the mean osteoblast and osteoclast are shown in Table 1.

Table 1. Mean and standard deviation of each group

Groups	Mean ± SD Osteoblast	Mean ± SD Osteoklast
K- 14	2.57 ± 1.512	3.86 ± 1.952
K- 28	3.86 ± 1.574	4.57 ± 1.397
K+ 14	5.71 ± 2.138	13.86 ± 2.410
K+ 28	6.14 ± 1.574	14.29 ± 1.496
P 14	7.14 ± 1.676	7.00 ± 1.528
P 28	8.57 ± 1.272	6.57 ± 1.512

(K-14) Negative control group using an implant without inflammation for 14 days; (K-28) negative control group using implant without inflammation for 28 days; (K+14) positive control group implant with inflammation for 14 days; (K+28) positive control group using an implant with inflammation for 28 days; (P14) treatment group using bone graft AG for inflamed for 14 days; (P28) treatment group using bone graft AG for inflamed for 28 days.

The average numbers of osteoblasts and osteoclasts in the K-14 group were as many as 2.57 and 3.86, and in the K-28 group, they were as many as 3.86 and 4.57. The numbers of osteoblasts and osteoclasts in the K+14 group were 5.71 and 13.86, and in the K+28 group, they were as many as 6.14 and 14.29. In the

P14 group, the numbers of osteoblasts and osteoclasts were 7.14 and 7.00, while the numbers of osteoblasts and osteoclasts in the P28 group were 8.57 and 6.57. Post-Hoc analysis of osteoblast and osteoclast numbers are is shown in Table 2 and Table 3 (respectively).

Table 2. One Way ANOVA analysis between groups of osteoblast

Osteoblast number	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	165.905	5	33.181	12.260	0.001
Within Groups	97.429	36	2.706		
Total	263.333	41			

Table 3. Results of post hoc LSD analysis concentration of osteoblast

Groups	K-14	K-28	K+14	K+28	P14	P28
K-14						
K-28	0.996					
K+14	0.012*	0.003*				
K+28	0.304	0.124	0.690			
P14	0.588	0.863	0.000*	0.008*		
P28	0.028*	0.088*	0.000*	0.000*	0.588	

*p<0.05 = significant differences

Table 3 shows significant difference in osteoblast between control and treatment groups. The significant difference obtained was less than 0.05 (p<0.05). Several therapies for treating peri-implantitis, one of which is the administration of bone graft from AG. The content produced from the synthesis of bone graft AG one of which is hydroxyapatite tricalcium phosphate, can help the secretion of growth factors produced by macrophages 2.[20] Growth factors can reduce the secretion of inflammatory factors from macrophages to accelerate bone regeneration. Growth factors will also increase the speed of pre-osteoblast differentiation activity and stimulate RUNX2 to induce the formation of mature osteoblasts.[25]

Table 4. One Way ANOVA analysis between groups of osteoclast

Osteoclast number	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	735.071	5	147.014	47.865	0.001
Within Groups	110.571	36	3.071		
Total	845.643	41			

Table 5 shows the number of osteoclasts in the K-14 group, which has a significant difference with K+14, K+28, and P14, with a smaller number of osteoclasts. K-28 has a significant difference with K+14 and K+28, with fewer osteoclasts. K+14 has a considerable difference with K-14, K-28, P14, and P28, which have more osteoclasts. K+28 has a significant difference with K-14, K-28, P14, and P28, which have a more significant difference of osteoclasts.

Table 5. Results of post hoc LSD analysis concentration of osteoclast

Groups	K-14	K-28	K+14	K+28	P14	P28
K-14		0.972	0.000*	0.000*	0.021*	0.065
K-28			0.000*	0.000*	0.125	0.293
K+14				0.997	0.000*	0.000*
K+28					0.000*	0.000*
P14						0.997
P28						

*p<0.05 = significant difference

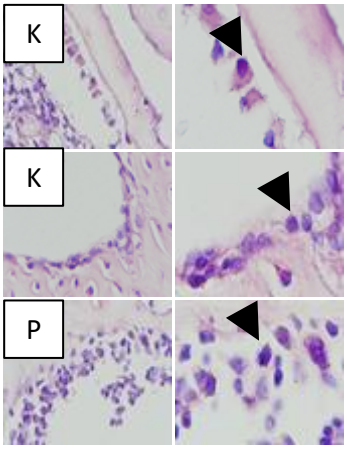

Figure 1: Hematoxylin Eosin (HE) examination of the Number of osteoblasts in the peri-implantitis area; (K-) a small number of osteoblasts in the control negative groups; (K+) a number of osteoblasts in control positive groups using LPS; (P) a number of osteoblasts using Anadara Granosa bone graft in a peri-implantitis condition

The differences in the numbers of osteoblasts and osteoclasts were detected among the groups through Hematoxylin Eosin (HE) staining (Figures 1 and 2). Figure 1 shows around the peri-implant region of the negative control group (K-), and only a small number of osteoblasts were seen. On the other hand, the positive control group (K+), which was induced by LPS, demonstrated a greater number of osteoblasts, although their distribution was irregular and less organized. The experimental group (P), which was given AG bone graft, not only demonstrated a higher number of osteoblasts than both control groups but also displayed osteoblasts which were oriented in a row and actively bone forming along the newly formed bone surface, indicating active bone formation.

Fourteen and twenty-eight days after application on the bone defect, the animals were killed and the femur preparation was taken and fed into a 10% PFA buffer solution to prevent the tissue from decomposing, tissue hardening, increasing the refractive index of various tissue components, and increasing the affinity of the tissue against the stain. After the process of tissue fixation, the process of decalcification was done using EDTA for 2 months. Femur specimens were prepared in the form of transversal preparations with hematoxylin-eosin (HE). After that, we observed the number of osteoblast and osteoclast cells with a light microscope at 400 magnification.

Histological section of HE examination with and without application of AG bone graft in bone defect showed that osteoblasts in the peri-implantitis area (K+) are a small number than control positive group (P). Differences found in the (K-) showed a number of osteoblasts smaller than other groups. HE examination for osteoclast showed an increase from (K-), (K+) and (P). The increase in osteoclast counted on day 28 was lower than day 3 in each treatment group.

Figure 2 shows minimal bone resorption demonstrated by the lack of osteoclasts in the negative control group (K-). An increase in the number of osteoclasts is notable in the positive control group (K+), along with multinucleated osteoclasts actively present on the resorption lacunae. Compared to the K+ group, the AG bone graft group (P) shows fewer osteoclasts, whereas osteoblast activity continued to dominate, indicating a more balanced bone remodeling.

Table 2 and table 4 show that the one-way ANOVA yielded a p-value of 0.000 (<0.05) indicating significant differences between groups. Post Hoc LSD was used to analyze whether there were any significant differences in each group (Table 3 and Table 5). Post-Hoc LSD results indicated significant differences in osteoblast number in peri-implantitis condition between K+14 to P14 and K+28 to P28. This table also determines significance differences in osteoclast number at K+14 and K+28 compared to P14 and P28.

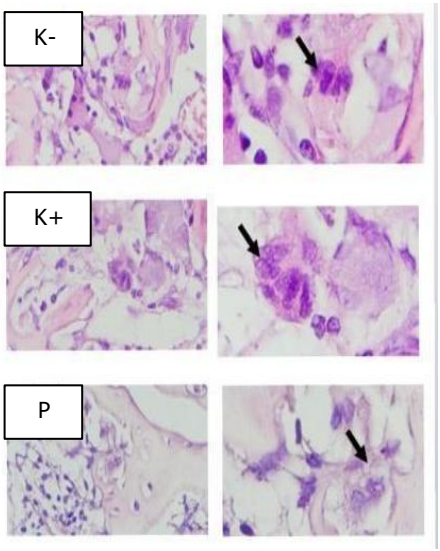

Figure 2: Hematoxylin Eosin (HE) examination of the number of osteoblasts n the peri-implantitis area; (K-) a small number of osteoclasts in the control negative groups; (K+) a number of osteodasts in control positive groups using LPS; (P) A number of osteoclasts using AG bone graft in a peri-implantitis condition

DISCUSSION

Implant failure may result from biological causes (peri-implantitis and mucositis), technical factors (e.g., immediate implant instances), age (elderly patients), hormonal changes (e.g., menopause), immunocompromised states (e.g., diabetes), and the use of non-biocompatible biomaterials. Peri-implantitis can be challenging to treat. While peri-implant mucositis can often be managed effectively, non-surgical treatment of peri-implantitis frequently leads to recurrence.[26] Reducing inflammation surrounding the implants and enhancing osseointegration are two essential elements for achieving the clinical success of dental implantation.[27]

The use of AG bone graft therapy, which contains hydroxyapatite (HA) and is combined with tricalcium phosphate (TCP), is expected to help accelerate bone regeneration by increasing the number of cells and reducing osteoclasts in P14 and P28 (table 1). In peri-implantitis, the local immune-inflammatory process is associated with a disturbed bone remodelling. Induced osteoclastogenesis is a significant determinant in the uncoupled bone resorption to bone formation, ending in the loss of the supporting alveolar bone and implant failure. Bone is a dynamic process that involves a delicate balance between bone-forming osteoblasts and bone-resorbing osteoclasts.[28] This study demonstrated that AG bone grafting improves this balance by increasing the number of osteoblasts and decreasing osteoclast activity in peri-implantitis-induced bone defects.

The average number of osteoblasts increased across all groups with extended observation periods (Table 1). The treatment group P28 showed the highest osteoblast count (8.57 ± 1.272), followed by P14 (7.14 ± 1.676). In contrast, the negative control (K–) groups showed the lowest osteoblasts counts at both 14 and 28 days (2.57 and 3.86, respectively). It is determined that AG bone graft can increase the number of osteoblast in bone defect caused by peri-implantitis. P14 and P28 show no significant differences but there is an increase in the number of osteoblast, as the inflammatory response resolved by 28 days.[29] One of the pro-inflammatory mediators released during the phagocytosis process is IL-6. Interleukin-6 induces the differentiation of T helper 17 (Th17) cells, which play a key role in interaction with metal implants as part of the adaptive immune response.[30] IL-6 levels on day 0 were higher on days 14 and 28. It is because the peak concentration of IL-6 occurs within 2–48 hours post-surgery and begins to

decline within 48–72 hours.[31]

Post hoc analysis (Table 3) revealed statistically significant differences in osteoblast numbers between K–14 and K+14, K-28, and K+28 ($p<0.05$), suggesting that the use of AG bone graft improves osteoblast activity compared to both inflamed and non-inflamed control groups. Anderson et al. (2008) stated that the body's response following implantation triggers a series of continuous processes, including tissue injury, blood-implant interaction, acute inflammation, chronic inflammation, foreign body reaction, and fibrosis. Metal implants elicit immune responses, both innate and adaptive, that can activate acute inflammatory responses primarily through phagocyte activation, chemotaxis, and protein adhesion to the implant surface.[32]

In group K28, there were more osteoblasts than in K14. This occurred during the proliferation phase in the inflammatory process in rat. Proliferation phase lasted from 3 to 14 days, marked by inflammatory cells, collagen, fibroblast and blood vessel (Angiogenesis). Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thereby facilitating the resolution of inflammation. In this way, macrophages promote the transition to the proliferative phase of healing.[33] Macrophages, neutrophils and platelets release pro-inflammatory cytokines such as TNF-alpha, BMP-s, PDGF, TGF-beta, VEGF and interleukins. This cytokine stimulates cell interactions in areas of bone damage.[34]

Fibroblast cells are essential in forming connective tissue during the proliferation phase. Fibroblast cells in the connective tissue play a role in synthesizing extracellular matrix components (collagen, elastin, and reticular fibers).[35] The next remodeling phase occurs after 14 days, where osteoblast cell maturation will occur in this phase.

In histopathological examination, the remodeling phase is characterized by the emergence of mature osteoblasts. This results in group K14 having fewer osteoblasts than group K28. According to Hafizha, IL-6 levels on day 0 represented the body's response to the implant. IL-6 levels on day 0 were higher on days 14 and 28. It is because the peak concentration of IL-6 occurs within 2-48 hours post-surgery and begins to decline within 48-72 hours.[29] The remodeling phase will occur until the healing process is complete, usually starting on the 21st day. Group K28 has a more significant number of osteoblasts than group K14, which can also be caused by the activity of osteoblasts and osteoclasts, which will change woven bone into mature bone.[36]

Group K-14 had fewer osteoblasts than group K14. This may be caused by inflammation in the area around the bone of group K-14 given LPS. LPS-induced inflammation can induce apoptosis and inhibit osteoblast differentiation in preosteoblastic cells. Therefore, LPS can inhibit osteoblastogenesis activity, thereby reducing the number of osteoblasts.[37]

Excessive secretion of pro-inflammatory cytokines can increase the number of osteoclasts and decrease the number of osteoblasts.[38] A bone graft that is synthesized produces hydroxyapatite-tricalcium phosphate, which can increase macrophage 2 by stimulating osteoblast differentiation through BMP2.[39] Macrophages 2 have a role in the secretion of anti-inflammatory cytokines and growth factors. Anti-inflammatory cytokines can increase growth factors to accelerate bone regeneration. Growth factors will increase the speed of preosteoblast differentiation activity. Anti-inflammatory cytokines will also signal osteoprogenitors in the form of per-osteoblasts and will stimulate RUNX2 to induce the formation of mature osteoblasts.[40]

The number of osteoblasts in the K-28 group was lower than in the K+28 group because osteoclasts continued to form in the area experiencing inflammation by stimulating RANKL in osteoblasts.[38] Inflammation caused by LPS induction can result in apoptosis and inhibit osteoblast differentiation in preosteoblastic cells. Therefore, LPS can inhibit osteoblastogenesis activity, thereby reducing the number of osteoblasts.[37]

Administration of AG bone graft can help the secretion of TGF-β growth factor produced by macrophages 2. TGF-β is the most significant growth factor in human bones.[41] Growth factors produced from AG bone grafts regulate RUNX2 expression. RUNX2 is required for the differentiation of mesenchymal cells into pre-osteoblasts. As a downstream gene of RUNX2, Osx (Osterix) is necessary to differentiate preosteoblasts into mature osteoblasts. Osx is expressed explicitly in all osteoblasts.[42] Growth factors can reduce the secretion of inflammatory factors from macrophages to increase the speed of wound healing.[43] The combination of HA-TCP produced from AG bone graft is a perfect blend in osteoblast cell formation, which plays an essential role in bone formation.[44]

Therapy using AG bone graft synthesized into HA-TCP can activate macrophages 2, which can secrete anti-inflammatory cytokines (IL-10) and induce the formation of cell migration and proliferation by activating AP1, so that it stimulates growth factors such as BMP-2, VEGF, and FGF. VEGF and FGF are angiogenic factors that form blood vessels as a supply of nutrients and oxygen, so that they can start the remodeling process.[45]

In contrast, the number of osteoclasts increased in the positive control groups (K+14 and K+28), showing values of 13.86 and 14.29, respectively (table 1), However, there is no significant difference between K+14 and K+28 in post hoc LSD ($p<0.05$) (table.3). This can be caused by the reversal phase that occurred. In this phase, cells that resemble macrophages and osteoclasts will gradually disappear.[47]

Histological examination (Figure 2) revealed multinucleated osteoclasts actively resorbing bone in these groups, consistent with the pathological bone destruction observed in peri-implantitis. Notably, the treatment groups (P14 and P28) showed reducing numbers of osteoclasts (7.00 ± 1.528 and 6.57 ± 1.512, respectively) when compared to the positive controls. In (P), the number of osteoclasts was higher than in group K-, due to inflammation in P groups which produced pro-inflammatory cytokine during the inflammatory process. Pro-inflammatory cytokines such as TNF-α and IL-1β, can induce RANKL expression in osteoblasts. Pro-inflammatory cytokine will increase osteoclastogenesis activity so that pre-osteoclast will release RANK which will form osteoclast.[40] This influences the increase in osteoclasts in a group (P) more than in group K-28.

The K-28 group with P28 showed almost the same number of osteoclasts as the K-28 group without P28. AG bone graft therapy in P28, can promote the secretion of growth factors, such as FGF, IGF and TGF-β thereby reducing the secretion of inflammatory factors from macrophage one and inhibiting osteoclast formation.[26]

On the P14 and P28 days, there was a reduction in the number of osteoclasts, which was not too significant based on post hoc LSD (table 3). This can be caused by the reversal phase that occurred. In this phase, cells that resemble macrophages and osteoclasts will gradually disappear.[46] This phase lasts from days 7-14, marked by the return of bone absorption to formation. After 2-4 weeks, a resorption phase occurs, characterized by the disappearance of osteoclasts by apoptosis.[47]

In summary, these findings suggest that AG bone graft promotes osteoblast function while inhibiting overactive osteoclasts, thereby creating a more favorable environment for bone repair in cases of peri-implantitis-related bone loss. This underscores its promise as an affordable, biocompatible alternative to traditional bone grafts for implant restoration. The limitation of this study is the need for higher bone graft dosage and a longer application and duration of this experimental investigation.

CONCLUSION

Osteoblasts increase in bone remodelling post bone defect caused by peri-implantitis conditions were on days 14 and 28, with this increase most common

on day 28. Meanwhile, AG bone graft also effectively reduces the number of osteoclasts in bone remodelling process after bone defect caused by peri-implantitis conditions on days 14 and 28. The implication of this research is that more AG bonegraft experiments about cytotoxicity when the dose is increased.

Acknowledgement: This research was funded and supported by Hang Tuah University Surabaya, Indonesia.
Author Contributions: Conceptualization, V.A. and N.H.; methodology, V.A.; software, H.K.; validation, C.P., M.N.A. and C.D.N.; formal analysis, A.A.; investigation, R.W.A.; resources, R.P.S.; data curation, E.H.; writing original draft preparation, V.A.; writing review and editing, B.F.; visualization, H.K.; supervision, N.H.; project administration, M.M.; funding acquisition, N.H. All authors have read and agreed to the published version of the manuscript.
Funding: This work was supported by the Research Fund of the Faculty of Dentistry, Hang Tuah University
Institutional Review Board Statement: Not Applicable
Informed Consent Statement: Not Applicable
Data Availability Statement:
https://docs.google.com/document/d/1xrAp_4rR1GKyDGjcbtKsDYE6KPYC7q8d/edit?usp=sharing&ouid=109291680061237768411&rtpof=true&sd=true
Conflict of Interest: The authors declare no conflict of interest, and the funders have no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

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