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**Research Report**

# Number of Osteoblasts and Osteoclasts in Combination Carbonate Hydroxyapatite, Platelet Rich Fibrin (PRF) and Antioxidant in Socket Rats Wistar After Tooth Extraction

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## ABSTRACT

**Background:** Bone graft materials have been used extensively to support bone healing after tooth extraction. Bone healing could be increased reactive oxygen species (ROS) that prolong the phase of the inflammatory and delay reparative phase. Antioxidants are substances that can improve and reduce the number and the damage produced by ROS. Hence, the utilization of antioxidant which is utilized in conjunction to Carbonate *hydroxyapatite* is expected to increase the success of bone healing. **Purpose:** The aim of this study is to compare the number of osteoblasts and osteoclast in the process of bone healing after employing *Carbonate Hydroxyapatite* and antioxidant in the Wistar rats' incisor tooth extraction socket. **Methods:** Twenty-seven male Rattus norvegicus strain Wistar rats were divided into 3 treatment groups. This study uses a post-test only control design. Sample of 27 rats were divided into 3 groups. Mandibular incisive is extraction. Group 1, socket is lefted to fill with blood (control). Group 2, socket is filled bonegraft and antioxidants and group III, socket is filled antioxidants, bonegraft and platelet rich fibrin (PRF). After that, the wound is sutured. On day 14, the mice are terminated then viewed in microscopy of osteoblasts. The preparation of the bone tissues was given the staining by hematoxylin-eosin and then the numbers of the osteoblasts and osteoclast were calculated. **Results:** Statistical testing by using one-way ANOVA has proved that there are significant differences in the number of osteoblasts in all 3 groups ($p = 0.000$). The highest numbers of osteoblasts were found in the group that was given *Carbonate Hydroxyapatite* combined with antioxidant and PRF and the lowest numbers of osteoblasts were found in the control group. **Conclusion:** Combination bonegraft, antioxidants and PRF could be increased the highest number of osteoblasts and could be decreased the lowest number of osteoclasts compared with control group and the group bonegraft and antioxidants.

**Keywords:** antioxidant; osteoblast; osteoclast; periodontal disease

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## INTRODUCTION

Various materials and techniques used as such bonegraft regenerative therapy, growth factors or other materials that play a role in the growth and differentiation of the cells periodontal.[1] Bone is composed of protein and minerals. The main component of bone is a protein called collagen and bone mineral (calcium phosphate). Bone consists of four types of bone cells are osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts involved in bone healing. Bonegraft can help the process of bone regeneration through two mechanisms: osteoinduction and osteoconduction.[2]

Platelet rich fibrin (PRF) is rich in growth factors and cytokines which increase the potential for hard tissue and soft tissue healing. Two prominent growth factors on bone healing are platelet derived growth factor (PDGF) and transforming growth factor beta (TGF-β). growth factor is the most important mediator for the stimulation osteogenesis.[2]

Osteoblasts plays an important role in the mineralization process. Osteoblasts regulates calcium and phosphate concentrations. In addition, osteoblasts also express alkaline phosphatase in high quantities to the plasma membrane. Alkaline phosphatase is also important in the process of bone mineralization. Osteoblasts as secretory metabolically active, producing a number of bone morphogenetic protein (BMP) 2, BMP 7 and a growth factor, in addition to insulin growth factor (IGF) I and IGF II, PDGF, fibroblast growth factor (FGF), TGF-β, interleukin (IL) I and PDGF as well as osteoid which mostly consists of collagen type I. osteoblasts also express receptor nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG). expression products of osteoblasts occurs during bone remodeling.[3–5]


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Osteoclasts are multinuclear cells along the surface of the bone where the resorption, bone remodeling and repair. Osteoclast function is bone resorption during remodeling. Osteoclasts attach to the bone matrix and form a microenvironment of the bone resorption process. Osteoclasts have as carbonic anhydrase enzyme which serves to lower the pH and dissolve the bone matrix, and lysosomal enzymes are collagenase and cathepsin K issued which then digest bone matrix. Osteoclasts produce acid, collagenase and other proteolytic enzymes that dissolve the bone matrix and damaging substances ground.[5,6]

Severe oxidative stress has been implicated in many chronic and degenerative diseases, including osteoporosis, cancer, aging, and neurodegenerative disease periodontitis. Many studies have focused on the use of antioxidants to repair the damaging effects of oxidative stress. There is evidence that reactive oxygen species play a role in the process of bone healing in periodontal disease.[7]

Osteoblast has been shown to produce such glutathione peroxidase (GPx) antioxidant enzymes that protect against the damaging effects of reactive oxygen species (ROS). The nuclear factor kappa B (NFκB) activation is essential for osteoclast formation and increased concentrations of ROS and decreased antioxidant. GPx reduction has been shown to increase osteoclast and increase RANKL. GPx is one of the enzymes produced by antioxidants.[8]

Bone is regulated by osteoblasts and osteoclasts, osteoblasts responsible for bone formation and osteoclasts regulate bone resorption. During the pathological process, the balance between bone resorption and formation led to increased resorption. Differentiation and activity of osteoclasts stimulated by the activity of the RANKL and receptor activity of osteoblasts nuclear factor kappa-B (RANK) on osteoclast precursors with the macrophage colony stimulating factor (M-CSF). Binding of RANK and RANKL induces signal transduction through the NFκB, which started the differentiation of precursor cells into osteoclasts preosteoclasts. Some inflammatory cytokines, including IL-1, tumor necrosis factor-alpha (TNF-α), and IL-6 can increase the production of RANKL which indicates that the inflammation that causes bone damage. In addition, TNF-α can increase osteoclast activity by inducing RANKL signaling.[8–10]

There are three phases in bone healing including reactive phase (Phase inflammation and granulation tissue formation), reparative phase (cartilage callus formation and deposition of lamellar bone) and remodeling phase (formation of bone contour).[11] Phase inflammation indicates the high concentration of ROS. ROS is an oxygen species containing molecules and free radicals, including the hydroxyl (OH) and superoxide radicals (O2), hydrogen peroxide (H2O2) and lipid peroxides. Several recent studies have reported the effects of oxidative stress on osteoclast differentiation and lead to increased bone resorption. Recent in vitro studies have demonstrated the role of ROS were not good on osteoblast activity.[8,10,12] ROS can stimulate bone resorption. Bone resorption occurs with bond oxidation and decreased pH around osteoclasts, inhibiting the function of the enzyme superoxide dismutase (SOD), an enzyme catalase (CAT) and GPx. in animal studies, ROS have been shown to stimulate osteoclast differentiation and bone resorption activity. ROS with high concentrations can damage the osteoblast cells. H2O2 of ROS reduce osteoblast cells with activating NFκB which decreased regulation of osteoblast differentiation.[6,8] The aim of this study is to compare the number of osteoblasts and osteoclast in the process of bone healing after employing *Carbonate Hydroxyapatite* and antioxidant in the Wistar rats incisor tooth extraction socket.

## MATERIALS AND METHODS

The study consists of three groups with three different treatments. In the first group, incisive mandibular teeth of Wistar rats are extraction then sockets left. In second group, incisive mandibular teeth of Wistar rats are extraction then sockets filled with antioxidants and bonegraft. In third group, incisive mandibular teeth of Wistar rats are extraction then sockets filled bonegraft, antioxidants and PRF.

On day 14, the experimental animals were sacrificed by injection of ketamine 50 mg / kg. After stopping the breath, jaw rats were taken by a small cut using saws. Having obtained the tissue, the jaw is stored in 70% buffered formalin solution. After the first 48 hours fixative replaced with a new and smaller to cut tissue fixation can penetrate evenly. In the second stage is left in the solution for 48 hours. Tissue was rinsed with water for 6-9 hours and then put in a solution of 5% decalcification nitric acid (HNO3) for 1 hour after that made a preparation.

The number of osteoblasts and osteoclasts were observed using Hematoxylin-eosin staining (HE), then the results were calculated using a microscope with a magnification of 1000x recorded. The study was used one-way ANOVA and Tukey HSD test.

## RESULTS

Based on research in three groups: control group, the combination group and the group of antioxidants and antioxidant combination bonegraft, bonegraft and PRF obtained the following results. Table 1 and Figure 1 showed that the numbers of osteoblasts were found in the group that the highest numbers were given *Carbonate Hydroxyapatite* combined with antioxidant and PRF (mean osteoblast: 15.17), the lowest numbers of osteoblasts were found in the control group (mean osteoblast: 3.96). Table 2 and Figure 2 showed that the lowest numbers of osteoclasts were found in the group that was given *Carbonate Hydroxyapatite* combined with antioxidant and PRF (mean osteoclast: 15.17) and the highest numbers of osteoclasts were found in the control group (mean osteoclast: 3.96).

Test for normality using the Kolmogorov-Smirnov test. The results obtained show that ni p = above values below 0.05, which means that all the data has a normal distribution


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**Table 1.** Number of osteoblast in socket extraction after application bonegraft, PRF and antioxidant

| Treatments | N | Mean |
|---|---|---|
| Control | 9 | 3.96 |
| Bonegraft and antioxidant | 9 | 9.31 |
| Bonegraft, antioxidant and PRF | 9 | 15.17 |

**Table 2.** Number of osteoclast in socket extraction after application bonegraft, PRF and antioxidant

| Treatments | N | Mean |
|---|---|---|
| Control | 9 | 14.56 |
| Bonegraft and antioxidant | 9 | 9.67 |
| Bonegraft, antioxidant and PRF | 9 | 5.00 |

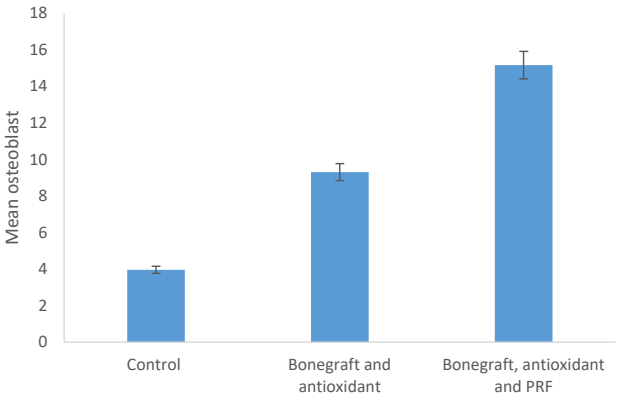


**Figure 1.** Bar chart number of osteoblasts.

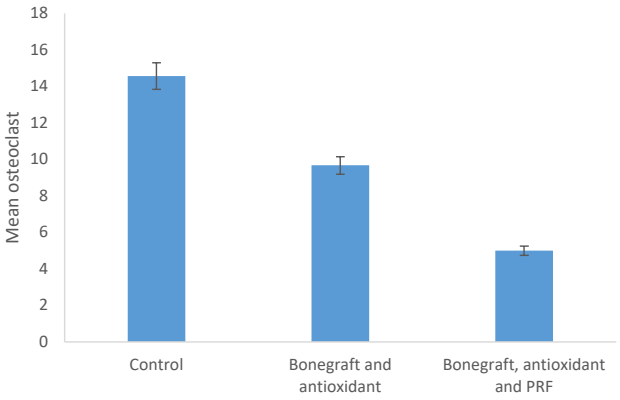


**Figure 2.** Bar chart number of osteoclasts.

(p> 0.05). Homogeneity test using test levene test values obtained osteoblasts (p = 0.169) and osteoclasts (p = 0.132), which means that research data are homogeneous (p> 0.05). Furthermore, the parametric test and one-way ANOVA p value = 0.000 (p <0.05), which means shows that there are significant differences in each treatment group of osteoblasts and osteoclasts. Then, the Tukey HSD post hoc test showed that the treatment in each group significant difference. It shows that the increased number of osteoblasts and osteoclasts decreased antioxidant contained in the combination group, bonegraft and PRF.

## DISCUSSION

This study aims to determine the number of osteoblasts and osteoclasts on the combination of antioxidant administration, bonegraft and PRF. Bonegraft often used in periodontal flap surgical procedure is expected to help the process of bone growth.[1]

Besides bone graft as a scaffold, bone healing process is influenced by the growth factor. growth factors involved in the formation of osteoblasts through IGF, PDGF and TGF-B.[2]

PRF can promote the growth of osteoblasts and proliferation PRF able to increase the production of collagen-related protein that is used in cell regeneration. PRF is a fibrin membrane which can lead to efficient cell migration and proliferation. More recently, research has shown that the growth factor PRF very slow release of about 1 week to 28 days which means that the PRF can stimulate the environment for wound healing and proliferation.[13]

In the inflammatory phase is accompanied by an increase in ROS. ROS inhibits the healing process by causing damage to osteoblasts and fibroblasts which in turn inhibits the formation of granulation tissue and reparative phase delays. Besides the inflammatory phase can be maintained over time and prevent entry into the reparative phase. [8,11]

ROS affect antioxidant defense systems of osteoblasts and osteoclasts. Osteoclast is differentiation and activation when RANK and RANKL is bond. the bond between RANK and RANKL signaling induces NF-KB causing differentiation and maturation of osteoclast precursor cells into mature osteoclasts. In the inflammatory phase also occurs activation of pro-inflammatory cytokines include IL-1, IL-6, IL-11, IL-17 and TNF-α.[6]

Hosts generate endogenous antioxidants, but in inflammatory conditions the immune system cannot produce endogenous antioxidant in sufficient quantities. Therefore, required exogenous antioxidants to reduce the damaging effects of ROS. Antioxidants can promote osteogenic transcripts and synthesis collagen. Antioxidants can express osteoblast activity and reduce the damaging effects of ROS. antioxidants can increase the number of OPG. OPG block osteoclast formation and bone resorption by inhibiting the bond between RANK and RANKL and osteoclastogenesis not happen.[6,8]

The results of the study at day 14 showed that the number of osteoblasts highest in the group of antioxidants, bonegraft and PRF. The lowest number of osteoclasts in the antioxidant group, bonegraft and PRF, in this case it takes antioxidants as a structural support to reduce the amount of excessive ROS so that the inflammatory phase is not too long and accelerate its entry into the reparative phase. Antioxidants may provide stability to the healing process and may prevent the damaging effects of ROS.[6,8]

There are significant differences in the number of osteoblasts by using one-way ANOVA test. in this study


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showed osteoblasts and osteoclasts respond well to antioxidants in the healing process socket of Wistar rats. The combination of antioxidants, bonegraft and PRF can be used for periodontal regeneration as regenerative therapy.

## CONCLUSION

The combination of bone graft, antioxidants and PRF can increase the number of osteoblasts and decrease the number of osteoclasts compared to the control group in socket Wistar rats after extraction.

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