# 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

Utari Kresnoadi[1]*, Teguh Setio Yuli Prabowo[2]

1. Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Resident, Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

**Abstract**

Preserving an effective ridge during prosthodontic treatment constitutes a prerequisite of successful denture production. An innovative material combining propolis extract with bovine bone graft is regarded as effective in inducing expression of TGF-β and IL-1β, in addition to proliferation of osteoblasts and osteoclasts.

To determine the combination effect of propolis extract and bovine bone graft on the expression of IL-1 and TGF-β.

28 Cavia cobaya subjects were divided into four groups, each containing seven members. Group I subjects were administered poly ethylene glycol (PEG), Group II subjects received bovine bone graft with PEG, Group III subjects were treated with propolis extract with PEG, and Group IV subjects were given a combination of bovine bone graft, propolis extract and PEG. On day 30, the subjects were sacrificed and paraffin blocks were prepared for immunohistochemical examination. The expressions of IL-1 and TGF-β were subsequently counted with a light microscope at 400x magnification. Finally, one-way ANOVA and Tukey HSD tests were performed ($p > 0.05$).

Significant differences existed in the expression of IL-1β and TGF-β between the four groups, the highest expression of TGF-β and the lowest expression of IL-1β both occurring in Group IV.

The combination of propolis extract and bovine bone graft increases TGF-β expression in osteoblast cell formation, reduces IL-1β expression, and decreases the number of osteoclast cells during alveolar bone regeneration.

**Experimental article (J Int Dent Med Res 2020; 13(3): 935-938)**

**Keywords:** Propolis extract, bovine bone graft, IL-1, TGF-β.

**Received date:** 26 April 2020 **Accept date:** 18 July 2020

## Introduction

Preserving the ridge following extraction represents an essential procedure necessary to provide support, retention and stabilization during denture manufacture. Post-extraction reduction of alveolar bone can be as high as 50%, while the condition can endure for as long as six months.[1] Diminished levels of alveolar bone are extremely detrimental to the effective treatment of prostodontia because support, retention, and stabilization will be severely compromised or, in extreme cases, completely absent. Therefore, preserving alveolar ridge volume during the manufacture of dentures is very important.[2]

The numerous ways of preserving alveolar bone are, at present, being widely studied, with a considerable body of research suggesting that one means of maintaining the ridge is through the application of graft material. The formation of new bone from graft material is extremely time-dependent. Therefore, it requires innovative materials that can stimulate the graft to accelerate bone formation.[3,4] According to research from Sularsih et al. (2019), the combination of chitosan-ethanol extracted Aloe vera has the suitable potential to be a scaffold for bone tissue engineering. However, the biological activity of chitosan depends on its molecular weight.[5] Propolis promotes anti-inflammatory activity due to its caffeic acid phenetyl ester (CAPE) content which is capable of inhibiting RANKL induced by the nuclear transcription activity of kappa b factor (NF-kB) during the

***Corresponding author:***
Utari Kresnoadi,
Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjend. Prof. Dr. Moestopo 47, Surabaya 60132, Indonesia.
E-mail: utari-k@fkg.unair.ac.id

osteoclast formation process.[6] Propolis also has antibacterial effect against gram positive and gram negative bacteria.[7] According to study by Mooduto et al. (2019), propolis was also found less cytototoxic against human periodontal ligament fibroblast cells (HPDLFc) compared to others irrigation solutions (sodium hypochlorite and chlorhexidine).[8] In previous studies, propolis extract combined with bovine bone graft proved highly effective in inhibiting osteoclast cells, while increasing the number of osteoblast cells.[9] The formation of osteoclast cells commences with inflammation, induced by pro-inflammatory mediators such as Interleukin IL-1, which stimulates the mitogenic precursors of osteoclasts. The function of IL-1 reinforced by TNF-α is synergistic. IL-6 is an acute phase protein that strengthens bone resorption together with IL-1 and TNF-α through mitogenic stimulation of osteoclastic cells.[10]

Elements of the literature referred to above proposed further investigation into the induction of a combination of propolis extract and bovine bone graft in IL-1 and TGF-β expression in tooth extraction sockets in relation to alveolar bone regeneration. The aim of this study was to determine the combined effect of propolis extract and bovine bone graft on the expression of IL-1 and TGF-β.

## Materials and methods

This study commenced following approval from the Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga number 595 / HRECC.FODM / IX / 2019. This study constituted experimental research incorporating the use of animal subjects with a randomized post-test only control group design. The combination of 2% propolis extract and bovine bone graft indicates that the active ingredient was 2%. The bovine bone graft supplied by the Tissue Bank of RSUD Dr. Soetomo was in powder form, ranging in size from 150 to 335 µm and weighing 500 mg.

The inclusion criteria for experimental subjects were that they must be healthy, male 3-3.5 month-old, Cavia cobaya, weighing 300-350 grams. They were anesthetized intravenously through the administering of ketamine 0.1cc / 300g BB. The incisor tooth was extracted with special needle pliers (needle holder) and the sockets subsequently filled with specific material according to the group: polyethyleneglycol (PEG), bovine bone graft, propolis extract, or a combination of propolis extract and bovine bone graft. Up to 0.1cc of the materials were inserted depending on the volume of the extraction socket, before all sockets were sewn up.

The study was conducted within a maximum period of 30 days because, according to Kresnoadi et al. (2017), the highest yield of osteoblast cells after the induction of a combination of mangosteen peel extract and demineralized freeze-dried bovine bone xenograft into the tooth socket occurs on day 30.[11] After that period, the *Cavia cobaya* were sacrificed prior to their jaws being decalcified with EDTA for 30 days[1] and then cut to make paraffin block preparations. Finally, immunohistochemical examination of IL-1 and TGF-β expression was performed.

## Results

During this study, increasing expression of TGF-β and decreasing expression of IL-1 occurred.

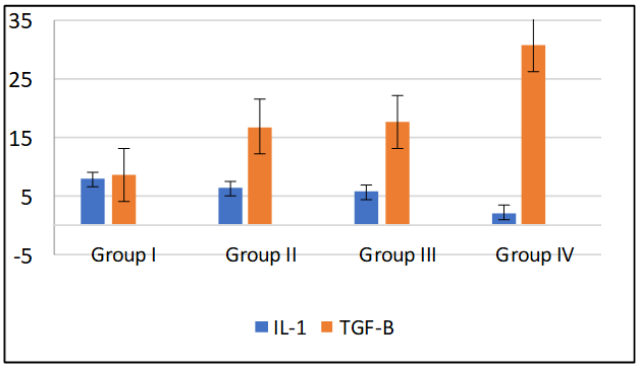


**Figure 1.** Bar chart of average results relating to the expressions of IL-1 and TGF-β on the 30th day. Group I: PEG only (control), Group II: bovine bone graft+PEG, Group III: propolis+PEG, and Group IV: bovine bone graft+propolis extract+PEG.

From the contents of the diagram, it is clear that the highest TGF-β level of 30.7 occurred in Group IV, while the lowest level of IL-1, (2.1), was that of Group IV. The results of the one-way ANOVA tests both between groups and within the treatment group showed a significant difference of $p = 0.000$ ($\alpha < 0.05$). The results of the Tukey HSD test with regard to the amount of IL-1β showed significant differences across all

groups. This pattern matched that of the results for the number of TGF-β in all treatments, in that there were significant differences between all groups.

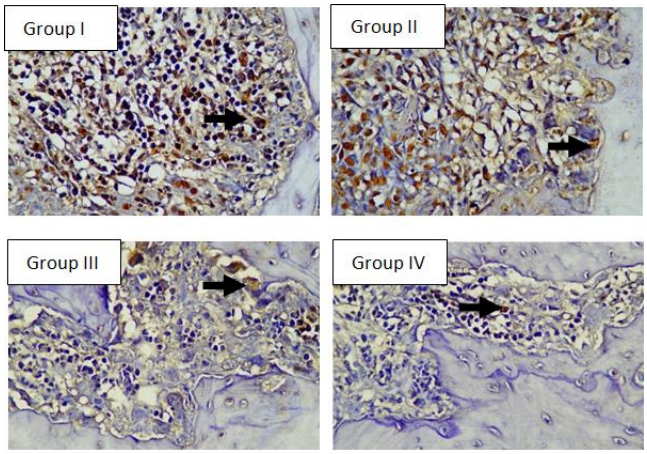


**Figure 2.** Microscopic expression of IL-1 β. Group I: PEG only (control), Group II: bovine bone graft+PEG, Group III: propolis+PEG, Group IV: bovine bone graft+ propolis extract +PEG.

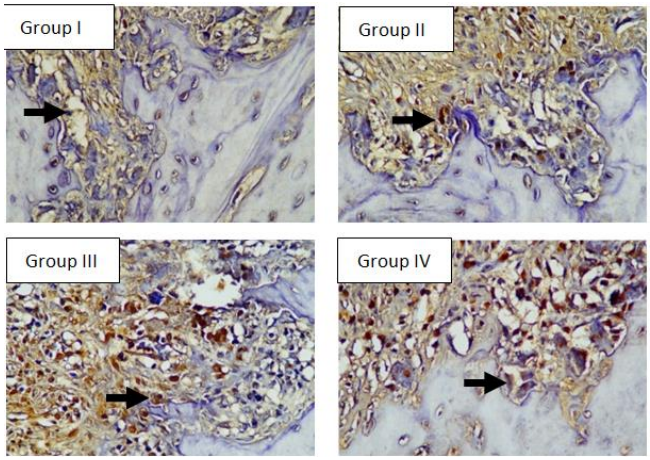


**Figure 3.** Microscopic expression of TGF-β. Group I: PEG only (control), Group II: bovine bone graft+PEG, Group III: propolis+PEG, Group IV: bovine bone graft+ propolis extract +PEG.

## Discussion

Cytokines that play a role in the process of bone resorption and remodeling include IL-1, IL-6, and TNF-α. IL-1 plays a major role in bone resorption, stimulating osteogenic mitogenic precursors.[12,13] The function of IL-1 is strengthened by TNF-α, both of which work synergistically triggering osteoclast formation by increasing the regulation of RANKL expression on the surface of bone marrow stromal cells and immature osteoblasts. RANKL is attached to RANK receptors on the surface of osteoclast precursors. The nuclear factor kappa B (NF-κB) pathway and Jun N-Terminal Kinase (JNK) trigger osteoclast formation and promote osteoclast survival.[14] Furthermore, as a proinflammatory cytokine, IL-1 is produced abundantly in the inflamed area. Besides being a mediator, IL-1 induces bone damage under inflammatory conditions such as rheumatoid arthritis and periodontitis. Thus, IL-1 exerts a stimulating effect on osteoclast survival and plays a role in activating osteoclast function.[10]

Propolis extract produces antioxidant effects that can increase alveolar bone density and accelerate the bone formation process. The CAPE content of these extracts possesses extremely strong properties which support the growth and development of human bones and activate osteoblast progenitor cells in increasing collagen formation. Propolis extract also promotes osteoclast formation and activity. However, under certain circumstances, propolis extract can also inhibit osteoclast formation.[6,15] CAPE is a natural NF-kβ inhibitor, possesses anti-tumor and anti-inflammatory properties which affect pro-inflammatory mediators. It is known that the inhibition of NF-κB and the nuclear factor of CAPE-activated T cells (NFAT) results in weakened osteoclastogenesis.[10,16]

A combination of propolis extract and bovine bone graft reduces IL-1β expression and osteoclast cell growth. TGF-β will differentiate into bone morphogenic proteins (BMPs), growth differentiation factors (GDFs), activin, and mullerian-inhibiting substances. The specifications of these various substances include: BMPs (2-8), GDFs (1,5,8, and 10) and TGFβ (1-3) which all play a role in the ossification process (intramembranous and endochondral) during the bone remodeling process. CAPE in propolis can also stimulate the production of TGF-β during the bone remodeling process.[17] The combination of propolis extract and bovine bone graft, which is a scaffold, can increase TGF-β expression. TGF-β is produced by osteoblasts, stored in sufficient quantities in the bone matrix and is an important regulator of bone development and bone metabolism homeostasis. TGF-β will differentiate into BMP, GDF, activin, and a mullerian-inhibiting substance during the bone remodeling process. The increase in TGF-β can enhance the process

of bone remodeling.[18] TGF-β plays a major role in development and treatment which affects both cartilage and hard bone metabolism, including that of the alveolar bone. The TGF-β-induced bone formation process is driven by osteoblast chemotactic attraction and increased osteoblast proliferation through the production of a protein, thereby stimulating the release of type II collagen and proteoglycan synthesis by chondrocyte cell precursors, and suppressing hematopoietic precursor cell proliferation.[19]

This results is consistent with bone healing research according to Maduratna et al. (2019). Hydroxyapatite carbonate combined with hyaluronic acid was significantly increased the number of osteoblasts, OPG and TGF-β1 in new bone formations, therefore it is a potential treatment for tissue-based periodontal defects.[20]

## Conclusions

A combination of propolis extract with bovine bone graft increases TGF-β expression in osteoblast cell formation, reduces IL-1β expression, and decreases the number of osteoclast cells in alveolar bone regeneration.

## Declaration of Interest

The authors report no conflict of interest.

## References

1. Kresnoadi U. The increasing of fibroblast growth factor 2, osteocalcin, and osteoblast due to the induction of the combination of Aloe vera and 2% xenograft concelous bovine. *Dental Journal (Majalah Kedokteran Gigi)*. 2012;45(4):228.
2. Kahnberg KE, Rasmusson L, Zellin G. *Bone Grafting Techniques for Maxillary Implants*. (Kahnberg K-E, ed.). Oxford, UK: Blackwell Munksgaard; 2005.
3. Elo JA, Herford AS, Boyne PJ. Implant success in distracted bone versus autogenous bone-grafted sites. *The Journal of oral implantology*. 2009;35(4):181–184.
4. Lupavici JA. Histologic and Clinical Results of DFDBA With Lecithin Carrier Used in Dental Implant Applications: Three Case Reports. *Practical Procedures and Aesthetic Dentistry*. 2009;21(4):223–230.
5. Sularsih, Soetjipto, Rahayu RP. The Fabrication and Characterization of Chitosan-Ethanol Extracted Aloe vera Scaffold for Alveolar Bone Healing Application. *J Int Dent Med Res*. 2019;12(4):1376–1381.
6. Guney A, Karaman I, Oner M, Yerer MB. Effects of propolis on fracture healing: An experimental study. *Phytotherapy Research*. 2011;25(11):1648–1652.
7. Al-Bayaty FH, bin Ismail IH, Zain Z binti M, Nasruddin NA binti, Suradi NF binti. Formulation and Evaluation of New Biodegradable Periodontal Chips from Malaysian Propolis in Chitosan Base. *Journal of International Dental and Medical Research*. 2017;10(2):292–298.
8. Mooduto L, Fredline C, Sampoerno G, Goenharto S, Puteri FH, Wahjuningrum DA. Cytotoxicity of Sodium Hypochlorite, Chlorhexidine and Propolis on Human Periodontal Ligament Fibroblast Cell. *Journal of International Dental and Medical Research*. 2019;12(2):476–480.
9. Lunardhi LC, Kresnoadi U, Agustono B. The effect of a combination of propolis extract and bovine bone graft on the quantity of fibroblasts , osteoblasts and osteoclasts in tooth extraction sockets. *Dental Journal (Majalah Kedokteran Gigi)*. 2019;52(32):126–132.
10. Jules J, Zhang P, Ashley JW, Wei S, Shi Z, Liu J, Michalek SM, Feng X. Molecular basis of requirement of receptor activator of nuclear factor κB signaling for interleukin 1-mediated osteoclastogenesis. *Journal of Biological Chemistry*. 2012;287(19):15728–15738.
11. Kresnoadi U, Ariani MD, Djulaeha E, Hendrijantini N. The potential of mangosteen (Garcinia mangostana) peel extract, combined with demineralized freeze-dried bovine bone xenograft, to reduce ridge resorption and alveolar bone regeneration in preserving the tooth extraction socket. *Journal of Indian Prosthodontist Society*. 2017;17(3):282–288.
12. Duan W, Wang Q, Li F, Xiang C, Zhou L, Xu J, Feng H, Wei X. Anti-catabolic effect of caffeic acid phenethyl ester, an active component of honeybee propolis on bone loss in ovariectomized mice: a micro-computed tomography study and histological analysis. *Chinese medical journal*. 2014;127(22):3932–6.
13. Fathy Al-Saeed H, Yonis Mohamed N. *The Possible Therapeutic Effects of Propolis on Osteoporosis in Diabetic Male Rats*.; 2015.
14. Putri MD. Caffeic acid phenethyl ester (CAPE) propolis dan Matrix Metalloproteinase 8 (MMP-8) dalam proses inflamasi. 2014.
15. Pileggi R, Antony K, Johnson K, Zuo J, Shannon Holliday L. Propolis inhibits osteoclast maturation. *Dental Traumatology*. 2009;25(6):584–588.
16. Ang ESM, Pavlos NJ, Chai LY, Qi M, Cheng TS, Steer JH, Joyce DA, Zheng MH, Xu J. Caffeic acid phenethyl ester, an active component of honeybee propolis attenuates osteoclastogenesis and bone resorption via the suppression of RANKL-induced NF-κB and NFAT activity. *Journal of Cellular Physiology*. 2009;221(3):642–649.
17. Al-Molla, Al-Ghaban N, Taher A. Immunohistochemical evaluation: The effects of propolis on osseointegration of dental implants in rabbit's tibia. *Journal of Dental Research and Review*. 2014;1(3):123.
18. Chen G, Deng C, Li Y. TGF- β and BMP Signaling in Osteoblast Differentiation and Bone Formation. *International Journal of Biological Science*. 2012;8(3):272–288.
19. Kim S Il, Kwak JH, Zachariah M, He Y, Wang L, Choi ME. TGF-β-activated kinase 1 and TAK1-binding protein 1 cooperate to mediate TGF-β1-induced MKK3-p38 MAPK activation and stimulation of type I collagen. *American Journal of Physiology - Renal Physiology*. 2007;292(5):F1471-8.
20. Setiawatie EM, Widiyanti P, Ryan M, Rubianto M. Carbonate Hydroxyapatite-Hyaluronic Acid as Bone http://www.jidmr.com Ernie Maduratna Setiawatie and et al Carbonate Hydroxyapatite-Hyaluronic Acid as Bone Healing Accelerator: in-vitro and in-vivo Studies on the Alveolar Bone of Wistar Rats. *Journal of International Dental and Medical Research*. 2019;12(4):1280–1286.