Original Article

Effects of mangosteen peel extract combined with demineralized freeze-dried bovine bone xenograft on osteocalcin, collagen 1, and osteoblast as alveolar bone regeneration in socket preservation

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Abstract

Background: Tooth extraction will provoke changes in alveolar bone morphology and dimensions. Postextraction bone resorption can lead to significant problems for restorative dentistry. Therefore, the extracted tooth socket needs to be preserved to reduce alveolar ridge bone resorption. This research aimed to analyze the expression and levels of osteocalcin, collagen 1, and osteoblasts in extracted tooth sockets filled with a combination of mangosteen peel extract and demineralized freeze-dried bovine bone xenograft (DFDBBX).

Material and Methods: Fifty-six *Cavia cobaya*, whose lower left incisors had been extracted, were divided into eight groups according to the substance used to fill their sockets on days 7 and 30, Poly ethylene glycol, DFDBBX, mangosteen peel extract, or a combination of mangosteen peel extract and DFDBBX. This research was conducted in several stages; the application of mangosteen peel extract combined with graft material was performed as the form of tooth extraction socket preservation. The *C. cobaya* rats were subsequently examined by immunohistochemical methods to measure osteocalcin and collagen 1 expressions, whereas histological examination was conducted to calculate the number of osteoblasts in accordance with the duration of the research.

Results: On days 7 and 30, the group treated with a combination of DFDBBX and mangosteen peel extract which had the highest expression and levels of osteocalcin, collagen 1, and osteoblasts.

Conclusion: The administration of mangosteen peel extract combined with DFDBBX as a means of tooth extraction socket preservation can increase osteocalcin and collagen 1 expression. Consequently, osteoblasts as a means of alveolar bone regeneration will increase in number.

Keywords: Alveolar bone, collagen 1, demineralized freeze-dried bovine bone xenograft, mangosteen peel extract, osteocalcin, socket preservation

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INTRODUCTION

Tooth extraction usually results in trauma leading to inflammation which, in turn, stimulates the development of osteoclasts and the resorption of alveolar bone. Subsequently, the postextraction resorption of alveolar bone can cause significant problems for restorative dentistry since removal of the tooth may result in changes of the morphology and dimensions of the alveolar bone. Thus, preservation of the extracted tooth socket is required to decrease the occurrence of alveolar bone ridge resorption. Unfortunately, many cases of extraction are not followed by appropriate and effective alveolar ridge management.

In the field of medicine and dentistry, grafts are often used to repair bone defects and augmentation.[1-6] The bone graft material often used in the field of dentistry is xenograft since it is relatively easy to apply.[7] The most commonly used type is bovine-derived bone xenograft made from demineralized bovine bone whose organic mineral has been removed.[8] Demineralized freeze-dried bovine bone xenograft (DFBBX) is a product of Batan whose immunogenic properties have been removed. DFBBX is also free of soft tissue.

Bone composition consists of minerals, organic matrix and cells at a ratio of 65% and water at ratio 35%. The proportion of organic matrix is about 35% of the weight of the bone when in a dry state. It consists of 90% collagen as the most common bone protein and other noncollagen proteins, namely osteonectin, osteocalcin, osteopontin, and sialoprotein. Osteocalcin and collagen 1 represent the most abundant products in osteoblast cells. Furthermore, osteoblasts also synthesize other proteins in the bone matrix, namely osteocalcin and osteonectin, constituting 40%–50% of the noncollagen proteins in bone.[9]

A significant number of natural ingredients are currently used in the field of medicine, including mangosteen (*Garcinia mangostana* L.). One of the substances contained in mangosteen is xanton which acts as an anti-inflammatory, anti-oxidant, anti-bacterial, antitumor, and anticancer agent.[10]

Mangosteen peel extract combined with DFDBBX, according to previous research performed by Kresnoadi *et al*., can increase the number of osteoblasts at a concentration of 2%.[11] On the other hand, Guskuma *et al*. analyzed protein expression during the osteogenesis process from day 7 to day 30. The research shows that protein is expressed on day 7 of the regeneration period, reaching a peak of bone formation on day 30.[12] Therefore, this research aimed to identify the effects of mangosteen peel extract combined with DFDBBX on the expressions of osteocalcin and collagen 1, the most prominent osteoblast-produced markers of alveolar bone regeneration, on days 7 and 30.

MATERIALS AND METHODS

Research design and animal model

Ethical clearance for this research was issued by the Committee of Health Research Ethics of Faculty of Dental Medicine No. 008/HRECCFODM/I/2017. This research was an experimental investigation incorporating randomized posttest control group design. It involved the use of healthy, active male *Cavia cabaya* weighing 300–350 g and aged 3–3.5 month as the research sample which was divided into eight groups, each consisting of seven subjects. In Groups I and II, the teeth of the *Cavia cabaya* were removed and the cavities filled with poly ethyl glycol (PEG) as controls which were subsequently examined on days 7 and 30. Moreover, the teeth of the *Cavia cabaya* in Groups III and IV were extracted, the sockets being filled with DFDBBX + PEG, and then checked on days 7 and 30. The teeth of the *Cavia cabaya* in Groups V and VI were similarly extracted. However, the resulting sockets were filled with manggosteen peel extract + PEG, with observation being carried out on days 7 and 30. Meanwhile, in Groups VII and VIII, the sockets of the extracted teeth of the Cavia cabaya were filled with manggosteen peel extract + DFDBBX + PEG, before being examined on days 7 and 30.

Research procedure

The fillers used as independent variables in this research were 25 g of PEG, 0.5 g of DFDBBX + 24.5 g of PEG, 0.5 g of mangosteen peel extract + 24.5 g of PEG, in addition to 0.5 g of mangosteen peel extract + 0.5 g of DFDBBX + 24 g of PEG as a carrier.[13] The identification of mangosteen peel extract was then performed at Laboratory Research and Industrial Consultants, Surabaya, Indonesia. The mangosteen used in this research was classified as Kingdom Plantae, Spermatophyta Division, Angiospermae Subdivison, Dicotyledoneae Class, Guttiferae Family, Garcinia Genum, and *Garcinia mangostana L* species. The mangosteen peel extract employed was composed of 1.22% flavonoid, 2.88% tanin, 2.56% xanton, and 3.01% mangosteen.

In addition, sterile aquadest, DFDBBX produced by Batan, absolute alcohol, 70% alcohol, anti-osteocalcin, as well as anti-Collagen Type 1 (Santa Cruzz) monoclonal antibodies were used. This research also utilized an immunostaining kit reagent (Licca Novocastra). To conduct an analysis of the specimen, this research also used H and E reagent,

micropipette, tip (yellow, white, and blue), light microscopy, object glass coated with polylysine, and covered glass.

Osteocalcin and collagen 1 as products expressed by osteoblast cells were examined by means of the immunohistochemistry method, using anti-osteocalcin monoclonal antibodies and anti-Collagen 1 monoclonal antibodies. Osteocalcin and Collagen type I expression was then analyzed using a light microscope with a view field of ×10 and a magnification of ×400.

RESULTS

The mean and standard deviation of the osteocalcin and collagen type 1 expressions examined on days 7 and 30 can be seen in Figure 1.

The mean number of osteocalcin and collagen 1 expressions increased from the control group, the group filled with DFDBBX, the group filled with mangosteen peel extract, to the group filled with the combination of mangosteen peel extract and DFDBBX on both days 7 and30. The mean increases of osteoblasts, osteocalcin, and collagen 1 on days 7 and 30 is shown in Figures 2 and 3.

The highest mean number of osteoblast, osteocalcin, and collagen 1 expressions was found in the group whose cavities had been filled with a combination of mangosteen peel extract and DFDBBX. The expression of collagen 1 and osteocalcin can be found in Figures 4 and 5.

The results of the ANOVA analysis of osteocalcin expression on days 7 and 30 indicated that there were significant differences in the expression of osteocalcin between the control group and the treatment groups, each of which had been induced with DFDBBX, mangosteen peel extract, and the combination of mangosteen peel extract and DFDBBX ($P < 0.05$). Moreover, the Tukey's HSD results confirmed significant differences in the expression of osteocalcin between the treatment groups each of which was induced with DFDBBX, mangosteen peel extract, and the combination of mangosteen peel extract and DFDBBX ($P < 0.05$).

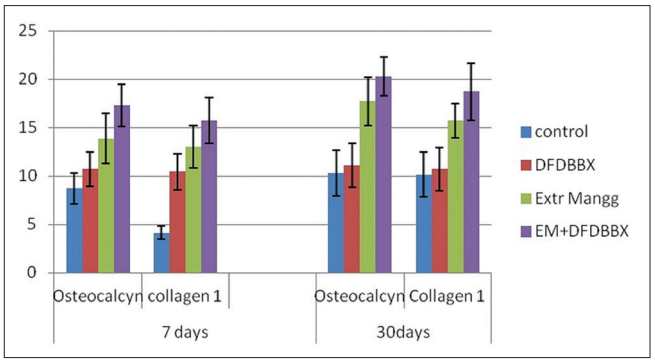

Figure 1: Bar charts of the average of osteocalcin and collagen 1 expressions on days 7 and 30 during the immunohistochemistry examination

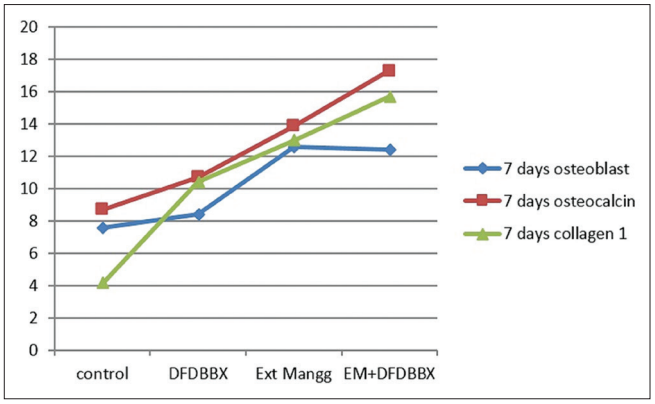

Figure 2: Graphic of the average osteoblasts, osteocalcin, and collagen 1 over 7 days of examination. Increases in osteoblast, osteocalcin, and collagen 1 occurred

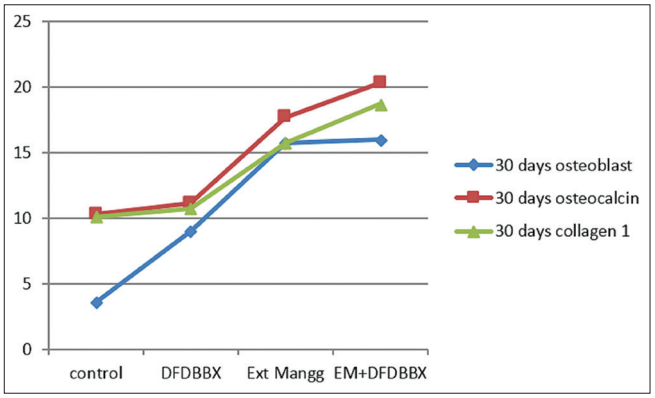

Figure 3: Graphic of the average osteoblasts, osteocalcin, and collagen 1 on 30-day examination. Increases in osteoblasts, osteocalcin, and collagen 1 occurred

DISCUSSION

The wound healing process after tooth extraction involves certain stages, namely, early protein expression, cell apposition, remodeling, and saturation maturation revocation. At a microscope level, the bone healing process begins with coagulate formation followed by inflammatory cell infiltration initiating necrotic tissue removal. Subsequently, loose connective tissue will migrate to stabilize the extracellular matrix. Thereafter, the fibrous connective tissue will be replaced by woven bone which then develops into bone and marrow,[14] a process referred to as ossification or osteogenesis.[15]

In this research, postextraction socket preservation was performed by means of DFDBBX material combined with mangosteen peel extract used as filling

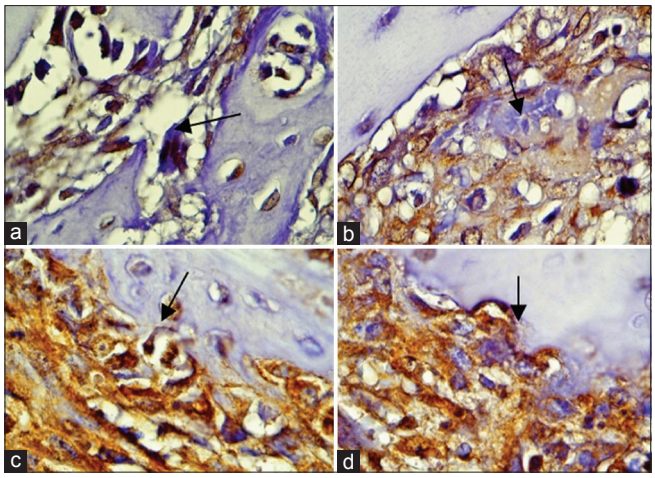

Figure 4: Collagen 1 expressions on day 30 were depicted in the following images. (a) Collagen 1 expression in the control group filled only with poly ethyl glycol on day 30. (b) Collagen 1 expression in the group filled with demineralized freeze-dried bovine bone xenograft + poly ethyl glycol on day 30. (c) Collagen 1 expression in the group filled with mangosteen peel extract + poly ethyl glycol on day 30. (d) Collagen 1 expression in the group filled with mangosteen peel extract + demineralized freeze-dried bovine bone xenograft + poly ethyl glycol on day 30

in the extraction socket of those *Cavia cobaya* rats whose lower left incisors had been removed. Selection of the *C. cobaya* rats had been on the basis of their hormonal and immunological response (thymus and bone marrow, innate and complementary immune systems, respiratory physiology, response to corticosteroids, exogenous Vitamin C presence, and delayed-type hypersensitivity after exposure to infection which was similar to that in humans. It is also suggested a resemblance between the chromosome of *C. cobayas* and that possessed by humans.[16]

Xenograft contains hydroxyapatite which bears some similarity to human bone in terms of the following characteristics; it covers the inner surface area, it is porous, its crystalline size, its calcium-phosphorus ratio leading to revascularization, and its being replaced by new bone. Similarly, research conducted by Jamjoom and Cohen indicated that xenograft can be used as an alternative to hard tissue transfer in patients susceptible to infectious diseases. Xenograft also possesses osteoconduction properties and can, therefore, serve as a scaffold for the surrounding cells to infiltrate and migrate into the graft.[17] Thus, the insignificant difference in the degree of osteocalcin expression between the control group and the group with DFDBBX indicates that the administration of DFDBBX as a socket preservation graft has no effect on osteocalcin expression. DFDBBX merely functions as a scaffold.

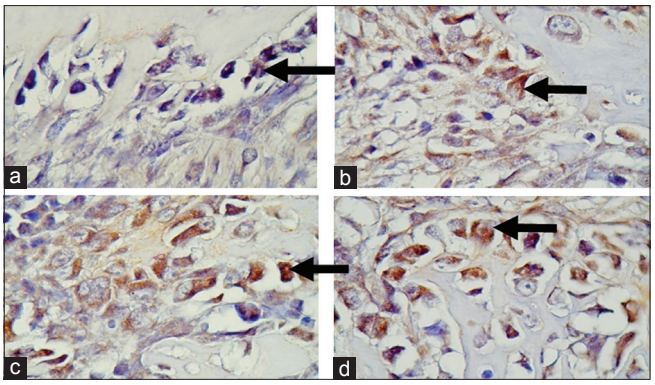

Figure 5: Osteocalcin expressions on day 30 were depicted in the following images. (a) Osteocalcin expression in the control group filled only with poly ethyl glycol on day 30. (b) Osteocalcin expression in the group filled with demineralized freeze-dried bovine bone xenograft + poly ethyl glycol on day 30. (c) Osteocalcin expression in the group filled with mangosteen peel extract and poly ethyl glycol on day 30. (d) Osteocalcin expression in the group filled with mangosteen peel extract and demineralized freeze-dried bovine bone xenograft + poly ethyl glycol on day 30

These results were also supported by previous research undertaken by Mardiana showing that mangosteen extract is composed of xanton-a cyclic polyphenol ketone compound. There are about forty species of xanton in mangosteen peel.[18] The most useful types of xanthones in mangosteen peel are α-mangostin and γ-mangostin. γ-mangostin exert a potentially inhibitory effect on the synthesis mechanism of prostaglandin E2 (PGE2). γ-mangostin in cyclooxygenase enzyme activity even can inhibit the conversion of arachidonic acid into PGE2.[19] Nakatani *et al.* also showed that γ-mangostin is able to inhibit the activity of IκB kinase inhibitor which will activate nuclear factor-κB (NF-κB).[20]

Therefore, as with previous research, the results of this investigation revealed that application of mangosteen peel extract to the extraction socket will suppress inflammatory response, thereby reducing the activity of inflammatory cells. For instance, the γ-mangostin in mangosteen peel extract can lead indirectly to a decrease in the activity of NF-κB which, in turn, will cause the number of osteoblast progenitor cells to increase.[19,20] Consequently, the osteoblast differentiation process will be more rapid and will improve the osteogenesis process.

Werner and Grose asserted that, on the first day after injury, the wound will be filled with frozen blood and neutrophils will then invade the blood clots. On the following days 3–7, neutrophils will experience apoptosis. As a result, numerous macrophages will be present in the wound tissue, and endothelial cells will subsequently migrate, develop, and form new blood vessels. Thereafter, fibroblasts will migrate into the wound tissue, multiply, and form new

tissues known as granulation tissues. One to two weeks after injury, the wound will be filled by granulation tissue. When fibroblasts evolve into myofibroblasts, this can lead to contractions and collagen deposition. The greatest product of osteoblast cells is collagen type-1 which will form collagen fibrils.[21] Furthermore, osteoblasts also synthesize other proteins in the bone matrix, namely, osteocalcin and osteonectin, which constitute 40%–50% of the noncollagen proteins in bone.[9] Therefore, it can be said that osteocalcin and collagen 1 are products of osteoblasts.

According to the findings of research performed by Scheller and Frerich, osteocalcin represents a bone marker with the result that increased bone formation will coincide with higher expressions of both osteocalcin and collagen 1.[22]

CONCLUSION

Postextraction ridge preservation involving the application of fillings consisting of a combination of mangosteen peel extract and DFDBBX can potentially increase osteocalcin and collagen1 expressions, as producers of osteoblasts, thereby accelerating alveolar bone regeneration.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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