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Research Report

BMP-7 expression after induction of hydroxyapatite bovine tooth graft gel as a socket preservation of the alveolar bone

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ABSTRACT

Background: *Hemisection is an endodontic surgical procedure that can leave alveolar bone defects. Changes in the dimensions of the alveolar bone can be minimized by preserving the alveolar bone socket using a bone graft. Induction of Hydroxyapatite Bovine Tooth Graft (HAp-BTG) in the alveolar bone socket is expected to increase the expression of BMP-7 which is a growth factor in osteogenesis.* ***Purpose:*** *To describe the increase in BMP-7 expression after induction of hydroxyapatite bovine tooth graft gel as a socket preservation of the alveolar bone of Wistar rats on the 14th and 28th days.* ***Methods:*** *28 Wistar rats were extracted on the lower left incisor. Post-extraction sockets were induced by Polyethylene Glycol (PEG) as the control group and HAp-BTG + PEG as the treatment group. On the 14th and 28th day the Wistar rats were euthanized and the mandibles were taken. By using immunohistochemical staining on anatomic histopathological examination, BMP-7 expression was observed using a microscope with 1000× magnification. The research results were analyzed using One Way ANOVA.* ***Results:*** *There was a significant increase in BMP-7 expression between the control group and the treatment group.* ***Conclusion:*** *BMP-7 expression after induction of hydroxyapatite bovine tooth graft gel as a socket preservation of the alveolar bone of Wistar rats increased on the 14th and 28th days.*

Keywords: *bovine tooth graft; socket preservation; BMP-7; osteogenesis; dental health*

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INTRODUCTION

Endodontic surgical procedures such as hemisection can leave a bone defect in the form of a socket. Sockets after endodontic surgery cause changes in the width and height of the bone dimensions so that they are included in the alveolar bone defects.[1] Alveolar bone defects require treatment to prevent damage and alveolar bone resorption.[2] This is a challenge for dentists because the process of repairing alveolar bone defects is often disrupted or even fails.[3,4]

Socket preservation is a procedure that allows socket healing without bone loss and changes in ridge dimensions.[5] In this procedure, a grafting material is administered to accelerate the healing process of the alveolar bone socket and improve the prognosis of the tooth.[2] Various previous studies have proven that administering bone grafts into the alveolar bone socket can accelerate the process of bone formation or osteogenesis.[6,7]

Bone graft functions as a filling material for augmentation so that it can help with bone reconstruction and stimulate the process of bone formation.[8] Previous studies have shown that several types of animal teeth can be used as alternative materials, including teeth of cattle, horses, primates, pigs, and sharks.[2] Grafts from bovine teeth containing hydroxyapatite or also known as hydroxyapatite bovine tooth graft have osteogenic properties and contain growth factors, making them a widely used choice as a filling material for human tooth replacement.[9]

One of the growth factors in the process of osteogenesis is Bone Morphogenetic Protein-7. BMP-7 has an important role in the proliferation, migration, and differentiation of Mesenchymal Stem Cells (MSC).[10] BMP-7 is a strong osteoinductor because it has a role in ossification, osteogenic cell differentiation, stimulates osteoblasts, and helps accumulate collagen until bone formation or osteogenesis occurs.[11]

Hydroxyapatite bovine tooth graft can be applied into the alveolar bone socket with a combination of PEG as a carrier material.[12] The combination of 80% PEG 400 and 20% PEG 4000 will produce a gel consistency so that HAp-BTG in powder form can adhere and apply properly to the alveolar bone socket. A laboratory experimental study was conducted to observe the expression of BMP-7 in the alveolar bone sockets of Wistar rats after being induced with HAp-BTG gel on the 14th and 28th-day post-extraction.

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MATERIALS AND METHODS

This study was conducted as an animal experimental laboratory in Wistar rats with a randomized post-test only control group design and was approved by the ethical committee of the Faculty of Dentistry, Airlangga University (No. 442/HRECC.FODM/VII/2022). This study used 28 male Wistar rats (*Rattus norvegicus* strain Wistar) aged 3-3.5 months and weighing 250-300 grams. All experimental animals were in good health and had no injuries or defects. Wistar rats were divided into two groups, namely the treatment and control groups. Rats were anesthetized with a combination of ketamine and xylazine in a 1:1 ratio intramuscularly in the right posterior femoral region. Then the lower left of the tooth was extracted with forceps. Post-extraction sockets were induced by PEG as the control group and the HAp-BTG+PEG combination as the treatment group. Then stitched and evaluated for 14 and 28 days.

On the 14th and 28th-day, 7 Wistar rats from each group euthanized and the left mandible was taken. Then the tissue is immersed in formalin liquid for tissue fixation. The mandibular tissue was made of paraffin block preparations and cut 4 micrometers thick. Then the slides were stained with immunohistochemistry (IHC). BMP-7 expression was observed using a light microscope with a 20× field of view and 1000× magnification. BMP-7 is expressed in a brownish color.

The data is then processed using the Shapiro Wilk test to find out whether the collected data is normally distributed. Levene's Test as a variance homogeneity test to test whether the data variance is homogeneous. One-Way ANOVA was used as a test of difference, and a post-hoc test was performed using Tukey HSD.

RESULTS

The data of this study were obtained from histological images showing BMP-7 expression (Figure 1 and 2 in arrows). Figure 1 shows that the expression of BMP-7 in the 14th-day of treatment group was significantly higher than the 14th-day of control group. In addition, in Figure 2 there is a significant difference in the expression of BMP-7 in the treatment group on day 28 and the control group on day 28.

The results showed that the average expression of BMP-7 increased significantly over time (day 28 versus day 14), both in the treatment and control groups (One Way ANOVA test, $p<0.05$). Then the Tukey HSD test was carried out at a significant level of 0.05. There were significant differences between the treatment group (higher) versus

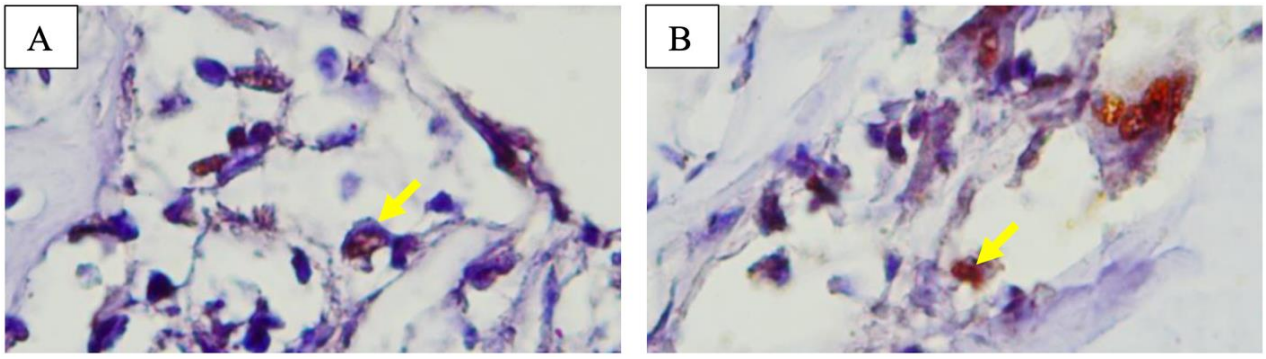

Figure 1. Microscopic view with 1000× magnification of BMP-7 (yellow arrow) in the 14th-day control group (A) and the 14th-day treatment group (B).

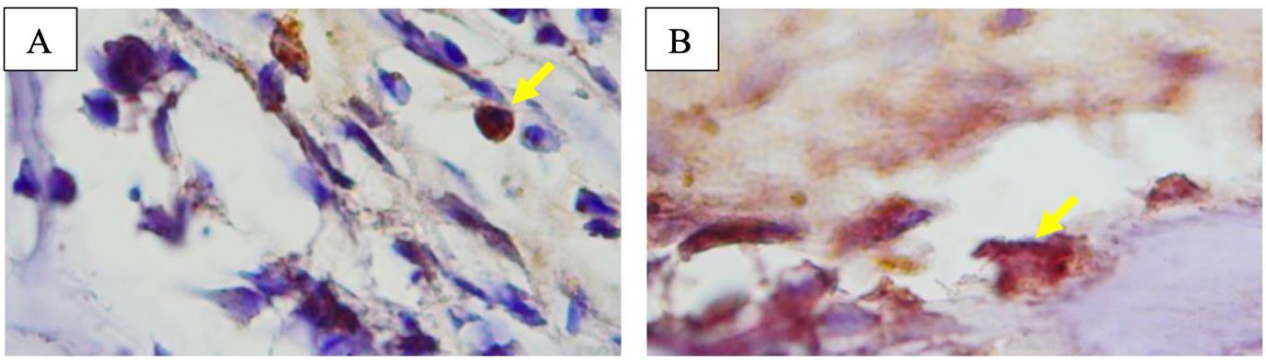

Figure 2. Microscopic examination with 1000× magnification of BMP-7 (yellow arrow) in the 28th-day control group (A) and the 28th-day treatment group (B).

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Table 1. The average number of BMP-7 expression in 14th and 28th days, and between control and treatment groups

Sample Group	n	Mean	Standard Deviation
14th-day Control Group	7	3.14	1.21
14th-day Treatment Group	7	10.14	1.46
28th-day Control Group	7	6.57	1.72
28th-day Treatment Group	7	12.71	1.60

Table 2. The significance among the number of BMP-7 expression, of 14 versus 28 days, and between the control and treatment group

Sample Group	14th-day Control Group	14th-day Treatment Group	28th-day Control Group	28th-day Treatment Group
14th-day Control Group				
14th-day Treatment Group	0.000			
28th-day Control Group	0.002	0.001		
28th-day Treatment Group	0.000	0.020	0.000	

the control group and on day 28 versus day 14 (shown by Table 1 and 2)

DISCUSSION

Bone Morphogenetic Protein-7 or osteogenic protein-1 is a growth factor that can mediate bone formation and repair as well as MSC differentiation. BMP-7 has osteoinductive properties because it can stimulate the expression of substances needed for the process of new bone growth. In this study, the treatment group was induced with HAp-BTG gel. The gel was made from a combination of PEG 400 and PEG 4000. PEG was used as a carrier so HAp-BTG could adhere to the alveolar bone socket. PEG has the characteristic of being soluble in water, benzene, methanol and dichloromethane. PEG is also a flexible polymer and has a low toxic content.[13] The addition of PEG in several studies is considered to be able to increase adhesive properties so that it can assist in attachment to the surface of the substrate.[14]

The results showed that post-extraction BMP-7 expression in the alveolar bone socket increased significantly ($p<0.05$) on day 28 compared to day 14, both in the control group and the treatment group. Studies on bone healing state that BMP-7 shows a period of expression during bone healing, when soft callus ossification occurs, osteoblast proliferation, until the fusion of the two ends of the bone occurs.[15,16] In the early period of new bone formation after socket formation, BMP plays a role in encouraging MSC differentiation into osteogenic cells to form new bone.[17] Furthermore, BMP-7 expression continued to increase until the 28th day after the formation of the alveolar bone socket, even according to the study of Sun et al. BMP-7 still shows its expression for more than 28 days.[18]

This study also showed that the average expression of BMP-7 in the treatment group on day 14 and 28 was higher than the average in the control group on days 14 and 28. This suggests a role for HAp-BTG gel in increasing BMP-7 expression during osteogenesis. HAp-BTG contains hydroxyapatite, collagen, and several growth factors such as IGF, TGF-β, and BMP which play a role in the process of osteogenesis. Hydroxyapatite has osteoinductive and osteoconductive properties so that it can stimulate osteoblast differentiation and bone formation. The microporous surface produced by HAp-BTG will expand the absorption area of growth factors such as BMP-7 so that the average expression of BMP-7 in the socket induced by HAp-BTG gel becomes higher. These results are in line with research conducted by Kresnoadi et al. that there was an increase in BMP-7 expression after administration of a combination of Ethanol Extract of Propolis (EEP) and Bovine Bone Graft (BBG) in the post-extraction alveolar bone socket.[19] In addition, research conducted by Fauzia et al. stated that bone grafts containing hydroxyapatite are potential BMP carriers.[20] In conclusion, our study showed that induction of HAp-BTG can increase BMP-7 expression. HAp-BTG can accelerate the osteogenesis in the alveolar bone socket by inducing BMP-7.

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