Conservative Dentistry Journal Vol. 13 No. 2 July-December 2023; 56-59

Research Report

Effects of hydroxyapatite bovine tooth graft (HAp-BTG) and polyethylene glycol (PEG) combinations in post extraction sockets on the amount of osteoid

Nanik Zubaidah[1], Raden Ajeng Wulandini[2], Nur Hikmatus Sa'adah[2], Tyas Ramadhini Arrianti[2], Nirawati Pribadi[1]
[1]Department of Conservative Dentistry, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia
[2]Undergraduate Student of Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia

ABSTRACT

Background: *One of the actions in the field of tooth conservation such as hemisection can result in changes, especially in hard tissue due to the extraction of tooth roots and part of the tooth crown. After tooth extraction, socket wound healing will occur which ends with the process of osteogenesis. This process can reduce the dimensions of the socket due to alveolar bone resorption. Socket preservation can prevent dimension reduction and bone resorption to achieve maximum treatment results. The administration of Hydroxyapatite bovine tooth graft (HAp-BTG) into the post-extraction socket is expected to increase the formation of osteoid matrix which is important in the formation of new bone.* ***Purpose:*** *Proving the effect of adding a combination of HAp-BTG and PEG into the post-extraction socket on increasing the number of osteoid.* ***Methods:*** *32 wistar rats were divided into control and treatment groups. Then the lower left incisor was extracted, the post-extraction socket was filled with PEG for the control group and a combination of HAp-BTG and PEG for the treatment group. On the 14th and 28th day the wistar rats were terminated and the mandibles were taken to make tissue preparations. HE staining was performed on the samples and observing the extent of the osteoid using a microscope with 400x magnification.* ***Results:*** *There was a significant difference between the control group and the treatment group on the 14th and 28th days.* ***Conclusion:*** *Administration of a combination of HAp-BTG and PEG into the post-extraction socket increased the number of osteoid on the 14th and 28th days.*

Keywords: *Hydroxyapatite bovine tooth graft (HAp-BTG), osteoid, osteogenesis*

Correspondence: Nanik Zubaidah, Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjen. Prof. Dr. Moestopo No.47 Surabaya, 60132 Indonesia. Email: nanik-z@fkg.unair.ac.id

INTRODUCTION

One of the treatments in the field of conservation is endodontic surgery. This treatment is performed when a case cannot be treated with conventional endodontic treatment with the aim of eliminating the infection and creating an optimal environment for healing of the periapical tissue and bone. One of them is the act of hemisection or removal of the root and part of the crown.[1] After the tooth is extracted, a series of biological events occur continuously in the alveolus to close the wound and maintain tissue homeostasis which is called socket healing.[2,3] Socket wound healing begins with the formation of a blood clot soon after the tooth is extracted, then migration of inflammatory cells, formation of granulation tissue and new blood vessels, synthesis of osteoid matrix by osteoblasts, and mineralization of the matrix into new bone.[3,4] Osteoid synthesis by osteoblasts begins in the 1st to 2nd week. After 2 weeks, the matrix mineralizes and forms woven bone which can be observed especially in the lateral and apical parts of the socket.[2,3,5] Woven bone will be replaced by lamellar bone, this process is called bone remodeling which begins after the 28th day of bone healing. The post-extraction socket healing process is considered complete after the formation of new bone trabeculae (osteogenesis) in the tooth alveoli.[5,6]

The process of alveolar bone resorption results in a reduction in the dimensions of the socket along with the healing process.[7] Placement of the graft can improve the stability of the blood clot that forms and will be replaced with a temporary connective tissue matrix, woven and lamellar bone, and bone marrow. In addition, the graft in the socket can prevent the possibility of reducing the hard tissue volume and become a scaffold for the growth of the vascular and cellular components needed to form new bone with sufficient quality and quantity.[7,8]

There are several types of bone grafts for socket preservation, including autografts, allografts, xenografts, and alloplastic.[9–12] Bovine teeth are one of the choices of graft materials because they contain a lot of hydroxyapatite[13]. Hydroxyapatite is often used as a bone graft material because of its ability to repair and as a good biocompatible material for hard tissues such as bone and exhibits osteoconductive

DOI: https://doi.org/10.20473/cdj.v13i2.2023.56-59

and non-toxic properties.[13,14] Mixing PEG with HAp-BTG material serves as a carrier material for graft particles into the socket.[15,16] Previous research by Guarnieri et al. showed osteoid formation of 26.85% in extracted tooth sockets after being given bovine xenografts.[11] The aim of this study was to prove the effect of giving a combination of HAp-BTG and PEG in the post-extraction sockets of Wistar rats on increasing the number of osteoid on days 14th and 28th.

MATERIALS AND METHODS

This research was conducted at the Airlangga University Biochemistry Laboratory and was approved by the ethics committee of the Faculty of Dentistry, Airlangga University (No. 534/HRECC.FODM/VIII/2022). This research is an experimental analytical study with a randomized post-test only control group design in vivo on Rattus norvegicus Wistar strain (Wistar rats). The samples used were male wistar rats, aged 3 – 3.5 months, weighing 250 – 300 grams, healthy and agile, and had no injuries to all parts of the body or tooth decay. This study used 32 wistar rats which were randomly divided into 16 rats each for the control and treatment groups. Wistar rats are kept in plastic cages for 5-7 days, given a mat of husks and covered with wire mesh on the top, and placed in a room with sufficient air and light.[17,18]

The combination of PEG 400 and 4000 was made following a ratio of 80% : 20% to get a total of 25 grams of PEG, PEG 4000 was heated over a water bath until it melted, then PEG 400 was added and stirred until homogeneous. Then 0.5 grams of powder type HAp-BTG was mixed with 24.5 grams of PEG combination as a carrier material. Wistar rats were anesthetized with a combination of ketamine and xylazine intramuscularly and then the lower left incisor was extracted. The post extraction socket was given a HAp-BTG and PEG combination for the treatment group and a PEG combination for the control group as much as 0.1 mL using a syringe and the socket was sutured with 3-0 nonabsorbable black silk thread to prevent the combination from coming out of the socket.[19] On the 14th and 28th day the rats were terminated with a combination of ketamine and xylazine anesthesia and then the mandible was cut to make tissue preparations. The mandible was fixed and decalcified with 10% EDTA solution then embedded in a paraffin block, then the block was cut 4 µm thick using a microtome to be placed on a slide and stained with hematoxylin eosin (HE).[18] Osteoid was observed using a light microscope with a magnification of 400x at 5 fields of view. The osteoid calculation is obtained from the area of the osteoid divided by the total area of measurement then expressed in percent. Data analysis was performed using SPSS v.25.

RESULTS

General data in this study were obtained from the calculation of the observed osteoid area of the socket preparations after tooth extraction of Wistar rats in the control and treatment groups on the 14th and 28th days. In Figures 1 and 2, the pink irregularly shaped osteoid matrix are shown by arrows, the osteoid areas are surrounded by osteoblasts.

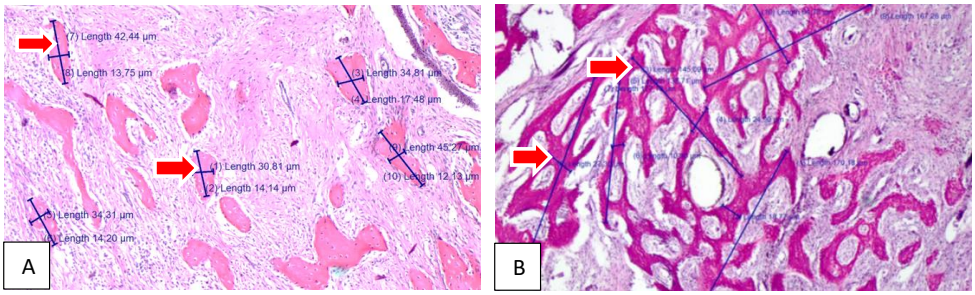

Figure 1. Osteoid matrix (red arrow) with 400x magnification in the control group on day 14 (A) and the treatment group on day 14 (B).

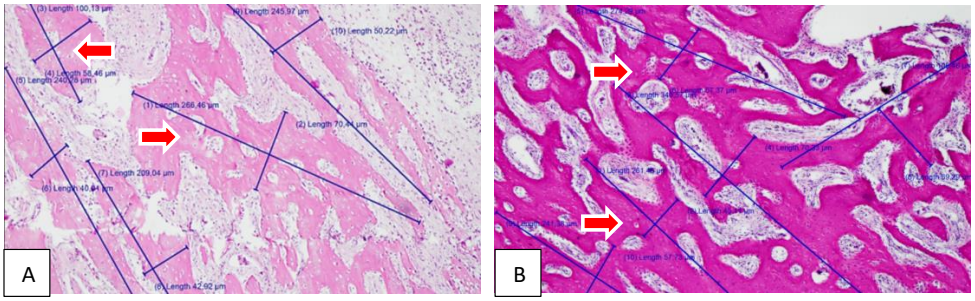

Figure 2. Osteoid matrix (red arrow) with 400x magnification in the control group on day 28 (A) and the treatment group on day 28 (B).

Table 1. Results of osteoid observations on the 14th and 28th days

Sample Group	N	Mean ± SD
14th-day Control Group	8	0.133 ± 0.13
14th-day Treatment Group	8	0.335 ± 0.10
28th-day Control Group	8	0.911 ± 0.34
28th-day Treatment Group	8	1.437 ± 0.42

Table 2. Results of data analysis of the control and treatment groups on the 14th and 28th days.

	14th-day Treatment Group	28th-day Treatment Group
14th-day Control Group	0.015*	-
28th-day Control Group	-	0.017*

* Significantly different

The results in Table 1 showed that the average osteoid area in the treatment group on day 14 (0.335 ± 0.10) was greater than the control group on day 14. The result for the average osteoid area in the treatment group on day 28 (1.437 ± 0.42) was also greater than the control group on day 28.

The research data were normally distributed for the 28th day (p=0.168) whereas for the 14th day they were not normally distributed (p=0.000) so the Mann Whitney test was carried out for the 14th day group and the Independent T-Test for the 28th day. Table 2 showed the results of the analysis for the 14th day group (p=0.015) and the 28th day group (p=0.017), all results stated p<0.05 so it can be concluded that there was a significant difference between the control and treatment groups.

DISCUSSION

The results showed the formation of an osteoid matrix in each group. On the 14th day osteoid formed by 0.335% in the treatment group and 0.133% in the control group. On the 28th day osteoid formed by 1.437% in the treatment group and 0.911% in the control group. There was a significant difference in the area of osteoid formed between the control group and the treatment group on day 14 and day 28. There was an increase in osteoid formation in the 14th and 28th day treatment group, this is in accordance with previous studies by Guarnieri et al. which showed the formation of an osteoid matrix of 26.85% in post-extraction sockets given bovine xenografts.[11] In accordance with the statement by Cardaropoli et al. who conducted a study regarding the administration of bovine bone mineral graft material and collagen membrane into the socket after tooth extraction, after 4 months all samples showed a variety of new bone formation with histological observations of osteoid areas surrounded by osteoblasts. The greatest bone formation occurs in the socket provided with the graft material.[8]

The results showed that the formation of bone trabeculae varied in the control and treatment groups, this is in accordance with the statement by Saima et al. who stated that after the post-extraction socket is given graft material, osteoblasts from the edge of the bone defect will use the graft as a framework for new bone growth.[20] As new bone is formed, the graft material is replaced and integrated into the new bone.[20] In the first week after tooth extraction after the formation of a blood clot, there is migration and infiltration of inflammatory cells into the socket. After that, the process of forming new blood vessels and fibroblast cells will replace the blood clot into granulation tissue. Then in the 1st to 2nd week the osteoblasts will synthesize osteoid to form a temporary matrix.[2,3] According to research by Vieira et al, the number of osteoblasts tends to stabilize on days 7 to 14.[6] In addition, it was found that RUNX expression reached its peak on day 14 in response to stimulation of BMP factors. The RUNX factor plays a role in the process of osteoblast differentiation. Osteoblasts that have differentiated will produce osteoid matrix, then osteoblasts will be surrounded by collagen fibers and become osteocytes. After the 2nd week or 14th day this osteoid matrix begins to mineralize into woven bone. Trabeculae of new bone can be observed especially in the lateral and apical parts of the socket. This bone formation occurs starting from the 7th day and continues to increase until the 21st day. Then after the 28th day of the healing process the bone remodeling process begins, namely the process of replacing woven bone into lamellar bone. The remodeling process occurs 3-4 weeks after tooth extraction, characterized by a constant number of osteoblasts and an increase in the number of osteoclasts in this period.[2,3,5,6]

The use of hydroxyapatite bovine tooth graft (HAp-BTG) as a graft material in this study is because bovine teeth contain a lot of hydroxyapatites. Hydroxyapatite itself has osteoconductive properties and is biocompatible with bone so that it can be a good attachment site for osteoblast cells and stimulates the formation of new bone (osteogenesis). Based on the statement by Zubaidah et al., precipitating calcium phosphate in hydroxyapatite will form apatite crystals, these crystals will form microporous.[18] The porous hydroxyapatite structure plays a role in the vascularization process and binds cytokines as well as being a good medium for the differentiation of mesenchymal stem cells into cells needed in the process of osteogenesis. In addition, bovine teeth also contain growth factor BMP which plays a role in the process of differentiation of mesenchymal stem cells into osteoblasts which will synthesize osteoid matrix.[13,18,21] Another study by Zubaidah et al. proved that there was an increase in osteoblasts in the treatment group that was given

DOI: https://doi.org/10.20473/cdj.v13i2.2023.56-59

a combination of hydroxyapatite bovine tooth graft (HAp-BTG) and PEG compared to the control group, especially on day 28.[19] The more osteoblasts, the greater the amount of osteoid matrix formed, this indicates that HAp-BTG plays a role in promoting the process of osteogenesis.[19]

According to Catros et al., the PEG combination has the potential to not cause negative reactions and is sufficiently adaptive to become a carrier material.[22] As per statement by Zubaidah et al. and Titsinides et al., PEG is used as a carrier material so that the graft material can reach the apical socket of the alveolar bone because one of the factors for successful integration of the graft material is adequate fixation at the place where the graft material is given.[19,23] As new bone is formed, the graft material is replaced and integrated into the new bone.[20] The process of bone healing after tooth extraction is considered complete when new bone trabeculae (osteogenesis) have formed in the socket after tooth extraction.[6] Based on the results of this study, administration of the combination of HAp-BTG and PEG into the post-extraction socket of Wistar rats can increase the number of osteoid on day 14 and day 28.

ACKNOWLEDGEMENTS

We thank the Mr. Heri and Mr. Ari who have helped us in the research process. And we thank Dental Conservative Department, Faculty of Dentistry Airlangga University, which has provided support in this research.

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