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EXPRESSION OF OSTEOBLASTS IN PERIODONTITIS WITH MATERIAL PRESERVATION POCKET TECHNIQUE GENGIGEL® (HYALURONIC ACID 0.2%)

Dwi Wahyu Indrawati[1], Ernie Maduratna Setyawatie[2], A. Retno Pudji Rahayu[3], Rizky Briliant Syah Manurung[4]
[1,2,3,4] Universitas Airlangga, Indonesia
Email: indrawatidwi55@gmail.com, Erniemaduratna@gmail.com, a-retno-p-r@fkg.unair.ac.id, brilyantm@gmail.com

ABSTRACT

Introduction: Hyaluronic acid as a socket preservation material can absorb large amounts of hydration and regulate appropriate pressure in the surrounding tissue, resulting in expansion of the extracellular space. The socket preservation technique used is a solution to bone loss due to tooth extraction. However, research on the hyaluronic acid (Gengigel) material in sockets with periodontitis has not yet been clearly studied. In this study, we will see an increase in the number of osteoblasts in the alveolar bone after tooth extraction in Wistar rats with periodontitis. Objective: To determine the increase in the number of osteoblasts after administering Hyaluronic acid to the tooth sockets of Wistar rats with periodontitis. Methods: An increase in the number of osteoblast cells was observed using a multi-head light microscope in 5 areas of the preparation at 400x magnification. The measurement results from 5 areas were averaged and a calculation was obtained for one preparation. There are 2 groups that will be used in this research, namely the Control group (without treatment), HA (given Gengigel). Each group was tested at 14 and 21 days after administering the material. Results: The research results showed that the Hyaluronic Acid (Gengigel) group had a higher number of osteoblast cells than the control group. The one-way ANOVA test showed an average significance result of 0.000 ($p < 0.05$) for all groups. This indicates that there was a significant change in each treatment group. Conclusion: Giving hyaluronic acid to the tooth sockets of Wistar rats with periodontitis can increase the number of osteoblast cells.

KEYWORDS *Osteoblasts, Hyaluronic Acid, Periodontitis, Socket Preservation*

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INTRODUCTION

Periodontitis is a highly prevalent disease, with approximately 10.5% to 12% of the human population experiencing periodontitis. Periodontitis causes inflammation of the tooth supporting tissue, loss of tooth attachment to the supporting tissue, and also alveolar bone resorption. Periodontitis is a severe condition because it can cause damage to the periodontal tissue, resulting in the tooth not having retention in the socket, so that the tooth can be categorized as hopeless and must be extracted (Carranza et al., 2019).

Previous research states that extraction of teeth that have a hopeless prognosis is highly recommended, because hopeless teeth have the potential to lose alveolar bone tissue 10 times faster if they are retained, compared to hopeless teeth that are extracted (Lin et al., 2019). Recent literature suggests that horizontal bone loss is around 29-63%, with vertical bone loss of 11-22% 6 months after tooth extraction without the use of socket preservation techniques (Kanning et al., 2015). The socket preservation technique can be used as a solution to the occurrence of bone loss due to tooth extraction. The socket preservation technique uses the principle of Guided Bone Regeneration (GBR) which functions as a filling material for post-extraction tooth sockets, so as to stimulate regeneration of bone tissue and minimize resorption of alveolar bone (Helmy, 2017).

Hyaluronic acid (HA) plays an important role in periodontal tissues. HA contains collagen-like structures, elastic fibers, and reticular fibers in a glycosaminoglycan matrix (Mani et al., 2016). HA can absorb large amounts of hydration and regulate the appropriate pressure on the surrounding tissue, resulting in expansion of the extracellular space. The function of HA is to buffer the chewing power of the periodontal ligament, is bacteriostatic, and anti-inflammatory which plays an important role in the early stages of the wound healing process (Romanò et al., 2017).

Osteoblasts are cells that function as bone apposition, osteoblasts are needed in the formation of new bone tissue through the process of osteogenesis which is regulated by Bone Morphogenic Proteins (BMPs) and Transforming Growth Factor-beta (TGF-β). The number of osteoblasts will decrease with age and will affect the balance of bone apposition and resorption, potentially causing osteopenia, bone loss, or osteoporosis (Jiang et al., 2016).

There have been many studies on the incorporation of Hyaluronic Acid in dental sockets, however, this study will look at the increase in the number of osteoblasts in alveolar bone after tooth extraction with periodontitis. This study will examine whether there is a significant growth in the number of osteoblasts, so as to repair the alveolar bone damage caused by periodontitis.

This study aims to determine whether the administration of Gengigel® (Hyaluronic Acid 0.2%) can increase the number of osteoblasts in the tooth sockets of Wistar rats that experience periodontitis. This study is expected to make a theoretical contribution by providing scientific references related to osteogenesis in alveolar bone sockets given Gengigel®. Practically, this study is useful as a contribution of thought for students majoring in Dental Education Universitas Airlangga, especially related to the role of Gengigel® in the healing process of alveolar bone.

Literature Review

Periodontal Tissue Regeneration

In the process of periodontal tissue regeneration, inflammation has a function that is regulated by several endogenous interactions with host cells. Acute inflammation plays a temporary role to maintain homeostasis. The first sensors of the inflammatory response include epithelial cells and innate immune cells that migrate to the wound site and stromal cells. *Polymorphonuclear leukocytes* (PMNs) or neutrophils are the cellular branch of the first line of defense of innate immune cells. Infiltration of PMNs is followed by an influx of mononuclear cells, monocytes, and activated macrophages into the inflammatory area to clear cell debris, bacteria, and apoptosis of PMNs by phagocytosis without prolonging the inflammatory period. Simultaneously, innate cells trigger the production of inflammatory mediators. Neutrophils, macrophages, dendrite cells and mast cells produce proteins called cytokines that regulate the initiation, maintenance and duration of the inflammatory response. By clearing the wound area of imprints, the healing process is accelerated due to the loss of local factors that can cause infection in the tissue.

Regeneration of periodontal tissues can be assisted by tissue engineering therapies. These therapies allow the tissue to be assisted in the repair process. There are several requirements that must be possessed by the material to be used for therapy for the success of tissue regeneration.

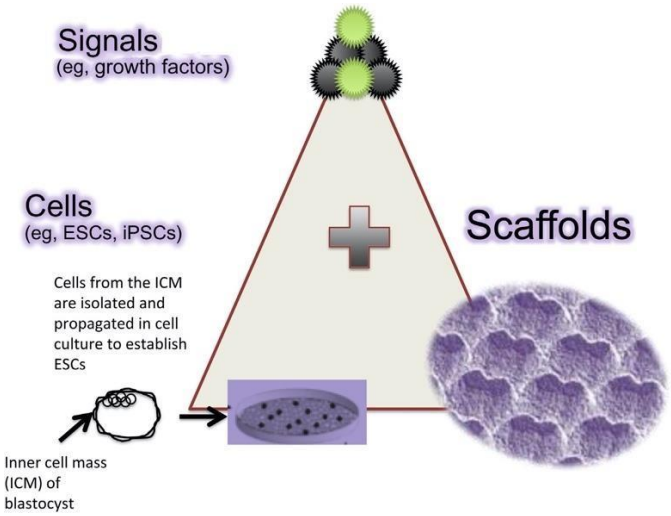


Figure 1. Key components in tissue engineering therapy.

Cells produce new tissue, while the scaffold provides an appropriate environment for cell proliferation and functioning. Signaling serves to promote cells to produce new tissue. The three components of tissue engineering are very important to complement each other for the perfection of the therapy (Akter, 2016). Scaffolds act as an artificial extracellular matrix that supports the formation of new tissues. Scaffolds have properties like natural extracellular matrices that support cell proliferation, differentiation, and synthesis. The source of cells is important in tissue engineering regenerative therapy, there are 3 types of cells that are generally

used, namely autologous (cells owned by the patient himself), allogenic (cells taken from other individuals belonging to 1 species), and xenogenic (cells taken from other species). Signaling exemplified in the chart is growth factors that have a function to control cellular responses through specific binding of receptors on target cells. Growth factors applied to a combination of cells and scaffolds can help promote tissue regeneration when compared to not using growth factors.

Alveolar Bone Healing

Bone healing that occurs due to tooth extraction takes a long time, for bone formation from the post-tooth extraction process generally takes about 16 weeks. Therefore, it is necessary to have a mechanism that can accelerate the bone healing process. Many studies have focused on therapies that can accelerate the healing process and the development of technologies for acute and chronic wound management.

Healing begins right after injury and involves cell migration, extracellular matrix and the action of soluble mediators. The mechanisms that occur in the healing process include inflammatory mediators and growth factors, cell-cell and extracellular matrix-cell interactions to trigger cell proliferation, migration and differentiation, supported by epithelialization, fibroplasia, and angiogenesis, as well as wound contraction and remodelling. These mechanisms occur immediately after physical injury and continue during the healing process.

The cells that play the most role in the healing process are macrophage cells, which have the function of secreting pro-inflammatory and anti-inflammatory cytokines and growth factors, fibroblasts and their ability to synthesize collagen which affects the tensile strength of the wound and replenishes the tissue to return to its original shape, followed by keratinocyte cells that divide and migrate to re-epithelialize and close the wound area (Primadina et al., 2019).

Additional proteins that play a role in bone healing such as RUNX2, OST, OCN, and OPN, OPG and RANKL are a class of tumor necrosis factors that are induced by cells during bone remodeling. The ratio of OPG to RANKL expression indicates whether the tissue responds to bone healing with predominance of OPG or bone resorption with increased RANKL (Hassumi et al., 2018).

The alveolar bone healing phase after tooth extraction is divided into four stages. The first stage is coagulation and homeostasis, which occurs within the first 24 hours after extraction. The second stage is inflammation, which begins after 24 hours post-extraction. The proliferation phase, which is the third stage, lasts about 14 to 21 days and is the main phase in the healing process. The final stage is modeling and remodeling, which focuses on restoring the shape and function of the alveolar bone, lasting for several months.

In periodontitis, bone resorption often occurs due to pathogens, genetic factors, and habits such as smoking. The process of bone regeneration in periodontitis aims to repair damaged tooth-supporting tissues, including the gingiva, cementum, periodontal ligament and alveolar bone. Various methods, such as bone grafts, scaffolds, stem cells, and growth factors, have been developed to aid osteogenesis. After biofilm removal and periodontal surgery, the infected tooth root surface can be filled with new cells, which then form the new tooth support tissue.

Hyaluronic Acid

Hyaluronic Acid naturally has a structure containing *Polyanionic Disaccharide Units* of glucuronic acid and *N-acetyl glucosamine* connected with b1-3 and b1-4 bonds. Hyaluronic Acid is a linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchyme, vitreous humor, skin, and many other organs and tissues (Casale et al., 2016).

Hyaluronic Acid (HA) acts as both an active and passive molecule in the body, depending on its molecular weight. High molecular weight (HMW) HA plays a passive role in maintaining osmotic balance and hydrating tissues through its physicochemical properties. In addition, HA also functions as an active molecule by interacting with binding proteins, which affect inflammatory processes, cell migration, and cell division and differentiation. The mechanism of interaction of these molecules varies, both in an autocrine and paracrine manner, through receptor binding in the same cell or in the surrounding tissue, which triggers various intracellular responses.

In dentistry, HA is often used as a biomaterial due to its uniform chemical structure in all tissues of the species. Its applications include maxillofacial surgical procedures, orthopedics, as well as periodontal therapy such as treatment of gingivitis, periodontitis, and implant placement. HA aids wound healing, reduces inflammation, accelerates cell regeneration, and prevents infection by inhibiting bacterial growth. Topical application of HA in postoperative cases has been shown to be effective in accelerating healing and reducing bacterial contamination of the affected area.

The main functions of HA in periodontal tissues include cellular and extracellular interactions, maintaining tissue structural integrity, lubricating and stabilizing tissues. HA also helps oxygen supply to neutralize periodontal tissue damage, and has angiogenic effects with low molecular weight and osteoconductive effects with high molecular weight. In addition, HA supports tissue regeneration by protecting the tissue surface and preserving space during periodontal regenerative procedures, and reduces the risk of infection after surgery.

Conceptual Framework

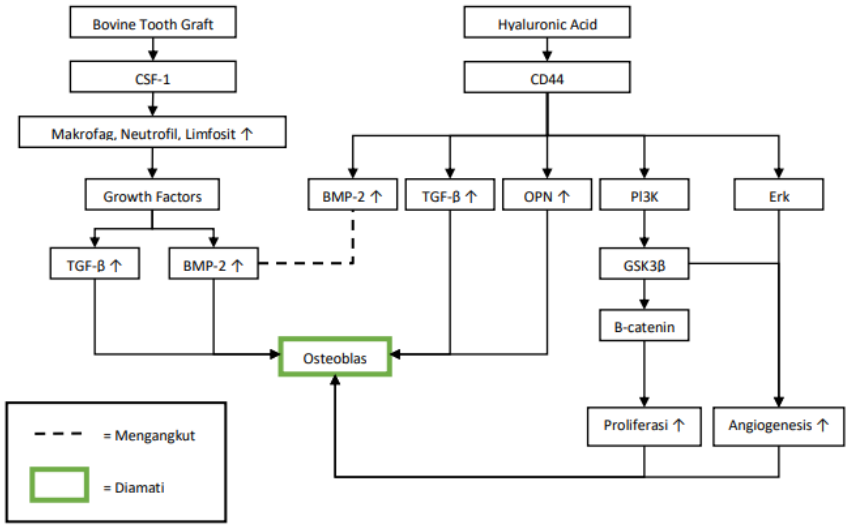


Chart 1. Conceptual Framework

Conceptual Framework Description

Tooth extraction is one of the actions in the field of dentistry that can cause trauma, such as alveolar bone damage and the formation of wound tissue. The tissue response to the trauma caused by tooth extraction will cause an inflammatory response that will continue into the socket healing process which will generally occur alveolar ridge resorption. Resorption can be minimized by adding regenerative material, which in this study is exemplified using Gengigel® (Hyaluronic Acid 0.2%) into the socket after tooth extraction. This material fills the socket caused by tooth extraction so that it can support the process of bone regeneration.

Gengigel® (Hyaluronic Acid 0.2%) binds to CD44. CD44 will increase the production of TGF-β and OPN as a product of HA. OPN and TGF-β will interact and produce osteoblasts. BMP-2 in the tissue will be transported by Gengigel® Hyaluronic Acid 0.2% in order to accelerate the process of bone regeneration. Gengigel® Hyaluronic Acid 0.2% also helps to signaling process with PlK3 effector to increase tissue proliferation. Erk pathway signaling also occurs in the CD44 pathway to stimulate angiogenesis. The process of angiogenesis and also the increase in tissue proliferation can support the increase in the number of osteoblast cells in the tissue. In the healing process, *growth factors* play an important role. TGF-β and BMP-2 will differentiate into osteoblasts. Application of Gengigel® Hyaluronic Acid 0.2% can support the process of tissue regeneration after post-tooth extraction procedures by increasing the number of osteoblasts.

Research Hypothesis

There was an increase in the number of osteoblasts after the administration of Gengigel® Hyaluronic Acid 0.2% in the tooth sockets of Wistar rats with periodontitis.

RESEARCH METHOD

This study is an in vivo laboratory experimental study conducted on Wistar rats. The research design used was post-test only control group design, which compared the control group with the group treated with Hyaluronic Acid (HA) to see an increase in the number of osteoblasts. This study was designed to determine the effect of Gengigel® (Hyaluronic Acid 0.2%) in accelerating healing in the tooth socket of rats experiencing periodontitis.

The research sample used Wistar male rats weighing 200-250 grams. Male rats were selected to avoid the influence of estrogen and progesterone hormones that can affect wound healing. Based on calculations with the Lemeshow formula, each group requires at least 2 samples, but in this study 4 rats were used per group. There were 8 sample groups consisting of the control group and the Hyaluronic Acid-treated group, which were observed on day 14 and day 21.

The independent variable in this study was Gengigel® (Hyaluronic Acid 0.2%), while the dependent variable was the number of osteoblasts measured on days 14 and 21. Controlled variables included factors such as mouse breed, body weight, research procedures, dosage of Porphyromonas gingivalis bacteria, and treatment duration. This study controlled a number of aspects to ensure valid and measurable results in the effect of Hyaluronic Acid on the number of osteoblasts.

RESULT AND DISCUSSION

Research Data

This study was conducted in an experimental laboratory with the aim of observing the increase in the number of osteoblast cells in the tooth socket of Wistar rats (*Rattus Norvegicus*) in a state of periodontitis after the administration of *Hyaluronic Acid*, *Bovine Tooth Graft*, and a combination of both. Observations were made in the control and treatment groups on days 14 and 21.

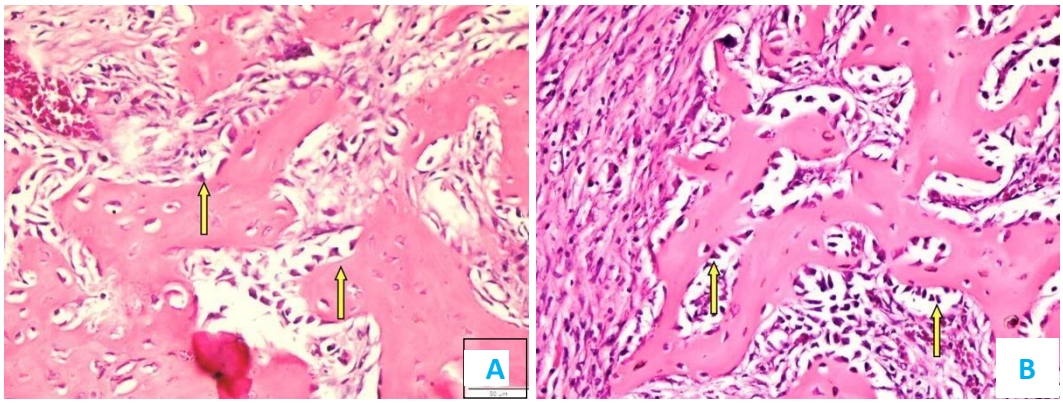


Figure 2. Results of osteoblast cell examination of HE preparations on day 14. The top left image (A) shows the results of osteoblast cells in the Control group, the top right image (B) shows the results of osteoblast cells in the HA group.

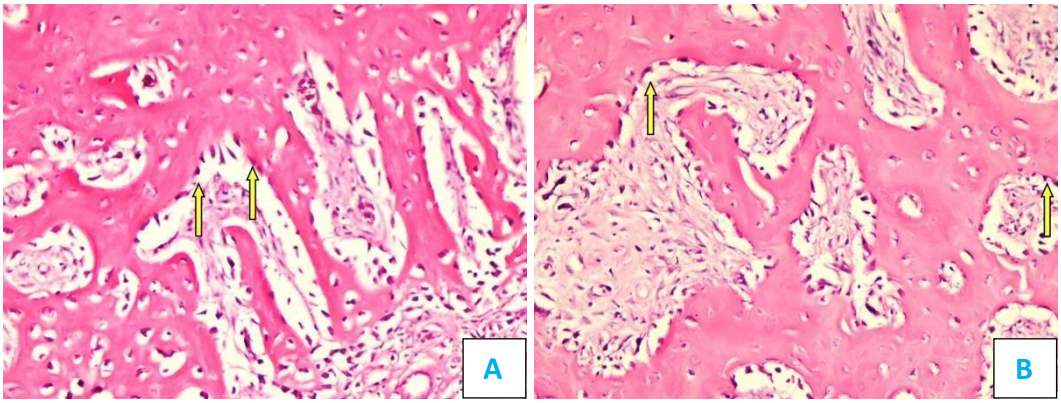


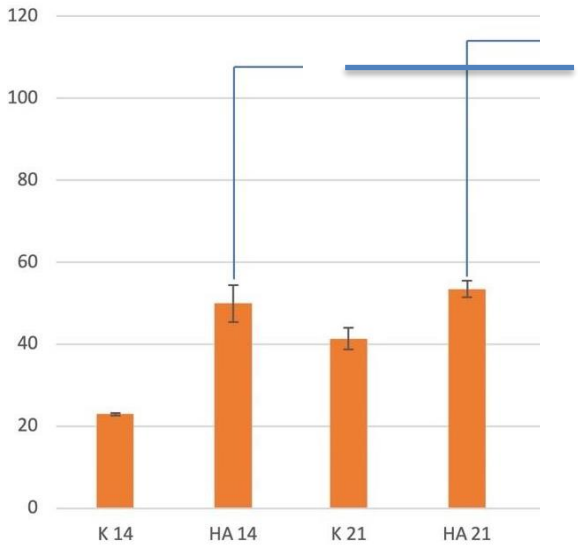
Figure 3. Results of osteoblast cell examination of HE preparations on day 21. The top left image (A) shows the results of osteoblast cells in the Control group, the top right image (B) shows the results of osteoblast cells in the HA group.

Preparations that have been given HE staining are viewed under a *multi head* light microscope. The results of each group were read out the number of osteoblast cells at 400x magnification. One preparation was observed in 5 areas, counting the number of osteoblast cells using a manual counter operated by the operator who read the preparation. The average results of the 5 areas observed in the preparations of each group are shown in the following table.

Table 1. Results of osteoblast cell counts.

Group	N	Average	Std. Deviation
K 14	3	22,933	0,3055
HA 14	3	49,933	4,5181
K 21	3	41,333	2,6633
HA 21	3	53,467	2,0429

Mean Number of Osteoclasts (Starting Table)



* p < 0,05

Figure 4. Graph of the mean number of osteoblast cells in each group.

Table 5.1 shows the average number of osteoblast cells in each group. It can be seen that the number of osteoblast cells in the group given the material is higher than the control group which is not given Hyaluronic Acid material.

Research Data Analysis

Data from the number of osteoblasts were tabulated and *one-way ANOVA* statistical analysis was performed to determine the difference in the number of osteoblast cells in each group on day 14 and day 21. Initially, before being tested using *one-way ANOVA* statistical analysis, the data must be tested for normality and homogeneity so that it can meet the requirements for using *one-way ANOVA* statistical analysis which will be followed by the *Tukey HSD* test.

Normality test (Table 2) using *Shapiro-Wilk* with the results of significance p> 0.05 in all groups, so it can be said that the sample is normally distributed.

Table 2. Normality test using *Shapiro-Wilk*

	Group	**Significance**
Day 14 Osteoblasts	Control	0,637
	HA	0,829
Day 21 Osteoblasts	Control	0,583
	HA	0,281

Furthermore, the homogeneity test was carried out using *Levenne statistics* along with the *one-way ANOVA test.* The homogeneity test (Table 3) produces a

significance value of p> 0.05 which indicates that the sample is homogeneous, so that it can meet the requirements for conducting the *one-way ANOVA* statistical test.

Table 3. Homogeneity test with *Levenne statistic*

	Levene Statistic	**df1**	**df2**	**Sig.**
Day 14 Osteoblasts	2,693	3	8	0,117
Day 21 Osteoblasts	1,828	3	8	0,220

Furthermore, the *one-way ANOVA* test and Tukey HSD *post hoc test* (Table 4) aims to compare the average of each group and determine the differences between groups. The results of the *one-way ANOVA* test obtained a significance of p < 0.05, indicating that the differences in each group were significant for the number of osteoblast cells counted. Tukey HSD *post hoc test* was used to compare the mean difference and significance between groups.

Table 4. Statistical test results of *one-way ANOVA* and *Post hoc test* Tukey HSD.

	Group		**Average Difference**	**Sig.**
Day 14 Osteoblasts	Control	HA	-27,0000*	0,000
	HA	Control	27,0000*	0,000
Osteoblasts Day 21	Control	HA	-12,1333*	0,031
	HA	Control	12,1333*	0,031

Discussion

In conditions such as tooth extraction and periodontal disease, bone defects may occur and cannot be avoided. What can be done to deal with the problem of bone defects can be done with bone graft therapy which aims to increase the amount of new bone production by the process of osteogenesis. The main requirements for bone graft material are biocompatible, strong enough to maintain bone condition, and have a low degradation rate (Mohammed et al., 2021). There are several options for performing bone graft therapy in the selection of materials, several types of bone graft that can be used are autograft, allograft, xenograft, and alloplast (Saima et al., 2016). The use of autograft is currently limited, due to the lack of availability of materials and the fear of morbidity in the patient's donor bone area. The use of allograft is more common in some cases, but there is concern about the possible immune reaction and transmission of infection at the time of application. To overcome this, various synthetic bone materials made of metals, ceramics, polymers, etc. have been introduced to accelerate and improve the regeneration process of alveolar bone (Setiawatie et al., 2019).

Periodontal tissues include soft tissues such as the gingiva and periodontal ligament and hard tissues such as alveolar bone. Periodontitis is generally accompanied by bone resorption and periodontal tissue destruction which can be caused by acute or chronic inflammation. Periodontitis is a problem that not only involves oral health, but systemic health can also affect or be affected by periodontitis. The main treatments for periodontal tissue inflammation include

scaling and root planing as well as periodontal surgery to reconstruct damaged periodontal tissue (Ouchi & Nakagawa, 2020). One of the efforts that can be made in periodontal surgery which aims to reconstruct tissue, namely by performing bone grafting on alveolar bone that has been destroyed due to periodontitis.

Bone substitution with bone graft material has an important role in regenerative dentistry therapy, bone graft material has a role as a frame or scaffold so that bone cells can proliferate. The content of hydroxyapatite in bovine tooth graft is osteoinductive and can be absorbed well by the body, so hydroxyapatite is recommended for use as bone tissue repair (Senra et al., 2020).

Hyaluronic Acid is a high molecular weight carbohydrate polymer containing Polyanionic Disaccharide Units of glucuronic acid and N- acetyl glucosamine connected with b1-3 and b1-4 bonds. HA is a linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchyme, vitreous humor, skin, and many other organs and tissues. In general, all cells in the human body can synthesize HA that occurs in cell membranes. In the early stages of the inflammatory phase, tissues containing HA have a role as antimicrobial agents. HA can accelerate tissue regeneration by using chemotaxis, proliferation, mesenchymal cell differentiation, and inducing osteogenic agents such as Bone Morphogenic Protein-2 (BMP-2) and osteopontin. Hyaluronic acid with the right molecular weight and dose can increase the osteoinductive and osteoconductive properties of bone graft material and stimulate the formation of osteoblast cells.

The combination of Hyaluronic acid and Bovine tooth graft can be categorized as a hydrogel. Hyaluronic acid-mediated hydrogels can be applied to alveolar bone defects aiming to form scaffolds with high water content. Hyaluronic acid has viscoelastic material properties that are very suitable for the bone regeneration process. The advantage of using hydrogels is that this material can act as a carrier material for the bone regeneration process, one of which is carrying BMPs to increase bone proliferation. Hyaluronic acid and hydroxyapatite hydrogel application in post-extraction sockets in vivo in wistar rats can increase the expression of osteoprotegerin (OPG) and TGF-β 5-fold compared to the control group.

Research says that signaling between CD44 and HA can induce β-defensin 2 (HβD2) which can act as an anti-microbial defense (Hill et al., 2013). The expression of CD44 also increases when the wound heals, the function of HA-CD44 interaction can provide a scaffold for tissue repair, another study also said that LMW and HMW HA are found in the wound area and may have a regenerating effect on the area (Müller et al., 2014).

Tissue engineering in dentistry is composed by 3 main things, namely signaling, scaffold, and cells (Akter, 2016). In this study, tissue engineering has been carried out with a combination of signaling and scaffold components using Hyaluronic acid and Bovine tooth graft materials, it is hoped that the combination of these two materials can help the healing process of alveolar bone defects to be better in the future.

Osteoblasts are cells derived from mesenchymal stem cells (progenitor cells) from bone marrow stromal cells and function as bone matrix synthesis and mineralization, osteoblast cells can also function as a counterweight to osteoclasts

and bone matrix decomposition. Osteoblasts contain alkaline phosphatase, organic phosphate-breaking enzymes, parathyroid and estrogen hormone receptors, growth factors (Setiawati & Rahardjo, 2019).

In vivo research that has been done, shows an increase in the number of osteoblast cells in bone defect extraction in Wistar rats with periodontitis. The treatment group given Hyaluronic Acid material showed a 5-fold increase in the number of osteoblasts compared to the control group, this suggests that the combination of Hyaluronic Acid material can increase the rate of proliferation of osteoblast cells through TGF-β and BMP pathways that function as an induction of new bone tissue growth. In previous studies, Hyaluronic Acid has been shown to increase new bone formation, but has not been tested for cases of inflammation, the group with the administration of a combination of Hyaluronic Acid ingredients experienced a significant increase in the number of osteoblast cells, it can be concluded that therapy using Gengigel material (hyaluronic acid 0.2%) can increase the formation of new bone tissue due to the osteoinductive process in it, even in periodontitis conditions.

CONCLUSION

The conclusion of this study is the administration of Gengigel (Hyaluronic Acid 0.2%) and Bovine Tooth Graft in the tooth socket of Wistar rats with periodontitis can increase the number of osteoblast cells.

Based on the results of research on increasing the number of osteoblasts after the administration of Gengigel (Hyaluronic Acid 0.2%) in the tooth sockets of Wistar rats with periodontitis, the authors have suggestions that should be done, namely further research on the need to test the biomolecular mechanism of the research that has been done.

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