THE INFLUENCE OF *Aloe vera* AND XENOGRAFT (*XCB*) TOWARD OF BONE MORPHO PROTEIN 2 (BMP2) EXPRESSION AND AMOUNT OF OSTEOBLAST OF ALVEOLAR BONE INDUCED INTO TOOTH EXTRACTION SOCKETS (*Cavia cobaya*)

Utari Kresnoadi* and Retno Pudji Rahayu**
*Department of Prosthodontics, Faculty of Dentistry, Airlangga University, Surabaya 60132. Indonesia
**Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Airlangga University, Surabaya 60132, Indonesia
Corresponding Author email: ut.kres@yahoo.com

ABSTRACT

Tooth extraction can cause inflammation leading to alveolar ridge resorption. In addition, prominent ridge has crucial role for making denture successfully. Thus, socket preservation is needed to prevent greater alveolar ridge resorption. An innovative material, a combination of ***Aloe vera*** *and xenograft (XCB), is then considered as a biogenic stimulator that can reduce inflammation, as a result, the growth of alveolar bone is expected to be improved. This research is aimed to prove whether the mixture of* ***Aloe vera*** *and xenograft can stimulate BMP2 and increase osteoblasts. Forty-eight* ***Cavia cobaya*** *animals were divided into eight groups each of which consisted of six animals. The mandibular incisors of those* ***Cavia cobaya*** *animals were then extracted and filled with PEG as Group Control, XCB as Group XCB,* ***Aloe vera*** *as Group* ***Aloe vera****, and a combination of* ***Aloe vera*** *+XCB as Group* ***Aloe vera*** *+XCB. Next, the first four groups were sacrificed seven days after extraction, and the second four groups were sacrificed 30 days after extraction. And then, immunohistochemical and histopathology examinations were conducted to examine BMP2 expression and osteoblasts. Based on the result known that the mixture of* ***Aloe vera*** *and xenograft can increase BMP2 expression and amount of osteoblasts. It can be concluded that the mixture of* ***Aloe vera*** *and xenograft can increase BMP2 expression and amount of osteoblast cel . It can be used as an alternative material to increase the growth of alveolar bone after extraction.*

Keywords: Aloe vera*, BMP2, Extraction Sockets ,Osteoblasts, Xenograft*

INTRODUCTION

The making of dentures can be considered as successful if they are retentive, not easily separated, stable, and comfortable to chew. In other words, the success of the making of dentures actually depends on retention factor, stabilization factor, and convenience factor, which can be achieved by supporting anatomical condition related to prominent ridge. Therefore, to achieve a good state of the ridge, extraction socket preservation should be conducted as soon as after tooth extraction.

Actually, extraction socket preservation is aimed to prevent bone resorption risks caused by alveol revocation trauma. It is because tooth extraction may generally cause trauma, triggering inflammation and later stimulating the growth of osteoclasts and the resorption of alveol bone. Inflammation in tooth extraction cases may result in narrow and shortened residual ridge triggering bone jaw atrophy (Oktavia, 2005). If this condition is not addressed soon, it can make the making of dentures not optimal. It is because several researches show that sufficient bone volume can make the use of dentures or implants satisfying.

Thus, bone regeneration, according to Istiati (2013), is important to rebuild the bone around the tooth sockets for planting implants after tooth extraction or during the making process of prosthetic dentures. Besides that, it can also aim to fill bone defects after the removal of the tooth roots and odontectomy. The use of bovine graft for the addition or augmentation of the bone defects has often been conducted, but until now the use of bovine graft can not give satisfactory results since the use of it in long-term can make it less stable (Pachene *et al* in Lanza *et al*, 2007). Therefore, an innovative material is needed as biogenic stimulator to stimulate bovine graft and accelerate bone growth.

In addition, BMPs (Bone Morpho Proteins) are a group of growth factors also known as sitogen and metablogens, which function to induce the formation of bone and cartilage (Istiati, 2013). Bone Morpho Protein 2, moreover, is produced by primitive mesensimal cells, osteoprogenitor cells, fibroblasts, and chondrocit cell proliferation (Lieberman and Friedlander, 2005). *BMP2* has a function as disulfide-linked homodimer inducing bone and cartilage formation. In short, *BMP2* can be considered as a mediator of retinoid playing an important role in osteoblast differentiation (Istiati, 2013).

In the other hand, *Aloe vera* is considered to have functions as a biogenic stimulator and hormones stimulating wound healing activity. *Aloe vera* liquid even can

prevent scar tissue at the time of incision, and when the gel is used after surgery, the incision will be healed faster (Rostita *et al*, 2008). Moreover, *Aloe vera,* according to Wolf (2001) cited in Ariyani dan Koeswanto (2008), has important roles as anti-inflammatory, anti-bacteria, and anti-virus so that *Aloe vera* can improve body immune and accelerate wound healing process by stimulating cell regeneration. It is because *Aloe vera,* according to Reni and Titik (2006), contains several sterols, salicylic acid, aloemannan and acemannan, and glycoprotein fractions that have several pharmacological effects including anti-inflammatory effects.

Therefore, a statement of problem is raised whether the mixture of *Aloe vera* and xenograft concelous bovine induced into tooth extraction sockets can increase BMP2 as osteoblast progenitors in accelerating alveol bone remodeling process. Thus, this research is aimed to determine whether the induction of the mixture of *Aloe vera* and xenograft concelous bovine into tooth extraction sockets can increase *BMP2* and improve osteoblasts to acelerate alveol bone remodeling process.

MATERIALS AND METHOD

Research Design and Animal Model

The research subjects in this research were determined by calculating compared data, namely continuous data with a population of more than two, and six replication. The animals used in this research were 3-3.5 months male *Cavia cobaya* (gui-nea pig) with the weight of those animals was 300 -350 grams, and also healthy and active. This research was conducted in Biochemistry Laboratory of Medical Faculty Airlangga University for those experimental animals had been taken care in. However, the making of tissue preparations used in this research was conducted in Anatomical Pathology Laboratory of Dr. Soetomo Hospital. Meanwhile the making of *Aloe vera* extracts was conducted in Physical Chemistry Laboratory of Pharmacy Faculty, Airlangga University, while the making of freeze dried *Aloe vera* was conducted in Biology Laboratory of Science and Technology Faculty, Airlangga University. And, the processes of immunohistochemical staining and HE staining for immunohistochemical examination were conducted in Biochemistry and Biomolecular Engineering Laboratory of Medical Faculty, Brawijaya University.

Those animals were divided into eight groups. First, in Group I, the extraction sockets of those animals were filled with *PEG* as a controll group, and then examined on day 7. Second, in Group II, the extraction sockets of those animals were filled with *PEG* as a controll group, and then examined on day 30. Third, in Group III, the extraction sockets of those animals were filled with *XCB* + *PEG*, and then examined on day 7. Fourth, in Group IV, the extraction sockets of those animals were filled with *XCB* + *PEG*, and then examined on day 30. Fifth, in Group V, the extraction sockets of those animals were filled with *Aloe vera* + *PEG*, and then examined on day 7. Sixth, in Group VI, the extraction sockets of those animals were filled with *Aloe vera* + *PEG*, and then as examined on day 30. Seventh, in Group VII, the extraction sockets of those animals were filled with *Aloe vera* + XCB+ PEG, and then examined on day 7. Eighth, in Group VIII, the extraction sockets of those animals were filled with *Aloe vera* + XCB+PEG, and then examined on day 30.

In addition, there are many variables in the research. First, free variables involve the mixture of *Xenograft Concelous Bovine* (*XCB*) 0.5 g and *Aloe vera* 0.5 g, 24 (PEG 4000+ PEG 400, ratio 1:1), and PEG 400 (with ratio 1:1). Second, dependent variables involve *BMP2* expression, osteoblasts. Third, controlled variable was *Cavia cobaya* (guinea pig) with body weight of 300-350 g at the age of 3-3.5 months. Those animals were fed with corn, carrots, and distilled water.

Administration Procedure

The procedures of this research consisted of several steps. Firstly, forty-eight *Cavia cobaya* animals were divided into eight groups, each of which consisted of six animals. Secondly, before their lower right incisors were extracted by using a special pliers (known as needle holder), they had intravenously been anaesthetized with ketamine 0.2 cc / 300 g BW (Kusumawati, 2004). Thirdly, their extraction sockets were induced with either *PEG, PEG-+XCB, Aloe vera* + *PEG*, and combination of *Aloe vera* + *XCB+PEG* as much as 0.1 cc of the appropriate volume of the extraction socket, and then stitched.

In other words, the sockets of those animals in Group 1 and Group 2 were induced with polyethylene glycol (PEG), and then examined on day 7 and day 30 after the treatment. Meanwhile, the sockets of those animals in Group 3 and Group 4 were induced with *XCB* + PEG, and then examined on day 7 and day 30 after the treatment. On the other hand, the sockets of those animals in Group 5 and Group 6 were induced with *Aloe vera* and PEG, and then examined on day 7 and day 30 after the treatment. And, the sockets of those animals in Group 7 and Group 8 were induced with the mixture of *Aloe vera* 500 mg + *XCB* 500 mg + PEG 24 g, and then examined on day 7 and day 30 after the treatment.

Histopathologic specimen preparation

Fourthly, after 7 days and 30 days, those animals were killed, and their jaws were cut off. Fifthly, the preparation consisted of hard materials was decalcified first with nitric acid 2% for approximately 14 days, and then paraffin

block preparations were made. Sixthly, those paraffin blocks were cut with a rotary microtome with a thickness of about 4 microns, and placed on a glass object. Seventhly, deparaffinization was conducted by dissolving in xylol for 2 x 3 minutes. Eighthly, the rest xylol was washed with absolute alcohol 99%, 95%, 90%, 80%, and 70% for 2 x 1 minutes. Ninthly, the residual alcohol was washed with running water.

The examination of specimen, furthermore, were conducted by making slide preparation used for HE staining and immunohistochemical staining. The examination of *BMP2* expression and osteoblasts was then conducted by using a light microscope. For the purposes of calculating, moreover, the code of the already coded slides was closed, and a new number was given randomly for each slide. Each slide was then observed with 1000x magnification and 10 fields of view.

Statistical Analysis

All the results of the observation were written on the worksheet and calculated for their average value per field of view. Next, the results of the calculation were tabulated. Finally, the result data were tested with Kolmogorov-Smirnov test and *ANOVA* test, and then tested with Tuckey HSD to compare the data among the groups.

RESULTS

The Mean and Standard Deviation of *BMP2* Expression and osteoblast cels on Day 7 and Day 30

The results of the mean and standard deviations of BMP2 expression and osteoblast cells (Figure 1), its known that there was significant improvement either in Group Control, in Group *XCB*, in Group *Aloe vera*, or in Group *Aloe vera* and *XCB* on day 7 and on day 30. Nevertheless, the highest BMP2 expression and osteoblasts cells was in Group *Aloe vera* and *XCB* on day 30. The y-axis shows the value of osteoblasts and expression of BMP2, while the x-axis shows the kinds of treatment: control, filled of XCB, filled Aloe vera and filled of combination of Aloe vera and XCB on 7 days and 30 days

The Results of Anova Test on *BMP2* Expression on Day 7 and Day 30

Based on the results of Oneway ANOVA test, it is known that there were significant differences of *BMP2* expression between Group Control and Group *XCB*, Group *Aloe vera,* as well as Group *Aloe vera* and *XCB*, with p = 0.000. Then tested with Tuckey HSD ,the result that there are significant difference of control group with group of filled XCB, *Aloe vera* and combination of *Aloe vera* and XCB .

Pictures of *BMP2* Expression of osteoblast cells Day 30

Based on the figure 2., BMP2 expression, shown in brown on osteoblasts in cytoplasma cells, the arrows indicate BMP2 expression.

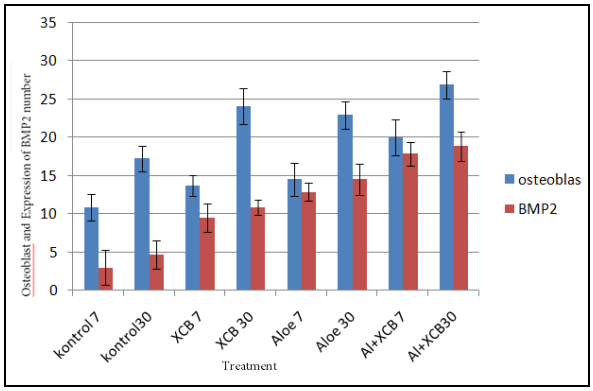

Figure 1. The Mean and Standard Deviation of *BMP2* Expression and osteoblast cels on Day 7 and Day 30

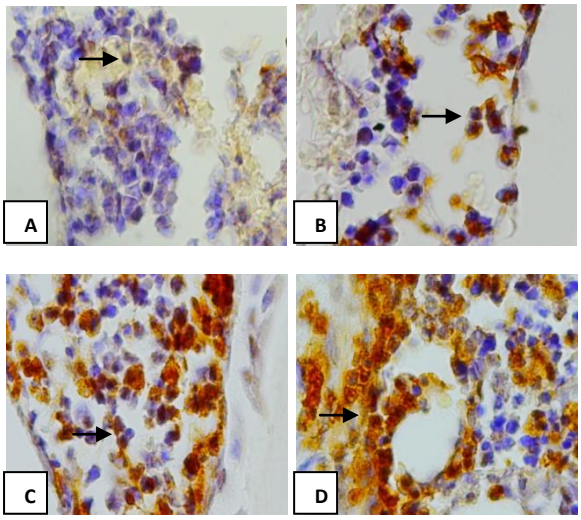

Figure 2. The Pictures of *BMP2* Expression of osteoblast cels in 30 Days. shown in brown on osteoblasts in cytoplasma cells, the arrows indicate BMP2 expression. A. *BMP2* Expression of osteoblast cell in the Group Control after the Immunohistochemical Examination on Day 30; b. *BMP2* Expression of osteoblast cel; in the Treatment Group induced with *XCB* after the Immunohistochemical Examination on Day 30; c. *BMP2* Expression of osteoblast cel in the Treatment Group induced with *Aloe vera* after the Immunohistochemical Examination on Day 30; and D. *BMP2* Expression of osteoblast cel in the Treatment Group induced with *XCB* and *Aloe vera* after the Immunohistochemical Examination on Day 30.

Table 1. The Results of Anova Test on *BMP2* Expression on Day 7 and Day 30

Variation	DF	F	Significancy
Among the Groups	7	47.774	0.000
Within the Groups	40		
Total	47		

DISCUSSION

Based on the results, it is known that the highest *BMP2* expression was in the group with the mixture of *Aloe vera* and *XCB* after 30 days of examination. Meanwhile, the highest osteoblasts were also in the group with the mixture of *Aloe vera* and *XCB* after 30 days. This is because antthraquinones components, namely aloin, aloe emodin, and barbaloin, contained in *Aloe vera,* have important roles as anti-inflammatory, antibacterial, anti-viral. Thus, *Aloe vera* can reduce inflammation risks in tooth extraction trauma and also induce wound healing process. Furthermore, *Aloe vera* can also stimulate the growth of fibroblast and osteoblast tissues (Vasquez *et al*, 1996; Reni and Titik, 2005; Chun-shu Yu *et al*, 2006; Hamman, 2008; Mi-Young Park *et al*, 2009; Lawrence *et al,* 2009; Moghaddasi and Verma, 2011). The growth is also stimulated with Aloeride polysaccharides contained in *Aloe vera* which has a high molecular weight and a very strong immunostimulator activity (Pugh *et al*, 2008).

In general, on the first day after injury, the wound, according to Werner and Grose (2003), is physiologically filled with clotted blood, then neutrophils invades into the blood clot, and 3-7 days after, the neutrophils gets apoptosis. At the same time, macrophages become abundant in the injured tissue, and endothelial cells migrate, proliferate and then form new blood vessels. In the next process, fibroblasts migrate into the wound tissue, proliferate, and then form new tissue, called granulation tissue. Approximately one to two weeks after injury, the wound is then filled by granulation tissue since fibroblasts have transformed into myofibroblast, causing contraction and collagen deposition.

In this research, the results show that in the extraction socket induced with the mixture of *Aloe vera* and *XCB,* the healing process occurs faster than physiological healing process. This is because acemannan considered as carbohydrate contained in *Aloe vera* has anti-inflammatory effect (Davis, 2002). *Aloe vera,* moreover, also contains pure carbohydrate mannan, acetyl glucomannan, alkaline phosphatase enzyme, and bradikinase enzyme considered as anti-inflammatory, auxin, gibberellins and saponins hormones stimulating healing process, and proteins accelerating the healing process and stimulating the growth of bone tissue (Pachanon, 2005; Roostita *et al*, 2008). According to Davis (2002), in other words, it can be said that *Aloe vera* can inhibit pain and inflammation, and also stimulate fibroblasts to produce functional collagen and proteoglycan 2. However, macrophages can also release similar substances that can stimulate fibroblasts as the result of *Aloe vera*'s stimulation. It is because when the skin is injured, fibroblasts will migrate into the wound, and then proliferate and produce collagen and proteoglycans. Next, proteoglycans form the ground substance in which collagen fibers are embedded. This is then known as a connective tissue remodeling in which the cells in the wound area connecting each other stimulated by growth factors. It means that the growth of *Aloe* will pull wound area and bind fibroblasts and IGF receptor in order to produce collagen and proteoglycans and improve the tissue.

Similarly, a research conducted by Steiner *et al* (2008) states that a week after tooth extraction, a tissue composed of fibrin is formed, and degeneration of early granulation tissue occurs. It is because after the development of fibrin, granulation tissue is formed containing blood vessels, fibroblasts and chronic inflammatory cells. Next, on day 8 after the extraction, the formation of new bone can be seen throughout the alveolar bone, part of which is on the bottom wall of the socket, but still not on the surface of the former extraction socket. Then on day 12 after the extraction, new bone is formed along the socket wall, and in the trabecular area around the extraction area there are trabecular bone woven at the periphery sockets, as well as osteoprogenitor cells, preosteoblasts and osteoblasts surrounded by the trabecular bone. Besides that, it is also known that periodontal ligament moves to the middle socket, but it does not attach to the wall socket, and then is vanished. Afterwards, collagen density increases gradually, and then is replaced by bone. After the resorption phase of the bone, freezing, granulation and collagenase phases of healing process can be skipped, so regeneration occurs in the extraction socket.

Therefore, the highest *BMP2* was found in the tooth extraction sockets induced with the mixture of *Aloe vera* and Xenograft Concelous Bovine (*XCB*) since *Aloe vera* combined with Xenograft Concelous Bovine (*XCB*) considered as osteoinduction can accelerate healing process and new bone growth. Similarly with the research, that graft as osteoconduction can accelerate healing process and new bone (Ferdiansyah *et al* cit Elly 2002; Khan *et al*, 2005; De Boix *et al*, 2006; Fickl *et al*, 2009; Pelegrine *at al*, 2009) Thus, it can be said that the induction of the mixture of *Aloe vera* and *XCB* into tooth extraction socket through TLR-2 receptor can stimulate osteoblast progenitor, known as BMP-2, triggering osteoblastogenesis process and stimulating Osteoblastic Specific Factor (OSF-2) or Core Binding Factor *1* (CBFA-1) later. Finally, osteoblast products, collagen 1, and osteocalcin products, non-collagen, as a result, will be increased, and then stimulate new alveol bone growth (Lorenzo, 2008).

Based on the result, it can be concluded that the mixture of *Aloe vera* and *XCB* induced into tooth extraction socket may increase *BMP2* expression and osteoblasts cell of alveol bone. Thus, the mixture of *Aloe vera* and *XCB*

can become an alternative material to improve alveol bone growth after tooth extraction.

ACKNOWLEDGEMENT.

It is thankful for the Rector of Airlangga University under the Education Minister for decentralized research funding managed by Community Research and Service Institute (CRSI), Airlangga University as the Excellent Research Institution of Higher Education, No. 965 / UN3 / 2014, dated February 28, 2014.

REFERENCES

Ariyani, E and Khoswanto, C., 2008. The use 90 % *Aloe vera* Frezze Dryiing as Modulator of Collagen Density in Exctraction Socket of Incisivus *Cavia Cobaya*, *Dental Journal*, 41(2): 74-76.

Chun-Shu, Y., Fu-Shun, Y., Kai-Sheng C. J., Te-Mao, L., Song-Shei, L., Ssu-Ching, C., Te-Chun, H., Yung-Hsien, C and Jing-Gung, C., 2006. Aloe-emodin affect the level of Cytokines and Functions of leukocytes from Sprague-Dawley Rat. *In vivo* 20: 505-510

Davis, R.H., 2002. Conductor-Orchestra of Aloe vera. A Long, Ilustrious History -Dating From Biblical Times, Complete story of *Aloe vera*, Copyright 1996-2002. The International Aloe Science Council. Acessed August 23, 2012

Hamman, J.H. 2008. Composition and Application of *Aloe vera* Leaf Gel, *Molecule* 13: 1599-1616

Istiati, S. 2013. *Regenerasi dan Penyembuhan untuk kedokteran gigi*, Sagung Seto, cetakan I, Jakarta

Kurniawati, R and Marminah, T.M. 2006. Efek Anti Inflamasi Gel Lidah Buaya (*Aloe vera* Linn) Terhadap Tikus Putih. *Prosiding Seminar Nasional Tumbuhan Obat Indonesia XXIX, Solo, Indonesia:* 82-89

Kusumawati, D., 2004. **Bersahabat dengan hewan coba, cetakan pertama**. Gajah Mada University Press. Yogjakarta

Lanza, R.P., Langer, R and Vacanti, J. 2000. **Principles of Tissue Engineering**, 2nd ed, Academic Press, San Diego-San Francisco,

Lawrence, R., Tripathi, P and Jeyakumar, E. 2009. Isolation, Purification and Evaluation of Antibacterial Agents from *Aloe vera*. *Brazilian Journal of Microbiology*, 40(4): 906-15

Lieberman, J. R and Friedlaender, G. E. 2005. *Bone Regeneration and Repair*, 1 th ed. Humana Press: Totowa-New Jersey

Mi-Young, P., Hoon-Jeong, K and Mi-Kyung, S. 2009. Evaluation of Aloin and Aloe-Emodin as Anti Inflammatory Agents in Aloae by using Murine Macrophages. *Biosci Biotechnol Biochem*, 73(4): 828-832

Moghadasi, S.M and Verma, S.K. 2011. *Aloe vera* their chemicals composition and applications : A Review. *Int J Biol Med Res* , 2(1) 466-471

Munadziaroh, E, Hendriyantini, N and Indrasari, M. 2002. Mempertahankan Tinggi Tulang Alveolar setelah Pencabutan Gigi Menggunakan Demineralize Frezze Dried Bone Allograft. *Majalah Ilmiah Kedokteran Gigi, Edisi Khusus Foril* : 321-324

Oktavia, R. 2005. *Tehnik Bedah Peninggian Lingir Alveolar Sebagai Salah Satu Penunjang Keberhasilan di bidang Prosthetik*. Tesis. Fakultas Kedokteran Gigi Universitas Sumatera Utara

Pachanon, C. 2005. **A Study the Element in Aloevera Powder by Neutron Activation Analysis.** Thesis the Degree of Master of Science Radiological Science**.** Faculty of Graduates Studies, Mahidol University

Pugh, N.D., Tamta, H., Balachandram, P., Xiangmei, W., Howell, J.L, Dayan, F.E and Pasco, D.S. 2008. The Majority of in Vitro Macrophage Activation Exhibited by Extract of Some Immune Enhancing Botanicals is Due to Bacterial Lipoproteins and Lipopoly saccharides. *Int Immunopharmacol*, 8 (7): 1023-32.

Roostita and Tim Redaksi Qanita, 2008. *Lidah buaya*, Cetakan I, Penerbit Qanita ,PT. Mizan Pustaka, Bandung, Indonesia

Steiner, G.G., Francis, W., Burrell, R., Kallet, M.P., Steiner, D.M., Macias, R. 2008. The Healing Socket and Socket Regeneration, *J. Compendium*, 29(2): 1-11.

Vasquez, B., Avila, G., Segura, D and Escalante, B. 1996. Anti-inflammatory activity of extract from *Aloe vera* gel. *Journal of Ethnopharmacology*, 55 : 69-75

Werner , S and Grose, R. 2003. Regulation of wound healing by growth factor and cytokine. *Physiol review*, 83: 835-66.