Dental Journal
Majalah Kedokteran Gigi
Vol. 44. No. 4 December 2011

Research Report

Stimulation of osteoblast activity by induction of *Aloe vera* and xenograft combination

Utari Kresnoadi[1] and **Retno Pudji Rahayu**[2]
[1]Department of Prosthodontics
[2]Department of Oral Biology
Faculty of Dentistry, Airlangga University
Surabaya - Indonesia

ABSTRACT

Background: *Tooth extraction is generally followed by alveolar ridge resorption that later can cause flat ridge. Aloe vera have biogenic stimulator and hormone activities for wound healing.* ***Purpose:*** *This study was aimed to know osteoblast activities in alveolar bone after induction of Aloe vera and XCB combination.* ***Methods:*** *Fifty four of Cavia cabaya were divided into three main groups. Group I was control group. Group II was filled with xenograft concelous bovine (XCB) and group III was filled with the combination of Aloe vera gel and XCB. Then, each group was divided into three sub groups according to timing, they are 14, 30, and 60 days after tooth extraction and application. Histology and morphology examination were performed on the harvested specimens.* ***Results:*** *There were significant differences between the control group and the other groups filled with the combination of Aloe vera and XCB.* ***Conclusion:*** *In conclusion, the application of Aloe vera gel and xenograft combination decrease the number of osteoclast and increase the number of osteoblast in post tooth extraction alveolar bone structure indicating the new growth of alveolar bone.*

Key words: *Osteoblast, osteoclast, Aloe vera, xenograft concelous bovine, tooth extraction socket*

ABSTRAK

Latar belakang: *Pencabutan gigi pada umumnya selalu diikuti resopsi tulang alveolar, sehingga bila terjadi dalam waktu yang lama ridge akan menjadi flat. Aloe vera adalah bahan stimulasi biogenik dan mempunyai aktivitas hormon untuk proses penyembuhan luka.* ***Tujuan:*** *Tujuan dari penelitian ini adalah untuk mengetahui aktivitas osteoblas pada tulang alveol dengan pemberian kombinasi Aloe vera gel dan xenograft concelous bovine (XCB).* ***Metode:*** *Lima puluh empat ekor Cavia cabaya, dibagi menjadi 3 kelompok besar, kelompok pertama adalah kelompok kontrol yaitu hanya dilakukan pencabutan saja tanpa perlakuan, kelompok ke-2 yaitu kelompok yang setelah dicabut diberi XCB saja dan kelompok ke-3 yaitu kelompok yang setelah pencabutan diberi kombinasi Aloe vera gel dengan XCB pada luka bekas pencabutan gigi. Kemudian masing-masing kelompok besar ini dibagi lagi berdasarkan waktu menjadi 3 sub kelompok yaitu setelah 14, 30 dan 60 hari. Kemudian dilakukan pemeriksaan histology dan morfologi pada specimen hewan coba.* ***Hasil:*** *Terdapat perbedaan bermakna antara kelompok kontrol dan kelompok yang diberi kombinasi Aloe vera dan XCB.* ***Kesimpulan:*** *Disimpulkan bahwa pemberian kombinasi Aloe vera gel dan xenograft menyebabkan penurunan jumlah osteoklas dan peningkatan jumlah osteoblas pada struktur tulang alveol pasca pencabutan gigi yang menunjukkan adanya pertumbuhan tulang alveol baru.*

Kata kunci: *Osteoblas, osteoklas, Aloe vera, xenograft concellous bovine, soket pencabutan gigi*

Correspondence: Utari Kresnoadi, c/o: Departemen Prostodonsia, Fakultas Kedokteran Gigi Universitas Airlangga. Jl. Mayjend. Prof. Dr. Moestopo 47 Surabaya 60132, Indonesia. E-mail: utari.kresnoadi@yahoo.com

INTRODUCTION

The preparation of complete denture will be successful if the denture is retentive, stable, and comfortable for patients. However, the success of the denture is also related to other factors, such as the skills of operators as well as anatomical factors of supporting tissues, one of which is the alveolar bone. Patients with longer tooth loss which is not replaced by artificial tooth or dentures may have alveolar bone resorption or residual ridge resorption, and later can cause the prevalence of increased alveolar bone resorption.

The initial phase of residual ridge is actually started from tooth loss though periodontal membrane which has the ability to form bone. It is because if the alveolar bone loss occurs in labio and vertical lingual, the residual ridge will become narrower. In some cases, it can even be sharp as a knife edge and retracted. These conditions will not only cause alveolar process disappeared, but can also cause lower residual ridge, rounded, or flat. If this resorption condition continuously occurs, basal bone can disappear, so the shortening of ridge occurs in mouth.[1]

Generally, dento alveolar component is a system showing degree of bone health that is able to withstand constant strength and strain. Tooth extraction, on the other hand, can not only lead to the existence of narrow residual margin, but can also lead to shortening and jawbone atrophy.[2,3] If this condition is not solved, then it can affect the manufacture of artificial teeth that are not adequate. Thus, to prevent alveolar bone resorption, it requires the development of an innovative technique of alveolar ridge augmentation elevation.[2] Therefore an effort is needed to prevent post-tooth-extraction sockets from resorption, so periodical relining action is not required.

The compositions of bone actually consists of mineral, organic matrix, cells, and water at a ratio of 65% minerals and 35% both organic matrix, such as osteoblasts, osteocytes and osteoclasts, and water. Organic matrix, about 35% of bone weight in a dry state, consists of 90% collagen, the highest bone protein, and non-collagen bone protein, such as osteonectin, osteocalcin, osteopontin and, sialoprotein. Osteoblasts produce bone matrix proteins. Osteoblasts also synthesize other proteins in the bone matrix, such as osteonectin and osteocalsin, about 40-50% of bone non-collagen protein.[4] Canalis *cit.* Lindawati[4] even said that other bone protein produced by osteoblasts are glycosaminoglycan, osteopontin, sialoprotein, fibronectin, vitronectin, and trombospondin that serve as glue interacting with integrins. The most common result of osteoblasts is collagen type 1, which will form collagen fibrils. [4]

The use of bovine graft in this study is aimed to repair bone defect or augmentation oftenly performed in the fields of general surgery and oral surgery that still requires an innovative modification of bio-products to produce maximal bone growth. Therefore an innovation of material modification used as biogenic stimulators in order to stimulate alveolar bone and to accelerate the growth of the bone is needed.

Aloevera is considered to have biogenic stimulator and hormone activities for wound healing. Liquid derived from *Aloe vera* can prevent scar tissue ocured at the time of incision, thus, when *Aloe vera* gel is used after surgery, the incision will heal quickly.[5,6] *Aloe vera* contains with two liquid, clear and yellow-colored liquid. The clear liquid, jelly-like liquid, contains with anti-bacterial and anti fungal stimulating fibroblasts or skin cells that serve to heal wounds, while the yellow-colored liquid contains *aloin* derived from latex skin of *Aloe vera*.[7]

Aloevera gel is a gel made from the meat leaves of Spama simplicia plants, such as aloe, succus aloe insipissatus from familia Liliaceae, containing chemical aloin and aloe emodine that have anti-inflammatory properties.[5,6] *Aloe vera* can not only be used as an anti-inflammatory, antibacterial, antifungal, and antiallergy, but can also increase immunity and accelerate the process of wound healing by increasing cell regeneration. *Aloevera* is a natural material that is necessary to be tested for the safe extract dose used for this study.[8]

It is needed to know whether the use of *Aloe vera* gel and xenograft cancellous bovine (XCB) combination on post tooth extraction sockets can accelerate the growth of alveolar bone, thus, it can prevent the occurrence of alveolar bone resorption. As a result, the making of artificial tooth can be conducted well. Therefore, this study is aimed to examine osteoblast activities in alveolar bone after introduction of *Aloe vera* and XCB combination.

MATERIALS AND METHODS

This study was conducted on experimental animals, *Cavia cabaya*, with randomized post test only control group design. The number of samples of each group is 6 from the total number of 54 *Cavia cabaya* with inclusion criteria: male, 3 months old, 300–350 grams, healthy and active.

The samples were divided into nine treatment groups. Group I, II, III consisted of *Cavia cabaya* which teeth were extracted, but without treatment. Meanwhile, groups IV, V, VI consisted of *Cavia cabaya* which teeth were extracted, but treated with xenograft concelous bovine only. Group VII, VIII, IX consisted of *Cavia cabay*a which teeth were extracted, and treated with the combination of *Aloe vera* gel and XCB. All of them were examined 14, 30, and 60 days after the tooth extraction and treatment.

Materials used in this study were *Aloe vera* gel, this gel was produced from 1000 grams of *Aloe vera* leaves blending and made into extract. This Aloe vera extract was freeze dried and stored in special tube. One gram of this extract was mixed with 1 ml sterile aquadest, filtered and centrifuged at 4000 rpm. The result was filtered using milipor 0.045 µml to get 0.5 ml. *Aloe vera* extract was done in 70%, 85% and 100%. These samples were sterilized with ultraviolet for 15 minutes before testing. *Aloe vera* toxicity testing was conducted in 70%, 85% and 100% concentration on fibroblast cells using MTT assay.

Combination of *Aloe vera* gel and XCB was produced by mixing 0.5gram of *Aloe vera* from freeze drying with XCB, and mixed with 99ml polyethylene glycol (PEG 4000 and PEG 400). This mixture was put into sterile container for application. This gel was made to ease application into tooth sockets.

Cavia cabaya was taken and anaesthetized using inhalation anesthetic ether, with 1 ml/1kg weight dose,[8] then the incisive tooth was extracted. Post tooth extraction sockets were filled according to grouping and then stitched. After 14, 30, and 60 days, *Cavia cabaya* were harvested, and the jaws were cut to make paraffin block preparation after decalcification process with fixation material of 2% nitric acid, for a week. After that, the paraffin block was cut, mounted on its slide, and coloured with hematoxylin-eosin (HE) staining.

Next, the checking and calculating processes were conducted separately with double blind technique by 2 (two) different examiners. Each slide was examined with 1000× magnification for about ten fields of view. After that, the results were recorded, and the mean value per field of view was calculated. The calculation results were recorded and then tabulated. Afterwards, statistical analysis was conducted by using analysis of variance (ANOVA) test, and then Tukey-HSD test was also conduceted to compare among the best groups.

RESULTS

The mean results of toxicity test of *Aloe vera* extract with concentrations of 70%, 85%, and 100% on day 1, 2 and 3 can be seen in table 1.

Table 1. The mean results of toxicity test of *Aloe vera* extracts with concentrations of 70%, 85% and 100% on the first, second and third day in optical density

Concentration/day	First day	Second day	Third day
70% *Aloe vera*	1.90	1.87	1.90
85% *Aloe vera*	1.91	1.91	1.814
100% *Aloe vera*	1.90	1.81	1.925
Control	1.814	1.838	1.820

Through One-Way ANOVA statistical analysis, it is known that on the first day value of $p \geq 0.05$, while on the second day value $p > 0.05$. It means that there was no significant difference. Meanwhile, on the third day there was significant difference, value of $p < 0.05$. It means that there was significant difference in toxicity tests by using MTT assay.

Based on the results of multiple comparison Tukey HSD test, it is also known that on the first day, the value of $p > 0.05$. It means that there was no significant difference between the three *Aloe vera* extract groups and the control groups. It is also known that on the second day the value of $p > 0.05$, means that there was also no significant difference between the three *Aloe vera* extract groups and the control groups. On the third day, the value of $p > 0.05$, means that there was no significant difference between the control groups and the three *Aloe vera* extract groups with concentrations 70%, 85%, and 100%. Thus, it can be indicated that *Aloe vera* extract with concentration 100% can only be used on the first day and the second day, while on the third day it should not be used.

Histological examination was conducted by observing the morphology of alveolar bone structure, it can also be known that the descriptions of osteoclasts and osteoblasts can be seen in figure 1.

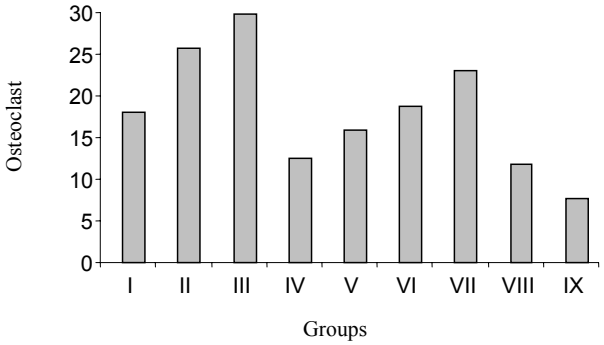

Figure 1. The number of osteoclast from histological examination.

There is an increase mean of osteoclast number from control group I, II, III after extraction, but on group IV, V, VI with XCB only there is increased number of osteoclast even though not significant. On group VII, VIII, IX with Aloe vera and XCB there is significant decrease of osteoclast number after extraction. Statistical ANOVA analysis on osteoclasts among group I-IX showed significant differences on osteoclast number ($p < 0.05$).

Multiple comparison growth of osteoclasts showed no significant difference among 14 day groups after the tooth extraction, which were between group I (control) and group IV (with XCB application), with significance of $p > 0.005$, and between group I (control) and group VII (with Aloe vera and XCB application), with significance $p > 0.005$.

In the 30 day groups, after the tooth extraction, there was significant difference between group II (control) and group V (with XCB application only), with significance $p \leq 0.05$. Similarly, there was also significant difference between group II (control) and group VIII (with *Aloe vera* and XCB application), with significance $p < 0.05$. In the 60 day groups, furthermore, the tooth extraction there was significant difference between group III (control) and group VI (with XCB application only) with significance $p < 0.05$. Similarly, there was significant difference between group III (control) and group IX (with the administration of *Aloe vera* and XCB application), with significance $p < 0.05$.

Decrease number of osteoblast on group I, II, III which were 14 days after tooth extraction (Figure 2). In group IV, V, VI (with XCB application only) there were increased number of osteoblast. In group VII,VIII, IX (with Aloe vera and XCB application) there were significant increase of

Table 2. Multiple comparison growth of osteoclasts (HPA)

Groups	I	II	III	IV	V	VI	VII	VIII	IX
I			*						
II				*	*			*	*
III				*	*	*		*	*
IV		*	*				*		
V		*	*						*
VI			*						*
VII	*							*	*
VIII		*	*				*		
IX	*	*	*		*	*	*		

Note: Group I: 14 days after the tooth extraction without any treatment (control); Group II: 30 days after the tooth extraction without any treatment (control); Group III: 60 days after the tooth extraction without any treatment (control); Group IV: 14 days after the tooth extraction with filled in XCB; Group V: 30 days after the tooth extraction with filled in XCB; Group VI: 60 days after the tooth extraction with filled in XCB; Group VII: 14 days after the tooth extraction with filled in *Aloe vera* + XCB; Group VIII: 30 days after the tooth extraction with filled in *Aloe vera* + XCB; Group IX: 60 days after the tooth extraction with filled in *Aloe vera* + XCB; *: Significance

osteoblast number in group VII eventhough in group VIII and IX there were slight decrease of osteoblast number but if compared to control group there were still significant increase. Statistical ANOVA analysis on osteoblasts among group I-IX showed significant differences on osteocblast number ($p < 0.05$).

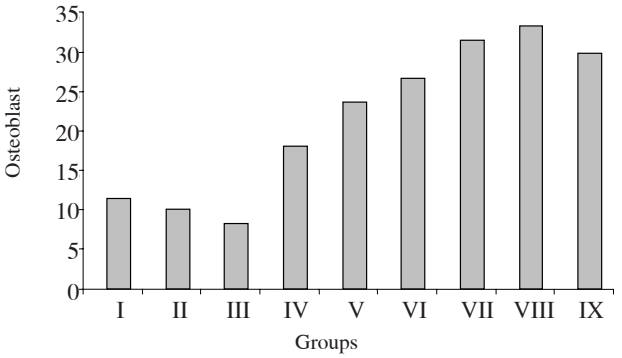

Figure 2. The number of osteoblasts from histological examination.

Multiple comparison calculation of osteoblasts showed significant differences among 14 day groups after the tooth extraction, which was between group I (control) and group IV, with significance $p < 0.005$, and also between group I (control) and group VII, with significance $p < 0.005$. In the 30 day groups, after the tooth extraction there were significant differences between group II (control) and group V with significance $p < 0.05$, as well as between group II and group VIII with significance $p < 0.05$. Then, in the 60 day groups, after the tooth extraction there were also significant differences between group III (control), group VI, and group IX with significance $p < 0.05$.

Table 3. Multiple comparison calculation of osteoblasts (HPA)

Groups	I	II	III	IV	V	VI	VII	VIII	IX
I				*	*	*	*	*	*
II				*	*	*	*	*	*
III				*	*	*	*	*	*
IV	*	*	*		*	*	*	*	*
V	*	*	*	*			*	*	
VI	*	*	*	*				*	
VII	*	*	*	*	*	*			
VIII	*	*	*	*	*	*	*		
IX	*	*	*	*					

Note: Group I: 14 days after the tooth extraction without any treatment (control); Group II: 30 days after the tooth extraction without any treatment (control); Group III: 60 days after the tooth extraction without any treatment (control); Group IV: 14 days after the tooth extraction with filled in XCB; Group V: 30 days after the tooth extraction with filled in XCB; Group VI: 60 days after the tooth extraction with filled in XCB; Group VII: 14 days after the tooth extraction with filled in *Aloe vera* + XCB; Group VIII: 30 days after the tooth extraction with filled in *Aloe vera* + XCB; Group IX: 60 days after the tooth extraction with filled in *Aloe vera* + XCB; *: Significance

DISCUSSION

In this research, MTT assay toxicity testing was performed to find out *Aloe vera*'s toxicity. The result was there was no significant difference on One-Way ANOVA analysis on the first and second day but on the third day there was significant difference. Multiple comparison test with Tukey-HSD showed no significant difference among the first, second and third day. Therefore 70%, 85% and 100% *Aloe vera* extract are safe to use against fibroblast cells.

Aloe vera is a plant with anti inflammatory effect, promote wound healing, and increase blood supply on wounds. [6] Histological finding on figure 1 and 2 showed that there are decrease number of osteoclast and significant increase number of osteoblast. This finding showed that *Aloe vera* is a biogenic stimulator which can promote XCB to activate inside alveolar bone socket. This research is in accordance to some opinions that *aloe* is a widely used traditional medical plant with various conditions. *Aloe* is also called barbaloin is a yellow crystal with bitter taste and derivative of C-glycoside from anthraquinone. A C-glycoside if hydrolyzed will form aloe-emodin, anthrone which can auto-oxidated forming quinine, aloe emodin. Aloin and aloe-emodin has not only laxative effect but also anti bacterial, anti virus, hepatoprotective and anti cancer effects. Aloin and *aloe*-emodin contains polyphenol which has anti inflammatory effect.[11]

Traumatic tooth extraction can lead to inflammation causing osteoclastogenesis, the growing process of osteoclasts induced with inflammation due to traumatic tooth extraction. The mature osteoclasts have a capacity for bone resorption.[12] In the process of osteoclastogenesis,

the differentiation of osteoclasts and the activation of the combination of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor ligand (RANKL) occur. Precusors of osteoclasts replicate and induce RANK in inflammatory condition produced by B and T lymphocyte.

The role of *Aloe vera* gel, on the other hand, is to prevent inflammation that osteoclastogenesis can be decreased, so bone cell resorption does not occur.[12] This is in accordance with the opinion of Kyung *et al*.,[13] that osteoclasts are cells responsible for bone resorption process, which is formed from haemopoetic stem cell. The regulation of osteoclastogenesis in inflammation, such as arthritis, proinflammatory cytokines (IL1, IL 6 and TNF-α), and RANKL, can stimulate osteoclasts and bone damage.

Similarly, the opinion of Regina[14] on her research on scar removal stated that by adding DBM (dimineralized bone matrix), the allograft DBM powder can not only reduce the expression of gelatinase A, but can also increase the expression of TIMP-2 so that the DBM stimulates the healing process of gingival wound. [14]

Aloe vera and XCB combination can reduce osteoclast because *Aloe vera* has antiinflammatory effect from antraguinon (Aloe, Aloe emodin) substance and saponin hormone which faster extraction wound healing and osteoblast formation (biogenic stimulator). XCB is an osteoinduction substance which can induce osteoblast in new bone formation.

Therefore, the combination of *Aloe vera* and XCB can not only accelerate the healing of scar removal, but can also enable the growth of bone cells by osteoblast cells. Finally, it then can be concluded that the addition of the combination of *Aloe vera* and XCB on post tooth extraction socket in this study not only can increase osteoblasts, but also can decrease osteoclasts indicating the new growth of alveolar bone.

REFERENCES

1. Nishimura I, Hosokawa R, Attwood DA. **Knive edge tendency in** mandibular ridge in women. J Prosthed Dent 1992; 67: 820–6.
2. Oktavia R. Tehnik bedah peninggian lingir alveolar sebagai salah satu penunjang keberhasilan di bidang prosthetic. **Karya tulis akhir** pendidikan dokter gigi spesialis. Sumatera Utara: FKG USU; 2005. p. 28
3. Gupta A, Tiwari B, Goel H, Shekawat H. Residual ridge resorption: A Review. Indian J Dent Sci 2010; 2(2): 7–11.
4. Khudhany LS. Penentuan Indeks densitas tulang mandibula perempuan pasca maenopause dengan memperhatikan beberapa faktor risiko terjadinya osteoporosis. Disertation. Jakarta: Pascasarjana Universitas Indonesia; 2003.
5. Sudibyo M. Alam, sumber kesehatan, manfaat dan kegunaan. **Jakarta:** Penerbit Balai Pustaka; 1998. p. 250.
6. Rostita, Tim Redaksi Qanita. Lidah buaya. Cetakan I. Bandung: PT Mizan Pustaka; 2008. p. 19–38.
7. Marthanthi R. Uji sitoksisitas aloe vera gel 100% metode freze drying terhadap sel fibroblas ditinjau dari waktu inkubasi. **Skripsi. Surabaya:** Fakultas Kedokteran Gigi Universitas Airlangga; 2007. p. 7–10.
8. Ariyani E, Khoswanto C. The use 90% *Aloe vera* frezze drying as modulator of collagen density in exctraction socket of incisivus in *Cavia cabaya*. Maj Ked. Gigi (Dent J) 2008; 41(2): 74–6.
9. Kusumawati D. Bersahabat dengan hewan coba. Cetakan pertama. Jogjakarta: Gajah Mada University Press; 2004. p. 67.
10. Kurniawati R, Marminah MT. Efek anti imflamasi gel lidah buaya (*Aloe vera Linn*) terhadap tikus putih. Solo: Prosiding Seminar Nasional Tumbuhan Obat Indonesia XXIX; 2006. p. 82–89.
11. Park MY, Kwon HJ, Sun MK. **Evaluation of aloin and aloe emodin** as anti inflammatory agents in aloe by using murin macrophage. J of Biosci Biotech Biochem 2009; 73(4): 828–32.
12. Lorenzo J. Osteoimmunology: Interaction of the bone and immune system. J of Endocrine Review 2008; 29(4): 403–40.
13. Kyung TW, Lee JE, Shin HH, Choi HS. Rutin inhibits osteoclast formation by decresing reactivee oxygen species and TNF-α by inhibiting activation of NFκB. J Experiment and Molecular Medicine 2008; 40(1): 52–8.
14. Tandelilin RTC. DBM augmentation on mandibular incisivus extraction wound healing. J of Dentistry 2006; 13(3): 190–6.