Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019, 19:e4537
DOI: http://doi.org/10.4034/PBOCI.2019.191.74
ISSN 1519-0501

ORIGINAL ARTICLE

Histometry Analysis of Osteoclasts, Osteoblasts and Fibroblasts in Immediate Placement Implants with Addition of Aloe Vera Extract

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Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 21 November 2018 / Accepted: 19 March 2019 / Published: 11 April 2019

Abstract

Objective: To analyze the effect of immediate placement of implants with extract from the new bone formation histometically. **Material and Methods:** In this true-experimental design with randomized post test control group, 9 mongrel dogs weighing 10 to 12 kg were used, which were divided into 3 groups, based on observation time of 14 days, 28 days and 56 days. On the installation of implants (⌀3.5x10 mm) sequentially, the former socket extraction of the lower jaw's right second premolar tooth in the study sample injected 10% Aloe vera gel extract and left second left premolar tooth without injection of 10% Aloe vera extract. To compare independent groups use the Mann-Whitney test. All analysis were carried out using SPSS version 20. **Results:** There was an increase in the number of osteoblast cells in both treatment and control groups, but the value of the treatment group was greater. There were significant differences in the number of osteoblast cells between the treatment and control groups 14 days ($p=0.019$), 28 days: ($p=0.018$), and 56 days ($p=0.009$). There were no significant differences in the number of fibroblast cells between the treatment and control groups ($p>0.05$). But at observations 28 and 56 days, it was showed a significant difference in the number of fibroblast cells between the treatment and control groups ($p=0.353$ and $p=0.024$, respectively). **Conclusion:** Immediate placement of implants with 10% Aloe vera extract gel on extracted socket increases the number of osteoblasts and suppresses the number of osteoclasts and fibroblasts.

Keywords: Dental Implantation, Endosseous; Connective Tissue Cells; Aloe.

Introduction

The success of a dental implant treatment is strongly influenced by the formation of osseointegration, which plays an important role in the stabilization, and retention of implants to the jaw bone. Osseointegration can be interpreted as a state of fixation of fixture implantin the jaw bone after implant placement, because the triggering of the jaw bone cells multiplies so that it can be interdigitated with the surface of the implant mechanically [1].

Osseointegration will form in the third to sixth month after the implant is placed into the jawbone. The process of osseointegration requires the role of osteoblasts, when osteoblasts are active for a certain period of time forming osteocytes [2]. The role of osteoblasts cannot be released from the function of fibroblasts, which are the initial growth cells of the new reinforcement process. Osteoblasts are cells that are responsible for bone formation in the bone matrix mineralization process by secreting collagen type l and releasing calcium, magnesium, and phosphate ions [3].

A previous study showed remodeling physiological bone in dogs beagle after inserting immediate implants in newly extracted areas and concluded that the procedure had a role in reducing alveolar buccal bone resorption [4].

A number of medicinal plants have a key role in the development of modern studies because of the biological activities of their bioactive substances. The use of medicinal plants in medicine is believed to contain only a small amount of toxicity and has biological activity as well as potential for the application of healing to a disease, one of which is Aloe vera [5]. The chemical composition of Aloe vera contains aloin, aloe-emodin, baroin, amino acids, and anthraquinone. Anthraquinone has several beneficial properties such as anti-inflammatory, analgesic, anti-microbial effects, and anesthetics, and laxative effects. It is also known that aloe-emodin can improve tissue healing and healing processes and minimizes pain [6].

The purpose of this study was to analyze the effect of immediate implant installation by adding Aloe vera extract to new bone formation by histometry.

Material and Methods

Study Design and Sample

The research was true-experimental design with randomized post test control group with a sample of 9 mongrel dogs with a weight of 10 to12 kg, which were divided into 3 groups, based on observation time of 14 days, 28 days and 56 days.

The following inclusion criteria were adopted: male mongrel dogs 1 year old, weighing about 10 kilograms, healthy (hair not dull, fall out, bald, and active), while the exclusion criteria comprised: a weight loss of more than 10% after the adaptation period at the place of animal breeders, and the dog dies before data collection.

Experimental Procedure

On the installation of implants (Ø3.5x10 mm; Osstem Implant Co., Ltd., Seoul, South Korea), the former socket extraction of the lower jaw's right second premolar tooth in the study sample

injected 10% Aloe vera gel extract and left second left premolar tooth without injection of 10% Aloe vera extract. After 14 days the first group was examined, then the group was 28 days and the last group was 56 days.

Data Analyzis

The Shapiro-Wilk test have been used to test data normality. Comparing independent groups, data that are not normally distributed use the Mann-Whitney test while determining the difference between two or more groups of independent variables with the dependent variable using Kruskal-Wallis. Data processing and analysis using the SPSS version 20.0 program (SPSS Inc., Chicago, IL, USA).

Ethical Aspects

All procedures have been carried out in this study of ethical and ethical clearance by the ethics commission of the Faculty of Dentistry, University of Hasanuddin, Makassar (Protocol No. 031 / PL.09 / KEPK FKG-RSGM UNHAS / 2018).

Results

There was an increase in the number of osteoblast cells in both treatment and control groups, but the value of the treatment group was greater (Figure 1). Figures 2 and 3 show a decrease in the number of osteoclasts and fibroblasts in both the treatment and control groups, but the value of the treatment group was smaller.

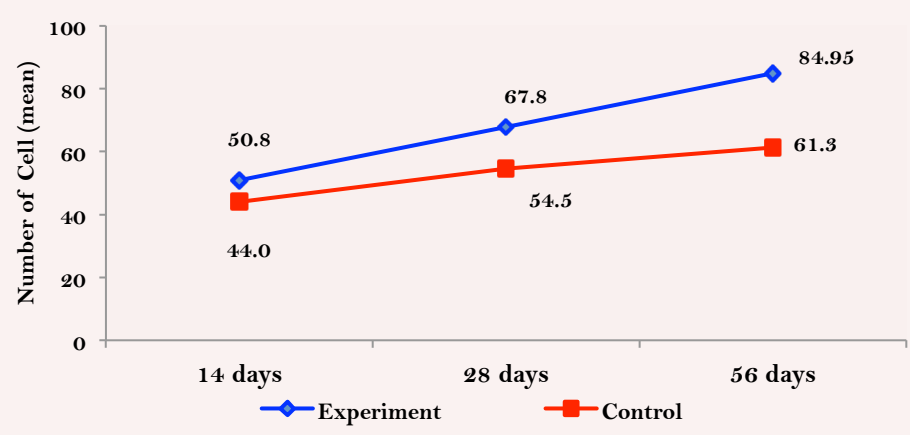

Figure 1. Distribution of osteoblasts and control at observation 14, 28, and 56 days.

In observing the average value of osteoblast cells 14, 28 and 56 days it was seen that the type of osteoblast cells in the treatment group was higher than the control group. Statistical results showed that there were significant differences in the number of osteoblast cells between the treatment and control groups 14 days (p=0.019), 28 days (p=0.018), and 56 days (p=0.009) respectively (Table 1).

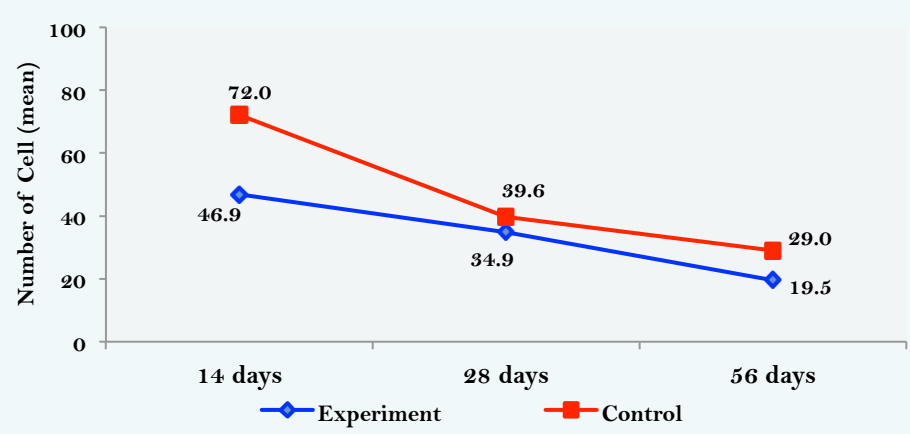

Figure 2. Distribution of osteoclasts and control at observation 14, 28, and 56 days.

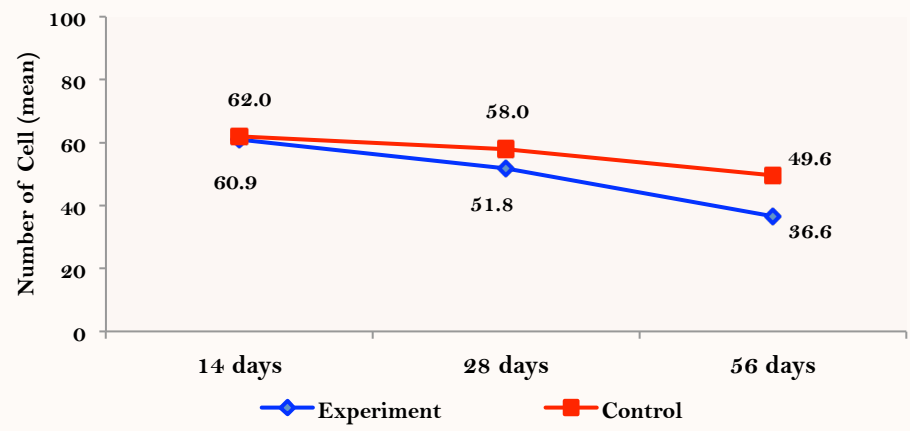

Figure 3. Distribution of fibroblasts and control at observation 14, 28, and 56 days.

Table 1. Differences in the number of average osteoblast, osteoclast, and fibroblast cells between treatment and control groups at the time of observation 14 days, 28 days, and 56 days.

Cell Type	Time Observation	Groups		p-value
		Treatment Mean ± SD	Control Mean ± SD	
Osteoblasts	14 Days	50.80 ± 0.28	44.00 ± 0.00	0.019*
	28 Days	67.80 ± 2.54	54.50 ± 0.00	0.018*
	56 Days	84.95 ± 0.49	61.30 ± 0.00	0.009*
Osteoclasts	14 Days	46.95 ± 0.92	72.00 ± 0.00	0.013*
	28 Days	34.90 ± 0.57	39.60 ± 0.00	0.004*
	56 Days	19.55 ± 1.06	29.00 ± 0.00	0.040*
Fibroblasts	14 Days	60.95 ± 0.92	63.00 ± 0.00	0.353
	28 Days	51.80 ± 5.37	58.00 ± 0.00	0.024*
	56 Days	36.65 ± 3.32	49.60 ± 0.00	0.011*

Normality test, Shapiro-Wilk test: $p<0.05$; Normal data distribution. *Mann-Whitney-U test: $p<0.05$; significant.

The results also showed the number of osteoclast cells in the two groups. At the time of observations 14, 28 and 56 days, the number of osteoclasts in the treatment group was less than the control group. Statistical results showed that there were significant differences in the number of osteoclasts between the treatment and control groups at the time of observation 14 days ($p=0.013$), 28 days ($p=0.004$), and 56 days ($p=0.040$) (Table 1).

At observations 14, 28 and 56 days, the number of fibroblast cells in the control group was higher than the treatment group. However, based on the results of the statistical test, there were no significant differences in the number of fibroblast cells between the treatment and control groups ($p>0.05$). But at observations 28 and 56 days, the results of statistical tests showed a significant difference in the number of fibroblast cells between the treatment and control groups ($p=0.353$ and $p=0.024$, respectively) (Table 1).

Table 2 shows the differences in the number of osteoblast cells, osteoclasts, and fibroblasts based on the time of observation. The results showed that the number of osteoblast cells at 14 days of observation reached 50.80. It was seen that there was an increase in the number of osteoblasts at the time of observation of 28 days and 58 days, namely 67.80 at 28 days and 84.95 at 56 days. The results showed that there were significant differences in the number of osteoblast cells between 14 days, 28 days and 56 days ($p<0.001$).

The inversely occurs in osteoclasts, which is a decrease in the number of cells from 14 days to 28 days and again decreases after 56 days. In succession, the number of osteoclasts decreased from 46.95 to 34.90 and finally at 19.55. There were significant differences in the number of osteoclasts between 14 days, 28 days, and 56 days of observation ($p<0.05$).

In fibroblast cell types, the results of the study show that the number of cells decreased from 60.95 on day 14 to 51.80 on day 28 and again declined on day 56 to 36.65. The results of statistical tests also showed that there were significant differences in the number of fibroblasts between the observation time of 14 days, 28 days, and 56 days ($p=0.016$).

Table 2. Differences in the number of osteoblasts, osteoclasts, and fibroblasts based on the time observation.

Cell Type	Time Observation			p-value
	14 Days Mean ± SD	28 Days Mean ± SD	56 Days Mean ± SD	
Osteoblasts	50.80 ± 0.28	67.80 ± 2.54	84.95 ± 0.49	0.000*
Osteoclasts	46.95 ± 0.92	34.90 ± 0.57	19.55 ± 1.06	0.000*
Fibroblasts	60.95 ± 0.92	51.80 ± 5.37	36.65 ± 3.32	0.016*

Normality test, Shapiro-Wilk test: $p<0.05$; Normal data distribution. *Kruskal-Wallis test: $p<0.05$; significant.

Table 3 shows the results of further differences in the number of osteoblast cells, osteoclasts, and fibroblasts between groups of immersion time 14 days, 28 days, and 56 days. The results showed that there were significant differences in the number of osteoblast cells between 14 days and 28 days of observation ($p=0.001$); between 14 days and 56 days ($p<0.05$); and between 28 days and 56 days ($p=0.001$). Thus, increasing the application of the addition of Aloe vera extract has a significant effect on increasing odontoblast cells along with the time of observation.

Furthermore, the results showed a significant difference in the number of osteoclasts between 14 days and 28 days ($p=0.001$); between 14 days and 56 days ($p<0.05$); and between 28 days and 56 days ($p<0.05$). There was a significant decrease in the number of osteoclasts as the time of

observation increased. This shows that the application of Aloe vera is also effective in reducing the number of osteoclasts.

In observing fibroblast cells, there was no significant difference in the number of fibroblast cells between 14 days and 28 days of observation (p=0.089). However, there were significant differences between the number of cells at the time of observation 14 days and 56 days (p=0.007), and between the observations of 28 days and 56 days (p=0.026). This shows that the application of Aloe vera has not shown its effectiveness on day 14, but on day 28 and is more effective on day 56.

Table 3. The results of further differences in the number of osteoblasts, osteoclasts, and fibroblasts between groups of 14 days, 28 days, and 56 days immersion time.

Cell Type	Time Observation	(j)	Mean Difference (ij)	95% CI (Min - Max)	p -value
Osteoblasts	14 Days	28 Days	-17.000	-21.793 – -12.206	0.001*
		56 Days	-34.150	-38.943 – -29.356	0.000*
	28 Days	56 Days	-17.150	-21.943 – -12.356	0.001*
Osteoclasts	14 Days	28 Days	12.050	9.269 – 14.830	0.001*
		56 Days	27.400	24.619 – 30.180	0.000*
	28 Days	56 Days	15.350	12.569 – 18.130	0.000*
Ffibroblasts	14 Days	28 Days	9.150	-2.582 – 20.882	0.089
		56 Days	24.300	12.568 – 36.032	0.007*
	28 Days	56 Days	15.150	3.418 – 26.882	0.026*

*Post Hoc test: LSD test; p<0.05: significant.

Discussion

Early bone formation plays a role in creating the stability of secondary implants. In this study the average number of osteoblasts was greater in the treatment group than in the control group. This result is in line with the research conducted before, which states that osteoblast cell proliferation occurs in low cytotoxicity conditions in the concentration of Aloe vera extract (accemanan) which is higher, where the higher the concentration of Aloe vera extract is given, the more osteoblast cells resulting from [7]. Previous research has shown that there was a decrease in osteoblast growth in the untreated control group, compared to the treatment group. In the mixture Aloe vera post-extraction there was a significant increase in osteoblast cell growth [8]. This shows that there is an increase in bone growth activity carried out by osteoblasts.

While the average value of osteoclast cells is smaller in the treatment group than in the control group, so that it can minimize the process of bone resorption. The anthraquinone glycoside content called emodin in Aloe vera extract suppresses bone resorption activity from osteoclasts by inhibiting RANKL [9]. It has been demonstrated that there was an increase in the number of osteoclasts in the untreated extraction group and in the group with filling mixture Aloe vera there was a significant decrease in the number of osteoclasts in the group 14 days after extraction and group 30 days and the most decrease in the group 60 days after extraction [6].

A similar aspect was found in the average value of fibroblasts, which was smaller in the treatment group than in the control group. The content of mannose and gibberellin glucomannan

from Aloe vera extract increases collagen synthesis in the process of wound remodeling. Glucomannan as a glycoprotein in Aloe vera contains mannose and gibberellin, a growth hormone that contributes to wound healing by stimulating the activity of fibroblasts (connective tissue cells) to increase collagen synthesis in the process of wound remodeling [10]. From the results of this study assume that when using the same material will cause osteoblasts to increase and have a good effect on the healing process.

Aloe vera is a biogenic stimulator that can stimulate cells to act on the alveolar bone and also accelerate the healing of former extractions [11]. In addition to examining cell histology, analysis of initial bone formation can also be seen by surface morphology using electron microscopy. Histometric evaluation of implant surface is a common method and is applied to most standard research procedures to evaluate bone formation on the implant surface [12].

Osteoblast and osteoclast cells are the main elements that are very influential in the process remodeling of alveolar bone. Osteoblasts play a role in the process of new bone formation, while osteoclasts play a role in the process of bone resorption [13]. Osteoblasts in bone remodeling will cooperate with calcium minerals to form bone calcifications. The addition of Aloe vera extract as a bio-active ingredient to the installation of imediat implants could increase calcium mineral elements and improve the calcium-phosphorus ratio [14], showing that there is an increase in bone growth activity carried out by osteoblasts after the addition of Aloe vera.

The process of healing soft tissue with hard tissue is very different. In the process of wound healing, the main cell involved is fibroblasts. Fibroblasts are cellular elements found in many gingival connective tissues that proliferate and actively synthesize matrix components in the process of wound healing and repair of damaged tissue [15]. Fibroblasts are a basic ingredient in the formation of scar tissue and collagen, which provides a range of strength in soft tissue, wound healing. When the tissue is inflamed, fibroblasts will migrate immediately to the wound, proliferate and produce collagen matrix to repair damaged tissue [16]. In this study fibroblasts decreased because their position was replaced by differentiated osteoblasts.

Conclusion

Immediate placement implants with addition 10% Aloe vera extract gel to the extraction socket can increase osteoblasts and suppress osteoclasts and reduce fibroblasts so that the formation of new bone is better than the installation of immediate implants without giving Aloe vera extract gel to the extraction socket.

Financial Support: None.

Conflict of Interest: The authors declare no conflicts of interest.

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