

Socket Preservation with Hydroxyapatite Gypsum Puger Scaffold and Aloe vera on Fibroblast and Type 1 Collagen Cells

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ABSTRACT

Background: The prevalence of tooth extraction was 7.29% according to RISKESDAS 2018. Post-tooth extraction will cause injury and bone resorption. Scaffold material combination of hydroxyapatite gypsum puger (HAGP) and aloe vera (AV) can be used for preservation of extraction socket. **Objective:** To analyze fibroblast and type 1 collagen cells in the extraction socket after being induction by a combination of hydroxyapatite gypsum puger scaffold and aloe vera. **Methods:** Making a combination of gypsum puger hydroxyapatite scaffold and aloe vera, divided into four groups, namely negative control, aloe vera scaffold, HAGP scaffold, and HAGP+AV scaffold. Extraction of the mandibular 1st molar in Wistar rats, the application on the extraction socket was waited for 3, 5, 7, and 14 days. Preparation of preparations using Hemaktocillin Eosin staining method. **Results:** LSD test on the HAGP+AV scaffold group between days 3 and 5, days 3 and 7, days 3 and 14, days 5 and 7, days 5 and 14, days 7, and 14 were obtained ($p=0.000$), this shows a significant difference between the treatment groups. The results of type 1 collagen in the HAGP+AV scaffold group between days 3 and 14 were obtained ($p=0.005$) showing a significant difference. Each group on day 14 showed the highest number of fibroblast and type 1 collagen cells. **Conclusion:** The combination of HAGP+AV+gelatin scaffold can increase fibroblast cells and type 1 collagen after tooth extraction. The socket healing process is getting faster on day 14.

Keywords: Aloe vera, Gelatin, Gypsum Puger Hydroxyapatite (HAGP), Scaffold, Tooth Extraction

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INTRODUCTION

The act of tooth extraction occupies the second-highest position after drug consumption. The prevalence of tooth extraction shows a figure of 7.29% according to RISKESDAS 2018. Post-tooth extraction will cause injury and the occurrence of alveolar bone resorption which can affect the readiness of the dental tissue in the installation of dentures or implants.¹ In this case, it is necessary to preserve the extraction socket with bone graft material to accelerate the wound healing process and prevent bone resorption.

Bone graft material that can be used as an alternative for extraction socket preservation is a combination of gypsum puger hydroxyapatite scaffold and Aloe vera. Hydroxyapatite (HA) is a biomaterial which has the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA has good biocompatibility and bioactivity properties. Crystallographically and chemically, HA is close to the structure of bones and teeth, and HA functions can be bound directly to tissue and can stimulate tissue growth. Based on the research conducted by Naini (2014), it was found that Puger gypsum material in the Jember area was successfully synthesized into hydroxyapatite called Hydroxyapatite Gypsum Puger (HAGP). The characterization of HAGP by XRD and FTIR tests had the same results with HA 200 from Japan as the standard for comparison.^{2,3} According to Salim (2015), the activity of new bone formation or tissue regeneration will occur optimally with the addition of bone graft material in the form of a scaffold.⁴

Aloe vera is a natural ingredient that is combined with gypsum puger hydroxyapatite to maximize the healing process. Aloe vera contains tannins, flavonoids, saponins, steroids, terpenoids, anthraquinone glycosides, and glucomannan compounds.⁵ Glucomannan compounds include polysaccharides (β 1,4)-mannosylation) which can accelerate the wound healing process. Glucomannan compounds are able to stimulate the activity and proliferation of fibroblast cells and can increase collagen production.⁶ Cells that

play an important role in the wound healing process are fibroblast cells, these cells play a role in the formation of connective tissue and support the formation of new bone after tooth extraction.⁷

In the process of tooth extraction, there will be mechanical trauma to the alveolar bone and an extraction socket will be formed, if there is an injury then hydroxyapatite is applied, it will cause inflammation in the tissue. In the inflammatory process, it will activate macrophages that secrete cytokines such as IL-1, IL-6, TNF- α .⁸ TNF- α as a homing stem cell chemoattractant, secretes cytokines and attracts endogenous mesenchymal stem cells so that attachment occurs and then proliferation and differentiation stimulate osteoprogenitors to become preosteoblasts, then differentiates preosteoblasts into osteoblasts. Osteoblasts secrete osteocalcin, alkaline phosphatase and type 1 collagen.^{3,9} Type 1 collagen is the most abundant collagen fiber found in adult connective tissue, bones, and teeth. This hydroxyapatite material can function as an osteoconductive.¹⁰

The difference is on this research author used gelatin as a gelling agent. Gelatin has a quality of biocompatibility that effectively increases support cell adhesion, also doesn't result in dangerous products at the time of enzyme degradation. Gelatin that is added for the manufacture of scaffolds can be biodegradable and has good porosity. If the gelatin is combined with another natural polymer or even the synthetic, the gelatin will show an increasing mechanical behavior and affinity on the scaffold.¹¹ The goal of this research is to analyze the fibroblast cell and collagen type 1 on extraction socket after induced by the combination of gypsum puger hydroxyapatite scaffold, aloe vera, and gelatin.

MATERIALS AND METHODS

This research was an experimental laboratory research with post test only control group design. This research has been approved by the Health Research Ethics Commission

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The combination of HAGP scaffold, Aloe vera, and gelatin (from cow skin, Pro Analysis, Merck, catalog number 104078, Malincord, JT. Baker) was made with a ratio of 1:1. HAGP material was a hydroxyapatite material that was successfully synthesized from gypsum from Mount Gamping Jember based on previous research according to Naini (2019) in the following way. Preparation of HAGP hydroxyapatite samples, weighing 0.5 grams of gypsum, 0.5 grams of DHP, 500 ml of distilled water, mixed into a tube, then placed in an oven at 100°C for 30 minutes. The solution was washed, filtered, and dried in a microwave at 50°C.¹² To form the aloe vera gel, we blended the aloe vera ingredients until smooth. Then mix 5 grams of HAGP powder with aloe vera gel in a ratio of 1:1 (w/v), after that add 10 ml of gelatin solution and mixed until homogeneous. Then the material is put in a scaffold mold and cooled in a freezer at -60°C for 24 hours and then freeze-drying was carried out at a temperature of -84°C for 24 hours.

This study used 32 males *Rattus norvegicus* rats which were divided into 16 study groups (4 control groups and 12 treatment groups). The sample size in each treatment group was two rats according to the Federer formula.¹² The control group was a negative control in the form of extraction of the mandibular first molar teeth of rats without being given scaffold material. The treatment group consisted of extraction of the mandibular first molar teeth of rats and induction of scaffold material (PI group: Aloe vera (AV) scaffold, PII group: Gypsum Puger hydroxyapatite scaffold (HAGP), and PIII group: HAGP+AV scaffold.

Experimental animals were anesthetized using ketamine injection at a dose of 0.04 - 0.08 ml/200kg body weight rats.³ The extraction of the rat's teeth was carried out by a simple extraction method using a Half moon sonde and an excavator. After the tooth was extracted completely, the socket was irrigated using sterile distilled water and given scaffold material until it

was completely filled. The experimental animals were then reared and euthanized on the 3rd day, 5th day, 7th day, and 14th day. Tissue decapitation in the left lower jaw of rats. Then decalcification using 3% nitric acid, then processing the tissue into histological preparations using Hematoxylin Eosin staining.

The calculation of the number of fibroblasts and the density of type 1 collagen were observed using a binocular microscope (EC1152 Euromex Ecoblue, Holland) with a magnification of 400x. Calculations were carried out in 3 fields of view and then tabulated the average number of fibroblast cells and type 1 collagen density.¹³ The average number of fibroblasts and type 1 collagen density was analyzed using One Way Anova and the Least Significant Different (LSD) test with a significance value of $p < 0.05$.

RESULTS

The preservation of extraction sockets by induction of a combination of Hydroxyapatite Gypsum Puger scaffold, Aloe vera, and gelatin (HAGP+AV+Gelatin) on the number of fibroblast cells and the density of type 1 collagen in the socket was carried out by histological observation of the rat sample preparations using a light microscope. Microscopic appearance of fibroblast cells in each group can be seen in Figure 1, while the microscopic image of the density of type 1 collagen in each group can be seen in figure 2.

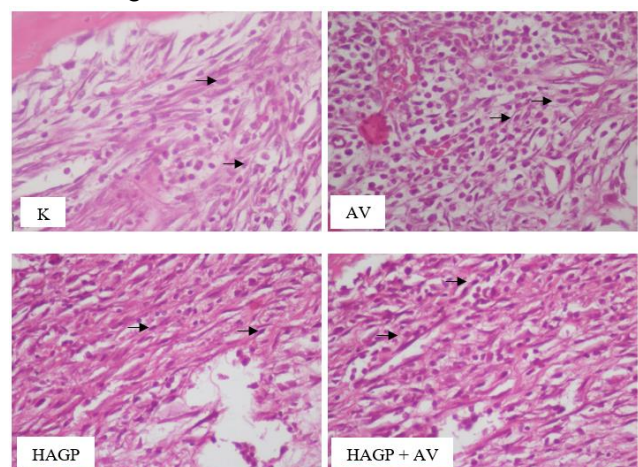


Figure 1. Microscopic image of fibroblast cells in control group (K), Aloe vera + gelatin (AV) scaffold

treatment group, HAGP + gelatin (HAGP) scaffold treatment group, and HAGP+AV+gelatin (HAGP+AV) scaffold treatment group (HE stains, light microscopy, 400x). Calculated cells are marked with arrows.

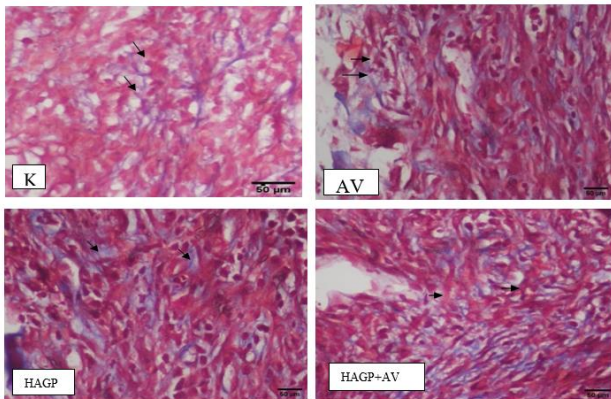


Figure 2. Microscopic image of Collagen density type 1 Control group (K), Aloe vera + gelatin (AV) scaffold treatment group, HAGP + gelatin (HAGP) scaffold treatment group, and HAGP+AV+gelatin (HAGP+AV) scaffold treatment group (HE stains, light microscopy, 400x). Calculated cells are marked with arrows.

Figures 1 and 2 showed an overview of fibroblast cells and type 1 collagen density taken in one field of view of each research group after tooth extraction. The calculation of the number of fibroblast cells, the density of type 1 collagen, and the *Least significant Different* (LSD) test results can be seen in table 1.

Table 1. The calculation of the number of fibroblast cells, the density of type 1 collagen, and LSD test results

Group	The number of fibroblast cells (X ± SD) and LSD				The density of type 1 collagen (X ± SD) and LSD			
	Day 3	Day 5	Day 7	Day 14	Day 3	Day 5	Day 7	Day 14
Control	8,38±0,54	11,83±0,72	16,17±0,39	18,24±1,08	15,86±2,13	31,14±6,7	34,73±13,4	80,41±10,9
	(5,7,14) (PI,PII,PIII)	(3,7,14) (PI,PII,PIII)	(3,5,14) (PI,PII,PIII)	(3,5,7) (PI,PII,PIII)	(4) (PI,PII,PIII)	(-) (PI,PII,PIII)	(-) (PI,PII,PIII)	(-) (PI,PII,PIII)
Scaffold Aloe vera + gelatin (PI)	13,33±0,47	16,67±1,41	17,72±1,33	21,65±0,49	34,05±12,8	43,07±4,7	72,85±24,4	80,41±22,2
	(5,7,14) (K,PII,PII)	(3,14) (K,PIII)	(3,14) (K,PII,PIII)	(3,5,7) (K,PII,PIII)	(3,4) (K,PII,PIII)	(4) (K,PII,PIII)	(1) (K,PII,PIII)	(1,2) (K,PII,PIII)
Scaffold HAGP + gelatin (PII)	17,16±1,64	18,16±1,64	19,89±0,94	23,5±0,24	36,58±24,5	42,17±19	76,07±28,1	79,65±19,9
	(7,14) (K,PI,PIII)	(7,14) (K,PIII)	(3,5,14) (K,PI,PIII)	(3,5,7) (K,PI,PIII)	(3,4) (K,PI,PIII)	(4) (K,PI,PIII)	(1) (K,PI,PIII)	(1,2) (K,PI,PIII)
Scaffold HAGP + AV+gelatin (PIII)	23,83±1,18	28,16±1,18	31,5±2,58	34,83±1,18	36,67±9,3	58,4±20,8	68,53±18,3	93,01±7,5
	(5,7,14) (K,PI,PII)	(3,7,14) (K,PI,PII)	(3,5,14) (K,PI,PII)	(3,5,7) (K,PI,PII)	(4) (K,PI,PII)	(-) (K,PI,PII)	(-) (K,PI,PII)	(1) (K,PI,PII)

Description:

X ± SD: Mean fibroblast cell count ± Standard Deviation

(3,5,7,14) : significantly different based on the day of treatment in one group of treatment materials
(³) significantly different from the 3rd day, (⁵) significantly different from the 5th day, (⁷) significantly different from the 7th day, (¹⁴) significantly different from the 14th day

(K,PI,PII,PIII) : significantly different based on the treatment material group in one treatment day group
(^K) significantly different from group K, (^{PI}) significantly different from group PI, (^{PII}) significantly different from group PII, (^{PIII}) significantly different from group PIII).

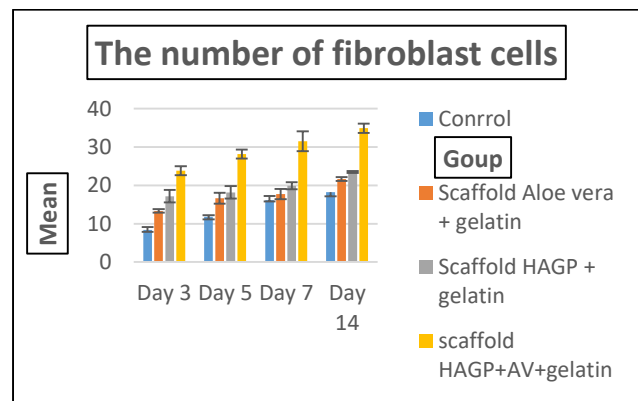


Figure 3. Histogram of the calculation results of the average number of fibroblast cells, (PI) The group given the scaffold Aloe vera+gelatin, (PII) the group giving the scaffold HAGP+gelatin, and (PIII) the group giving the scaffold HAGP+AV+gelatin.



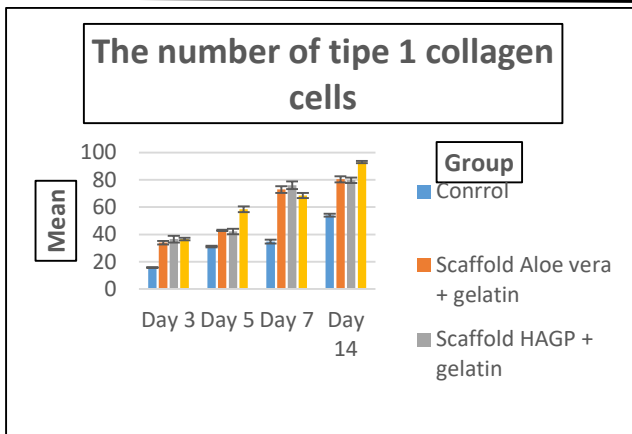


Figure 4. Histogram of the calculation results of the average number of type 1 collagen, the group control, (PI) The group given the scaffold Aloe vera+gelatin, (PII) the group giving the scaffold HAGP+gelatin, and (PIII) the group giving the scaffold HAGP+AV+gelatin.

Figures 3 and 4 are the average number of fibroblasts and type 1 collagen cells in the control and treatment groups. The fibroblast cell data showed that the largest increase was in the PIII group, that is the scaffold group with a combination of HAGP, gelatin, and Aloe vera. While the control group had the lowest fibroblast cell count value, although it experienced a significant increase in value from day 3 to day 14. In addition, the trendline also shows that the Aloe vera and gelatin (AV) scaffold group showed an insignificant increase in the number of fibroblasts from day 5 to day 7. Meanwhile, in the Hydroxyapatite Gypsum Puger and gelatin (HAGP) scaffold group, there was an insignificant increase in the number of cells on the 3rd to 5th day.

Analysis of the data on the number of fibroblasts was tested for normality using the Shapiro-Wilk test with the results of the data being normally distributed with $p > 0.05$. Then the homogeneity test was carried out using Levene's test and showed that the data were homogeneously distributed with a P-value of 0.374 so that the data had a p value > 0.05 . Furthermore, the One-way Anova parametric statistical test was carried out with the results of a P-value of 0.000 which meant that there was a significant difference in the average number of fibroblasts between the study groups due to the

$p < 0.05$. Furthermore, the LSD test was carried out with results showing that most of the data between groups had significantly different values ($p < 0.05$) both based on the length of treatment day group and the treatment material group (table 1).

Based on the results of this study, the average density of type 1 collagen was found to be the highest on day 14 in all groups, the denser the collagen density increased (figure 3). Data analysis was carried out by normality test using the Shapiro Wilk test which obtained results that were normally distributed, carried out by homogeneity test with the Levene test to obtain homogeneous data, then the One-way Anova test was carried out showing a P-value of 0.000 which means there is a significant difference between the research groups because of the p -value < 0.05 . Furthermore, an LSD test showed that most of the data between groups had significantly different values ($p < 0.05$) both based on the treatment day group and the treatment group (table 1).

DISCUSSION

Socket preservation is still often used to accelerate wound healing and the occurrence of alveolar bone resorption after tooth extraction. In this study, a combination of hydroxyapatite gypsum puger scaffold, aloe vera, and gelatin was used for fibroblast cells and type 1 collagen.

The use of scaffolds in tooth extraction sockets has important benefits in the treatment group. Scaffold is a porous structure that acts as a substrate for cells to migrate, adhere, grow, proliferate and differentiate. In addition, the scaffold can accelerate the bone healing process by acting as a delivery vehicle for cells, providing space for vascularization, new tissue formation, and bone remodeling. With biocompatible and biodegradable scaffold properties to support cell growth such as fibroblasts and type 1 collagen.¹¹

Aloe vera and gelatin scaffold treatment group showed a higher average number of fibroblast cells than the control group based on the results of the research that had been done.

Aloe vera is a natural polymer gel that has excellent biocompatibility and is non-toxic. Active components of Aloe vera such as acemannan can bind special ligands to mannose receptors found on the surface of fibroblasts and macrophages so this will trigger cell proliferation and growth factors.¹⁴ In addition to acemannan, there is another polysaccharide component, namely glucomannan which has healing properties by influencing the secretion of fibroblast growth factors, stimulating cell proliferation, and increasing the production of type 1 collagen through increasing the transformation of growth factor TGF- β in the wound area.¹⁵

Aloe vera will activate macrophage cells that are present in the inflammatory phase of the wound healing process. So along with the increased activation of macrophage cells, it increases the stimulation of the release of growth factors such as Fibroblast Growth Factor (FGF) and Transforming Growth Factor (TGF- β) as well as other important cytokines in accelerating wound healing by increasing the proliferation of fibroblast cells and collagen type 1.⁷ This is why the results of the study on the Aloe vera scaffold group showed no significant difference between the 5th and 7th-day groups. Macrophages that increase their cell activation in the inflammatory phase will increase the migration of fibroblast cells to the wound area at the beginning of the proliferative phase so that the number of fibroblast cells increases significantly on day 3 and day 5. While on day 5 to day 7 the number of fibroblasts will tend to be stable.

The provision of HAGP scaffold and gelatin showed an increase in the number of fibroblast cells when compared to the control group. Hydroxyapatite (HA) has been widely used as a tooth and bone repair material because it can chemically interact and blend with bone due to its non-toxic, non-allergenic, non-mutagenic, osteoconductive, and weak osteoinductive properties. So that the use of HAGP scaffold can increase the number of

fibroblast cells and type 1 collagen in the post-extraction socket.

The main property of the HAGP scaffold is its osteoconductivity which can support bone growth above the surface and direct new bone formation through the matrix support, acting as a scaffold that can be absorbed and replaced by bone tissue. HAGP has a major role as an interconnected porous structure. Its osteoconductive properties can attract healing cells to be able to adhere to the porous space. So, it can be seen from the results of the study that between day 3 and day 5 there was no significant difference in the number of fibroblast cells due to the main ability of HAGP only as a framework to facilitate cells adhesion. Thus, the proliferation of fibroblasts was not seen to increase in the early phase of wound healing.

Hydroxyapatite content in the HAGP scaffold has an important role related to the ideal properties of the scaffold. Despite having only osteoconductive properties, hydroxyapatite can provide a microenvironment that resembles the physiological environment so that stem cells can interpret biomaterial instructions and change according to their fate. The topographical and chemical properties of HAGP are almost the same as hydroxyapatite in human bone, so HAGP is able to send special signals to cells that will translate the code into biochemical signals.¹⁶ So in wound healing, especially seen from the increase in the number of fibroblast cells and type 1 collagen, the HAGP scaffold has a higher average cell count compared to the Aloe vera scaffold as seen in the research results.

The scaffold group with the combination of HAGP, gelatin and Aloe vera increased the number of fibroblasts and type 1 collagen in the tooth extraction socket compared to the treatment group without the combination and the control group. This is because the combination of properties of the two materials gives a synergistic effect as a scaffolding material. Hydroxyapatite is a complex inorganic material with weak osteoinductive properties and does not have sufficient ability to stimulate vascularization thus limiting its application.

Therefore, hydroxyapatite must be combined with other materials that can optimize the potency of the material.¹⁷ The osteoinduction properties of Aloe vera will be able to optimize the properties of the hydroxyapatite material when combined in a scaffold.¹⁸ So that the combination of HAGP and Aloe vera into a scaffold can accelerate the healing process in bone by enabling osteoblast cell proliferation, cell differentiation, as well as inorganic apatite deposition.

Aloe vera combined with HAGP to become a scaffold material will be able to increase the biocompatibility of the scaffold. Aloe vera is not only able to increase the number of cells attached to the scaffold but also able to increase the growth and proliferation of cells.¹⁸ Based on research conducted by Prabakaran et al. (2020), the substitution of mineral HA with natural ingredients Aloe vera can increase collagen protein deposition in the first week and second week after extraction so that this scaffold can induce connective tissue in the damaged bone area.

In this research, it can be seen an increase in the number of fibroblast cells between the treatment group and the control group on the 3rd, 5th, 7th, and 14th days. The 3rd day after tooth extraction was the day group that had the lowest number of fibroblast cells in the control group and the treatment group, this was because the 3rd post-extraction day was the beginning of the proliferation phase which overlapped with the end of the inflammatory phase so that Fibroblasts begin to migrate and are seen to secrete their extracellular matrix proteins.¹⁹ Migration of fibroblasts to the wound area reaches its peak until the 5th day after extraction to prepare for the synthesis and maturation of the matrix so that it will replace the blood clot with the formation of granulation tissue.²⁰

Granulation tissue will completely replace the blood clot on the 7th day after tooth extraction.¹⁹ When the fibroblasts have filled the wound area, the fibroblast cells will proliferate and make the matrix of the injured area rich in

collagen by secreting extracellular matrix proteins.^{18,20} However, in the results of this study, the number of fibroblast cells in the tooth extraction socket was more visible on the 14th day of the treatment group than on the 7th day of treatment. This is because the 14th day after extraction is the peak of the proliferation phase and the highest amount of connective tissue accumulation. So at this time fibroblast cells will appear to be formed a lot because they have fully migrated and are active.²¹

In this research also seen an increase in the density of type 1 collagen between the treatment group and the control group on the 3rd, 5th, 7th and 14th days. These ingredients contain glucomannan and hydroxyapatite compounds that can activate growth factors to accelerate fibroblast cell proliferation, angiogenesis, and re-epithelialization. TGF β -1 and FGF can increase mitosis from fibroblast cells thereby stimulating the formation of extracellular matrices such as fibronectin, proteoglycans, and collagen.

The socket healing process after tooth extraction consists of three phases, the first one is the inflammatory phase, after that the proliferative phase, and the maturation phase. In the proliferative phase, fibroblasts play an important role in forming granulation tissue and secrete collagen to form the extracellular matrix.²² So that if fibroblasts have increased cell proliferation, it can indicate a gradual wound healing process.

The use of a combination of HAGP, Aloe vera, and gelatin will form a signaling complex that can stimulate macrophages to release important growth factors in bone healing such as VEGF, FGF, TGF- β . Increased expression of growth factors will also stimulate an increase in fibroblast cell proliferation. So that it can be seen in the combined scaffold group which has the highest mean number of fibroblast cells on the 14th day after tooth extraction and significantly increases the number of fibroblast cells from the 3rd day to the 14th day. This result is different from the scaffold group without the combination, namely the group treated with Aloe vera plus

gelatin scaffold and HAGP+gelatin scaffold. The mean number of fibroblast cells was found to be not significantly different between the HAGP+gelatin scaffold group and the Aloe vera+gelatin scaffold group on day 5. This is because the scaffold made from a single material is not able to meet the ideal scaffold requirements, so it provides a less than optimal function.

CONCLUSION

The combination of gypsum puger hydroxyapatite scaffold, aloe vera, and gelatin can increase fibroblast cells and type 1 collagen cells after tooth extraction. With the combination of this scaffold, the socket healing was faster until it entered the 14th day when the mean number of fibroblasts was 34.83 and type 1 collagen was 93.01.

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