

# The effect of Avocado leaf extract (*Persea americana* Mill.) on the fibroblast cells of post-extraction dental sockets in Wistar rats

*by* Christian Khoswanto

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Research Report

## 1 The effect of Avocado leaf extract (*Persea americana Mill.*) on the fibroblast cells of post-extraction dental sockets in Wistar rats

Christian Khoswanto, Wisnu Setyari Jullastuti, and Karina Awanis Adla  
 Department of Oral Biology,  
 Faculty of Dental Medicine, Universitas Airlangga  
 Surabaya – Indonesia

### ABSTRACT

**Background:** Tooth extraction, a common practice among the dental profession, causes trauma to the blood vessels during the wound healing process. The acceleration of wound healing, within which fibroblasts play an important role, is influenced by nutrition. Avocado leaves contain a variety of chemicals, including flavonoid compounds, tannins, catechins, quercetin, saponin and steroidal triterpenoid. Avocado leaves also contain glycosides, cyanogenic, alkaloids and phenols which function as anti-inflammatory, antibacterial and antioxidant agents. This avocado leaf content could be used as an alternative medicine to accelerate the wound healing process in post-tooth extraction sockets. **Purpose:** To determine the role of avocado leaves (*Persea americana Mill.*) in accelerating fibroblast cells proliferation in tooth socket post-extraction. **Methods:** The sample was divided into four groups, a control group and three treatment groups. The treatment groups used avocado leaf extract and 3% CMC Na solution which was inserted into the tooth sockets of Wistar rats. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3<sup>rd</sup> and 7<sup>th</sup> days of the experiment. Mandibular decapitation and tooth extraction socket were prepared by HPA (Histology Pathology Anatomy) with Hematoxylin Eosin (HE) staining. The fibroblast proliferation was analyzed by means of a light microscope at 400x magnification. The obtained data was analyzed using a t-Test. **Result:** The t-Test obtained a significance value 0.001 ( $p < 0.05$ ) between the control and treatment groups. The number of fibroblast cells increased in the group treated on the third day and decreased in the group treated on the seventh day. **Conclusion:** Avocado leaf extract (*Persea americana Mill.*) accelerates proliferation of fibroblast cells in Wistar rats post-tooth extraction.

**Keywords:** avocado leaf extract; wound healing; fibroblast

**Correspondence:** Christian Khoswanto, Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjend. Prof. Dr. Moestopo no. 47, Surabaya 60132. E-mail: christiankhoswanto@hotmail.com

### INTRODUCTION

Within the dental profession, one of the most common procedures performed is tooth extraction which may cause trauma to the blood vessels. After trauma occurs to the blood vessels, the hemostasis process, involving blood clotting on the walls of damaged blood vessels in order to prevent bleeding, commences. The process of post-extraction wound healing can occasionally cause infections, possibly even leading to complications.<sup>1-4</sup> Patients require appropriate post-extraction management in order to reduce the possibility of complications and accelerate blood clotting, thereby promoting wound healing after extraction. The wound healing process itself is relatively complex,

consisting of various processes and assisted by many cells, one of them being fibroblasts. Fibroblasts are cells found in connective tissue responsible for the phagocytosis of bacteria. TGF- $\beta$  (transforming growth factor  $\beta$ ) and PDGF (platelet-derived growth factor) stimulate fibroblast structures to become miofibroblasts located at the edges of ECM which promote wound closure in tissues. Fibroblasts will appear in the wound area after three days with the number of fibroblast cells peaking on the seventh day after trauma.<sup>5-8</sup>

The avocado plant possesses the benefits of traditional remedies<sup>9</sup> since almost all its constituent parts possess properties akin to those of such medicines. The leaves, fruit and seeds all have a high nutrient content. Avocado

leaves contain a variety of chemicals, including: flavonoid compounds, tannins, katekat, kuinon, saponin, steroids/triterpenoids, glycosides, cyanogenic compounds, alkaloids and phenols.<sup>7-9</sup>

The aim of this study was to determine the effect of avocado leaf extract on fibroblast proliferation rates and inflammation indicators.

## MATERIALS AND METHODS

This study used rodent subjects to evaluate wound healing activity indicated by fibroblast proliferation. Approval by the ethical board was granted (304/ HRECC. FODM/XII/2017). This study used a post-test only control group design with 24 male Wistar rat subjects, 150-200 grams in weight and aged 2-3 months which were allowed to freely consume pellet food for one week.

The sample was divided into four groups, a control group (n=6) and the treatment groups (n=6). In the control group, the subjects were given a 3% CMC Na solution to synchronize the physiological state of their bodies which had no negative effect on their tissues or organs. The treatment groups had avocado leaf extract and 3% CMC Na solution as a 0.1cc solvent inserted into their tooth sockets. Both the control and treatment groups had their mandibula decapitated with all the required specimens being prepared on the 3<sup>rd</sup> and 7<sup>th</sup> days of the experiment period.

Fresh avocado leaves were obtained from and identified at UPT Materia Medica, Kota Batu, East Java. The leaves were washed thoroughly, dried and liquified in a blender with 96% ethanol solvent, placed in a tightly sealed jar for 24 hours and agitated in a digital agitator at 50 rpm. The resulting liquid extract was filtered by being passed through a cloth, inserted in an Erlenmeyer tube and subsequently evaporated in a rotary evaporator for 90 minutes and stored in a freezer until required.

A general anesthetic was administered to the subjects by means of chloroform inhalation. Tooth extraction was performed on the left mandibular incisor using pliers after which irrigation was carried out using sterile aquades to remove the remaining debris. In order to stop post-extraction bleeding, a sterile cotton roll was applied to the resulting socket. The treatment protocol adopted was that advocated by Krinke whereby, following removal of the teeth and discontinuation of bleeding from the sockets, the subjects were treated.<sup>10</sup>

The treatment group was selected to have its mandibula decapitated and made into preparations on the 3<sup>rd</sup> and 7<sup>th</sup> days. Decapitation of the mandible in the treatment group and preparation on the 3<sup>rd</sup> and 7<sup>th</sup> day were performed because fibroblasts appeared in the wound area three days after the trauma before peaking after seven days. On the 3<sup>rd</sup> and 7<sup>th</sup> days, a mandibular retrieval procedure was performed by anesthetizing the subjects in a glass gas chamber filled with 10% chloroform. The members of each group had their mandibula decapitated and

appropriately disposed of. The decapitated mandibles were made into tissue preparation, before being stained with HE (Haematoxylin Eosin) and observed. Histopathologic observation was performed by counting the number of fibroblasts under a light microscope at 400x magnification. Data was analyzed by means of a One-way Anova test with a 5% significance rate and subsequently with an LSD test to establish whether a significant difference existed.<sup>11,12</sup>

## RESULTS

The results in Table 1 show that after a 3-day experimental period the number of fibroblasts in the treatment group had increased compared to that in the control group (Figures 1 & 2). Conversely, after seven days the number of fibroblast cells in the treatment group was lower than that in the control group (Figures 3 & 4).

Table 1 shows the extent of fibroblast proliferation on day 3 was significantly different in the wounds in the treatment group and the control group, while on day 7 no such significant difference was observed between the two groups.

## DISCUSSION

Tooth extraction will result in a wound which then undergoes a healing process consisting of a series of complex processes involving a number of cells, cytokines, growth factors and extracellular components that play a role in repairing damage to the hard tissue and soft tissue.<sup>2</sup> The wound healing process is influenced by several factors including: bacterial infections, damage to the tissue, necrosis, hematoma (tissue bleeding), excessive movement of injured tissue, low blood supply and drug administration.<sup>8,13,14</sup> The injured tissue rapidly experiences an acute inflammatory reaction. The inflammatory phase precedes healing and wound immobilization. The instantaneous acute inflammatory phase is characterized by the exudation of plasma proteins and neutrophils. The chronic inflammatory phase is characterized by the presence of chronic inflammatory cells (macrophages, lymphocytes, and plasma cells).<sup>7</sup> In this study, it was observed that the

**Table 1.** Mean amount of fibroblast in the treatment group and control group

Group	X±SD Day 3	X±SD Day 7
K1	3.00 <sup>b</sup> ±0.89	14.33 <sup>b</sup> ±0.51
K2	20.33 <sup>a</sup> ±1.75	10.16 <sup>b</sup> ±0.75

**Note:** Different superscript showed significance difference (p < 0.001)

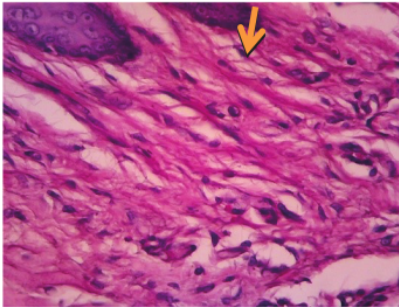
K1: Control group

K2: Treatment group

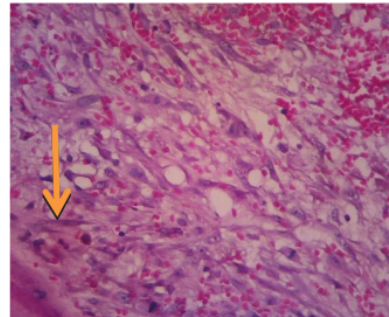
proliferation phase begins on the third day and can last for several weeks.<sup>2</sup> In the proliferation phase, neutrophil cells digest bacteria, then release intracellular enzymes into the surrounding matrix before expiring. Monocytes will move from the blood capillaries into the ECM, transforming into macrophages which are then mediated by the inflammatory mediator TGF  $\beta$ . TGF  $\beta$  activates fibroblast cells and stimulates collagen deposition by increasing collagen synthesis. With the synthesis of collagen by fibroblasts, the formation of the epithelial layer will be enhanced by regulating the balance between it and the granulation tissue.<sup>10,15,16</sup> As a result, the mucous epithelium and collagen layer will form.<sup>2</sup>

Acceleration of the wound healing process can be confirmed by the presence of several indicators, one of which is the number of fibroblasts. Fibroblasts are key to the proliferative phase of wound healing, such as destroying fibrin clot, forming collagen, elastin, glycosaminoglycan and proteoglycans induced by TGF- $\beta$  to form a new extracellular matrix to close the wound and affect the reepithelization process in the wound.<sup>10</sup> Thus, as indicated in this study, the more fibroblasts appear in the socket sample, the more rapid the wound healing process might be.<sup>2</sup>

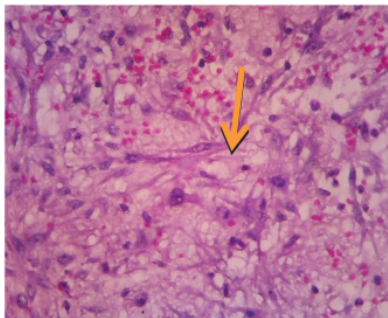
This study showed that on the third day an increase in the number of fibroblast cells occurred due to active substances such as flavonoids contained in the avocado leaves (*Persea americana* Mill) that have an anti-inflammatory effect through inhibition of cyclooxygenase and lipoxygenase. In this manner, they are able to limit the number of inflammatory cells that migrate to the wound area. Flavonoids play an important role in maintaining permeability and increasing capillary vascular resistance. Therefore, flavonoids are present in pathological conditions such as disruption to the permeability of the blood vessel walls. Flavonoids and phenol substances in avocado leaves accelerate wound healing through antioxidant mechanisms which inhibit the activity of free radicals to donate hydrogen atoms and bond to unstable free radicals that can cause damage to cell membranes and impede cell functioning. The existence of this bond will render free radicals more stable, thereby reducing damage to cell membranes and enabling the proliferation phase to proceed more rapidly. This reduces the duration of the inflammatory reaction, induces earlier TGF- $\beta$  proliferation and results in the production of fibroblasts. In addition, avocado leaves also contain tannins which are active substances that increase the formation of fibroblast cells and capillary blood vessels,



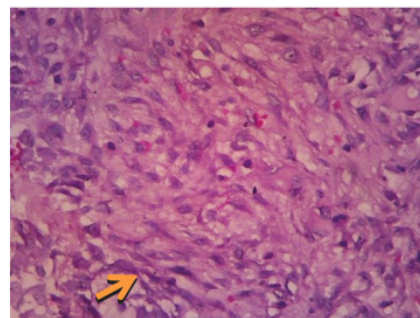
**Figure 1.** Fibroblasts HPA in control group; 3<sup>rd</sup> day (400x magnification)



**Figure 3.** Fibroblasts from the control group; 7<sup>th</sup> day (400x magnification)



**Figure 2.** Fibroblasts after the application of avocado leaves extract for 3 days (400x magnification)



**Figure 4.** Fibroblasts after the application of avocado leaves extract for 7 days (400x magnification)



causing growth factor to stimulate the proliferation of fibroblast cells.<sup>1,13,17</sup>

Other content of avocado leaves (*Persea americana* Mill) includes saponin, another active substance, which increases monocyte proliferation and can augment the number of macrophages that will secrete growth factors such as EGF, FGF, PDGF and TGF- $\beta$ . These, in turn, can stimulate the migration and proliferation of fibroblasts in the wound area in order to more rapidly synthesize collagen.<sup>1,15</sup>

This study showed a decrease in the number of fibroblast cells on the seventh day. Due to a significant increase in fibroblast cell production on day 3, fibroblasts are sufficient to synthesize collagen. This has the result that, on day 7, the number of fibroblast cells decreases as they are transformed into myofibroblasts located on the ECM margins of wound tissue closure.<sup>10,18</sup>

This study showed that avocado leaves (*Persea americana* Mill) topically applied to the post-extraction socket were capable of accelerating the amount of fibroblast present in the wound healing process in Wistar rat tooth sockets on day 3.

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