Potential Effect of Djambal Catfish *(Pangasius djambal) Gelatin as Biomaterial Product* on Healing Socket after Tooth Extraction in Rats

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Abstract

Purpose: Adequate wound healing after a tooth extraction is significant because the oral cavity has a masticatory function and plays a role in nutrient intake. Various scientific studies have been conducted to find the ideal biomaterial to support wound healing. This study aims to determine the wound healing activity using Djambal Catfish (*Pangasius djambal*) gelatin after tooth extraction.

Method: Gelatin was obtained from Djambal Catfish (*Pangasius djambal*). The sample was selected by random sampling, divided into 6 groups, namely the control group on the 3rd day (n=6), the control group on the 5th day (n=6), the control group on the 7th day (n=6), the treatment group on the 3rd day (n=6), the treatment group on the 5th day (n=6), and the treatment on the 7th day (n=6). Wound healing was examined by evaluating wound healing marker such as macrophages, fibroblasts, epithelialization, angiogenesis, and collagen. Data were processed using statistical analysis.

Results: The treatments had a positive result in wound healing as compared with the control groups. Histological assessment showed a remarkable sign of an increased number of macrophage, fibroblast, epithelialization, and collagen deposition.

Conclusion: Gelatin derived from Djambal Catfish (*Pangasius djambal*) can accelerate the wound healing process thus could be a component therapeutic potential product that may be beneficial in wound healing in the future.

Keyword*: gelatin, Djambal catfish, Pangasius djambal, wound healing, tooth extraction*

Introduction

The wound healing process after a tooth extraction is influenced by various factors, both internal and external. The healing process can be different for each individual. Generally, the wound healing process after tooth extraction includes the following stages: coagulation, inflammation, cell proliferation and migration, angiogenesis, matrix synthesis, remodelling, and wound contraction[1]. Various studies have reported the use of various materials to accelerate the tissue healing process, such as the use of hemosponge, bone graft, platelet-rich plasma, and platelet-rich fibrin[2,3,4].

Wound healing is the process of replacing and repairing damaged tissue function. The speed of wound healing depends on the location, severity, and extent of the injury. There are 4 main phases overlap between one and the other phase, namely the haemostasis phase, inflammatory phase, proliferation phase, and maturation phase. To ensure that the wound healing process runs well, several parameters can be used, including fibroblast proliferation, angiogenesis, collagen synthesis, epithelialization, and macrophages. Various

factors influence the acceleration of wound healing, such as the presence of local factors from intraoral and systemic factors[5,6,7].

This research aimed to obtain biomaterial products that can help accelerate the wound healing process after tooth extraction. This research applied gelatin gel material from Djambal Catfish (*Pangasius djambal*) in which the amino acid content consists of arginine, glutamine and glycine which can accelerate wound healing. There are no commercial products on the market that use Djambal Catfish as the basic ingredient, and there has been no research focused on wound healing using *Pangasius djambal*. Thus, this researcher was challenged and interested in conducting this research to determine the potential effect of applying gelatin gel made of Djambal Catfish on the tooth socket.

Materials and Methods

This research is a laboratory experimental research with Randomized Post Test Only Control Group Design in the laboratory in vivo to determine the effect of Djambal Catfish gelatin on the wound healing process in the socket after tooth extraction in Wistar strain rats with Ethical Clearance of Brawijaya University Number 399A/ EC/ KEPK-FKG/ 12/ 2017. The independent variable in this research was Djambal Catfish gelatin (*Pangasius djambal*) with a concentration of 100% while the dependent variables included the distributions of macrophages, fibroblasts, epithelialization, collagen, and angiogenesis.

The experimental animals were selected based on the sample criteria, then divided into 4 groups, each consisting of 7 rats kept in the experimental animal rearing place. The rats were cared for and adapted in the laboratory for 7 days. Before the extraction of the mandibular left incisor tooth of Wistar strain rats, an anaesthetic injection was carried out using 0.3 ml of ketamine peritoneally.

The production of gelatin comes from Djambal Catfish skin which was initially taken and separated from the meat and fat attached to it. Djambal Catfish skin was then stored at -20° C. The stored Djambal Catfish skin was thawed at room temperature and cut to about 1 cm^2. After that, Djambal Catfish skin was rinsed with lemon water to remove any other material. A total of 100 gram of Djambal Catfish skin was rinsed and immersed in the citric acid solution for 12 hours to dilute the collagen fibers in order to be easily extracted. The sample was neutralized by washing it several times until the washing water was at a neutral pH (6-7). Djambal Catfish skin was extracted using a shaker water bath with distilled water at 60^0C for 6 hours. The gelatin solution was separated from the remaining skin using the Watchman filter cloth No. 1 and cooled at room temperature until the gelatin gel formed.

The administration of Djambal Catfish gelatin was performed by using a pipette and then the gel was slowly applied as much as 0.1 ml to the socket once after extraction and after the bleeding stop. Meanwhile, the control group was not treated. After being given Djambal Catfish gelatin (*Pangasius djambal*), the experimental animals were given a novalgin analgesic at a dose of 500 mg/ ml to reduce pain.

Tissue sampling was examined on the 3rd, 5th, and 7th day using ether. The rats were injected with a lethal dose of ketamine anaesthetic. The rats had the mandible decapitated where the tooth had been extracted. In the preparation, HPA staining was conducted by using a light microscope with a 400x magnification to ensure the location of the preparation to be counted. The sample on the preparation was divided into 5 fields of vision with the same size.

Results

Table 1. Results of Marker Calculation on Rats' Wound Healing in Control and Treatment Groups with *Pangasius djambal* gelatin

Group	Macrophage	Fibroblast	Epithelization	Angiogenesis	Collagen
C1	55.2 ± 7.92	2.9 ± 27.96	106.43 ± 13.63	1.76 ± 0.75	1.13 ± 0.35

Cont... **Table 1. Results of Marker Calculation on Rats' Wound Healing in Control and Treatment Groups with *Pangasius djambal* gelatin**

C2	42 ± 5.15	4.04 ± 32.04	96.66 ± 12.54	2.68 ± 0.72	1.76 ± 0.68
C3	33.2 ± 9.12	5.01 ± 36.52	125.28 ± 19.53	41.2 ± 0.30	-
T1	83.6 ± 4.83	6.44 ± 75.32	115.55 ± 24.33	2.92 ± 0.67	-
T2	65.6 ± 4.62	4.51 ± 88.4	131.66 ± 30.87	4.36 ± 1.29	1.66 ± 0.48
T3	33.2 ± 1.93	6.61 ± 91.02	187.29 ± 21.62	5.36 ± 0.99	2.93 ± 0.25

Note: Values were expressed as mean + SD. Values with the same letter in a row mean they are statistically significant different ($p<0.05$), and vice versa.

C1: control day 3 ; C2: control day 5 ; C3: control day 7 ; T1: treatment day 3 ; T2: treatment day 5; T3: treatment day 7

Examination Methods and Analysis of Macrophage

Based on Table 1, there was a difference in the number of macrophages between the control group not given and given Djambal Catfish gelatin. The one-way ANOVA test results showed a significance value of 0.000 ($p < 0.05$), indicating an increase in the number of macrophage cells in the wound after tooth extraction in rats between groups on the 3rd and 5th days. The results of the Post Hoc Tukey test (comparing the groups) showed that there was a significant difference in the mean number of macrophages between groups with a significant value of less than 0.05. However, several groups were being compared which had a greater value than 0.05, covering Group C1 and C2, C1 and C3, C2 and C3, and.T2 and T3.

Examination Methods and Analysis of Fibroblast

Based on Table 1, there is a difference in the number of fibroblasts between the control group not given and given Djambal Catfish gelatin. The one-way ANOVA test showed a significant value of 0.000 ($p < 0.05$), meaning that H_0 was rejected. This research indicated a difference in the number of fibroblasts in the wound after rat tooth extraction between the groups. The Post Hoc Tukey test results (comparing the groups) obtained that there was a significant difference in the mean number of fibroblasts between groups with a significant value of less than 0.05. However, several groups were being compared which had a greater value than 0.05, covering Group C1 and C2, C1 and C3, C2 and C3, and.T2 and T3.

Examination Methods and Analysis of Epithelization

Based on Table 1, there was a difference in the epithelial thickness in rats (*Rattus norvegicus*) post-tooth extraction socket between the treatment groups given and not given Djambal Catfish gelatin. The one-way ANOVA test showed a significant value of 0.000 ($p < 0.05$), meaning that H_0 was rejected. This research indicated that there was a difference in the average epithelium thickness of the wound after rat tooth extraction between the control groups and the treatment groups on the 3rd, 5th, and 7th days. The Pearson correlation test on the treatment group research data showed a correlation strength (r) of 0.761**, indicating a strong correlation between Djambal Catfish gelatin (*Pangasius djambal*) and epithelial thickness after rat tooth extraction. Furthermore, this correlation showed a positive direction, meaning that the increasing number of days (length of time) can thicken significantly the rat tooth epithelium after tooth extraction.

Examination Methods and Analysis of Angiogenesis

Based on Table 1, the administration of Djambal Catfish gelatin increased the number of blood vessels or angiogenesis in the treatment group of rats and accelerated the wound healing process in the treatment group. The One Way ANOVA test showed a significance value of less than 0.05 ($p < 0.05$). That is, the use of Djambal Catfish (*Pangasius djambal*) gelatin had a significant effect on angiogenesis in the wound healing process after tooth extraction of Wistar rats (*Rattus novergicus*). In other words, there was a significant difference in the number of blood vessels (angiogenesis) between groups on the 3rd, 5th, and 7th days. The Pearson correlation test on the research data showed a correlation strength (R) of 0.860 in the control groups and 0.736 in the treatment groups. Furthermore, this correlation had a positive direction, indicating a strong correlation between variables, in which the increasing number of days (length of time) can also increase the angiogenesis.

Examination Methods and Analysis of Collagen

The comparison between groups through the Kruskal-Wallis test was obtained a significant value (p) of 0.000, meaning that the null hypothesis was rejected. Hence, there was a significant difference in collagen formation in the healing process after tooth extraction in rats (Rattus norvegicus). The Spearman correlation test results showed that the correlation strength (r) on the 3rd day was 0.707*, indicating that Djambal Catfish (*Pangasius djambal*) gelatin was strongly correlated with collagen formation after tooth extraction in rats (Rattus norvegicus). This correlation on the 3rd day had a positive direction, meaning that the increasing number of days (length of time) can also significantly increase collagen formation after tooth extraction in rats (Rattus norvegicus). The correlation strength (r) on the 7th day reached 0.949**, indicating a very strong correlation between Djambal Catfish (*Pangasius djambal*) gelatin and collagen formation after tooth extraction in rats. This correlation on the 7th day had a positive direction, meaning that the increasing number of days (length of time) can also significantly increase collagen formation after tooth extraction in rats (Rattus norvegicus).

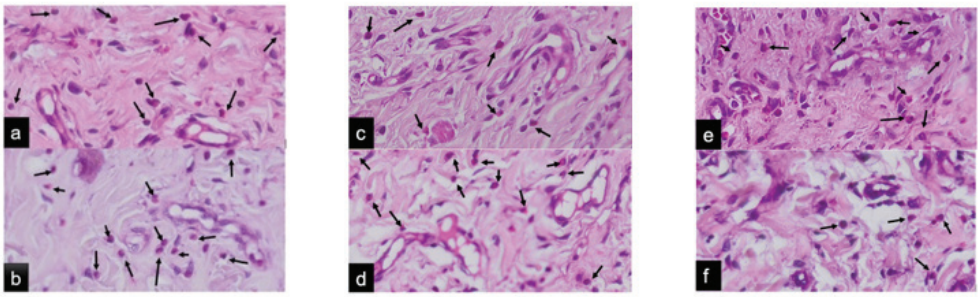

Figure 1. Histological view of macrophages using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

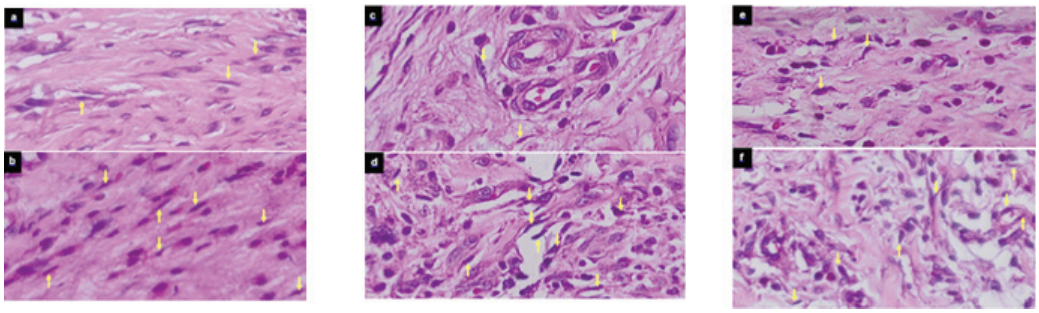

Figure 2. Histological view of fibroblasts using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

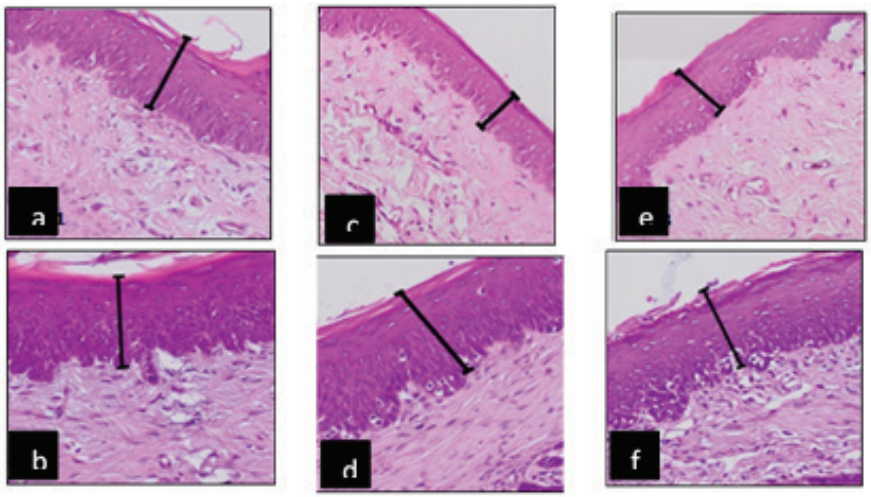

Figure 3. Histological view of epithelial thickness using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

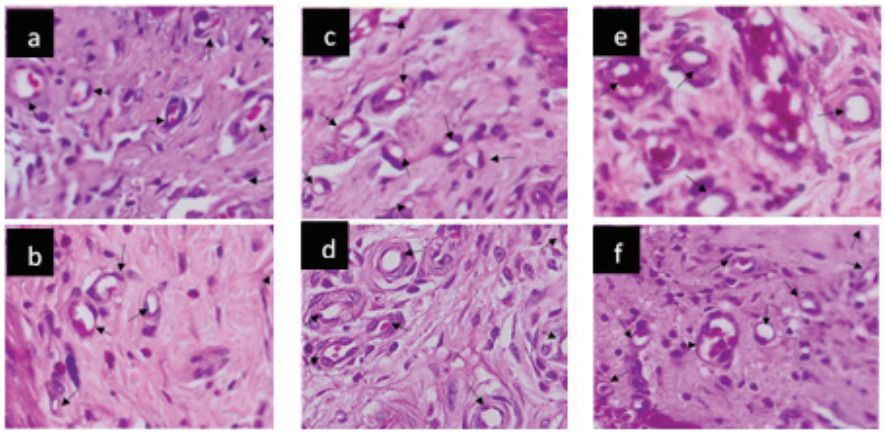

Figure 4. Histological view of the number of new blood vessels using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

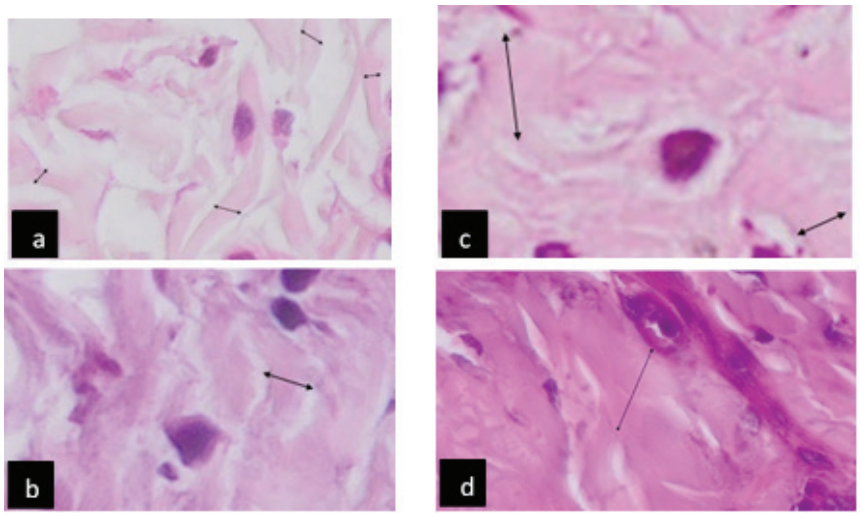

Figure 5. Histological view of collagen formation using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

Discussion

Based on the results of the study, the administration of Djambal Catfish (*Pangasius djambal*) gelatin in the post-tooth extraction wound resulted in an increase in the number of macrophages in the treatment groups compared to the control groups. When tissue damage occurs, macrophages will dominate the initial wound healing process for three to five days during the inflammatory phase[9,37]. Macrophages have a major role in the immune system, both as antimicrobial cells and immunoregulatory cells, which induce, suppress or modulate adaptive immune responses. In the immune response, this macrophage biology aspect is driven by the presence of macrophage arginine metabolic phenotype[10]. On the 7th day after the gelatin administration, the number of macrophages significantly decreased if compared to the control group. This is because the macrophages playing as phagocytic agents underwent apoptosis and replaced by fibroblasts. Besides that, the decreased number of macrophages can signify that the healing process has entered a proliferation phase, which is marked by the presence of fibroblasts and angiogenesis[8,12,13].

The increase in the number of fibroblasts after treatment using Djambal Catfish gelatin indicated an improvement in the wound healing process of the rat tooth socket. Fibroblasts began to emigrate to the wound area on the 3rd day after tooth extraction, so the lowest number of fibroblasts was on the 3rd day and increased to the 7th day[14,15]. The evaluation results showed a significant difference in the number of fibroblasts in the wound after rat tooth extraction, especially in the treatment groups (with the administration of Djambal Catfish (*Pangasius djambal*) gelatin), if compared with the control groups (without the administration of Djambal Catfish gelatin on the same day (C1 with T1, C2 with T2, and C3 with T3). This research suggests that the administration of Djambal Catfish (*Pangasius djambal*) gelatin can increase the number of fibroblasts in the wound after rat tooth extraction due to the glutamine and glycine amino acids which affect the wound healing process. The glutamine amino acid plays a role in increasing the fibroblast proliferation process and stimulates collagen formation[16]. Meanwhile, the glycine amino acid plays a role in regulating metabolism, anti-oxidative reactions, and neurological functions. The various beneficial effects of glycine make it an essential amino acid, including for mammals. Fibroblasts also influence the angiogenesis process. The absence of fibroblasts in combination with angiopoietin-1, angiogenin, hepatocyte growth factor, α transformation growth factor, and tumor necrosis factor will interfere the formation of blood vessel endothelial lumen[17,18,19,20,21]. The results of increasing fibroblasts make the wound healing process of the rat tooth socket would be faster with the use of Djambal Catfish gelatin.

The epithelization process begins at 12 hours after trauma with keratinocyte cell mitosis in the basal stratum. Keratinocytes will flatten and form bumps around it. These cells will lose the attachment of the hemidesmosomes to the surrounding basal cells and begin migrating at 24 hours after trauma. Within 48 hours, the proliferation of epithelial cells begins. Complete epithelial formation occurs on the fourth day to seventh days[22]. The presence of arginine plays a role to stimulate epithelial cell formation, strengthen the immune system and help synthesize amino acids. Arginine and glycine in protein can accelerate the maturation phase and the wound healing process[23,24].

In the proliferation of granulation phase starting from the 3rd to 7th day, the wound matrix will be occupied by proliferating endothelial cells. These cells will form new blood vessels in the process of angiogenesis, followed by the production of a temporary extracellular matrix and migrating from the wound edges to form layers covering the wound. The formation of blood vessels can occur from the 3rd to 7th day[26]. The findings of the increasing number of blood vessels (angiogenesis) are consistent with the study of Nofikasari (2016) showing that in the wound healing process on the 3rd day, the mean number of blood vessels seen on the histological preparation was higher than on the first day. The observation results showed that the highest mean number of blood vessels was on the 7th day because endothelial cells experienced peak proliferation[26]. The angiogenesis process also affects the formation of fibroblasts. The better vascularization of the wound area will increase fibroblast proliferation[27].

Glutamine is the main transporter of nitrogen from glutamine synthesis sites such as skeletal muscle, liver, and lungs to the endothelium. Endothelial glutamine together with asparagine and metabolism is significant

in the process of angiogenesis[25,28,29]. Arginine has the function of improving wound healing, triggering the release of insulin-like growth factor 1 (IGF-1), insulin, and prolactin[30]. Meanwhile, glycine together with VEGF increases the proliferation of endothelial cells and initiates angiogenesis both in vitro and in vivo[31]. The data analysis results from the angiogenesis examination based on the One Way Anova test on the control and treatment groups showed a significant difference in the number of blood vessels (angiogenesis). The higher number of blood vessels in the treatment group (P) indicated that the rats (*Rattus norvegicus*) experiencing tooth extraction and given Djambal Catfish (*Pangasius djambal*) gelatin affected the angiogenesis process.

Collagen is essential for the resilience and integrity of all tissues[32,33]. In the period of stress due to trauma, endogenous arginine synthesis is not sufficient to meet the increased demand for protein replacement. Thus, arginine supplementation becomes conditionally important in wound healing[34,36]. In this research, the collagen formation in the T3 group was higher than the C3 group. The mean collagen score of the T3 group was 2.93 while that of the C3 group was 1.76. This can occur because, at this stage, the inflammatory phase decreases and will change to the proliferation phase, in which growth factors will dominate. This process begins with the formation of fibroblast cells. Fibroblasts produce a basic substance, namely mucopolysaccharides, which unites collagen fibers. Collagen is useful in increasing strength in wound healing, accelerating the wound closure[35]. The higher collagen scoring in the treatment groups (T) than the control groups (C) was possibly due to the arginine contained in the Djambal Catfish gelatin.

Conclusion

Based on the research results and discussion on the use of Djambal Catfish (*Pangasius djambal*) gelatin after tooth extraction in rats (*Rattus norvegicus*), it can be concluded that Djambal Catfish (*Pangasius djambal*) has the ability to heal wounds, indicated from the activities of macrophages, fibroblasts, epithelialization, angiogenesis, and collagen formation. Although further research is still needed, it is learned that Djambal Catfish gelatin is likely to be an additional material used for wound healing therapy after tooth extraction.

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Declaration of Interest : The authors report no declarations of interest

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