# The effect of extract gel of pandan wangi leaves on level of CD34 post-gingival incision

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**Abstract**

An incision is a common procedure applied in dentistry. Incision leads to blood vessel damage in tissue, followed by wound healing process. Wound healing requires angiogenesis process in order to distribute nutrients to the tissues. One of the angiogenesis molecule markers is Cluster of differentiation 34 (CD34). CD34 is an antigen used as an indication of the new blood vessel growth by endothelial cells. Pandan Wangi leaves have antioxidant compounds that stimulates angiogenesis process and therefore accelerate formation of blood vessels. The purposes of this study were to determine the effect of Pandan Wangi leaves extract gel in various concentration (12.5%, 25%, and 50%) on CD34 levels in the wound healing process post-gingival wound incision of wistar rats. The study was experimental laboratory with posttest-only control group design, using 30 male rats Rattus Norvegicus wistar strain that separated into 5 groups, healthy control group (K0), negative control group (K1), treatment groups of 12,5% gel extract concentration (P1), 25% (P2), and 50% (P3). In each group, peripheral serum blood samples were taken on day seven to examine the CD34 levels using ELISA method. The results showed that there were significant differences among the treatment groups ($p<0.05$). Post-Hoc analysis showed significant differences between treatment group 2 and 3 and negative control group ($p<0.05$). However, there was no significant differences between treatment group 1 and negative control group ($p>0.05$). Following this study, it was concluded that an increase of CD34 levels in wound healing post-gingiva incision of Wistar rats were affected by application of pandan wangi leaves extract gel at concentration 25% and 50%.

**Keywords:** incision gingiva, wound healing, CD34, pandan wangi leaves

## Introduction

Wounds are a damage to body tissues that rupture the connective tissue, muscles, and skin as well as impair the function of nerve and blood vessels, resulting in bleeding. In dentistry, the most common type of wound is a gingival incision wound [1]. Gingiva is part of the periodontium network that is located at the peripheral part which functions as the oral cavity's barrier. When the tissue is injured, it will physiologically perform a wound healing process [2].

The complex walking wound healing process involves dissolved fluid, blood cells, extracellular matrix (ECM), and parenchymal cells which will result in a permanent anatomical repair and the restoration of tissue function integrity [3]. The stages of the wound healing process are divided into 3 phases, namely homeostatic and inflammatory phases, proliferation phase, maturation phase, and remodeling phase [4]. In the proliferation phase, the peak angiogenesis mechanism occurs on day 7. Angiogenesis is a mechanism for the proliferation and growth of new capillary blood vessels. These blood vessels carry oxygen and micronutrients to accelerate the healing process of wounds [5].

Angiogenesis can be detected through a marker molecule in the form of Cluster of differentiation 34 (CD34). CD34 is a glycoprotein antigen in the human hematopoietic progenitor cell which can be found in the vascular cell. CD34 also functions as a marker to identify specific tissues such as stem cells, muscle cells, and epidermal precursors. CD34 levels decrease in inflamed tissues due to the damage to endothelial cells, but this will change into the formation of new neovascularization that forms on day 2 to the peak of day 7 and ends on day 14 of the proliferation phase. This phase is characterized by the formation of capillary blood vessels and erythrocytes in the capillary lumen [6, 7, 8].

Wound healing in the oral cavity can be done by the means of pharmacological or non-pharmacological therapy. Researchers develop natural antioxidants obtained from nature to help the wound healing process to reduce the side effects of drug use [9]. One of natural source of antioxidant is a fragrant pandanus plant found in Indonesia [10, 11].

Antioxidants contained in fragrant pandanus leaves are secondary metabolites of polyphenol compounds which have anti-inflammatory and anti-cancer functions. Fragrant pandan leaves can increase new vascularization in the proliferation phase so as to accelerate the process of wound healing and type III collagen synthesis [12]. In a previous study, using 50% pandan leaf extract gel. The gel was applied to mice that experienced gingivectomy and the mice were also observed for the formation of a number of new vessels on days 1, 3, 7, and 14. The results showed that the gel with a 50% concentration could produce the most new blood vessels on day 7 [13].

Based on this, the researchers are interested in examining the potential of giving fragrant pandan leaf extract gel with various

concentrations of 12.5%, 25%, and 50% to the process of angiogenesis and wound healing through Cluster of differentiation 34 (CD34) markers after gingival incision. Blood sampling will be carried out at the peak of the proliferation phase on the 7th day [13].

## Material and methods

This research is an experimental laboratory research with post test-only with control group research design. Ethical eligibility certificates were obtained from the Research and Education Ethics Commission of Dr. Moewardi Hospital, Surakarta (No. 275 / III / HREC /2018, on 31 May 2018). Identification of fragrant pandan leaves was carried out in the Laboratory of Plant Structure and Development, Faculty of Biology of Jenderal Soedirman University. The manufacture of fragrant pandan leaf extract was carried out at the Pharmacetic and Therapeutic Laboratory of the Department of Pharmacy, Faculty of Health Sciences, Jenderal Soedirman University. The animal treatment process was carried out at the Laboratory of Pharmacology, Faculty of Medicine, Jenderal Soedirman University.

### The making of pandan wangi leaf extract

A total of 2 kg fragrant pandan leaves were washed, cut, and then dried in a drying cabinet at 48° C for 30 minutes. The dried leaves were then mashed using a blender. The pandan leaves powder was soaked in 96% ethanol for 24 hours. The filtered solution was macerated 3 times to get the fragrant pandan leaf filtrate. The filtrate was evaporated using a vacuum rotatory evaporator for 40 minutes and evaporated again using waterbath for 15 minutes.

### The production of fragrant pandan leaf gel extract

Na-CMC 2% was weighed as much as 2 grams as a base gel, then added 100 ml of distilled water to obtain a concentration of 2% (b / v). The manufacture of fragrant pandan leaf extract gel with a certain concentration was obtained with the comparison formulation in Table 1 as follows. The formulation ratio of the gel is heated and stirred for 10 minutes until evenly distributed, then transferred into a container and cooled into a gel.

**Table 1:** The comparison of the formulation of fragrant pandan leaf extract gel with different gel concentrations

| No | Gel Concentration | Extract Volume | Gel Volume of Na-CMC 2% |
|---|---|---|---|
| 1. | 12,5% | 1,25 ml | 8,75 ml |
| 2. | 25% | 2,5 ml | 7,5 ml |
| 3. | 50% | 5,0 ml | 5,0 ml |

*Source:* Processed Primary Data, 2018

### Experimental animal treatment

Mice anesthetized using ketamine at a dose of 80 mg / kg body weight were then incised on the attached mandibular gingiva along 5 mm using the scapel and blade on the mandibular gingival mucosa. Furthermore, mice were treated accordingly into their respective groups. K0 group was given 0.1 mL Na-CMC gel as much as 0.1 mL and groups P1, P2, and P3 were given 0.1 mL of fragrant pandan leaf gel with P1 concentration being (12, 5%), P2 being (25%), and P3 being (50%) for 1 minute every 12 hours using microbrush for 6 days. K mice are not injured because they are used as a healthy control.

### The extraction of mice serum

Blood samples were taken on day 7. Blood was taken as much as 2 mL using a microhematocrit pipette and then insert it into a non EDTA tube. Blood was centrifuged and the blood serum was taken using a micropipette and inserted into the eppendorf tube.

### Elisa measurement

Determination of CD34 levels was carried out using an ELISA reader with absorbance at a wavelength of 450 nm ± 2 nm.

## Results

The results of the average calculation of CD34 serum levels using the ELISA method in each treatment group can be seen in Table 2 as follows.

**Table 2:** The average calculation of CD34 serum levels using the ELISA method

| No | Treatment group | Average result of CD34 serum (mmol/L) |
|---|---|---|
| 1. | K | 5,5877 |
| 2. | K0 | 4,0915 |
| 3. | P1 | 4,5487 |
| 4. | P2 | 5,7398 |
| 5. | P3 | 5,8652 |

***Source:*** Processed Primary Data, 2018

The results showed the average number of CD34 levels in the 50% fragrant pandan gel treatment group had the highest yield. The results of the One-Way ANOVA statistical test obtained a significance value of $p = 0.002$ ($p < 0.05$) which showed that there were significant differences in serum CD34 levels between groups. These results indicate that there is an effect of administering 2% Na-CMC and fragrant pandan extract gel on the number of blood vessels in the area of gingival post-incision wound.

Post-Hoc Duncan test results In Table 3 shows the average difference between the fragrant pandan gel treatment group with the negative control group and healthy control groups.

**Table 3:** Post-Hoc Duncan test

| No | Treatment Group | Average of group | |
|---|---|---|---|
| | | 1 | 2 |
| 1. | K0 | 4,0915 | - |
| 2. | P1 | 4,5486 | - |
| 3. | K | - | 5,5876 |
| 4. | P2 | - | 5,7398 |
| 5. | P3 | - | 5,8651 |

***Source:*** Processed Primary Data, 2018

The Post-Hoc Duncan test results showed that P1 and K0 groups did not have significant differences because they were in the same column, but had significantly different results with groups K, P2, and P3.

## Discussion

The results showed that the average serum level of CD34 of group K without wounding was higher than the average of group K0 Na-CMC 2% and there was a statistically significant

difference between the two groups. 2% Na-CMC as a gel base does not stimulate growth factors in the vascular proliferation phase but has the effect of preventing bacterial growth in the area around the wound so as to help the wound avoid bacterial contaminants [14, 15].

Wound causes a decrease in the number of blood vessels within the tissue. The decrease in the number of blood vessels is caused by damage to the structure of blood vessel-forming endothelial cells due to injury resulting in dysfunction [16]. When endothelial cell formation is delayed, the function of tissue metabolism will also be inhibited, resulting in delays of the new blood vessel lumen formation. Delay in the formation of new blood vessels will result in serum CD34 expression in new tissues that fill the injury to also decreases. This happens because the migration of endothelial cell-forming progenitor cells that originate from the bone marrow becomes obstructed so that they cannot enter the wound tissue circulation [17].

The administration of the fragrant pandan leaf extract gel (P1, P2, and P3) in this study showed higher CD34 serum levels compared to negative control (K0). Previous research explained that the addition of fragrant pandan extract concentration will affect the tissue because the higher the concentration of fragrant pandan extract gel will result in an increase in tissue pH. Normal tissue has a pH ranging from 5 to 8. Along with the increase in the concentration, the pH produced will be better too. The pH concentration that is too low, especially in the wound condition, will make the tissue susceptible to bacterial contamination because the acidic atmosphere will then causes wound healing to be delayed [18, 19].

In addition, the lower concentration of a gel causes the lower antioxidant content to, so that neutral gel base in the form of Na-CMC is more influential compared to antioxidants. This has an effect on the speed of diffusion of the active substance in passing through the membrane so that it will affect the stimulation of new blood vessel formation. This is thought to cause the fragrant pandan leaf extract gel with a concentration of 12.5% (P1) to have a low increase compared to the concentration treatment group of 25% (P2) and 50% (P3) [18, 19].

The process of wounding results in inflammation and an increase of free radicals in the body in the form of superoxide anions (O2-) which can bind to NO produced by iNOS to form peroxynitrite (ONOO-). Peroxinitrite conducts oxidation and degradation of tetrahydro bioprotein (BH4) which is a cofactor of eNOS enzyme. This condition causes endothelial permeability to experience dysfunction. Formation of ONOO- which causes failure of endothelial cell formation can be prevented by giving exogenous antioxidants. Exogenous antioxidants are needed by the body to reduce O2- free radicals and prevent them from binding to NO which then forms ONOO - produced by iNOS [20].

There is a balance disorder when NO production and increased production of free radicals such as O2- will cause interference with blood vessel spasm. O2- molecules can inactivate NO and inhibit prostacyclin synthesis so as to interfere with the balance between relaxation and contraction factors released from the endothelium so that the endothelium experiences arterial spasm [21]. This shows that the endothelium has a very important role as a vascular regulator, as its main function as blood vessel formation. The formation of ONOO- can be prevented using natural antioxidant compounds such as fragrant pandan leaves that can protect target molecules from free radical attack [22].

Previous research shows that fragrant pandanus contain antioxidant substances that are useful in post-incision healing as angiogenesis stimulants namely flavonoids and saponins [13]. Flavonoids are able to limit the release of inflammatory mediators by inhibiting the signal in microglia cells in the brain so that the formation of inducible iNOS (sepsis) is inhibited [23]. When there are restrictions on the number of inflammatory cells that enter the wound area, it will stimulate the formation of VEGF which shortens the inflammatory phase. VEGF has a function to help activate growth factors such as PDGF, EGF, TGF-β, and FGF to stimulate blood vessel growth in the proliferation phase [24]. Saponins in fragrant pandan leaves can help endothelial cells to produce protease enzymes to inhibit the growth of extracellular matrix [25].

The formation of new blood vessels is an important process in wound healing involving growth factors, especially VEGF and endothelial progenitor cells. When the endothelium begins to form by VEGF stimulation, the endothelial cell molecule marker, CD34, appears on the wound tissue. This is a sign that at the time of wounding there is migration of endothelial-forming progenitor cells from the bone marrow into the circulation through damaged blood vessels. These progenitor cells will then form granulation tissue before finally differentiating into mature endothelium and forming new blood vessels. The migration of endothelial progenitor cells in the treatment group was faster because the stimulation of antioxidants contained in fragrant pandanus work as blood vessel formers. An increase in the number of blood vessels by endothelial cells will be followed by an increase in CD34 serum because CD34 can be secreted actively by cells that play a role in the immune system and one of them is endothelial cells. Therefore, increasing the concentration of fragrant pandan extract gel can increase CD34 serum levels [17].

Fragrant pandan leaf extract gel has been shown to increase CD34 levels as additional concentration can help accelerate wound healing. Previous research by Nofikasari *et al.* [13], showed that a fragrant pandan extract gel with a concentration of 50% could stimulate blood vessel growth and accelerate the proliferation phase [13].

In this study, the minimum concentration of fragrant pandan extract gel that has been able to increase serum CD34 levels after gingival incision was 25%. The gel effect of pandan leaf extract with 25% concentration had significantly different results with negative control (K1) but was not significantly different from healthy control (K0).

## Conclusion

This study suggests that there are effects of the administering the fragrant pandanus gel (*Pandanus amaryfollius Roxb.*) to the increasing CD34 levels in the wound healing process after gingival incision. The effective concentrations of fragrant pandanus extract gel (*Pandanus amaryfollius Roxb*.) on wound healing after gingival incision was 25% concentration.

## Compliance with ethical standards (WJS-I-Heading no numbering)

### Conflict of interest statement

The author declare no conflict of interest.

**Statement of ethical approval**

This research received approval from The Health Research Ethics Committees Dr. Moewardi General Hospital, School of Medicine Sebelas Maret University, Surakarta (No. 275 / III / HREC /2018, on 31 May 2018).

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