

RESEARCH ARTICLE

The Expression of IL-8 in Gingival *Rattus novergicus* After Exposed by Silver Gel Nano Particles 15 µg/ml

(Ekspresi IL-8 pada Gingiva *Rattus novergicus* Pasca Paparan Gel Nano-Partikel Perak 15 µg/ml)

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ABSTRACT

Background: The uses of silver nanoparticle are growing so fast, especially in the medical science, it's used in many kinds of concentration. In dentistry, it's used to decrease halitosis and periodontal diseases, and also wound healing process. It can affect the viability of the cells, give the bad effects to the human's health and environment if we use it in a long-term and in an uncertain concentration. **Purpose:** The aim of the study was to analysis the healing process of gingival fibroblast in *Rattus novergicus* after exposed by 15 µg/ml silver gel nanoparticles. **Materials and Methods:** This study uses 9 male wistar rats were divided into 3 groups. Group A: Cut (hurt it) in the oral gingiva and exposed to Ag-Np Gel 15 µg/ml for 3 days. After 3 days, they were sacrificed and cut the gingival fibroblast off 3x4 cm size with scalpel. Group B: cut in the oral gingiva and exposed to Ag-Np Gel 15 µg/ml for 5 days. After 5 days they were sacrificed and cut the gingival fibroblast off 3x4 cm size with scalpel. Group C: cut in the oral gingiva and exposed to none for 3 days then cut the gingival fibroblast off 3x4 cm size with scalpel. The expressions of IL-8 in the wound healing process were painted by Immunohistochemical method. This data was analyzed by using the t-test method. **Results:** General characteristic of IL-8 is shown in brown color immunoreactive expression on macrophage via immunohistochemical painting with monoclonal antibody anti IL-8. Mean expression numbers of IL-8 in the group A = 11,7; group B = 16,3; and group C (control) = 28,3. T-test sign.number of group A & C = 0,004; group B & C = 0,000. **Conclusion:** The exposure of 15 µg/ml silver gel nanoparticle to gingival fibroblast of *Rattus novergicus* reduces the expressions of IL-8 in the day-3 and day-5 post exposure. So, it may accelerate the wound healing process.

Keywords: Healing, silver, nanoparticles, IL-8

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INTRODUCTION

The uses of silver reminds us about the Egyptian's luxurious. Silver is one of the most popular metal in the world after gold and copper (Hill, 2009). It's used as the basic material in a saving water equipments and other liquids to keep its hygiene (Elzey & Grassian, 2009). The uses of silver are growing so fast, especially in the medical and nanobiotechnology science. In dental science, silver used in many kinds of concentration to decrease halitosis and periodontal diseases as an anti-bacteria and anti-fungal agents, and wound healing.

There are so many kinds concentration of silver nanoparticles in the market. We have to know how to use it properly. Ag-np shows an unexpected effect in the human health and environment if it's used in a long time and in incorrect ways and concentrations. Many researches have been done to examine the effect of Ag-np exposure, such as in the year of 2005, Hussain *et.al* try to examine it in the rat liver cells. In his research, 5-10 µg/ml Ag-np can decrease the mitochondrial function and the integrity of rat liver membran cells after 24 hours of incubation. The other in vitro research by Asharani *et.al* in the 2009, try to examine the effect of 5-25 µg/ml Ag-np to the human lung fibroblast cells. In his research, 24 hours and 48 hours after 10-25 µg/ml Ag-np exposure, can stimulate the secretion of pro-inflammatory cytokine and increase the level of ROS production. Hence, it can be a potential problem which causes a DNA cells damage.

Silver nanoparticles can easily migrate into the cell because of its nano size. Hence, if we don't manage the use, it can affect the viability of the

cells (Kumar *et.al*, 2007). It can induce the cell's death and involve in the biology system of a human. This research would be done to examine the capability of Ag-np gel 15 ppm as a healing process inductor in an apoptotic pathways in *Rattus novvergicus*.

OBJECTIVES

Learn the healing process of gingival fibroblast in *Rattus novvergicus* which is exposed with 15 ppm silver nanoparticles gel. Observe the expression number of pro-inflammatory cytokine IL-8.

MATERIALS and METHODS

This research uses the gingival fibroblast cells of *Rattus novvergicus*. The examined material in this research is the 15 ppm Ag-np gel. In a slide making proccess need some materials such as, formaline 10%, alcohol 70%, alcohol 85%, alcohol 90%, alcohol 95%, absolute alcohol, xylol, soft and hard parafin, gelatin 5%, dH₂O. Staining proccess use PBS pH 7,4, counterstain Mayer hematoxylen, dH₂O, micropipet Gilson, H₂O₂ 3%, serum 5% FBS 0,25% Triton X-100, caspase-3 primer antibody, caspase-3 seconder antibody biotin labeled, IL-8 primer antibody, IL-8 seconder antibody, SA-HRP (*Strep Avidin-Horse Radis Peroxidase*), DAB (*Diamono Benzidine*), *aquadest*.

This study was a pure laboratory experimental research approved by The Health Research Ethics Committee Airworthiness Faculty of Dentistry, University of Airlangga. 9 male wistar rats were divided into 3 groups. Group A: cut (hurt it) in the oral gingiva and exposed to Ag-Np Gel

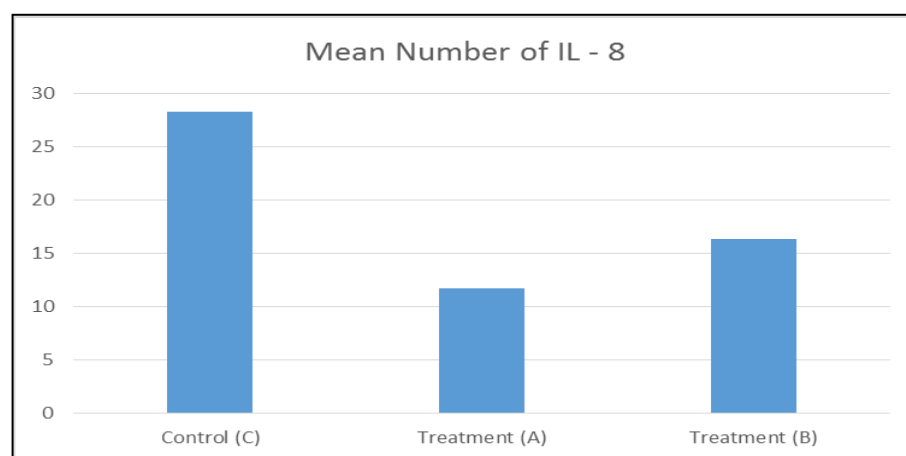
15 ppm for 3 days. After 3 days, they were sacrificed and cut the gingival gingival fibroblast off 3x4 cm size with scalpel. Group B: cut in the oral gingiva and exposed to Ag-Np Gel 15 ppm for 5 days. After 5 days they were sacrificed and cut the gingival fibroblast off 3x4 cm size with scalpel. Group C: cut in the oral gingiva and exposed to none for 3 days then cut the gingival fibroblast off 3x4 cm size with scalpel. The expressions of pro-inflammatory cytokine IL-8 in the wound healing process were analyzed by Immunohistochemical test. *T-test* was used to analyze the significance of the differences between the exposure and control groups.

RESULTS

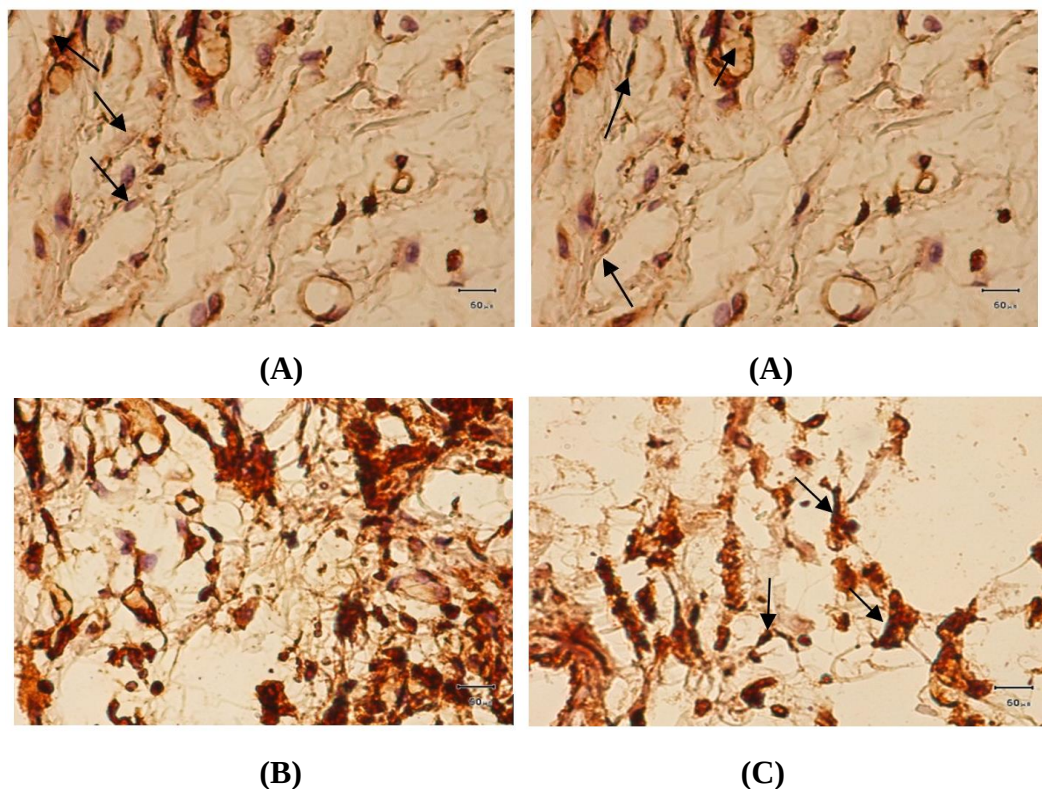
The exposure of 15 ppm silver gel nanoparticles to gingival fibroblast of *Rattus novergicus* reduce the expressions of pro-inflammatory cytokine IL-8 in the wound healing process. Based on the table, the mean number of IL-8 in the day-5 treatment group is more than the mean number in the day-3 treatment group. The mean number of IL-8 in the treatment group is less than the control group. Significance number $< 0,05$. The highest mean number of IL-8's expression is in the control group. The lowest one is in the treatment group day-3.

Table 1.1. Table of mean and std.deviation the expression of IL-8 in the day-3 and day-5 ($p < 0,05$)

Group	Name Of Group	Day-3			t-test sig.	Day-5			t-test sig.
		Mean	Standard Deviation			Mean	Standard Deviation		
Treatment	A	11,7	$\pm 1,528$		0,004	B	16,3	$\pm 1,528$	0,000
Control	C	28,3	$\pm 5,132$						



Picture 1.1 Bar chart of the IL-8 expression's mean number. Noted : A. Treatment group, day-3; B. Treatment group, day-5; C. Control group.



Picture 1.2. The expression of IL-8 in the fibroblast cells. Noted: A. Treatment group, day-3; B. Treatment group, day-5; C. Control group, day-3; D. Control group, day-5.

Table 1.2. *t*-test significance table of IL-8 in day-3 and day-5. $p < 0,05$

	Day-3	Day-5
<i>t</i> -test	0,004	0,000

DISCUSSION

Interleukin-8

Pro-inflammatory cytokine, in this case IL-8, involve in a bad outcome of a disease. The highest number of pro-inflammatory cytokine IL-8 will be expressed in the day 2-3 after injuries (Waxman, 2007). The repairing process after injuries will be held if it's supported by the proper and health environment. But, if the environment couldn't support the

injury's repairing process, the involved cells will be died by the apoptotic pathways or the necrosis pathways (Kumar *et.al*, 2007).

The result was shown that 3 days after 15 ppm gel Ag-np exposure to the gingival fibroblast cells, reduced the respon of cell's inflammation by the decreasing number of the IL-8 expression as a pro-inflammatory cytokine. Low expression number of IL-8 in the range of time ≥ 48 hours post injured informs that the inflammation phase which is the first

phase in the healing process, held faster than the control group without gel Ag-np exposure. Intrinsic pathway of apoptotic starts from the secretion of pro-inflammatory cytokine, in this case IL-8, because of the exposure. This condition may affect the increasing of ROS, sitosol's calcium, and the other biochemical products which come from the oxidative stress (Widya, 2008). So, if the number of pro-inflammatory cytokine IL-8 decrease, the production and activity of ROS will be decreased too. It is a potential phase which can cause a DNA disorder, decreasing of mitochondrial's function, then it potents in causing a cell death.

CONCLUSION

The exposure of 15 ppm silver nanoparticles gel to gingival fibroblast in *Rattus novergicus*, reduce the expressions of pro-inflammatory cytokyne IL-8 in the wound healing process. That indicates the proliferation of fibroblast will be

increased and this is going to accelerate the wound healing process.

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