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Original Article

Evaluation of Silver Nanoparticles Addition in Periodontal Dressing for Wound Tissue Healing by ^{99m}Tc-ciprofloxacin

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ABSTRACT

Objective: Periodontal dressing is a protective material that is used on post-surgical periodontal to protect the surface of the wound and provides a comfort sense to patient. This study was conducted to determine the effect of periodontal dressings containing silver nanoparticles by evaluating inflammatory parameters using ^{99m}Tc-ciprofloxacin; a radiopharmaceutical that can be used for diagnosing infection and inflammation. **Methods:** This research was carried out using purely experimental 24 male Sprague Dawley rats and divided into 4 groups. First group is a group without any treatment, second group was the positive control group (excision procedure and given CoePak(R)), third group was negative group (only excision procedure) and fourth group (excision procedure and given CoePak (R) with silver nanoparticles). On second and fourth days after the procedure, each group was observed of inflammation inside of the excision by injecting ^{99m}Tc-ciprofloxacin through vein and after one hour the rat was sacrificed and dextra palate organ of each rat was counted by Single Channel Analyzer to determine the accumulation of ^{99m}Tc-ciprofloxacin. **Results:** In rats given CoePak (R) with silver nanoparticles showed a tendency or inclination data which is good in giving effect to promote healing compare to positive control group on day 4 with less accumulation of ^{99m}Tc-ciprofloxacin in dextra palate. **Conclusion:** Addition of silver nanoparticles in periodontal dressing gave a good effect for wound tissue healing.

Key words: Periodontal dressing, Silver nanoparticles, Wound tissue healing, ^{99m}Tc-ciprofloxacin.

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INTRODUCTION

Nano technology is an industrial technology that currently developing now days. Silver nanoparticles have been widely used in medicine such as dressings from burns, contraceptives, layers of surgical instruments and basic ingredients of bone prostheses. Products from silver have been widely used as antimicrobials because of the silver ions can inhibit bacterial growth. On the previous studies show that silver nanoparticles have potential as antimicrobial, anti-inflammatory and accelerate wound healing.

The environmental condition of the oral cavity generally has full of bacteria. These bacteria are opportunistic, causes a disease when the oral environment is bad and pathogenic, directly causes a disease. The bacteria activity can cause acute or chronic infection and inhibits the wound healing.[1] Protecting the wound from bacterial infection using periodontal dressing or wound's cover is important in dentistry. The periodontal dressing generally used for postsurgical treatment.[2] Its function is preventing infection, bleed and also protecting surgical area from traumatic during mastication. Periodontal dressing generally does not have the healing effect. However, it supports the wound healing by keeping the injured tissue from trauma.[3,4] In contrary, the periodontal dressing can increase plaque accumulation and irritates the tissue which causes the inflammation.[5] This problem needs the efforts to repairing the mucosal tissue and restoring the function without pain and discomfort. Previously, the periodontal dressing containing silver Nano particles (NP) histologically can accelerate the gingival wound healing based on inflammatory parameters.[6] Another method used to evaluate the inflammatory is nuclear medicine diagnostic. It uses the radiopharmaceutical to trace the inflammation and infection site by emitting the radiation which will be captured by detector. One of the radiopharmaceuticals that useful to diagnose inflammation and infection is ^{99m}Tc-ciprofloxacin.[7]

The aim of this study was to evaluate the effect of periodontal dressings with silver NP to the wound healing in animal using ^{99m}Tc-ciprofloxacin and inflammatory parameters. This experiment is expected to give information to the clinicians and researchers about the periodontal dressings containing silver NP's effect.

MATERIALS AND METHODS

Materials

The material used in this experiment were periodontal dressing Coe-Pak$^{(R)}$ with addition of silver nanoparticle, Coe-Pak GC as control material, ketamine, xylazine, blade no.15c, Silk/nylon suture 6-0, Isoflurane and ^{99m} Tc-ciprofloxacin.

Synthesis Nanopartikel (NP) Silver

Synthesis of NP silver was made in Material Engineering Laboratory-Bandung Institute of Technology using the procedure by Kim *et al.* (2006). Silver elements were made from $AgNO_3$ that reduced using ethylene glycol and stabilized using polyvinyl alcohol.

AgNO3 was dissolved in its volume of ethylene glycol (EG) (5 mL) from the calculated ratio of moles of ethylene glycol (EG) to Ag + (50: 1). The solution was added with 20 mL PVA in concentration (1%), then heated to boiling temperature of 100°C in an Erlenmeyer flask. The mixture was stirred using a magnetic stirrer during the heating process until the

color became pale yellow. When the color changed, heating process was stopped however the stirring was carried out until the mixture temperature became room temperature and silver colloid nanoparticles formed.

Preparation of ^{99m}Tc-ciprofloxacin[8]

20 mg of ciprofloxacin. HCl was dissolved into 5 mL of NaCl, then 2 mg Sn-tartrate was dissolved into 40 mL of 1 N HCl and 1960 mL water was added. Afterward, aliquots of 1000 µL ciprofloxacin solution was added 800 µL Sn-tartrate and pH of the solution was adjusted to 3.0. The mixture was filtered using 0.22 µm milipore. A total of 900 µL of mixture was then taken and transferred to another vial, then added freshly eluted pertechnetate solution ($^{99m}TcO_4$) to 1100 µL (1 mCi / mL) and incubated for 15 minutes at room temperature. ^{99m}Tc-ciprofloxacin radiochemical purity was determined by using two kinds of chromatography systems. The first system is Whatman 1 paper (1 x 10 cm) as a stationary phase with methyl ethyl ketone as the mobile phase, then the second system is ITLC-SG (1 x 10 cm) as a stationary phase and a mixture of ethanol: water: ammonia with a ratio of 2 : 5: 1 as the mobile phase. After complete development, the two radiochromatograms were dried, cut into 1 cm pieces and separately counted using the NaI (Tl) scintillation counter to determine the ratio of ^{99m}Tc-ciprofloxacin, free $^{99m}TcO_4^-$ and hydrolyzed ^{99m}Tc (TcO_2) and. The radiochemical yield was calculated as the ratio of the radioactivity of the labeled product to the total radioactivity

Periodontal Dressing Protocol

Silver NP dressings were made by mixing the periodontal dressing Coe-Pak (R) (0.1 gram base and 0.1 gram catalyst) and 0,1ml silver nanoparticles colloid. The total weight is 0.3 grams, it is containing 25 ppm colloid of Silver NP.

Animal Model

All the procedures already accepted by the Institutional Animal Care and Use Committee of BATAN. This research used 200-300 gram of 24 male *Sprague dawley* rats from animal laboratories of the Center for Applied Science and Nuclear Technology (PSTNT) National Nuclear Energy Agency (BATAN). The experiment in this research were divided into 2 groups, 2 days and 4 days, each group was classified into 3 categories. The group with silver NP dressings, the group with a Coe-Pak (R) dressing (positive control), the group without dressing (negative control).

Rats were anesthetized using ketamine and xylazine by intraperitoneal injection. Each rat treated with excision in the dextra palate region with a size of 5x1 mm, regard to bleeding conditions and vital signs of the rat (Figure 1). Furthermore, interupted suturing was carried out to maintain the dressings in the palate (Figure 2).[15]

To observe the inflammation inside of the excision ^{99m}Tc-ciprofloxacin (100 µCi/ 100 µL) was injected through vein to each group and after one hour the rat was euthanized. The vital organs such as excision dextra palate and normal dextra palate were taken and counted by Single Channel Analyzer with Na (I) Tl detector to count accumulation of ^{99m}Tc-ciprofloxacin

The percentage injected dose for each sample (%ID/g) was then calculated. The differences was determined using two-way ANOVA and Box-plot for descriptive statistical.

RESULT

Quality control of the ^{99m}Tc- ciprofloxacin was assessed by thin layer paper chromatography to distinguish and quantify the amounts of radioactive contaminants (free $^{99m}TcO_4$, $^{99m}TcO_2$) Labeling efficiency of the ^{99m}Tc-ciprofloxacin provide high radiochemical yield > 90% and can be used to carry out *in vivo* test. The biodistribution study was performed 1 h post

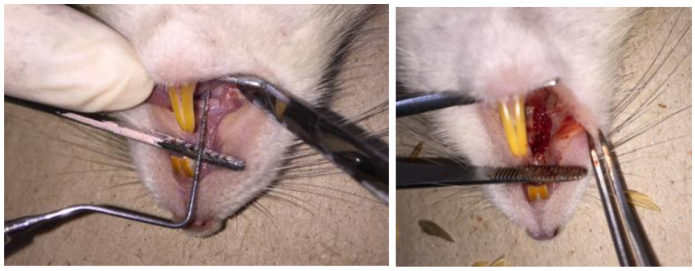

Figure 1: Excision region on dextra palate mice.

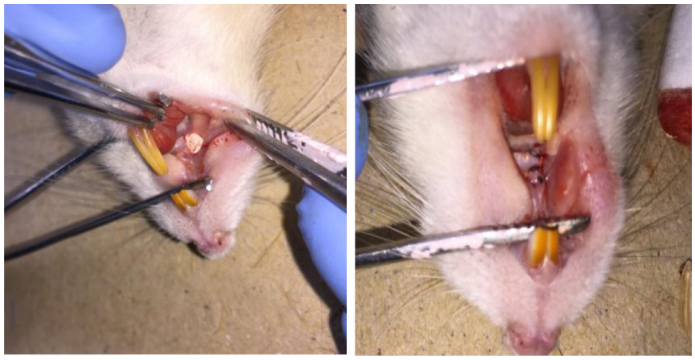

Figure 2: Placement technique of periodontal dressing

intraperitoneal injection. This time acquisition was gotten after performing preliminary study. Evaluating results of the biodistribution study by comparing the ratio between excision tissue as targeted organ and normal tissue as non-targeted organ. The results of biodistribution study were shown in Table 1 and Figure 3. The results of two-way ANOVA showed no significant differences between all groups in day-2 and day-4.

After performing two-way ANOVA, we performed descriptive statistic using boxplot analysis.

The descriptive analysis showed the mean data of the target to non-target (excision to normal tissue) ratio of each treatment. The mean value of day 2 showed dressing of silver nanoparticles (1.4)> positive control (0.63); range value: dressing silver nanoparticles (1.01)> positive control (0.14); and the difference between the mean and median values: dressing silver nanoparticles (0.005) <positive control (0.02). The mean value (mean) of the 4th day: silver nanoparticles dressing (0.84)> positive control (0.77); range values: dressing silver nanoparticles (0.3) <positive control (0.63); and the difference between the mean and median values: dressing silver nanoparticles (0.03) <positive control (0.62) (Table 2). These values are described in the boxplot analysis (Figure 4). We can see the ratio, excision to normal muscle as target to no target sample, silver nanoparticles dressing group have a range greater than the positive control group on observations day 2. However, it has a range value smaller than the positive control group on day 4 (Figure 5). The silver nanoparticles dressings group showed a more symmetrical shape or the data in this group was more evenly distributed. The mean ratio in silver nanoparticles group is almost coincident with the median value which can be seen from the dotted red line coincides with the black line in the middle of the boxplot. The ratio of T / NT silver nanoparticles dressings group was not far apart from the others. Analysis of boxplot descriptive statistical data on the silver nanoparticles dressing group had a better effect than positive groups and negative controls.

Table 1: Biodistribution results.

Groups	Organs	Day-2		Day-4	
		Mean	SD	Mean	SD
Dressing Np Silver	Excision	8.39	1.97	6.84	4.32
	Normal	6.51	2.80	4.88	1.90
	Excision to normal	1.40	0.51	1.27	0.71
Positive control	Excision	13.02	3.72	10.61	6.05
	Normal	20.63	5.40	17.26	10.59
	Excision to normal	0.63	0.08	0.43	0.31
Negative control	Excision	1.08	0.09	0.77	0.59
	Normal	1.07	0.75	0.97	0.19
	Excision to normal	1.64	1.42	1.36	0.31

Note: Excision to normal = Palatum Dextra Excision Tissue (Target)/ Normal Tissue Palatum Sinistra (Non target)

Table 2: Descriptive value ratio excision to normal (target/non-target).

Day 2	Negative control	Positive control	Dressing nanoparticle silver
Mean	1.64	0.63	1.40
Median	1.95	0.65	1.405
Minimum	0.63	0.58	0.90
Maximum	3.27	0.72	1.91
Range	2.64	0.14	1.01
Day 4	Negative control	Positive control	Dressing nanoparticle silver
Mean	0.68	0.77	0.84
Median	0.64	0.85	0.81
Minimum	0.41	0.46	0.66
Maximum	0.88	1.24	0.96
Range	0.47	0.78	0.30

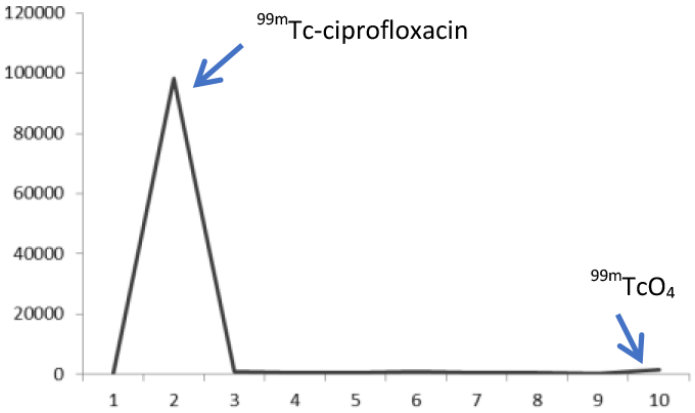

Figure 3: Chromatogram profile of ^{99m}Tc-ciprofloxacin and TcO_4^- with Whatman 1 MM/ methyl ethyl ketone.

DISCUSSION

The wound healing mechanism is a complex and dynamic process that consists of several interconnected stages.[17] The process stage begins with the inflammatory process, the migration of inflammatory mediator cells including accumulation of neutrophil cells and monocytes. The wound

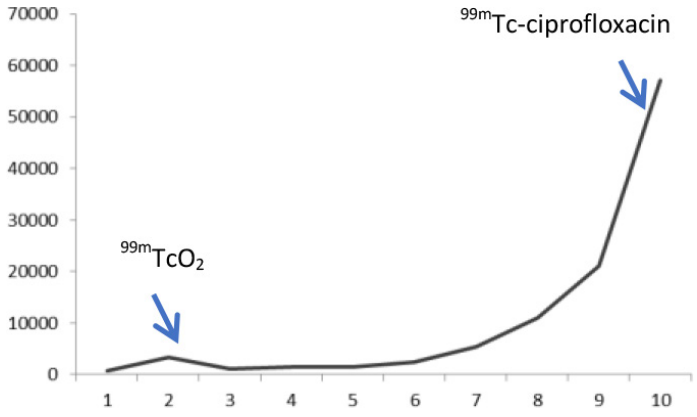

Figure 4: Chromatogram profile of ^{99m}Tc-ciprofloxacin and TcO_2 with ITLC-SG/ Et-OH/H_2O/NH_3 (2:5:1).

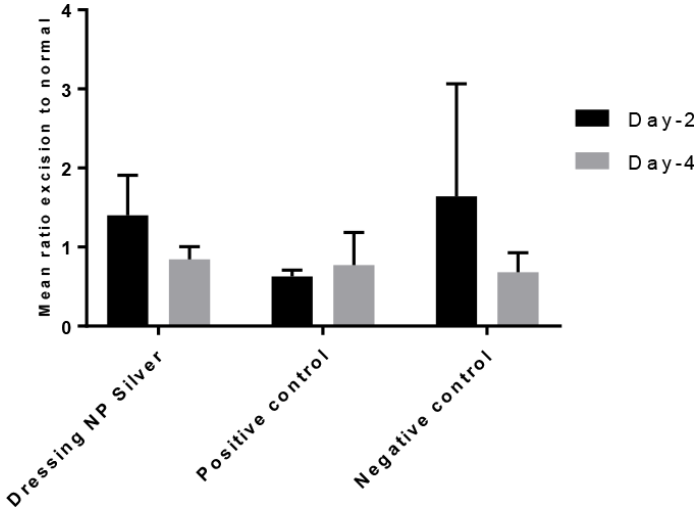

Figure 5: Pattern ratio of excision to normal muscle.

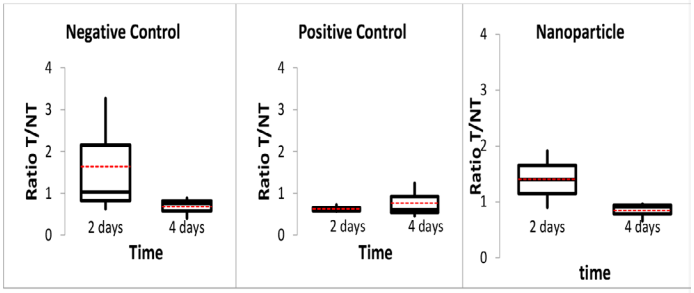

Figure 6: Boxplot diagrams of each treatment.

healing process can be seen from the parameters of the accumulation of inflammatory mediator cells. Observations in this study were carried out on the 2nd and 4th days to see the peak of inflammation and the end of inflammation in accordance with the time period of inflammation.[9] This research was conducted by looking at the accumulation of inflammatory mediator cells which was characterized by the presence of ^{99m}Tc-ciprofloxacin radiopharmaceutical compounds which are used as markers.

Observations on day 2 of the ratio T / NT from treatment of silver nanoparticles dressing group were higher than the treatment with

positive control (Coe-Pak (R)) group and boxplot descriptive statistical analysis of the range of treatment data in the positive control group was smaller than the silver nanoparticles dressing group. These observations showed the use of silver nanoparticles which are used as additional ingredients for periodontal dressings, has no anti-inflammatory effect and the body will respond to silver nanoparticles that are on periodontal dressing as foreign contents in the body so that the radiopharmaceutical value is higher. Observation of data on day 4, in the dressing group of silver nanoparticles experienced have a decreased difference compared to day 2. The positive control group experienced a difference in the accumulation of radiopharmaceutical compounds increased more than day 2, the condition of this observation is likely in accordance with the study of Stahl *et al.* (1969), Heaney and Appleton (1976), Bose *et al.* (2013) who stated that the use of periodontal dressing will cause more inflammatory conditions due to plaque retention. Local factors in the oral cavity such as plaque retention cause accumulation of bacteria and make factors that cause an increase in inflammatory conditions. The study of Newman and Addy (1978) also showed an increase in plaque accumulation in the use of periodontal dressings after flap surgery.[10] Observations in the silver nanoparticles dressing group showed that on the 4th day there was already an anti-inflammatory response from the body against silver nanoparticles. The response is based on the literature in the form of modulation of inflammatory mediator cells, namely by inhibiting IL-6 release as the initiator of the inflammatory phase and stimulating the release of IL-10 which serves to inhibit the synthesis of several chemokines so that the migration of inflammatory mediator cells is also inhibited.[11] The mechanism causes accumulation from ^{99m}Tc-ciprofloxacin radiopharmaceuticals compounds were lower than in day 2 observations. (Figure 6)

The data obtained from this study provide a response in accelerating wound healing in accordance with the literature presented, although statistically there are no significant differences in the ANOVA test data, but descriptively from boxplot analysis provide a good response on day 4 seen from more homogeneous data variation (small range). The accumulation of ^{99m}Tc-ciprofloxacin radiopharmaceutical compounds on the 4th day of the negative control group was lower than the other two treatment groups due to the placement of material dressing on the tissue and suturing in the two treatment groups causing tension, irritation from the tissue. This condition made the factors of the inflammation of the two treatment groups still high, thus showing data of high accumulation of compounds from ^{99m}Tc-ciprofloxacin radiopharmaceutical. Nezwek (1980) states that studies using periodontal dressings placed in animal tissues will always show the presence of inflammatory cells.

The limitations of this study are the characterization of silver nanoparticles and the concentration formed on the reduction reaction method so that it effects on the analysis of the response of silver nanoparticles in the wound healing response. The observation time cannot be extended cause the behavior of experimental animals that have high activity so that it will interfere with the placement of given periodontal dressing.

CONCLUSION

The results of this study showed coe pak (R) dressings with the addition of silver nanoparticles tended to give more effect to accelerate the tissue healing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ABBREVIATIONS

NP: Nano Particles; **T/NT:** Target/Non Target tissue; **TC:** Technetium.

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