Effect of Application of Silver Nanoparticle on Fibrin Density Post Dental Extraction (Trial at Sprague dawley Rats)

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Abstract: Post extraction bleeding is one of the most common in oral surgery procedures. It has been attributed to various factors that can be broadly classified as local and systemic factors. One of them is management bleeding complications is non-surgical interventions included haemostatic agents application. Silver nanoparticle has been widely used in medicine, for example as a burn dressing, contraceptives, layers of surgical instruments and basic ingredients of bone prostheses and as anti-microbes. This study aims to analyze the effect of Silver Nanoparticle (SNP) on the hemostasis process after tooth extraction of Sprague dawley rats as test animals. This study was a pure experimental study conducted on 27 animals of Sprague dawley rats after tooth extraction, which was divided into three groups of equal size. The first group was the control group, the second group was the treatment group that applied 250 ppm SNP gel, and the third group was the group that applied 500 ppm SNP gel. Tooth extraction and application of test materials on animal tooth sockets are carried out in anesthetized animals. After 5 minutes of application of the test material, the animal was euthanized and necropsied to take socket tissue samples for histopathological preparations and stained with Hematoxylin-Eosin. Assessment of the effectiveness of the test material was carried out based on assessment of fibrin density on histopathological preparations. The results showed the effect of SNP on the hemostasis process seen from fibrin density. This was demonstrated in the non-parametric Kruskal Wallis statistical test with Mann Whitney further test; fibrin density in 500 ppm SNP gel administration group described significant results. The conclusion of this study is that SNP gel can increase fibrin density after tooth extraction in Sprague dawley rats.

Keywords: silver nanoparticles (SNP), fibrin density, bleeding

1. Introduction

The use of silver in the field of dentistry has been developed since the early 18th century as a filler material better known as amalgam, which is still used till today. Research conducted by Martinez et al in 2014 found that Titanium abutments implants coated with silver nanoparticles (NPS) can reduce bone loss in cases of periimplantitis. NPS as an abutments coating material can control the development and progression of periimplantitis.¹

Bleeding is a common problem that must be faced by surgeons from various surgical specialities. Although the human body has a natural ability to stop bleeding at the site of the wound and is also able to keep the blood liquid in the blood vessels, which is known as the hemostasis process, but bleeding complications can occur. In the field of specialist oral surgery, bleeding complications that are often found everyday are after tooth extraction. Complications of dental extraction measures are divided into two, namely complication during dental extraction and complications after tooth extraction.² Bleeding complications after tooth extraction can be caused by local or systemic factors. Several techniques for controlling bleeding have been developed for various types of surgery. The principles of the management of bleeding after tooth extraction because of local factors are by doing a good emphasis or suturing, and if necessary with the administration of hemostatic agent drugs both locally and systemically.²³

Nano technology was one of technology that has been developed lately. NPS already used in medical, for example as a dressing in burns, contraception tools, as a coating material on a surgical tools and as a based on bone prosthetic⁴⁵.

2. Methods

Nano particle silver using product from Sigma-Aldrich® silver nanopowder and carried out at the Materials Engineering Laboratory, Bandung Technology Institute to dissolved to be 250 ppm and 500 ppm gel, where bacterial tests were previously carried out.
A total of 27 Sprague-Dawley rats were divided into 3 groups with each group consisting of 3 rats. Group I is a control group without treatment. Group II was treated with topical NPS gel with a concentration of 250 ppm. Group III was treated with topical NPS gel with a concentration of 500 ppm. All experimental animals were taken with anesthesia using 10% Ketamine hydrochloride at a dose of 45 mg / kg body weight, and 2% xylazine at 0.35 mg / kg body weight, intramuscularly on the abdominal muscles. Then the tooth the maxillary central incisors was extracted in each group, after tooth extraction apply the NPS 250 ppm at group II and 500 ppm at group III. S. dawley rats group I, II and III then necropsyzing in the 5th minute, after that take the socket tissue and soak it in Buffered Neutral Formalin (BNF) 10% for 3 days. Furthermore, decalcification was carried out in 10% nitric acid solution for 72 hours. The next step is to cut off the tissue, followed by the process of making a histopathological preparations, and stained it with Hematoxylin-Eosin staining. Next is an assessment of the fibrin density using a microscope. Assessment of fibrin density using a modified method from Greenhalgh's histological scoring system, where these parameters use a score of 0-3 to assess fibrin density, with the following scoring criteria: Score 0: there is no fibrin density in the socket, Score 1: if the fibrin density is small (less than 25% of the entire socket area), Score 2: if fibrin density is moderate (26-50% of all socket areas), Score 3: if the fibrin density is large (more than 51% of the entire socket area).

To test the hypothesis, a statistical test used comparison test with non-parametric Kruskal Wallis with Mann Whitney test

3. Results

Microscopic observation of fibrin density using a binocular light microscope and photographed with an MD 250® digital eyepiece camera. Microscopic observations found blood clots and fibrin threads in the tooth socket. Fibrin yarns appear in the form of fine fibers that bind to each other and are pink in color, which is fused with clots of erythrocytes and platelets (Figure 2). In Figure 3 shown the fibrin threads of the control group (which were not treated). Figure 4 shown the fibrin density in the treatment group with NPS application of 250 ppm, and Figure 5 shown a histology description of the treatment group of 500 ppm NPS application.
After doing the Kruskal Wallis test then proceed with further using 250 ppm NP with a minimum value of 2.0 and a maximum value of 3.0.

Table 1: Average Fibrin Density of *S. dawley* rat tooth sockets Fibrin Density Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>St.Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2</td>
<td>0.4</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>NS 250 ppm</td>
<td>1.3</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>NS 500 ppm</td>
<td>2.6</td>
<td>0.5</td>
<td>2.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The table 1 explains fibrin density after tooth extraction after being treated. Fibrin density in the control group had an average of 1.2 ± 0.4 with a minimum value of 1.0 and a maximum of 2.0. Fibrin density in the group given 250 ppm NP had an average value of 1.3 ± 0.5 with a minimum value of 1.0 and a maximum value of 2.0. Fibrin density in the group given 500 ppm NP had an average value of 2.6 ± 0.5 with a minimum value of 2.0 and a maximum value of 3.0.

The results of the Kruskal Wallis test obtained a p-value of 0.000 (p < 0.05) which means that there is a significant difference in the average fibrin density between groups using 250 ppm SNP and 500 ppm NPS.

Table 2: Kruskal Wallis Test Results of Fibrin Density

<table>
<thead>
<tr>
<th>Fibrin Density</th>
<th>Chi-Square</th>
<th>Df</th>
<th>Asymp. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16,887</td>
<td>2</td>
<td>.000</td>
</tr>
</tbody>
</table>

After doing the Kruskal Wallis test then proceed with further tests to find out which treatment group has significant differences. Post hoc test uses the Mann Whitney test as follows.

Table 3: Mann Whitney Advanced Test Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kontrol</td>
<td>1.22</td>
<td>0.44</td>
<td>0.609</td>
</tr>
<tr>
<td>Nano Silver 250 ppm</td>
<td>1.33</td>
<td>0.50</td>
<td>0.609</td>
</tr>
<tr>
<td>Kontrol</td>
<td>1.22</td>
<td>0.44</td>
<td>0.609</td>
</tr>
<tr>
<td>Nano Silver 500 ppm</td>
<td>2.67</td>
<td>0.50</td>
<td>0.000</td>
</tr>
<tr>
<td>Nano Silver 250 ppm</td>
<td>1.33</td>
<td>0.50</td>
<td>0.001</td>
</tr>
<tr>
<td>Nano Silver 500 ppm</td>
<td>2.67</td>
<td>0.50</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Based on the results of Mann Whitney’s advanced test, some conclusions can be explained, fibrin density in the control group was significantly different from the NPS group fibrin density of 500 ppm (p < 0.05) but not significantly different from the fibrin density using 250 ppm NPS (p > 0.05) Fibrin density in the group using 250 ppm NPS was significantly different from fibrin density using 500 ppm NPS (p < 0.05).

4. Discussion

The coagulation cascade consists of intrinsic and extrinsic components. The action of the extrinsic and intrinsic pathways produces thrombin, which in turn converts fibrinogen to fibrin. Fibrin is in the form of long fibers that are insoluble so that they will stick to the platelet collection to form structures such as nets. Fibrin fiber is sticky so it will collect platelets, red blood cells and passing white blood cells. After the wound is closed properly, a signal will be given which makes the blood clotting process stop. The process of platelets working in forming a wound blockage consists of several stages. The first stage is platelet adhesion, which is the attachment between platelets with endothelial tissue and injured tissue so that the wound is closed in the blood vessels. This attachment process will make the interaction between the surface of the platelets with the injured tissue increase the platelet adhesion and call other coagulation factors. The second stage is platelet aggregation, which is the ability of platelets to attach to each other to form blockages, platelets attached to injured tissue during the adhesion process will make other platelets attached to it so that the blockage closes the wound. But the formation of this blockage should not be excessive, because it will be dangerous and cause blockage of all blood vessels. The third stage is the release of platelets, which is the reaction to form a stable platelet coagulation. This process is triggered by the release of platelet granule contents, including ADP, collagen, epinephrine, etc., this release makes platelets change from disk to round shape. The last stage is platelet fusion, platelet fusion is a combined platelet reaction that is irreversible, this process is triggered due to high levels of ADP and other components that come out due to release reactions. The composition of fibrin will strengthen the new tissue formed in the wound area, and this platelet fusion is irreversible (cannot be returned).

In the present study Based on the results of the advanced Mann Whitney test on the results of fibrin density, the fibrin density of the control group was significantly different from the 500 ppm NPS group (p < 0.05), but not significantly different from the 250 ppm SNP group (p > 0.05). Fibrin density in the 250 ppm NPS group was significantly different from the 500 ppm NPS group. This was in accordance with the literature submitted. Jun et al. (2011) demonstrated that NPS induces coagulant activation by increasing intracellular calcium and increasing platelet aggregation. The use of silver nanoparticles with levels of 25 micrograms / ml by Laloy et al. (2014) did not affect platelet aggregation, while a concentration of 50 micrograms / ml showed an increase in platelet adhesion and a procoagulant effect. The other study from Choi et al. (2011) demonstrated that aqueous nanoparticle preparations released significantly more silver ions than micronsized particles, which correlated with increased hemolysis. Although significant size changes occurred to the silver particles due to interaction with media components, the
higher level of in vitro hemolysis observed with nanoparticles compared with micron-sized particles may be related to their greater surface area, increased silver ion release, and direct interaction with Red Blood Cells.  

5. Conclusion

As a result of the study we found 500 ppm NPS gel administration can increase fibrin density in hemostasis after tooth extraction in Sprague dawley rats, NPS Gel could be considered as an hemostatic agent due to the post extraction bleeding therapy.

6. Acknowledgement

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References


