Effectivity of ozone water application on the socket after posterior teeth extraction to accelerate clotting time phase

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ABSTRACT
After tooth extraction, dentists always place sterile tampons on the extracted socket to stop bleeding, to prevent infection and to reduce patients’ discomfort. Ozone is a biocompatible, bactericidal gas that can accelerate the healing of wounded soft tissue. In addition, it comes up with hemostasis effect to stop bleeding and may accelerate the clotting time and wound healing. Another advantage of ozone therapy is that it can be treated without making any trauma. The purpose of this research is to evaluate the effectivity of ozone water application in the socket to accelerate clotting time phase after posterior teeth extraction in 3 minutes, 6 minutes, and 10 minutes. This research was clinical experimental research with post-test only control group design. The research sampling was done through non-probability sampling. Then, sample was divided into two groups, each of which consisted of 15 patients treated with ozone tampons and 15 patients treated with sterile tampons after tooth extraction. Data were analyzed using chi-square test, and results showed a significant difference between the two groups. Ozone tampons show better result in accelerating clotting time after posterior teeth extraction than sterile tampons.

Keywords: clotting time; ozone; wound healing

INTRODUCTION
Tooth extraction is one of the widely practiced dental treatments in Indonesia. Tooth extraction is a process for removing a tooth from alveoli, since the tooth can no longer be restored anymore. Tooth extraction medically aims to prevent further complications in the future.1

Wound healing is a complex cellular and biochemical response to restore tissue integrity and functional capacity after injury. There are various intrinsic and extrinsic factors that can inhibit or accelerate the wound healing process. Intrinsic factor are factors that originates from the cell itself, such as the ability of fibroblast cells to divide, while extrinsic factors are factors that originate outside the cell that affect the healing process, such as location of the wound, size of trauma, temperature, nutrition, and hormonal factors.2

The best way to optimize health is by oxygenating each cell in our body. The more oxygen we have in our system, the more energy we produce and more efficiently we can eliminate waste in the body.3

Ozone is used in large quantities into the body so that it is unbound and circulating free singlet oxygen molecule. This molecule will invade all immature cells and defects that are foreign to the body, such as viruses, bacteria, fungi, etc as a way to improve the stability of normal cells. Ozone has several therapeutic effects in wound healing because of its new oxygen-releasing properties, which have been shown to have bactericidal ability and to stimulate antioxidants.3

Ozone is proven to be biocompatible, can improve the healing of soft tissues, and can be used in various aspects in dentistry field. The advantage of ozone therapy is that it do not cause any trauma.4 The aim of this research was to determine the effectivity of ozone water application on tampons to accelerate clotting time phase after posterior teeth extraction in 3 minutes, 6 minutes, and 10 minutes.
MATERIALS AND METHODS

This research used clinical experimental method with post-test only control group design. Research was done from April 2018 to June 2018, in Department of Oral and Maxillofacial Surgery with ethical approval No.504/TGL/KEPK.FK USU-RSUP HAM/2018. It involved 30 patients, divided into two groups, as the research population to carry out simple extraction in their posterior teeth. The first group was treatment group with ozone tampons applications into the socket, while the second was control group with sterile tampons application. The sampling method used was non-probability sampling method.

This study applied inclusion and exclusion criteria. Inclusion criteria included: posterior tooth extraction patients in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry University of Sumatera Utara, patients who did not have systemic disease or pregnant women and patients willing to participate in this study. While the exclusion criteria included: patients extracting anterior teeth, patients with complicated impacted dental surgery, and patients not willing to participate in this study. Criteria for assessing the formation of coagulation were observed after tooth extraction was carried out in normal coagulation time around 6-10 minutes.

Ozone water was made using Ozone maker. Ozone levels of 120-160μg can increase the healing effect of fibrinogen.9 This research used 133μg ozone in 2 ml water and it was injected into tampons for treatment group. Then, patients’ teeth were extracted according to the extraction procedure and tampons were placed in the socket according to their groups. The observation was made after three minutes, six minutes, and ten minutes. Data were analyzed using chi-square test with SPSS Statistic version 22 software.

RESULTS

Clot observation on tooth socket after 3 minutes, 6 minutes, and 10 minutes showed that there was a significant difference in clot formation (Figure 1). Chi-square test on three minutes showed that 40% of patients given ozone tampons after posterior teeth extraction had good results, in which there was blood coagulation seen in the socket three minutes post extraction. Meantime, the group with sterile tampons did not show any formation of blood coagulation. Based on the chi-square results, the p-value was 0.017 (p<0.05), indicating a significant difference between the effectivity of blood coagulation with ozone tampons and sterile tampons (Table 1).

Chi-square test on six minutes showed that 93.3% of patients given ozone tampons had good results, in which there was blood coagulation seen in the socket six minutes post extraction. However, the group with sterile tampons did not show any formation of blood. Based on the chi-square results, the p-value was 0.000001 (p<0.05), indicating a significant difference between the effectivity of blood coagulation with ozone tampons and sterile tampons (Table 2).

Chi-square test on ten minutes showed that all patients given ozone tampons had good results, in which there was blood coagulation seen in the

![Figure 1. Socket condition in three minutes (A), six minutes (B), and ten minutes (C) after ozone tampons application](image)
socket six minutes post extraction. However, only 40% of blood coagulation form on the group given sterile tampons. Based on the chi-square results, the p-value was 0.001 (p<0.05), indicating a significant difference between the effectivity of blood coagulation with ozone tampons and sterile tampons (Table 3).

**DISCUSSION**

Based on the results of the study, 40% of patients treated using ozone tampons on posterior (teeth) after extraction successfully formed coagulation in the 3rd minute, 93.3% of patients in the 6th minute, and 100% of patients in the 10th minute. While 0% of patients treated using sterile tampons on posterior (teeth) after extraction successfully formed coagulation in the 3rd minute, 0% of patients in the 6th minute, and 40% of patients in the 10th minute. From these results, we can conclude that there was a significant difference in effectivity between ozone tampons and sterile tampons.

Blood clots are hemostatic processes that involve cellular interactions between red blood cells, white blood cells, platelets and acellular components (plasma proteins) from the blood. Blood clot is formed from fibrin threads, which are interwoven in all directions with blood cells, platelets, and plasma in it. Fibrin threads are also attached to the surface of damaged blood vessels. Therefore, blood clots seal the ruptured blood vessels, so that they can prevent further blood loss.

Clotting time is the time blood takes to clot. Blood coagulation is a hemostasis process that prevents blood loss after the damage to blood vessels. Normal blood clots will shrink to around 40% of the original volume within 24 hours. Clotting time varies greatly. Normally formation of blood clot is 6-10 minutes. The test using ozone reveals that it has the significant effect of accelerating clotting time, since the formation of blood clots is seen in the 3rd minute. Ozone has been used successfully in the treatment of various diseases for more than a hundred years. Ozone has several therapeutic effects in wound healing because of its new oxygen-releasing properties, which have been shown to have bactericidal and antioxidants inducing effects. This is in accordance with ozone properties which are known to have hemostatic and anti-hypoxic effects. In addition, ozone can increase oxygen supply in tissues, causing a rapid healing process. Ozone can also reduce healing time after extraction by forming a pseudo-membrane above the socket and protecting it from physical and mechanical disruption.

In terms of the wound, hypoxic tissue is more easily infected leading to poor healing process.

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**Table 1. Effect of ozone tampons and sterile tampons three minutes after application**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood clot formed (%)</th>
<th>Blood clot didn’t formed (%)</th>
<th>Statistical analysis results (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone tampons</td>
<td>40</td>
<td>60</td>
<td>p = 0.017</td>
</tr>
<tr>
<td>Sterile tampons</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Effect of ozone tampons and sterile tampons six minutes after application**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood clot formed (%)</th>
<th>Blood clot didn’t formed (%)</th>
<th>Statistical analysis results (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone tampons</td>
<td>93.3</td>
<td>6.7</td>
<td>p = 0.000001</td>
</tr>
<tr>
<td>Sterile tampons</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Effect of ozone tampons and sterile tampons ten minutes after application**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood clot formed (%)</th>
<th>Blood clot didn’t formed (%)</th>
<th>Statistical analysis results (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone tampons</td>
<td>100</td>
<td>0</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Sterile tampons</td>
<td>40</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
because of the low proliferation of leukocytes and fibroblasts due to hypoxic tissue. One of the ozone mechanisms in wound healing is anti-hypoxia, where ozon produces an increase in \( \text{PO}_2 \) in the tissue and increases the transport of oxygen in the blood, which results in changes in the cell metabolism, activation of aerobics process (glycolysis, krebs cycle, B-oxidation of fatty acids) and use of the energy sources. Ozon acts as super oxygenator that carries oxygen to the tissues and helps the body in the natural healing process.10,11,12

Like other gases, ozone must be dissolved in water to interact with organic substrates. In the blood, ozone dissolves in plasma and instantly decomposes in the Reactive Oxygen Species (ROS) cascade, including hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), superoxide anion (\( \text{O}_2^- \)), hydroxyl radical (\( \text{HO} \)) and hypochloric acid (\( \text{HClO} \)). Repair or elimination with metabolic processes and or excretion is the main response of living organisms to damage that depends on ROS to biomolecules.11

Ozone therapy is reported to increase blood oxygen saturation, increase blood circulation, activate erythrocyte metabolism, increase tissue oxygenation and oxygen supply, and restore cell function, effectively increasing oxygen metabolism. Ozone is also reported to increase arterial and venous blood flow, increase erythrocyte elasticity, increase the ability of blood to pass through capillaries, and consequently increase oxygen supply to all organ systems. Ozone also reduces platelet aggregation and promotes the formation of \( \text{H}_2\text{O}_2 \) at the location of the thrombus, which damages thrombosis and cause thrombus disintegration.13

Ozone oxidation is chemically known to produce \( \text{H}_2\text{O}_2 \), which enters cells has and causes various effects. In red blood cells, it facilitates the temporary release of oxygen, and in leukocytes and endothelial cells, it can stimulate the production of interleukin, interferon, growth factors and nitric oxide. In platelets, it supports the release of growth factors in other cell types.14

Because of its unstable form, ozone measurements must be carried out in place. Ozone stored in a closed container in the refrigerator will last longer and prevent gas loss compared to that at room temperature.15 After being prepared for less than 1 hour, only half of the mixture is ozone and the other half turns into oxygen.4

High voltage electricity is widely used in industry and the Dielectric Barrier Discharge (DHF) method is considered the most efficient way to produce ozone. The process of forming ozone water can be produced alone using a high voltage ozone generator, where oxygen in the air will be converted to ozone.16 Ozone water levels of 120-160 \( \mu \text{g} \) can increase the healing effect of fibrinogen. To fulfill the levels of ozone gas in water, the ozonation period depends on the volume of the water and the gas flow.17

The production process is to fill the glass beaker with plain water and to insert condenser hose into it from a device that has an oxygen-converting component to ozone. Then, we should turn on the appliance, and set the required time to convert water into ozone water and finally to obtain ozonated water.16

**CONCLUSION**

Based on this research, it can be concluded that ozone tampons application on the socket after tooth extraction can accelerate clotting time by oxygenation using ozone rather than sterile tampons. Application of ozone tampons were effective in accelerates clotting time. In this study, the tooth that became the object of the research were not differentiated based on their diagnosis. Thus, it is advised that further studies discussed this matter.

**REFERENCES**
