Original Article

The effect of agitation techniques with increasing natrium hypochlorite temperature on bovine pulp tissue dissolution

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Abstract

Background: Root canal irrigation is one of the most determining factors that affect the success of a root canal treatment. Natrium hypochlorite (NaOCl) is the most commonly used root canal irrigants, which, in high concentrations, increases the risk of irritation to the periradicular tissues and oral mucosa if extrusion occurs. Low concentration NaOCl with increased temperature and agitation techniques could reduce the risk of irritation. **Objective:** To evaluate the effect of agitation techniques with the increased temperature of natrium hypochlorite on bovine pulp tissue dissolution capacity. **Method:** Twenty-five pulp tissues of bovine mandibular incisors (n = 5 per group) were weighed and then placed individually in Eppendorf test tubes containing the following irrigants: NaCl 0.9%; NaOCl 5% at 25°C; NaOCl 5% at 60°C. Group 1 NaCl 0.9% received no further treatment. Group 2 NaOCl 5% at 25°C agitated with sonic. Group 3 NaOCl 5% at 60°C agitated with sonic. Group 4 NaOCl 5% at 25°C agitated with ultrasonic. Lastly, Group 5 NaOCl 5% at 60°C agitated with ultrasonic. Dissolution speed was calculated by dividing the difference between initial pulp weight and after-treatment pulp weight (mg) by the period of time (sec). **Result:** NaOCl 5% at 60°C agitated with the ultrasonic group and showed the best result at dissolving pulp tissue. **Conclusion:** Increased temperature of natrium hypochlorite showed no significant difference toward bovine pulp tissue dissolution, while sonic and ultrasonic agitation techniques showed a significant difference toward bovine pulp tissue dissolution.

Keywords: *NaOCl, agitation techniques, temperature, tissue dissolution capacity*

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Background

Root canal treatment is a treatment aimed at teeth with a microorganism infected pulp, either on a vital tooth or non-vital tooth.[1] The success of the root canal treatment is directly tied to the root preparation, which involves cleaning and shaping the root canal, irrigation, which involves the cleaning of necrotic tissue and dentin debris that mechanical instruments cannot reach, and also obturation, which involves the filling of the prepared root canal.[2–4]

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Irrigation is the key to the success of a root canal treatment, because it is the only way to reach the wall area of the root canal that cannot be reached during the mechanical preparation phase.[3]

Natrium Hypochlorite solution (NaOCl) is an irrigation solution that is most commonly used and the most recommended in root canal treatment because of its high antimicrobial activity and capacity to dissolve organic tissues.[5] The efficacy of NaOCl in dissolving tissue is affected by several factors, which is concentration, temperature, volume, contact time with the tissue, agitation, and effective refreshment.[6]

The usage of NaOCl at a high concentration as an irrigant in root canal treatment will increase its antimicrobial properties; however, it will also increase its toxicity.[5] The capability of NaOCl to dissolve tissue can be maximized with refreshing and thorough agitation3. Among the tools of agitation, sonic and ultrasonic equipment has been used. Sonic activation results in mechanical agitation, especially on the tip of the file, and the activation ultrasonic equipment results in an acoustic streaming and cavitation7. Sonic agitation works on frequencies between 190 Hz–6kHz, and ultrasonic agitation works on frequencies between 25–30 kHz.[8]

According to previous research, the NaOCl group that works at 25°C can dissolve 96.94% of tissue with an average time of 985 seconds. The reaction that happens is slower in comparison with NaOCl at 60°C, where it can dissolve 98.31% of tissue at the rate of 389 seconds or 7 minutes.9 This proves that the temperature increase in NaOCl influences the ability to dissolve tissue.[10]

Research performed by Stoijicic et al. shows a significantly higher tissue dissolution time after a simultaneous action of temperature and agitation in comparison with one simultaneous action6; therefore, if NaOCl is used in maximum concentration and maximum temperature and continously moved by agitation, it will increase its ability to dissolve pulp tissue and reduce the risk of complication due to root canal treatment. The aim of this study was to evaluate the effect of agitation techniques with the increased temperature of natrium hypochlorite on bovine pulp tissue dissolution capacity.

MATERIALS AND METHODS

The sample in this research study uses pulp tissue from the mandibular incisor of the bovine. Sample retrieval is done immediately after the bovine is slaughtered at the Cakung Slaughterhouse, North Jakarta. The lower bovine incisor is prepared as a negative control (NaCl 0.9%) and NaOCl solution with 5% concentration as the active group, and it is continued with a 25°C and 60°C water bath. The pulp tissue is inserted in an Eppendorf tube that is filled with NaCl 0.9%, with a volume of 1 ml using tweezers, and the initial mass is weighed. Observation is performed to observe the changes that happen to the sample for 7 minutes.9 Then, they were extracted, and an outline was created using a mini grinder with a cutting disk with 2.2 cm diameter. An outline is created without contact with the pulp tissue.

After the outline was created using a cutting disk (Fig 1), the division of the tooth was performed using chisel and mallet. The pulp was extracted as a whole, and then, it was inserted on the sample bottle that was filled with fixation fluid Phosphate Buffered Saline (PBS) and frozen at a temperature of -22°C to prevent tissue damage. Sample mass equalization was performed by drying the pulp tissues and whatman filtration paper no. 1 for 5 minutes, and the pulp tissue was weighed and recorded. The sample was cut using scalpel and blade no 11, until a mass of ±150–170 mg is achieved, and then, the pulp tissue is placed in the fixation fluid PBS and frozen with a temperature of -22°C to prevent tissue damage.

Figure 1. Bovine Pulp Tissue Extraction

Research was performed in a laboratorium by increasing the sample temperature with room temperature (25°C). Several experimental methods used for measuring the dissolution capacity, such as measuring the time of tissue dissolution and visual examination. Afterwards, the remaining tissue that remained undissolved was weighed. As reported in other studies, the initial and final weights of the sample were compared.[11]

Eppendorf tube that was filled with 1 ml 5% NaOCl solution was inserted in a water bath by dividing into 2 treatment groups with temperatures of 25°C and 60°C, and the temperature of the NaOCl solution within the Eppendorf tube was conditioned with temperatures of 25°C and 60°C, and the solution was measured with a thermometer. After, the 5% NaOCl solution with temperatures of 25°C and 60°C pulp tissue was inserted within the Eppendorf tube using tweezers, and the initial mass was weighed. Samples of each temperatures were divided into 2 groups, which uses sonic agitator using EndoActivator® (2-3kHz) and Irrisafe® for ultrasonic (30kHz). Observational changes were performed on the sample for 7 minutes, according to previous study used stopwatch.[9] The quantification of pulp tissue dissolution has been carried out in several ways, for example by the evaluation of weight loss.[12,13] The remainder of the tissue within the Eppendorf tube that was unable to be dissolved dried using whatman filtration paper no.1 and weighed.

Research documentation was performed using Nikon DSLR D7000 camera using Nikkor 105 mm f/2,8 micro lens. Lighting was performed using softbox and light emitting diode (LED) lamps, using 5,500K temperature in order to get constant lighting.

Table 1. Pulp Mass Loss in NaCl 0.9% and NaOCl 5% Solution at Different Temperature with Different Agitation Techniques

Pulp Mass (mg)										
	NaCl 0.9%		NaOCl 5% Sonic Agitation				NaOCl 5% Ultrasonic Agitation			
			25°C		60°C		25°C		60°C	
No	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	158	103	179	58.2	176	39.1	151	42,7	172	9.2
2	167	122	169	53.1	172	60	172	15,4	172	7.9
3	170	143	163	51.2	175	11.2	174	1,1	178	8.4
4	173	113	173	68.1	169	73	169	18,1	179	2.3
5	163	147	171	51.2	157	50.1	154	15,7	153	5.1
$\bar{X}$	166.2	125.6	171	56.3	169.8	46.6	164	18.6	170.8	6.5

Table 2. Mean dissolution speed (mg/s) within the groups (Tissue Dissolution ± Standard Deviation).

Sample Group	Treatment	Mass Difference (mg)	Time (second)	Dissolution speed (mg/s) ($\bar{X}$± SD)
1	NaCl 0,9%	40.60	420	0.09 ± 0.04
2	NaOCl 5% 25°C Sonic Agitation	114.46	420	0.26 ± 0.01
3	NaOCl 5% 60°C Sonic Agitation	123.12	420	0.28 ± 0.06
4	NaOCl 5% 25°C Ultrasonic Agitation	145.40	420	0.34 ± 0.06
5	NaOCl 5% 60°C Ultrasonic Agitation	165.62	420	0.39 ± 0.02

RESULT

A comparison of mass after and before treatment with each group can be seen on table 1. The dissolving rate of the pulp tissue can be viewed on Table 2. The normality test of this research is performed using Shapiro-Wilk tests, in which the p value is $p > 0.05$, and the data distribution is considered to be normal. The homogenity test of this research study was performed using Levene's Test of Equality of Error Variances, in which the value is $p > 0,05$ and data distribution was considered to be homogenous. Two-Way ANOVA tests showed no significant differences between each group of increasing temperatures in dissolving rate; however, it showed significant differences in groups using sonic and ultrasonic agitation methods. Post-hoc Tukey tests showed that 5% NaOCl that was agitated using Irrisafe® ultrasonic tools exhibited the best capability to dissolve pulp tissue compared to 0.9% NaCl without agitation and 5% NaOCl that were agitated using EndoActivator® sonic appliances. 5% NaOCl that were agitated using EndoActivator® sonic appliances showed better capability to dissolve

pulp tissue compared to 0.9% NaCl without agitation (Table 3).

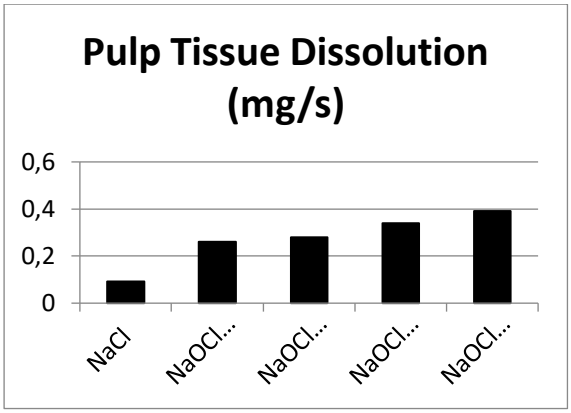

Figure 2. Pulp Tissue Dissolution Diagram

Table 3. Post Hoc Test

Agitation Technique		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Without Agitation	Sonic	-.1850*	.02606	.000	-.2509	-.1191
	Ultrasonic	-.2730*	.02606	.000	-.3389	-.2071
Sonic	Without Agitation	.1850*	.02606	.000	.1191	.2509
	Ultrasonic	-.0880*	.02128	.001	-.1418	-.0342
Ultrasonic	Without Agitation	.2730*	.02606	.000	.2071	.3389
	Sonic	.0880*	.02128	.001	.0342	.1418

DISCUSSION

Tissue dissolution outcome during the process of endodontic irrigation have tested. However, the capacity and efficiency of organic tissue degradation processes are related to the type, concentration, and how the irrigation solution is agitated.[11] Natrium hypochlorite is the main endodontic irrigant used worldwide, the concentrations ranging from 0.5% to 6% due to its antimicrobial and tissue-dissolving properties.[14] Research by Estrela et. al declared that the dissolving rate of pulp tissue can be seen by bubbles that were created by the saponification reaction. The saponification reaction happens when the natrium hypcholorite comes in contact with the pulp tissues and destroys the fatty acid structures, which results in soap and glicerol.[15] The bubbles that were formed were caused by oxidation reaction and hydrolisis that happens between NaOCl as an oxidator and strong hydrolisis.[16]

In this study, it was shown that temperatures increase of natrium hypochlorite have no significant difference in dissolving pulp tissue. Pulp tissue dissolve rate using natrium hypochlorite was influenced with the chlorine solution within the solution itself.[17] The increase of temperature of NaOCl above 25°C causes the chlorine content to demonstrate a tendency to evaporate.18 The decrease of chlorine solution from 5% NaOCl solution happens significantly at a temperature of 24°C. According to research by Cunningham and Balekjian (1980), data show a decrease in chlorine content from the NaOCl solution at a temperature of 37°C with a concentration of 2.5% as much as 4%; however, on 5% concentration as much as 9.5%.19 These factors can be linked with one of the factors that causes a temperature increase of NaOCl solution, which shows no significant difference to the dissolving rate of the pulp tissue.

According to the result of pulp dissolution by counted pulp mass and dissolution time, ultrasonic agitation shown significant difference compared with sonic agitation. Ultrasonic is simple and cost-effective irrigant activation method, works by delierivering an acoustic stream that creates shear stress sufficient enough to dislodge the debris of instrumented root canals.[20] Ultrasonic activation of NaOCl has been suggested because it is claimed to accelerate chemical reactions and promote superior cleaning action. Ultrasonic agitation methods results in vibration that causes in a current change in the NaOCl solution that occurs constantly. The vibration produced by the ultrasound devices provides continous flow of irrigation solution in root canal.[21] Those vibrations were directly connected with the acoustic streaming and cavitation that facilitates the increase of capability to clean debris and dissolve the pulp tissue within the root canal. Possible reason of ultrasonic agitation mechanism is the collapse of bubbles during transient cavitation that produces a pressure-vacuum effect, which sucks the canal content to the inside rather than pushing it further in the canal. This will be followed by diffusion of the irrigant in the main canal to substitute the space created. Another possibility is that the streaming around the activated file because of the cohesion between fluid particles inside the accessory canal and the irrigant in the main canal sucks the content of the accessory canals into the main canal with fluid flow toward the main canal.22 The agitation frequency ultrasonic (25–30kHz) is higher in comparison with sonic agitation (190Hz–6kHz). Those occurences can be connected with the speed of NaOCl solution and the friction between the irrigation solution and root canal, which increases the capability to clean debris and increases the dissolving rate of pulp tissue in comparison with sonic agitation.[14] Repetition several cycles of agitating the irrigant solution during chemomechanical preparation can promote the complete dissolution of the pulp tissue.[11]

CONCLUSION

No significant difference was found between the temperature increase of natrium hypochlorite between bovine pulp tissue; however, significant

differences were found between sonic agitation and ultrasonic agitation compared to the dissolving rate of bovine pulp tissue.

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