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The Effects of Sodium Hypochlorite on Organic Matters: Influences of Concentration, Renewal Frequency and Contact Area

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ABSTRACT

Introduction: Sodium hypochlorite (NaOCl) reacts mainly with proteins and its effectiveness depends on the substances chemical reactivity. It has been reported that volume, concentration, renewal, time, temperature and contact area affect the diffusion of NaOCl in the root canal. However, the relationship between some of these factors is not clear. The purpose of this study was to test the effect of volume, contact area, concentration and renewal frequency of 2.5% and 9.8% NaOCl solutions on their organic matter dissolving-capacity. **Methods and Materials:** Pieces of gelatine (18% w/v) with standardized weight, form and structure were either fully or partially exposed to a 2.5% or 9.8% NaOCl solution. In three successive studies, biological dissolution-capacity of NaOCl was tested under different conditions. In experiment 1 the effect of volume/time, in experiment 2 the time/concentration/renewal frequency and in experiment 3 the contact area/renewal frequency/concentration/time of 2.5% or 9.8% NaOCl solutions on dissolving-capacity of organic matter were studied. The weight loss of gelatine pieces over time was registered. The non-parametric tests of Mann-Whitney and Kruskal-Wallis at the 5% threshold were used for statistical analysis. **Results:** The differences between the two concentrations of NaOCl solution (2.5% and 9.8%) are statistically significant in the effects of different volumes on total dissolution time ($P<0.05$). Differences in weight loss according to the concentration of the NaOCl solution used (2.5% or 9.8%) were significant after 2 min of contact time ($P<0.05$). Differences in weight loss between the model and the tube are significant ($P<0.05$) when the solution is repeated every 30 sec and every 1 min after 2 min of contact. **Conclusion:** This *in vitro* study showed that using a more concentrated NaOCl solution would certainly improve the endodontic disinfection, but the biological risk in case of apical extrusion should be considered.

Keywords: Concentration; Dosage; Gelatine; Root Canal Irrigant; Sodium Hypochlorite

Introduction

Sodium hypochlorite (NaOCl) is the main irrigant used in endodontics because of its antimicrobial activity, tissue dissolving-capacity and, when properly used, the absence of clinical toxicity [1, 2]. This tissue dissolving-capacity is crucial because the mechanical instrumentation of the root canal cannot remove pulpal tissue from oval extensions, isthmuses or canal irregularities [3]. However, the tissue dissolving-capacity of a 5% or 6% NaOCl solution alone is not sufficient for *in vivo* removal of all tissues from isthmuses or the apical root canal [4-6].

The chemical effect of NaOCl can be influenced by its concentration, the contact area substrate-irrigant, exposure time, pH, temperature, interaction with other chemicals, irrigant activation, its volume, and are regulated by its convection or diffusion [7]. The diffusion is the random movement of individual particles in the fluid. Its effects increase as the diffusion distance decreases and the concentration gradient increases. In literature, tissue dissolving-capacity is often evaluated when the tissue is in full contact with a surplus of NaOCl, facilitating an optimal diffusion [8, 9]. Unfortunately, this is not the clinical reality where a complicated anatomy of the root canal system significantly restricts the NaOCl-tissue contact area, thus hampering the diffusion.

Figure 1. Test tube with gelatin block in complete immersion can be seen

This was confirmed by the results of *in vivo* study by Rosenfeld *et al.* [6] and the *in vitro* study by Moorer and Wesselink [10]. However, none of these authors did correlate the effect of the concentration with the renewal frequency of the irrigant.

The purpose of this study was to test the influence of concentration, renewal frequency, exposure time and the contact area on the organic matter dissolving capacity of NaOCl.

Materials and Methods

To evaluate the organic matter dissolving-capacity of NaOCI, gelatine in an 18% w/v concentration 18% w/v concentration (Difco^{tm} Becton, Dickinson and Company, Sparks, USA) was used as an organic protein collagen with standardized weight, form and structure. This holder was obtained by mixing 9 gr of gelatine powder (Technical PROLABO, VWR, Fontenay-sous-bois, France) with 50 mL of water. To allow its visualization during the experiment, four drops of methylene blue were added to the mixture, which was then heated under constant agitation. A plastic holder was used as a mold to make standardized parallelepiped blocks of 50 mg gelatine. The weight was verified using a balance (Ohaus Explorer, OHAUS, Parsippany, USA) with high precision. These blocks were used for all the tests.

NaOCI in berlingots at 36 chlorometric degree (9.8% Chlorine) for household use was used. Prior to its contact with gelatine, the concentration of NaOCl solutions was assessed by measuring the amount of total chlorine (% w/v) in the solution using a standard iodine-thiosulfate titration method [11]. The stability of NaOCl solutions is checked before each experiment by measuring the pH to check the stability of the active chlorine in contact with gelatine. Three studies were conducted.

Volume-time (Total dissolution time)

First, the relation volume-time that is needed to completely dissolve the gelatine was tested. Blocks of gelatine were submerged in test tubes (Elleplast of 100 mm/16 mm with Red Tops (Plastic Test Tubes LTD, Crewe, UK)) containing 4, 3, 2 or 1 mL of 2.5% or 9.8% of NaOCl solution. The time needed to completely dissolve the gelatine was recorded. Gelatine is considered dissolved if no trace is visible with a 6.4 binocular magnifier (Leica, Wild M3, Wetzlar, Germany).

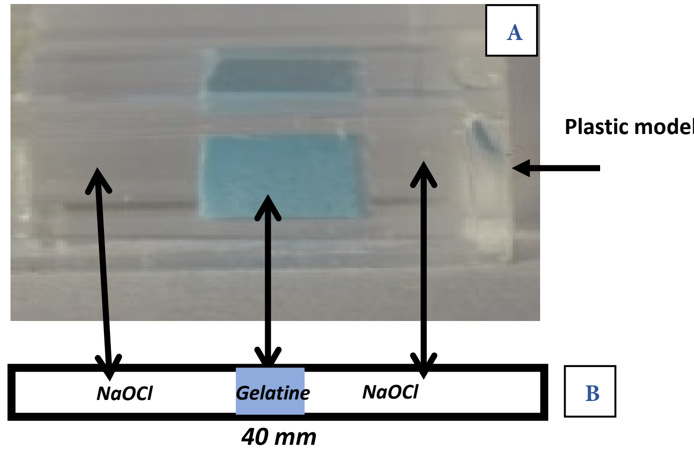

Figure 2. The gelatin block is in the middle of a plastic model; *A*) model photograph; *B*) model diagram

Dissolving capacity during full contact of NaOCl/gelatine: time/concentration/renewal frequency

The gelatine block was completely immersed in a test tube containing NaOCl (Figure 1). The dissolution-capacity of 3 mL of 2.5% and 9.8% NaOCl solution was tested.

The weight loss after 1, 2 or 3 min of contact with NaOCl without or with renewal every 30 or 60 sec was measured. For every test, a new block of gelatine was used. The weight was verified using a balance (Ohaus Explorer, OHAUS, Parsippany, USA).

Dissolving-capacity during restricted contact of NaOCl/gelatine: time/concentration/renewal frequency

All tests described in experiment 2 were repeated under the same conditions, except that blocks of gelatine were placed in a box of endodontic files cut out (L/l/h: 40 mm/3 mm/4 mm) so as to expose only two surfaces in contact with the NaOCl solution (Figure 2). Clinically, this situation simulates the typical anatomical situation of an isthmus.

All tests were performed in triplicate under same atmospheric conditions, NaOCl solutions were used at room temperature.

Statistical analysis

We used SPSS (Statistical Package for Social Science, SPSS, version 20.0, SPSS, Chicago, IL, USA) and for comparisons, the non-parametric tests of Mann-Whitney and Kruskal-Wallis at the 5% threshold were used.

Results

Experiment 1: *Volume-time*

The differences between the two concentrations of NaOCl solution (2.5% and 9.8%) are statistically significant in the effects of different volumes on total dissolution time ($P<0.05$). From 3 mL onwards, the volume/dissolution time ratios (≤47.3 min for 2.5% and ≤14.8 min for 9.8%) are similar to the clinical situation (duration of endodontic treatment) (Table 1).

Experiments 2 and 3: *Dissolving capacity during full and restricted contact of NaOCl/gelatine*

Differences in weight loss according to the concentration of the NaOCl solution used (2.5% or 9.8%) are significant from 2 min of contact time ($P<0.05$).

Differences in weight loss between the model and the tube are not significant ($P<0.05$) for each renewal mode after 1 min of contact.

Differences in weight loss between the model and the tube are significant ($P<0.05$) when the solution is repeated every 30 sec and every 1 min after 2 min of contact (Tables 2 and 3).

Table 1. Average time, in minutes, required for the complete dissolution of 5 mg of gelatine (18% w/v) with different volumes of NaOCl solution ($P<0.05$)

Volume of NaOCl solution in mL	Dissolution time in minute for 2.5% NaOCl	Dissolution time in minute for 9.8% NaOCl
1	> 120	> 120
2	106±59.05	15.25±5.02
3	47.30±58.09	14.88±23.33
4	47.10±3.10	10.75±5.25

Table 2. Mean weight loss (in mg) according to the NaOCl solution concentration and contact time

Contact time	Model type	Renewal mode	NaOCl 2.5%	NaOCl 9.8%	*P*-value
1 min	Model	Ren 0	0.1±0	1.2±0.2	0.057
		Ren 30s	0.9±0.2	7.4±1.4	
		Ren 1min	0.9±0.3	4.2±2.2	
	Tube	Ren 0	3.5±2.4	16.3±2	
		Ren 30s	6±2	17.4±2.5	
		Ren 1min	4.8±2.3	11.4±4.2	
2 min	Model	Ren0	2.1±0.2	7.6±2	0.004
		Ren 30s	4.3±1	16.6±2.6	
		Ren 1min	3.4±1.8	11.6±2	
	Tube	Ren 0	6,6±1	32.3±4	
		Ren 30s	10±1.8	35.4±4.2	
		Ren 1min	6.7±2	31.5±2.3	
3 min	Model	Ren 0	1.7±0.1	35.7±4.2	0.009
		Ren 30s	4.6±1.9	19.6±3	
		Ren 1min	2.8±1.3	13.3±3	
	Tube	Ren 0	7.9±1.8	12.5±1.4	
		Ren 30s	15.8±1.3	44±3.6	
		Ren 1min	9.1±3	40.5±4	

Ren 0=no renewal, Ren 30s= renewal every 30 s, Ren 1min = renewal every minute

Table 3. Average weight loss: comparison between the model and the tube according to renewal modes of the NaOCl solution after 1 or 2 min of contact

Contact time	Model types and Ren NaOCl	2.5% NaOCl	9.8% NaOCl
1 min	Model Ren 0	0.1±0	1.2±0.2
	Tube Ren 0	3.5±2.4	16.3±2
	P-value	0.333	0.333
	Model Ren 30s	0.9±2	7.4±1.4
	Tube Ren 30s	6±2	17.4±2.5
	P-value	0.333	0.333
2 min	Model Ren 0	2.1±0.2	7.6±2
	Tube Ren 0	6.6±1	32.3±4
	P-value	0.333	0.3 33
	Model Ren 30s	4.3±1	16.6±2.6
	Tube Ren 30s	10±1.8	35.4±4.2
	P-value	0.029	0.029
	Model Ren 1min	3.4±1.8	11.6±2
	Tube Ren 1min	6.7±2	31.5±2.3
	P-value	0.029	0.029

Ren 0=no renewal, Ren 30s= renewal every 30 sec, Ren 1min= renewal every min

Discussion

This study aimed to test the influence of concentration, renewal frequency, contact time and contact zone on the organic matter dissolution ability of NaOCl.

NaOCl is the most commonly used root canal irrigation solution due to its many properties, such as organic tissue dissolving capacity, antibacterial effect and lubricant effect [10]. However, its most effective concentration is controversial. Recently, Zang *et al.* [12] showed that, in its solution form, NaOCl at 2.5% and 5.25% completely kills bacteria in 10 min unlike NaOCl gel.

The present experimental study has been limited to the proteolytic action (the tissue dissolving-capacity) in testing two concentrations: 2.5% and 9.8%. The choice of the 9.8% NaOCl solution is based on the fact that the tissue dissolving-capacity of a 5% or 6% NaOCl solution alone is not sufficient for *in vivo* removal of all tissues from isthmii or the apical root canal [4-6]. In addition, a recent study highlighted a difference between the concentration of sodium hypochlorite indicated by the manufacturer and the actual concentration of household solution used by most practitioners [13]. In addition, this concentration could decrease significantly before use, depending on conservation [13]. The use of an apparently higher concentration of NaOCl (9.8%) would compensate for this loss.

In order to counteract the difficulty of standardizing dental pulp identified by Clarkson *et al.* [14], gelatin blocks of standardized form, structure and weight were used as organic matter for the present study.

With regard to the time required for gelatin dissolution, with 1 mL of solution, whatever the NaOCl concentration, the total dissolution of the gelatin is obtained beyond 2 h. From 2 mL of NaOCl solution, a significant difference is observed between the 2.5% and 9.8% solutions. The 9.8% solution diluted the gelatin in 15.2 min while the 2.5% solution is still at 106 min. The 2.5% solution provide acceptable dissolution time of 47.3 min from 3 mL. This time remain unchanged with 4 mL of solution while, with the 9.8% solution of NaOCl, at this volume, there was a much faster dissolution. This shows that the volume of the solution affects its tissue dissolution-capacity. The dissolution of gelatine is proportional to the concentration of the NaOCl solution: a 9.8% solution dissolves gelatine more quickly than a 2.5% solution. This is consistent with the literature data that have already shown that the NaOCI dissolving-action depends on its active chlorine content [2, 7].

The concentration is an important modulator of the reaction between the NaOCl and the organic tissue because it positively influences the diffusion rate. Therefore, the 9.8% NaOCl solution reacts in a direct proportion with the 2.5% NaOCl solution, resulting in around five times more chlorine consumption [7] and organic matter dissolving-capacity as shown here. A 5% NaOCl solution should react accordingly.

Results of this study show that a restriction of contact zone or contact area significantly reduces the dissolution capacity of 2.5% and 9.8% NaOCl solutions. Indeed, the digestion is more rapid when the gelatine is completely immerged than in restricted contact. However, it was only after 2 min of total immersion that statistically significant differences were observed when the solution is repeated every 30 sec or every min.

These results also showed that concentration and diffusion distance influence the level of diffusion. The higher the concentration, the more efficient the dissolving-capacity even if the contact area is restricted. Although enough free chlorine was present in the container, it could not effectively diffuse in the gelatine. This indicates that the diffusion distance hampered the transportation of free chlorine and consequently the dissolution effectiveness. A thorough analysis of weight loss averages at 2 min of contact per category of NaOCl concentrations, between sample types and according to renewal method, reveals that, frequent renewal does not compensate for a lower NaOCl concentration. Indeed, the chemical reactivity and the diffusion rate of a higher concentration will always be higher, as mentioned by Macedo *et al.* [7].

Probably, there is a zone of partially altered substance between the non-affected gelatine and the NaOCl solution when the latter is in contact with the gelatine. This zone is easily overcome by the 9.8% NaOCl solution, but not by the 2.5% NaOCl solution. This could partly explain why it is difficult to chemically remove all organic tissues from locations with restricted contact with the irrigant like the apical portion of the root canal, isthmuses or oval extensions during irrigation, even when the NaOCl solution is frequently renewed [5, 6].

The chemical reaction efficiency also depends on the volume of the NaOCl solution in relation to the amount of organic matter. A root canal of size 30/0.06 with a length of 15 mm has a volume of 7.42 mm^3, 0,007 mL. Although generally, 2 mL of NaOCl solution is delivered after each instrumentation, only 0.007 mL is available for the chemical action. Furthermore, the ratio volume/contact area is even lower, because a relatively small volume is in direct contact with the tissue to be dissolved. Additionally, due to its chemical reaction with the organic matter, the prolonged contact with the later will inactivate the NaOCl [15], hence the importance of the recommended frequent renewal. In this study, 50 mg of gelatine fully immersed in 1 mL NaOCl solution could not be completely dissolved in a time frame approximating an endodontic treatment duration. This indicates that in addition to the concentration, the delivered volume of solution is equally important. 4 mL NaOCl solution was used for 50mg of organic matter which is equivalent to 0,087 mg for 0.007 mL of solution.

It has been reported that the (average) quantity of pulp tissue, including the pulp chamber of molars and premolars, is 41.1 mg [9]. The form, weight and structure, would influence the reaction of a substrate with a chemical product and are difficult to standardize for pulp tissue or other tissue substitutes [13]. Gelatine was used in this study to overcome these problems. According to Sirtes *et al.* [9], one min of irrigation with 5 mL of a 1% NaOCl solution dissolves around 30% of the pulp tissue. In this study, weight loss is insignificant after 1 min of contact in total immersion for 2.5% NaOCl solution with or without renewal. A significant weight loss occurred after 2 min, especially in total immersion where we have a weight loss of 20% when renewals were more frequently made. A much higher digestion marks the effect of increasing the NaOCl solution's concentration over time and with renewal, under the same conditions with the 9.8% solution. However, it should be noted that, under clinical conditions, due to its toxicity, recommended NaOCl concentrations should not exceed 6% [2]. Already at 5.25%, Karkehabadi *et al.* [16] have observed *in vitro* a cytotoxicity of NaOCl on laboratory fibroblastic cells.

Conclusion

The frequent renewal does not compensate for a lower concentration. Limited contact area does severely hamper the dissolving of the organic matter. By attention to these results, the diffusing rate of 9.8% NaOCl solution is five times higher than 2.5% NaOCl solution resulting in a better organic matter dissolving-capacity. Without omitting clinical practice ethics, in the final rinse, using a more concentrated solution could be considered to improve the success rate of endodontic treatment of infected canals since success in endodontics is essentially based on root canal system disinfection. Using a more concentrated NaOCl solution would certainly improve the endodontic disinfection, but the biological risk in case of apical extrusion should be considered.

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Conflict of Interest: 'None declared'.

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