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In Vitro Evaluation of Dynamic Viscosity, Surface Tension and Dentin Wettability of Silver Nanoparticles as an Irrigation Solution

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ABSTRACT

Introduction: The aim of this study was to evaluate dynamic viscosity, surface tension and dentin wettability of a newly introduced imidazolium-based silver nanoparticle solution (Im AgNP) in comparison with three common root canal irrigants. **Methods and Materials:** The irrigants were Im AgNPs at 5.7×10^{-8} mol/L^{-1}, 5.25% Sodium hypochlorite (NaOCl), 2% Chlorhexidine (CHX) and 17% Ethylenediaminetetraacetic acid (EDTA) and distilled water (control group). Dynamic viscosity was measured using rotational digital viscometer at 25, 37, 45 and 60°C. Surface tension was evaluated using dynamic contact angle analyzer at room temperature (25°C). Wettability was assessed by contact angle measurement for five groups of 10 dentin samples after each group was treated in each irrigant for 10 min. One-way ANOVA, and post hoc Tukey's test were used for statistical analysis. Significance was set at $P<0.05$. **Results:** Dynamic viscosity of all irrigants decreased as the temperature increased. 17% EDTA was the most viscous solution in all examined temperatures ($P<0.05$). Viscosity of Im AgNP solution at 25, 37 and 45°C was significantly lower than that of 17% EDTA and 5.25% NaOCl ($P<0.05$). Im AgNPs exhibited a higher surface tension than other irrigants except distilled water. The wettability of dentin increased when it was in contact with 2% CHX and 5.25% NaOCl while Im AgNPs decreased the wettability of dentin surfaces ($P<0.05$). **Conclusion:** Im AgNP irrigant has the potential to reach apical portions of root canals due to its lower viscosity compared to the other tested irrigants. However, it may not bring better penetration inside dentinal tubules because of its higher surface tension. Furthermore, Im AgNPs can influence physiochemical properties of dentin by decreasing its surface wettability.

Keywords: Irrigant; Silver Nano Particle; Surface Tension; Viscosity; Wettability

Introduction

Efficient disinfection of root canal system is highly dependent on the ability of irrigants to penetrate dentin and its tubules [1]. The capability of irrigants to access apical third of the root canal system as well as penetration to dentinal tubules mainly depends on the irrigants' characteristics such as dynamic viscosity and surface tension. Dynamic viscosity is the resistance exhibited by a liquid as it is being reshaped by shear or tensile stresses; hence, less viscosity allows the fluid to flow easier [2]. Surface tension is defined as the force amongst molecules which yields an affinity for the surface area of a liquid to decline [3]. The greater the surface tension is, the lesser the contact angle of a liquid with a surface [4, 5].

Irrigation solutions can affect surface properties of dentin such as wetting ability, which in turn can influence the adhesion of bacteria and regulate the interaction between dentin and restorative materials [6]. Furthermore, wettability has an essential role in developing a suitable contact time of an irrigant with dentinal walls. This property is strongly relevant to chemical composition, roughness, and hydration state of dentin and can be influenced by tubule density [7]. Wettability correlates with surface tension on ideal surfaces (chemically homogeneous, flat, nonreactive, undeformable, and not swollen by the wetting liquid) and consequently with the surface properties of dentin [8].

To date, there is no endodontic irrigant with all desired properties and therefore the need to search for new irrigants is still ongoing. Sodium hypochlorite (NaOCl) is the most common root canal irrigant with an outstanding antimicrobial efficacy against microorganisms together with its potential to remove organic components. Previous studies also revealed that NaOCl increase dentin wettability [9-11]. However, due to its high surface tension, it may fail to reach microorganisms in the depth of tubules [3]. Furthermore, the ineffectiveness of NaOCl and Ethylenediaminetetraacetic acid (EDTA) in dissolving smear layer in the apical third of root canals can attribute to their high dynamic viscosity [12].

In recent years, interest in the use of silver nanoparticles (AgNPs) as root canal irrigants or medicaments has developed in dentistry especially in endodontics [13-17]. These particles appear to prevent the development of resistance by the organisms because of their rapid reaction between the target cells and them [18-20]. They are highly active against bacteria while compatible to human cells [21-23]. In a recent research, Abbaszadegan *et al.* [16] demonstrated that the positively charged imidazolium-based silver nanoparticles (Im AgNPs) is a favorable disinfectant for endodontic purposes due to their high level of antibacterial activity against *Enterococcus faecalis* (*E. faecalis*) and cytocompatibility against fibroblast cells. Therefore, it is relevant to investigated more about other properties of this newly introduced AgNP solution relative to common endodontic irrigants [24]. As a result, this study was designed to assess dynamic viscosity, surface tension and dentin wetting ability of this solution compared to three commonly used endodontic irrigants.

Materials and Methods

In this *in vitro* study, the tested irrigants were: 17% EDTA (Cerkamed, Pawłowski, Poland), 2% Chlorhexidine (CHX) (Cerkamed, Pawłowski, Poland), 5.25% NaOCl (Cerkamed, Pawłowski, Poland) and Im AgNPs at 5.7×10^{-8} which were synthesized according to the protocol previously suggested [16]. Distilled water was also used as control group.

To assess the surface wettability, roots of 25 human caries free permanent anterior teeth were removed from cementoenamel junction and then sectioned longitudinally under water to gain 50 dentin slices. To remove surface scratches dentin samples were polished by 400-, 600-, 800 and 1200-gritt polishing papers and finally ultrasonicated in deionized water.

Dynamic viscosity was determined using a rotational digital viscometer (Brookfield LVDV-II PRO, Middleboro, USA). A proper spindle for low viscosity liquids at 75 rpm was selected and a fluid volume of 15 mL was used. The measurements were repeated 10 times for each sample group at 25, 37, 45 and 60°C. Measurements of surface tension were conducted using Cahn DCA-322 dynamic contact angle analyzer (Gibertini Elettronica s.r.l. 20026 Novate, Italy) following Wilhelmy plate technique at 25°C. In brief, a glass slide was immersed in 15 mL of the test irrigants at the constant speed of 50 mic/sec until at least 2 cm of the slide was immersed. The surface tension of each irrigant was obtained through the ratio between the force at zero depth of immersion and the sample perimeter. The surface tension was calculated and reported by the instrument's software. These measurements were repeated 10 times for each sample.

For water contact angle assessment, 50 dentin samples were randomly allocated into 5 groups of 10 samples and treated with the test irrigants for 10 mins. Then, they were rinsed copiously by the deionized and distilled water. Water contact angles (q) were measured by sessile drop technique on dentin surfaces by using a VCA Optima Surface Analysis System (AST Products, Billerica, MA, USA). In brief, two drops (0.0002 mL/drop) of distilled water were deposited on each dentin surface with a microsyringe and images were captured subsequently by a micro video system. The acquired images were evaluated for calculation of contact angles using VCA-2500XE software (AST Products, Billerica, MA, USA).

Table 1. Means (SD) of dynamic viscosity of different irrigants at different temperatures. Read vertically, different letters in each column indicate a statistically significant difference

Irrigant	Temperature			
	25° C	37° C	45° C	60° C
NaOCl	2.73 (0.32) a	1.85 (0.43) a	0.80 (0.02) a	0.67 (0.05) a
CHX	1.00 (0.01) b	0.75 (0.02) b	0.60 (0.03) a	0.48 (0.03) a
Ag NPs	0.90 (0.02) b	0.66 (0.32) b	0.57 (0.02) a	0.47 (0.03) a
EDTA	12.1 (0.09) c	8.99 (0.03) c	6.13 (0.01) b	4.36 (0.07) b
Distilled water	0.89 (0.02) b	0.7 (0.03) b	0.56 (0.03) a	0.43 (0.07) a

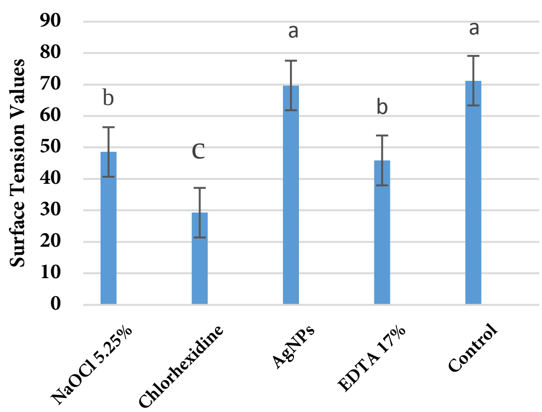

Figure1. Mean values of surface tension for each solution. Different letters in each column indicate a statistically significant difference

Statistical analysis

Dynamic viscosity, surface tension and water contact angle values were statistically analyzed by one-way analysis of variance, followed by a post hoc Tukey's test for pair-wise comparison. Statistical significance was set at 0.05.

Results

Table 1 shows the mean values of dynamic viscosity recorded for different irrigants at different temperatures. At 25˚C and 37˚C, there were no significant differences in dynamic viscosity of Im AgNPs, CHX and distilled water ($P>0.05$). At 45˚C and 60˚C, EDTA was significantly the most viscous solution ($P<0.05$), while no differences were observed amongst other irrigants.

Table 2 shows the water contact angle of each irrigant developed on dentin surfaces treated with different solutions. The contact angle on dentin surfaces treated with EDTA did not change significantly ($P>0.05$), while Im AgNPs resulted in significant increase in the angles ($P<0.05$). There was a significant reduction in water contact angles on samples treated with NaOCl and CHX ($P<0.05$).

Table 2. Means (SD) of water contact angles on dentin surfaces treated with different solutions. Different letters indicate a statistically significant difference

Irrigant	Water contact angle (degrees)
NaOCl	20.60 (3.36) [a]
CHX	19.49 (3.42)[a]
Ag NPs	56.62 (2.90) [b]
EDTA	51.15 (2.03) [c]
Distilled water	51.30 (2.52) [c]

Figure 1 demonstrates the surface tension observed for each irrigant at room temperature (25˚C). Im AgNPs had a high surface tension comparable to distilled water. CHX yielded the lowest value.

Discussion

Dynamic viscosity and surface tension are two important characteristic of the irrigants that determine their ability to flow and penetrate into capillary tubules [2, 4, 5]. However, there is insufficient data in endodontic literature to support the clinical advantages of surface tension reduction [25].

To date, there is no report in literature on dynamic viscosity and surface tension of AgNPs in endodontics. We found that Im AgNPs had lowest level of dynamic viscosity comparable to CHX 2% and distilled water at all temperatures tested and this was similar to those achieved for 5.25% NaOCl at 37˚C and 60˚C. Data concerning 5.25% NaOCl, 17% EDTA and 2% CHX were consistent to the findings reported by Gopikrishna *et al.* [26]. They also stated that ineffectiveness of NaOCl and EDTA in removing debris from the apical third of root canals might be a result of their high level of viscosity.

Many studies have revealed that heating can improve antimicrobial efficacy [27-29], tissue solubility [27, 30, 31], and flow characteristics [32] of NaOCl. Our study reached similar results in terms of viscosity for NaOCl; however, we found that heating had limited effect on flow property of Ag NPs.

In the present study, we demonstrated that AgNP irrigant has high surface tension similar to distilled water. Our findings concerning the surface tension of 5.25% NaOCl and 17% EDTA and distilled water were in line with reports by Tasmen *et al.* [3, 32], and Giardino *et al.* [3, 32]. Furthermore, CHX surface tension was observed to be 53.82 mJ/m^2 which was similar to the report determined by Tasmen *et al.* [3, 33] and Estrela *et al.* [3, 33]. The high surface tension value determined for the AgNP irrigant reflects the fact that it might not bring better dentinal tubule penetration in comparison with the three common irrigants. This finding was consistent with the findings of a recent study by Rodrigues *et al.* [34]. They tested antimicrobial efficacy of aqueous AgNPs relative to 2.5% NaOCl and 2% CHX against biofilm *E. faecalis* and in infected dentinal tubules after 5, 15 and 30 min using a confocal laser scanning microscope. They demonstrated that after 5 min irrigation with AgNPs, more viable bacteria were presented in biofilms than in the tubules but after 30 min irrigations, the number of viable bacteria was greater in dentinal tubules compared with the biofilm. These researchers concluded that AgNPs was not able to penetrate dentinal tubules and remove bacteria at all of the tested time intervals.

Some studies have suggested that using irrigants with lower surface tension could decrease the need for removing large amounts of root canal dentin to gain a debris free root canal [35]. However there is no evidence that root canal irrigation with low surface tension irrigants can clinically reduce the bacterial contamination of root canals [3]. Hence, role of surface tension on antibacterial efficiency of irrigants is still in doubt that needs to be investigated in future studies.

Wettability is one of the most important physicochemical properties of an irrigant. Contact angle measurements determine the wettability of a substratum. Low contact angles are an indication of high surface free energy, whereas high contact angles indicate low surface free energy [7]. Interfacial surface free energy has been postulated as a driving force for initial adhesion of microorganisms to a solid surface.

NaOCl is able to nonspecifically deproteinase the root canal dentin during endodontic treatment. Recently, NaOCl was also used to remove the collagen fibrils [36]. Attal *et al.* [37] and Hu *et al.* [9] have found that NaOCl increases the wettability of dentin surface. In contrast, Buzoglu *et al.* [38] have demonstrated that NaOCl decreases the wettability of dentin surface. Our study indicated that both 2% CHX and 5.25% NaOCl significantly increased the wettability of dentin surfaces while the AgNP irrigant significantly decreased the wettability of dentin surfaces. An increase in wettability after NaOCl treatment can be explained because deproteinzation leads to a hydrophilic surface [37]. Hogt *et al.* [39, 40] and Ludwicka *et al.* [39, 40] found that hydrophilic materials were more resistant to bacterial adhesion than hydrophobic materials. However, An *et al.* [41] found that hydrophobic UHMWPE disks attracted much less *Staphylococcus Epidermis* compared with the hydrophilic metal surface. Yilmaz *et al.* [42] reported that the use of low-surface-tensioned EDTA compounds alone or in combination with NaOCl increased the wettability of root canal dentin.

Iwanami *et al.* [36] also reported that surfactant-added EDTA solution increased the spreading ability of solution over the dentin surface. At the present time, there is no report on the evaluation of the action of 2% CHX on dentin wettability. However, Ricci *et al.* [43] revealed that there was no increase in surface wettability by using 0.2% CHX.

The increase in water contact angle of dentin samples treated with AgNPs in this study resulted in significant decrease of surface wettability and this might be attributed to the slightly hydrophobic character of the twelve carbons present in alkyl chain of imidazole molecules.

Conclusion

Im AgNP solution has obvious impact on physiochemical properties of dentin by decreasing its surface wettability. Besides, due to the lower viscosity of this solution in comparison with other irrigants, it has the potential to reach apical portions of root canal. However, it may not bring better penetration inside dentinal tubules due to its higher surface tension.

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Conflict of Interest: 'None declared'.

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