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Effect of a New Imidazolium-based Silver Nanoparticle Irrigant on the Bond Strength of Epoxy Resin Sealer to Root Canal Dentine

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ABSTRACT

Introduction: The aim of this study was to evaluate the effect of a new imidazolium-based silver nanoparticle (ImSNP) root canal irrigant on the bond strength of AH-Plus sealer to root canal dentine. **Methods and Materials**: Forty single-rooted extracted human teeth were used in this study. The crowns were resected and according to the irrigation solutions used during root canal preparation, the roots were divided into 5 groups (n=8): Group 1: normal saline (control group), Group 2: 2.5% Sodium Hypochlorite (NaOCl), Group 3: 2.5% NaOCl+17% ethylene diamin tetracetic acid (EDTA), Group 4: silver nanoparticles (AgNPs), Group 5: AgNPs +17% EDTA. After root canal instrumentation, the canals were filled with AH-Plus. Then, after 7 days, 2 or 3 dentine disks were obtained from the mid-root of each sample. Bond strength was measured by the push-out test. Additionally, failure patterns were classified as adhesive, cohesive and mixed. Data were statistically analyzed by one-way ANOVA and Tamhane post hoc tests. The level of significance was set at 0.05. **Results**: There was no statistically significant differences between groups (P>0.05). Groups 4 (AgNPs), 3 (2.5% NaOCl+17% EDTA) and 2 (2.5% NaOCl) showed statistically higher bond strength compared to group 1 (control group) (P<0.05). Also, Group 4 showed a significant difference with group 5 (AgNPs+17% EDTA) (P=0.017). The failure patterns were mainly cohesive. **Conclusion**: This *in vitro* study showed that, when used without EDTA, AgNPs improved the bond strength of AH-Plus to radicular dentine.

Keywords: AH-Plus Sealer; Push-out Test; Silver Nanoparticles; Sodium Hypochlorite

Introduction

Complete removal of debris from root canal walls is not only achieved by mechanical debridement. In fact, using root canal irrigants has become an integral part of root canal preparation [1, 2]. In addition to debris flushing out, irrigation solutions provide lubrication, destruction of microorganisms and dissolution of tissues [3].

Different chemicals have been suggested as efficient irrigant solutions for root canal disinfection. Amongst them, sodium hypochlorite (NaOCl) is the most widely used solution in endodontic treatments. NaOCl has shown to have effective antimicrobial activity and also, the ability to dissolve organic tissues [2]. On the other hand, NaOCl has some disadvantages including toxicity, odour, discolouration, and corrosion of dental equipment [4]. Furthermore, the use of NaOCl could affect the polymerization and penetration of the resin-based sealers into dentine. Additionally, this solution is a proteolytic agent and thus, can degenerate dentine by collagen dissolution [5]. NaOCl has also been shown to be associated with higher incidence of defects on root canal walls [6]. Therefore, studies are continually attempting to investigate and find other efficient disinfectants for root canal treatments.

In recent years, silver nanoparticles (AgNPs) have been suggested as potential endodontic irrigants. They have shown to have a broad spectrum of antibacterial activity [7-9] and high level of biocompatibility [10, 11]. The exact antibacterial mechanism of AgNPs is not known. However, it has been speculated that AgNPs can attach themselves to the cell wall of microorganisms and cause dysfunction in cellular permeability. Moreover, they may enter bacteria and interact with their DNA and enzymes, causing cell extermination [12, 13].

Recently, a new positively-charged silver nanoparticle (SNP) irrigant coated with imidazole (ImSNP) has been introduced in literature [14, 15]. This novel endodontic irrigant has shown a promising antibacterial activity against *Enterococcus faecalis* (*E. faecalis*) and exhibited a high level of cytocompatibility to L929 cells [14]. Furthermore, it has been reported that similar to 2.5% NaOCl, ImSNP was able to eliminate *E. faecalis* biofilm effectively [15].

Chemical irrigants applied for root canal preparation may alter the chemical composition of radicular dentine surface and affect its interaction with materials used for root canal obturation [16]. Studies showed that irrigation protocols affected the adhesion of materials to dentine [17-19]. A recent study has shown that this novel irrigant increases dentine roughness and therefore may affect adhesion of dental materials to root canal dentinal walls [16]. Moreover, in some studies, the application of a chelating agent, following root canal irrigation with NaOCl, resulted in higher adhesion values of sealers to dentine [20-22].

To the best of our knowledge, there has been no published study regarding the effect of AgNPs on the bond strength of resin-based sealers to root canal dentine. Therefore, this study was designed and conducted to evaluate the bond strength of a resin-based sealer (AH-Plus, Dentsply, Tulsa Dental, Tulsa, OK, USA) to root canal dentine, irrigated with ImSNP compared to NaOCl with and without the application of ethylene diamin tetracetic acid (EDTA) for smear layer removal.

Materials and Methods

For this *in vitro* study, forty extracted human single-rooted teeth were collected and soaked in 5.25% NaOCl (Golrang, Tehran, Iran) for 1 h to disinfect, and then, stored in normal saline. Teeth with a curved root, any sign of internal root resorption or calcification, or previous root canal therapy were excluded from the study. The teeth were decoronated 2mm coronal to the cementoenamel junction using a diamond disk and water spray. In the next step, a size 15 K-file (Mani, Tochigi, Japan) was introduced into the canals until it was visible at the apical foramen. The working length was determined at 1mm shorter than the apical foramen.

To prepare AgNPs solution, laboratory glassware was put in 1:3 of HCl/HNO3 solution, and rinsed three times by triply distilled water. Subsequently, a total of 1mL of 0.01M AgNO3 aqueous solution was added to 20mL of 6.2mM 1-dodecyl-3-methylimidazolium chloride ([C12mim][Cl]) aqueous solution. After stirring the resultant solution, the prepared 0.4M NaBH4 aqueous solution was added dropwise until the colour of the solution turned into golden. In order to remove the excess amount of ionic liquids, the colloidal solution was centrifuged for 20min. The prepared suspension was then stored at room temperature. The suspension concentration was 5.7×10^{-8} Mol/L [14].

The samples, according to the irrigation regimen, were randomly assigned to 5 groups (*n*=8): G1-The canals were irrigated with 1.5mL of saline before the insertion of each instrument; G2 and G3- The canals were irrigated as described in G1, but the irrigants were NaOCl 2.5% and AgNPs, respectively; G4 and G5- The canals were irrigated similar to G2 and G3, respectively, but then filled with 1mL of 17% EDTA (Aria Dent, Tehran, Iran) for 5 min.

In all groups, after enlarging the coronal part of each canal with #2 and #3 Gates Glidden drills (Mani, Tochigi, Japan), the root canals were shaped by means of ProTaper nickel-titanium rotary system (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 instrument. In the last step of canal preparation, Gates Glidden burs #4 and #5 were used 1mm short of the working length to eliminate its taper and achieve unified and round canals [17]. All groups received a final flush with 10mL of distilled water and were then dried with absorbent paper points.

In the next step, all specimens were filled with AH-Plus sealer (De Trey-Dentsply, Konstanz, Germany). The sealer was prepared according to the manufacturer's instructions and introduced into the root canals using a lentulo spiral. The specimens were kept in an incubator at 37°C and 100% humidity

Table 1. Mean push-out bond strength value (MPa), and standard deviation (SD) of samples for each group

Experimented groups	Mean (SD)
G1: Saline	1.85 (1.7) [ed]
G2: NaOCl	5.69 (3.03) [ab]
G3: NaOCl+EDTA	6.38 (3.98) [ab]
G4: AgNPs	8.03 (4.83) [bc]
G5: AgNPs+EDTA	3.25 (2.03) [ad]

Different letters indicate statistically significant difference.

Table 1. Different types of bond failure in experimented groups

Experimented groups	Adhesive	Cohesive	Mixed
G1:Normal saline	35%	65%	0
G2: NaOCl	29%	70%	0
G3: NaOCl+EDTA	13%	73%	0
G4: AgNPs	12%	75%	12%
G5: AgNPs+EDTA	35%	57%	7%

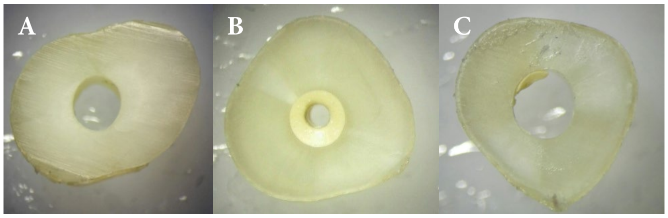

Figure 1. Different types of bond failure *A)* Adhesive, *B)* Cohesive and *C)* Mixed

in vertical position. After 7 days, the roots were sectioned transversally into 1.5±0.2mm thick slices using a water- cooled precision saw (Persi-T180, France). The slices were then examined under a light microscope (Dino-light, Hsinchu, Taiwan) at 20× magnification for the roundness of the lumens and presence of voids in the sealer mass or in the sealer-dentine interface. Finally, 16 slices in each group were selected and subjected to push-out test.

A universal testing machine (Zo20; ZwickRoell, Ulm, Germany) was used to measure the push-out bond strength of the samples. The slices were placed on a metal slab with a central hole to allow free motion of the plunger. A downward load was applied on the surface of the sealer using a 0.7mm diameter cylindrical stainless steel plunger at a speed of 1mm/min. The maximum load at the time of sealer dislodgement was registered in newtons (N) by a computer connected to the universal testing machine.

To express the bond strength in mega Pascals (MPa), the following formula was used: $MPa=N/2\pi rh$, where N is the maximum load for each sample, π is the constant 3.14, r is the root canal radius in mm, and h is the thickness of each slice in mm.

The modes of bond failure were evaluated under the light microscope (Dino-light; Hsinchu, Taiwan) under 40X magnification. Each sample was classified into one of the three failure modes as adhesive failure at the sealer and dentine interface, cohesive failure within the sealer, and mixed failure.

Statistical analysis

The data were analyzed using one-way ANOVA and Tamhane post hoc tests ($P<0.05$).

Results

Table 1 presents the mean and standard deviation of the bond strength of each group. The highest and lowest bond strength was observed in AgNPs and saline groups, respectively. While no significant differences were detected amongst AgNPs, NaOCl+EDTA, and NaOCl groups ($P>0.05$), they showed significantly higher bond strength compared with saline ($P<0.05$). Also, AgNPs showed a significantly higher bond strength compared with AgNPs+EDTA ($P=0.017$).

Microscopic evaluation of the samples revealed that the mode of bond failure was predominantly cohesive for all groups (Figure 1) .Table 2 shows bond failures in experimented groups.

Discussion

In the present study, the highest bond strength was detected in AgNPs group, which was not significantly different from those of NaOCl and NaOCl/EDTA groups. As dentine surface roughness might affect the adhesion of restorative material to root canal walls [23], our findings may be attributed to the effect of AgNPs on the dentine surface . In a recent study, the same AgNPs used in our research, significantly increased the surface roughness of dentine samples which were similar to those treated with 5.25% NaOCl and 17% EDTA [16].

On the other hand, when EDTA was used following AgNPs, the bond strength decreased significantly. Although the reason is unknown, one possible explanation is that EDTA may cause a collapse of the dentine matrix structure; which impedes sealer infiltration, and limits strong bonding to the dentine surface [24]. Nevertheless, the effect of EDTA following AgNPs on dentine surface should be more investigated in future.

The results of the current study also showed that the application of EDTA following the irrigation of canals with NaOCl to remove smear layer has no effect on the bond strength of AH-Plus sealer to dentine. There is strong controversy regarding the effect of the smear-layer removal on the bond strength of endodontic sealers. While some studies reported higher bond strength following the removal of smear layer [25, 26], others showed no significant difference or even higher bond strength when the smear layer was present [18, 27]. The discrepancy between the results of different studies has been attributed to the different smear layer removing protocols and various techniques used for evaluating the bond strength [28].

In the current study, we did not use any core materials and the canals were filled only with the sealer. In this method, only one interface (sealer and dentine) was present, which made it possible to solely evaluate the bond strength between the sealer and the dentine [29]. One of the limitations of the current study was that only one sealer was used. It has been reported that the effect of different irrigation protocols on adhesion differed amongst different types of sealers. Therefore, the results of our present study may not be extrapolated to the other sealers.

Conclusion

Under the limitations of this *in vitro* study, it seemed that irrigation of the root canal system with AgNPs had a promising effect on the bond strength of AH-Plus to dentine. However, using EDTA following AgNPs decreased the bond strength.

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Conflict of Interest: 'None declared'.

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