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The effect of dentin pretreatment using silver nanoparticles on shear bond strength of one self-etch and one etch-and-rinse resin cements

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ABSTRACT

Introduction: Metal nanoparticles may apply to the cavity walls as acceptable antibacterial agents. The present work was conducted to assess the effect of silver nanoparticles (NPs) on the resin cements Shear Bond Strength (SBS) using one self-etch and one etch and rinse resin cement.

Materials & Methods: Sixty intact noncarious extracted, human third molars were selected. All teeth were cut out in the middle of dentin thickness. Resin cement samples were randomly assigned to five groups: (A) Duolink cement without any further surface treatment; (B) Duolink cement with silver NPs (0.5%) dentin surface treatment after acid etching and before the One Step Plus (OSP) adhesive system; (C) Duolink cement with silver NPs (0.5%) dentin surface treatment before acid etching and OSP; (D) Panavia F2.0 cement without any further surface treatment; and (E) Panavia F2.0 cement with silver NPs (0.5%) dentin surface treatment. SBS was assessed by using universal testing machine. Then, human gingival fibroblasts (HGF) cells were treated with tested materials. The biocompatibility of resin cement was evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results: The maximum and minimum of the SBS at the presence of silver NPs were observed in group E (15.81±0.91, P-value<0.01) and group B (12.01±0.15, P-value <0.05), respectively. In comparison with the controls, the resin cements incorporated with 0, 0.2% and 0.5% silver NPs, 20.34%, 22.00% and 22.67% for Panavia F2.0 and 36.84%, 37.34% and 38.17% for Duolink flow - decreased in total cell number, respectively (P-value<0.01).

Conclusion: These findings demonstrated that Panavia F2.0 cement with silver NPs dentin surface treatment showed satisfactory result in the SBS compared to Panavia F2.0 cement without any further surface treatment.

Keywords: Nanoparticles, Shear Strength, Dental Etching, Resin Cements, Operative Dentistry, Cell Culture

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Introduction

The longevity of indirect restorations forcefully depends on the quality of bond strength of the cement. The adhesion between cement and dentin is more susceptible to failure than cement-enamel or cement-ceramic interfaces.[1,2] Most resin cements are based upon the use of self-etch or etch-and-rinse adhesives along with a low-viscosity resin composite.[3] When the cavity walls lack optimal resistance and retention form, the application of resin cement, can help to compensate for the shortcoming of traditional cement by its special characteristics such as high strength, lower solubility, and adhesion to the tooth structure.[4,5] Nevertheless, higher polymerization shrinkage stress and gap formation at the cavity cement interface can occur, because these cements have low filler content in comparison with conventional resin composites. These factors can cause failure to inhibit plaque formation and bacterial colonization.[6]

Secondary caries is one of the most important causes of restoration failures. Clinically, it is crucial for the crown margins of indirect restoration to precisely fit into the prepared tooth.[7] Because of the imperfect marginal adaptation of cemented crowns, they are susceptible to some degrees of gapping.[8,9] Different types of cement are applied to fill the gaps and serve as sealing agents; however, the breakdown of cement may occur and lead to the ingress of pathogenic microorganisms and oral fluid along with the restoration- tooth interfaces and result in microleakage.[10]

Inorganic nanoparticles (NPs) have been investigated for their antibacterial potential and have proven to be antimicrobial agents against both Gram-positive and Gram-negative bacteria. [11] The mode of action of silver depends on Ag+ ions that forcefully prevent bacterial growth through interference with DNA functions and suppression of respiratory enzymes. It seems that at very small concentrations, silver is non-toxic to humans. [12]

Acidogenic bacteria especially Streptococcus mutans and their byproducts are responsible for dental caries. [13] Thus, attempts have been made to create antibacterial restorative materials. For dental composites, it is favorable to incorporate Ag NPs with a high surface area into the resin to reduce the concentration necessary for efficacy.[14] The incorporation of silver NPs to resin composite materials may result in a reduction of adhering bacteria and an increase in the number of dead cells on the composite surfaces.[15] Along with the long-term antibacterial effects of up to six months with Ag-containing composites, silver NPs have less bacterial resistance and exhibits high biocompatibility in comparison with antibiotics.[16] Hernández- Sierra et al. demonstrated that silver NPs, require lower concentrations to prevent the development of the Streptococcus mutans strain compared with other inorganic ions, because of their high surface area.[17, 18] Resin cements compared with conventional GIC or RM-GIC, have a limited history of application and their biocompatibility is of concern. [19-22] Dental cements that release monomers may adversely affect clinical results. However, studies assessing the cytotoxic potential of dental cements are lacking. [23]

The objective of the current study was to evaluate the effect of dentin pretreatment with silver NPs on the dentin shear bond strength (SBS) of self-etch and etch and rinse resin cement and their cytotoxicity using culture media of human gingival fibroblasts (HGF). Since our knowledge around the cytotoxic effect of dental materials towards HGF is limited, so we evaluated the biocompatibility of two resin cement on HGF and compare it with the biocompatibility of resin cements with incorporated silver NPs. The null hypothesis of the study was that there would be a positive effect on SBS in silver pretreated groups compared to conventional resin cement groups.

Materials & Methods

Preparation of Specimens and Surface Treatments: This study was approved by the Ethical Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1397.187). The materials used in this study and their mode of application of them are shown in Table 1 and Table 2. Sixty intact noncarious extracted, human third molar teeth were collected and stored in 0.1% thymol for seven days. A diamond bur was used for the preparation of teeth using a high-speed handpiece and water coolant. All teeth were cut in the middle of dentin thickness and ground with a 600-grit silicon carbide abrasive paper under water cooling. No pulpal exposure should occur in the preparation site. The preparations were up to the nearest half of the DEJ to the pulp after polishing. 60 resin cement blocks of Panavia F2.0 (Kuraray, Kurashiki, Okayama, Japan) and Duolink (Bisco Inc., Schaumburg, USA) were fabricated on dentin surfaces and established in five different groups based on the surface treatments (12 samples in each group) using polyethylene molds (5mm inner diameter and 2mm height). Both resin cements were mixed based on the manufacturer's instructions and placed inside the molds on the dentin surfaces.

Table 1. Test materials name, manufacturers, and composition

Product	Manufacturer	Composition
Panavia F2.0 Paste A	Kuraray Medical Inc., Sakazu, Kurashiki, Okayama, Japan	Hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimethacrylate, sodium aromatic sulfinate, N, N-diethanol-p-toluidine, surface treated (functionalized) sodium fluoride, silanated barium glass
Panavia F2.0 Paste B	Kuraray Medical Inc., Sakazu, Kurashiki, Okayama, Japan	MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimethacrylate, silanated silica, benzoyl peroxide, dibenzoylperoxide
ED Primer 2.0	Kuraray Medical Inc., Sakazu, Kurashiki, Okayama, Japan	Liquid A: HEMA, MDP, 5-NMSA, Water, Accelerators Liquid B: 5-NMSA, Water, initiator, Accelerators
Doulink	Bisco Inc., Schaumburg, USA	Bisphenol A diglicidyl methacrylate, demethacrylate, triethyleneglycol glass filler, urethane dimethacrylate
One-Step Plus	Bisco Inc., Schaumburg, USA	Biphenyl dimethacrylate, 2-hydroxyethyl methacrylate, , p-dimethylaminobenzoic acid, glass fillers and acetone
Phosphoric Acid	3M, ESPE, St Paul, MN, USA	Phosphoric acid gel (35%)

Table 2. The mode of application of tested resin cement

Resin cement	Mode of application
Panavia F2.0	Mix ED Primer Liquids A and B in equal portions, apply to the tooth, wait 30s, gently air-dry. Mix equal amounts of Panavia F2.0 cement paste A&B for 20s, apply the mixture, light cure for 40s.
Doulink	Etch the dentin for 15s, rinse for 20s, Dispense 1-2 drops of One Step Plus adhesive into a mixing well. Apply a minimum of 2 generous coats to the entire preparation, Agitate slightly for 10-15 seconds each coat. Light cure for 10 seconds. Air-dry for 10s, Apply Duolink cement with a syringe, light cure 40s.

Resin cement samples were randomly assigned to five groups: (A) Duolink cement without any further surface treatment; (B) Duolink cement with silver NPs (0.5%) dentin surface treatment after acid etching and before the One Step Plus (OSP) (Bisco Inc., Schaumburg, USA) adhesive system; (C) Duolink cement with silver NPs (0.5%) dentin surface treatment before acid etching and OSP; (D) Panavia F2.0 cement without any further surface treatment; and (E) Panavia F2.0 cement with silver NPs (0.5%) dentin surface treatment; After the surface treatments, each sample was rinsed 60s. The surfaces of samples were polymerized with a LED curing light (EliparTM, 3M ESPE, St Paul, MN, USA) at 600 mW/cm2 for 20 s each. The sequences of dentin pretreatment steps were shown in Table 3.

Table 3. Sequence of treatment steps of different tested groups

	Study Groups			
Groups	Sequence of treatment steps			
A	Etching		One-Step Plus	Duolink
B	Etching	silver NPs	One-Step Plus	Duolink
C	silver NPs	Etching	One-Step Plus	Duolink
D			ED Primer	Panavia F2.0
E		silver NPs	ED Primer	Panavia F2.0

Shear Bond Strength Test: Specimens of each test group were stored in distilled water for one week. The SBS tests were done using a universal testing machine (Zwick/Roell Z020, Stuttgart, Germany) at a crosshead speed of 0.5mm/min. loads were applied to the resin cement-dentin interface of the specimens until failure occurred and the corresponding software showed the maximum forces in Newtons (N). Then SBS values were evaluated by converting Newtons into megapascals (MPa). 1 newton/square meter is equal to 10^{-6} MPa. [24]

Resin cement disc preparation: Metal type washers were prepared and placed on a glass slab. The washers had the 2-mm thickness and 5-mm inner hole diameter. Resin cement disks that contain different concentrations of silver NPs (0, 0.2% and 0.5%) were prepared. The samples were light-cured for 20 seconds (VIP Junior, Bisco, Schaumburg, IL, USA) at 600 mW/cm^2. Then, each sample was separated from the washer and sterilized in Iran׳s gamma radiation center with 25 kGy dosages.

Cell culture and restorative dental materials treatment: Thirty samples (2 mm thick and 5 mm in diameter) were prepared by placing the Panavia F2.0 and Duolink resin cement into a stainless steel mold. In half of the samples, 0, 0.2%, and 0.5% silver NPs were added to resin cement. The incorporation of silver NPs was done using both ultrasonic and manual techniques. HGF cell line was purchased from the National Cell Bank of Iran. HGF cells were cultured in roswell park memorial institute (RPMI-1640) medium supplemented with glutamine, 10% FBS, and antibiotics (Gibco, Paisley, Scotland) at 37ºC in an incubator containing 5% CO_2. The cells were seeded into 96-well plates (10000 cells/well) and incubated by samples at 24 h. [25,26]

Cell viability: The cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma, St. Louis, USA) assay. The MTT dye was solved in phosphate buffer saline (PBS). Then MTT solution was added to each well. After 5h incubation, the formazan was dissolved in 100 ml isopropanol (Merck, Darmstadt, Germany), and its optical density (OD) was read with an ELISA reader (Organon Teknika, Netherlands) at 570 nm. The percentage of cytotoxicity was evaluated based on the following formulas. [26]

$$\%\text{Cytotoxicity} = \frac{1 - \text{mean absorbance of toxicant treated cells}}{\text{mean absorbance of negative control}} \times 100$$

$$\%\text{Viability} = 100 - \%\text{Cytotoxicity}$$

Statistical analysis: Data analysis was done using SPSS 16.0 software (SPSS Inc. USA). The results of the study were analyzed using a two-way ANOVA test and an independent-sample *t*-test. Mean±SD of cytotoxicity of two different composite samples were analyzed by the Kruskal-Wallis test. P-value<0.05 was considered as the level of statistical significance.

Results

SBS measurement: Table 4 and Figure 1 illustrate the mean SBS and standard deviation values of the five test groups. The maximum and minimum of the SBS at the presence of silver NPs were observed in group E (15.81±0.91, P-value<0.01) and group B (12.01±0.15, P-value <0.05), respectively.

Table 4. Descriptive statistics of the groups and comparison of SBS values

Groups	n	Mean±SD [MPa]	Significance *P- value <0.05, ***P- value* <0.01
A	12	14.87±0.54	A,B*
B	12	12.01±0.15	B,A* B,D**B,E**
C	12	13.44±0.43	C,E**
D	12	15.17±0.98	D,B**
E	12	15.81±0.91	E,B** E,C**

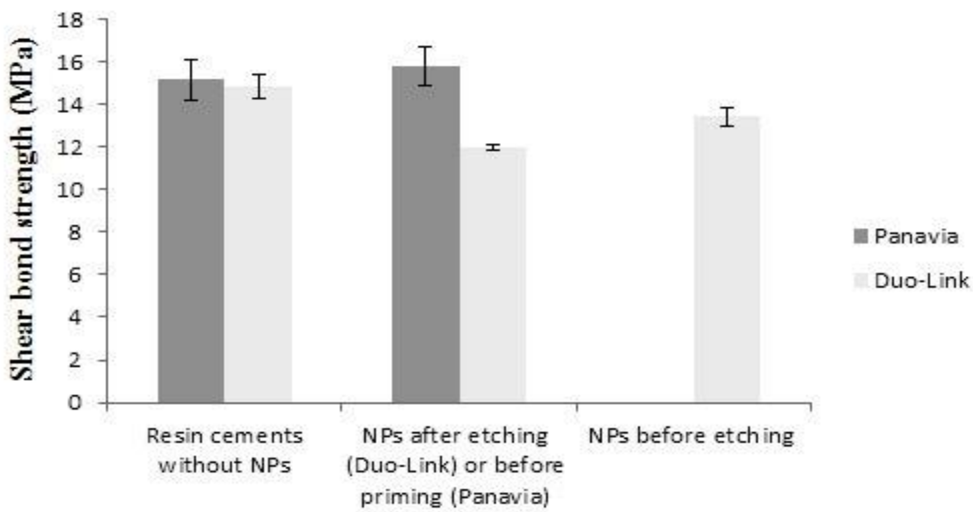

Figure 1. Means and standard deviations of SBS for the groups

Cell cytotoxicity: The current study characterized the cytotoxicity of Panavia F2.0 and Duolink resin cements pretreated with silver NPs by treating the HGF cells for 24 h followed by MTT assay. In comparison with the controls, the Panavia F2.0 incorporated with 0, 0.2% and 0.5% silver NPs, 20.34% (P-value<0.01), 22.00% (P-value<0.01) and 22.67% (P-value<0.01) - decreased in total cell number, respectively. Also, compared to the controls, the Duolink flow incorporated with 0 and 0.5% silver NPs, 36.84% (P-value<0.01), 37.34% (P-value<0.01) and 38.17% (P-value<0.01) - decreased in total cell number, respectively (Figure 2).

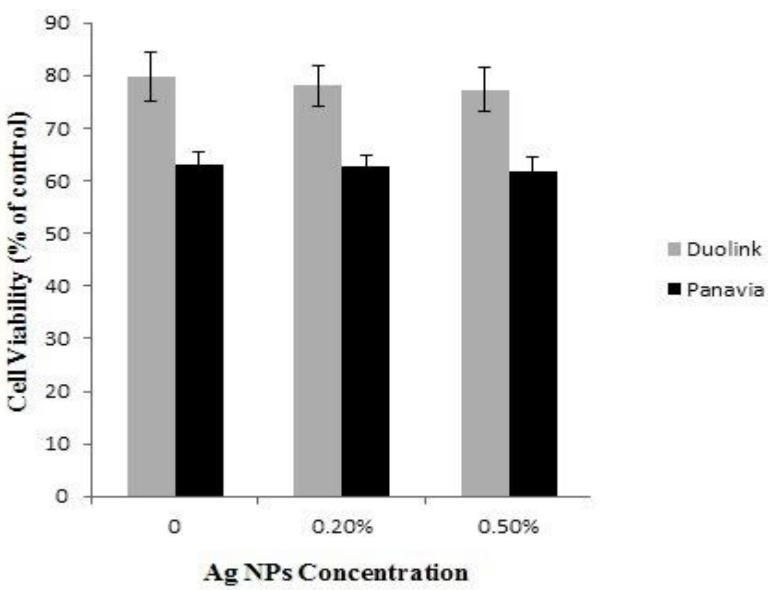

Figure 2. Effects of Panavia F2.0 and Duolink resin cements pretreated with silver NPs on the viability of HGF cells after 24 h. Data are expressed as the mean ± SD

Discussion

The ongoing study was conducted to assess the SBS of the two resin cement systems before and after pretreatment with silver NPs solutions. The maximum of the SBS was observed in group Panavia F2.0 and silver NPs pretreatment. The lowest mean SBS value was observed in the Duolink and silver NPs pretreatment after acid etching. Also, no negative effect was observed in resin cement incorporated with silver NPs on cell viability.

Resin cement is important dental material because of its highly adhesive characteristics. They also resist the dislodgement of the restoration. [27] Adding inorganic ions such as Ag in dental materials is an important issue in nanotechnology. [28] Silver is a broad-spectrum antibacterial agent that shows long-term antibacterial characteristics via sustained Ag ion release, low toxicity and good biocompatibility with human cells. [29] In this study, no negative effect was observed in resin cements incorporated with silver NPs on the HGF cell viability.

Furthermore, investigations showing micro gaps at tooth-restoration interfaces demonstrate that in clinical practice a complete sealing of the restoration-tooth interfaces is difficult to achieve.[30] Thus, an antibacterial surface pretreatment directly coating enamel and dentin surface could be beneficial to help eradicate the residual bacteria along the margins and disinfect the prepared tooth cavity.[31] As the conventional shear test requires minimal equipment and sample preparation, a lot of the available data on material adhesion still comes from the macro shear test, especially regarding the resin cement. [32] Thus the present work was conducted to determine the effect of dentin pretreatment with silver NPs on the SBS of etch-and-rinse and self-etch resin cements. The highest SBS were observed in group Panavia F2.0 and silver pretreatment. In the present study, the SBS of Panavia F2.0 self-etch resin cement was significantly higher than Doulink. This may be related to the different pH, composition, and chemical structure of the adhesives used with two resin cements. The OSP adhesive used with Doulink resin cement had a pH of about 4.61 and had lower acidity compared to Panavia F2.0 ED primer that is about 2.4. This lower acidity may lead to the lower dissolution of OSP and interfere with resin penetration and decrease the SBS. Moreover, it seems that using the silver NPs had a good effect on wetting the dentin surface and subsequent penetration of resin cement. Cekic-Nagas et al. showed that compared with the control group, the bond strength of Panavia F2.0 resin cement materials with silver NPs pretreatment was better. They attributed the higher durability of the bond strength to the MDP content of these materials.[33] On the other hand, one of the most important factors in determining SBS of resin cement is the presence of functional monomers specially MDP. Considering that Panavia F2.0 includes MDP monomers, it could interact with calcium ions of hydroxylapatite crystals and create a stable salt that could reinforce the adhesive layer and improve the SBS.

Our results showed that the least value of SBS was observed after acid etching in Duolink cement. This outcome may be related to the aggregation of silver NPs. The aggregation phenomenon has a more considerable effect when the silver NPs apply after etching. This may be related to the shortened rinsing time in the groups that the silver NPs apply after etching. According to the results of the present study, the dentin pretreatment in Panavia F2.0 self-etch group showed an improvement in SBS, albeit the difference was not significant.

The differences in the SBS of this study could be related to the different filler content of the resin cement and the fluid passage of different resin cements after polymerization. [33] Nanoparticle sizes can influence SBS. Evaluating the structural and mechanical characteristics of materials, color stability and flexural strength has been recently tested. Spyrou et al, on the other hand, observed that the size of NPs can influence the bond strength values. [34]

Two limitations of the present study were its in vitro nature and the use of higher thickness of resin cement. The effects of the hybrid and smear layer, aging, and thermal changes on the SBS were not investigated. The other limitation was the failure to consider the effect of C-factor and cement film thickness on SBS.

Therefore, the behavior of different NPs and their interaction with enamel and dentin and the protocol proposed by this investigation should be further investigated. Mechanical and antibacterial properties are essential factors to investigate. Further in vitro and in vivo studies are needed to investigate the effect of different NPs on dentin bond durability, and their long-term antibacterial and anticaries efficacy of them. Additionally, further investigations are needed to assess the effect of incorporating NPs into other types of resin cement and on other mechanical properties, such as the flexural strength, microleakage and elastic modulus.

Conclusion

Because of the beneficial antibacterial effects of silver NPs, using them is advisable in operative dentistry. It was shown that silver NPs pretreatment has no negative effect on the SBS of Panavia F2.0 self-etch resin cement but Duolink etch and rinse cement showed a mild reduction of the SBS values. The best outcome of silver NPs has been shown in the self-etch resin cement system. Also, no negative effect was observed in resin cement incorporated with silver NPs on cell viability.

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Conflicts of Interest

There is no conflict of interest.

Authors' Contribution

The study data were collected by Farzaneh Mohammadi. Fatemeh Koohpeima and Mohammad Javad Mokhtari designed the study, evaluated the results, edited and reviewed the article.

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