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Original Article
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Evaluation of Shear Bond Strength and Antimicrobial Effects of Resin-Modified Glass Ionomer Containing Titanium Oxide and Silver Nanoparticles

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Objectives Streptococcus mutans, as one of the most important cariogenic bacteria, is responsible for dental caries. To prevent and control dental caries, it is reasonable to use new materials and techniques for eradicating cariogenic bacteria. The present study aimed to evaluate and compare the antimicrobial effects of resin-modified glass ionomer (RMGI) containing silver and titanium oxide nanoparticles, on Streptococcus mutans (S. mutans) and to assess and compare the shear bond strength between coronal and radicular dentins.
Methods This study investigated the antibacterial properties of 16 RMGI discs, containing 3 weight percent (wt.%) of silver and titanium nanoparticles prepared for each group. Also, the shear bond strength of RMGI discs containing nanoparticles between the coronal and radicular dentins were compared. Kolmogorov-Smirnov test was used to evaluate the normal distribution of data. Normally distributed data were analyzed and compared between the groups, using two-way ANOVA test; otherwise, Kruskal-Wallis test was performed to analyze the data. The level of statistical significance was set at 0.05.
Results The mean colony count of S. mutans significantly reduced in both silver and titanium nanoparticle groups after 15 days compared to one-hour and 24-hour intervals ($P<0.0001$). The shear bond strength test revealed no significant differences between the two experimental groups ($P>0.05$).
Conclusion Incorporation of silver and titanium oxide nanoparticles at a concentration of 3 wt.% yielded optimal antibacterial characteristics, without causing any significant changes in the shear bond strength. Comparison between nanoparticle containing RMGI and RMGI showed that over time it has more ability to create antibacterial properties against S. mutans bacteria.
Keywords Shear bond strength; Glass ionomer; Dentin; Silver; Nanoparticles; Titanium oxide

Introduction

Dental caries is a microbial infectious disease, which destroys calcified tooth structures. This destructive process results from the activities of bacteria, which ferment carbohydrates, produce acids, and demineralize the tooth structure. [1] Dental caries is recognized as an infectious disease with a microbial origin, induced by several bacterial species in the oral cavity. One of the most important bacteria causing dental caries is Streptococcus mutans (S. mutans), which is the most important etiological agent of coronal dental caries. [2]

Today, with increased attention to oral hygiene, besides increased life expectancy, more natural teeth are retained by old age. Additionally, the incidence of root surface caries has increased in the elderly due to various factors, including decreased salivary flow and poor oral hygiene because of reduced finger skills, reduced motivation, and higher prevalence of gingival recession. [3] Therefore, it is reasonable to use new materials and techniques to destroy this cariogenic bacterial species for the prevention and management of this medical condition. The development of dental materials with antimicrobial properties is one of the most important objectives of dental materials science and dentistry, and many studies have been conducted on these materials, including glass ionomer. [4]

The use of nanoparticles (NPs) has opened new research areas in dentistry, with the aim of improving the mechanical properties and antibacterial effects by inducing changes in the aerobic and anaerobic processes of bacteria, dissolving DNA, disrupting bacterial cell wall synthesis, creating pores on the cell membrane, and increasing permeability to destroy bacteria. The combination of hydroxyapatite and fluorapatite nano-bioceramics with conventional glass ionomer has enhanced the mechanical properties and bond strength to dentin. Titanium dioxide (TiO2), as a mineral additive, improves the chemical stability and antibacterial properties of dental materials. NPs have been designed as reinforcing fillers for dental composites and epoxy resins. [5] Besides, the combination of glass ionomer with 3 weight percent (wt.%) of silver oxide (Ag2O) NPs significantly decreases bacterial biofilm formation, with no impact on the mechanical properties. [6]

Commonly, NPs are spherical particles, measuring 1-100 nm in length. They have a high surface-to-volume ratio, making them more reactive. They also exhibit unique physical and chemical properties, which can effectively

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solve biological and dental problems. Silver and titanium can prevent acid production by S. mutans. Some studies have shown the antimicrobial effects of these materials on gram-negative bacteria. [7] Despite the biocompatibility and cytotoxicity tests that can partially guarantee the safety of NPs for in vivo applications, changes in effective parameters, even insignificantly, are of great importance in minimizing the ensuing irreparable damage.

Cellular responses vary depending on properties, such as the size, shape, and surface properties of NPs. The severity of toxicity also depends on the type of cells exposed to NPs. Today, nanoscale toxicological studies are underway to describe the possible effects of nanomaterials in contact with living cells and to investigate their toxicity. However, there are many uncertainties about the mechanism of toxicity and different types of toxic nanomaterials. [8] Based on previous studies [9, 10], Silver NPs exhibit the best antibacterial properties. Titanium NPs possess the best mechanical properties after being combined with restorative materials; they also show antibacterial properties. However, no study has yet compared these two NPs in terms of their antibacterial and mechanical properties. In the present study, resin-modified glass ionomer (RMGI) was used. Nevertheless, only few studies have evaluated the properties of this ionomer in combination with NPs.

RMGI is the best material for carious and non-carious root surface lesions. [11] It has replaced conventional glass ionomer in most cases, with ever increasing applications. The present study aimed to evaluate and compare the antimicrobial effects of RMGI containing Ag2O and TiO2 against S. mutans and to assess and compare the shear bond strength in coronal and radicular dentins.

Methods and Materials

In this study, 12 groups of 16 samples and a total of 192 samples were prepared for the shear bond strength test in two areas twice for the three groups. The study protocol was approved by the Local Committee of Research and Ethics IR.SSU.REC.1398.092.

To examine the antibacterial properties of the samples on the serial dilution test, three main groups were prepared in three intervals; finally, nine groups of 16 samples and a total of 144 samples were prepared. For the disk diffusion test, a total of 64 samples were prepared in four groups of 16 to examine their antibacterial properties. In this in vitro study, the groups consisted of RMGI discs, containing 3 wt.% silver and titanium NPs and 96 human maxillary premolar teeth extracted for orthodontic reasons. From each tooth, one sample of the crown and one sample of the root were prepared for the shear bond strength test in different groups.

Preparation of RMGI

Ag_2O and TiO_2 containing NPs, measuring 30 nm (Fanavaran Danesh Gostar Company®, Tonekabon, Iran), along with liquid and powder RMGI (light-cured, reinforced glass-ionomer restorative, A2-GC®, Tokyo, Japan), were prepared and measured using a digital weighing machine (A&D EJ-303; accuracy= 0.0001 g) to obtain RMGI, containing 3 wt.% of NPs. Next, the required amounts of NPs were mixed with the powder component of RMGI. The mixture was triturated in an amalgamator (Dentin A16971®) at 2000 rpm in two five-second rounds with an interval of one minute to avoid heating of the powder component to achieve a homogenous powder. Subsequently, the powder and liquid were mixed on a Zhermack paper pad (Zhermack®, Germany) with a plastic spatula in a semi-dark environment.

For the light-curing procedure, an LED light-curing unit (DEMI, Kerr®, USA) was used at a light intensity of 800 mW/cm^2. Two random samples from each group underwent electron microscopy to ensure the homogenous distribution of NPs. Also, samples containing NPs were compared with RMGI samples without NPs (negative controls). A VITA classical shade guide was set clinically by restorative specialists as a pilot study to evaluate color changes by incorporating the mentioned NPs.

Antibacterial assessments

Sixteen discs in nine groups (a total of 144 samples) were prepared for the modified serial dilution test, and 64 samples were prepared for the disk diffusion test to evaluate the antibacterial properties of RMGI with and without Ag_2O and TiO_2 NPs, using a previously described technique. The samples were placed in plastic molds, measuring 6 mm in diameter and 1 mm in thickness. Streptococcus mutans (ATCC35668) was procured from the Persian Type Culture Collection (Tehran, Iran).

An antimicrobial sensitivity test was carried out using the disk diffusion method by preparing pure and fresh bacterial cultures in a test tube, containing physiological serum suspensions with turbidity of 0.5 McFarland. Next, the suspension was seeded on a blood agar medium, enriched with 5% sheep blood using a swab. RMGI containing 3% NPs was placed on the culture medium. The growth inhibition zone diameter around the samples was measured in millimeters, using a ruler after incubation at 37ºC for 24 hours. A penicillin disc was used as the positive control, and RMGI without NPs was used as the negative control (Figure 1).

Modified serial dilution test

The discs were placed in tubes containing BHI broth and *S.* mutans, equivalent to 0.5 McFarland, and incubated under CO_2 at 37°C. The glass ionomer discs were retrieved from tubes containing the culture medium in one-hour, 24-hour, and 15-day intervals (Figures 2-4) and rinsed with 1 mL of physiological serum for five minutes in a vortex® mixer at 1000 rpm to detach bacteria from the glass ionomer discs. At this stage, the discs were excluded from the study. To determine the bacterial count, the resulting irrigation solution was cultured on blood agar medium, containing sheep blood at 37°C for 24 hours, and the bacterial colony count was determined for each disc. [12]

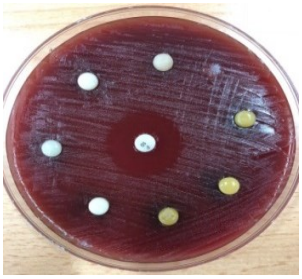

Figure 1: The disk diffusion control

Figure 2: Bacterial colony count after one hour

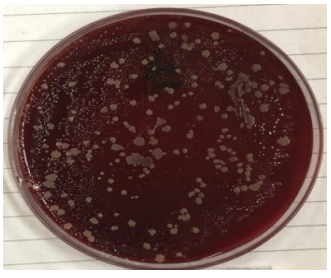

Figure 3: Bacterial colony count after 24 hours

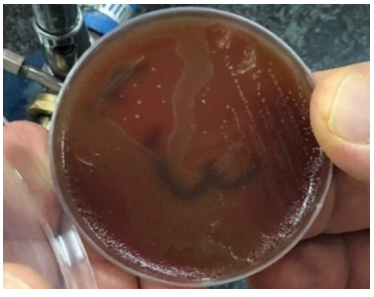

Figure 4: Bacterial colony count after 15 days

Sample preparation and shear bond strength test

To carry out this test, 96 sound human premolars without caries, cracks, abrasions, or developmental anomalies were collected. The tooth samples were extracted in less than three months ago. The teeth were mounted in cold-cured acrylic resin with the tooth's buccal surface half out of the acrylic resin. Next, they were cut in the mesiodistal direction on the buccal aspect, using a high-speed handpiece (High-Speed Air Turbine Handpiece, CH-∑®, Tokyo, Japan) and a medium-grit diamond bur (837XLG.FG.014, Jota®, Switzerland) under air-water spray to achieve a dentin surface on the root and crown for bonding with RMGI. From each tooth, one crown sample and one root sample were prepared (Figure 5).

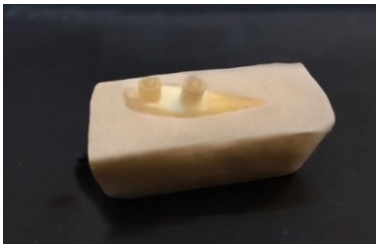

Figure 5: Samples prepared for the shear bond strength test

After preparing the surface, a cylindrical mold, measuring 3.5 mm in diameter and 4 mm in height, was placed on the crown and root, 2 mm away from the cemento-enamel junction on the middle of buccal surface. [13] Next, RMGI was packed into the mold in two 2-mm layers, using a composite resin spatula. One piece of Mylar matrix bond was placed on the last layer of RMGI, followed by the placement of a glass slab on it. After two minutes [14], excess RMGI was removed, followed by light curing (Kerr Demi LED System®, USA) of each layer separately for 20 seconds at a light intensity of 800 mW/cm^2 in four directions.

Samples for the shear bond strength test were prepared as follows: RMGI with Ag_2O NPs bonded to coronal dentin; RMGI with Ag_2O NPs bonded to radicular dentin; RMGI with TiO_2 NPs bonded to radicular dentin; RMGI with TiO_2 NPs bonded to coronal dentin; and RMGI without NPs bonded to coronal and radicular dentins as the controls. The shear bond strength test was carried out in a universal testing machine (ZwickRoell®, Ulm, Germany) at a strain rate of 1 mm/min, with a steel loop wire at the glass ionomer block-dentin interface until failure occurred. [15]

Scanning electron microscopic (SEM) evaluation

Two samples were randomly selected from each group of discs, which were prepared for the antibacterial test to ensure a homogenous distribution of NPs, and then, SEM (SEM-EDS ProX®, Switzerland) images were acquired.

Data Analysis

The collected data were analyzed in SPSS Version 23.

Kolmogorov-Smirnov test was used to evaluate the normal distribution of data. Normally distributed data were analyzed and compared between the groups using two-way ANOVA; otherwise, Kruskal-Wallis test was used for data analysis. Besides, t-test was performed for the analysis of data. The level of statistical significance was set at 0.05.

Results

Considering the normal distribution of data, ANOVA test was performed for the analysis of data pertaining to the shear bond strength (P=0.023). Tukey's test was also used for pairwise comparison of the groups concerning the shear bond strength, which indicated no significant difference between the groups (P=0.05). Table 1 presents descriptive data pertaining to the shear bond strength of coronal dentin (P=0.080).

Table 1- The results of shear bond strength test for tooth crown dentin

Coronal dentin shear bond strength	No.	Mean	SD	Min	Max
TiO2/24 hours	16	12.09	3.63	4.6	16.4
Ag2O/24 hours	16	11.91	3.64	4.5	16.2
Control/24 hours	16	12.31	3.70	4.8	17.0
TiO2/3 months	16	9.44	3.42	2.5	14.0
Ag2O/3 months	16	9.37	3.45	2.2	14.0
Control/3 months	16	9.47	3.44	2.4	14.5
Total	96	10.77	3.71	2.2	17.0

Ag_2O: Resin-modified glass ionomer containing silver nanoparticles.
TiO_2: Resin-modified glass ionomer containing titanium nanoparticles.

Table 2 presents the shear bond strength of the root dentin, indicating no significant difference between the groups (P=0.023).

Table 2- The results of shear bond strength test for the root dentin

Radicular dentin shear bond strength	No.	Mean	SD	Min	Max
TiO_2/24 hours	16	10.87	3.93	2.70	16.90
Ag_2O/24 hours	16	10.51	3.70	2.50	14.50
Control/24 hours	16	11.24	3.80	3.10	16.00
TiO_2/3 months	16	8.40	3.65	1.50	14.70
Ag_2O/3 months	16	8.56	3.55	1.40	14.80
Control/3 months	16	8.49	3.65	1.50	14.60
Total	96	9.68	3.82	1.40	16.90

The samples were evaluated under a stereomicroscope at 40× magnification to determine the failure modes. The evaluation of failure modes using Fisher's exact test did not indicate any significant differences between the groups in terms of failure modes (P=0.05). Moreover, pairwise comparisons of the groups were carried out using the disk diffusion method. The growth inhibition zone was significantly larger in the TiO_2 NP group compared to the negative control group, with no significant difference between the TiO_2 and Ag_2O NP groups. According to the antibacterial dilution test, the NP groups exhibited a significantly lower colony count in the 15-day interval compared to the 24-hour interval. Additionally, the colony count in the Np group was significantly lower in 15-day interval compared to the control group; there was no significant difference between the RMGI groups with TiO_2 and Ag_2O NPs.

Discussion

The present study aimed to evaluate and compare the antimicrobial effects of RMGI containing Ag2O and TiO2 NPs on S. mutans and to compare the shear bond strength between the coronal and radicular dentins. In this regard, Garcia et al. [7] attributed no antibacterial activity to conventional glass ionomers, glass ionomers containing TiO2, and Nano powders as bases, lines, and cores. Additionally, Sodagar et al. (2016) [16] evaluated the antibacterial activity of conventional orthodontic composite resins, containing silver and hydroxyapatite NPs and found that composite resin discs containing 5% and 10% silver and hydroxyapatite NPs produced growth inhibition zones for all bacterial species. The antimicrobial activity of eluted components from composite resin discs was quite variable, depending on the type of bacteria and NPs. [16] In the present study, growth inhibition zones were observed in the NP groups. The discrepancy between the present findings and the study by Sodagar et al. [16] can be attributed to the evaluated restorative materials and the use of different concentrations of NPs in their study.

Moreover, El-Wassety et al. (2017) [8] evaluated the effects of silver NPs, combined with glass ionomer cement on Staphylococcus aureus biofilm. The results showed that this combination could inhibit S. aureus biofilm formation [8], which is consistent with the present results concerning the antimicrobial effects of NPs in the RMGI structure. The only difference between these studies is that the bacterial species evaluated in the present study was S. mutans, which is the most important bacterial species involved in dental caries induction; the evaluation of this species might be considered the main strength of the present study compared to the mentioned research.

Additionally, Porenczuk et al. (2019) [17] evaluated the vitality and antibacterial activity of dental pulp stem cells in response to a disinfecting agent, containing gold and silver NPs, different bonding systems, and glass ionomer cement. It was concluded that the disinfecting agent was biocompatible for stem cells in terms of cytotoxicity and genotoxicity. The disinfecting agent and glass ionomer cement exhibited antibacterial activity against all

bacterial samples [17], which is consistent with the present findings; however, NPs used in their study differed from those of the present study.

The current findings showed no significant difference in the shear bond strength of RMGI and RMGI containing TiO2 and AgO2 NPs to the radicular and coronal dentin, which is consistent with the results of a study by Garcia et al. [7]. The advantage of the present study to the study by Garcia et al. is that we evaluated Ag2O NPs, in addition to TiO2 NPs. Moreover, Porenczuk et al. (2016) evaluated the shear bond strength of glass ionomer and dentin bonding agents, containing silver NPs and microscopically investigated their fracture modes. The results showed that incorporation of silver NPs did not significantly affect the shear bond strength to dentin. [18]

One of the strengths of the present study is the comparison of shear bond strength to radicular and coronal dentin. Besides, in this study, apart from the evaluation of silver NPs, the effects of titanium NPs were evaluated, as well. The fracture modes of RMGI bonds to dentin were also evaluated. In the present study, a wire was used to apply a shearing force between resin-modified glass ionomer and dentin. According to previous studies, stress concentration in the force application area can lead to a low bond strength [19], which may explain adhesive failure in most samples. Also, when there were no significant differences in bond strength between the groups, there were also no significant differences in the failure modes between the intervention and control groups.

Moreover, Hamid et al. (2018) [20] evaluated the effects of incorporating TiO2 NPs and cetylpyridinium chloride (CPC) on the compressive strength and antibacterial activity of conventional glass ionomer cement and reported that incorporation of 3 wt.% TiO2 NPs improved the compressive strength and antibacterial activity against S. mutans. [20] In the present study, the shear bond strength of TiO2 NPs was evaluated, and incorporation of this NPs had no adverse effects on the shear bond strength.

Additionally, Rezvani et al. (2019) [21] evaluated RMGI, with or without silica, regarding the microshear bond strength to permanent tooth dentin and concluded that 5 wt.% silica NPs changed the microshear bond strength compared to RMGI. [21] According to different studies, there are certain changes in the dentin structure. Besides, the tubular density and thickness of peritubular dentin are higher in the coronal dentin compared to the radicular dentin. Based on previous research, the dentin characteristics vary depending on location, and different structures create different characteristics. [22]

The mechanism of RMGI adhesion to the tooth structure is mainly chemical and is achieved through ionic exchange between the tooth and restorative material. Electron spectroscopic studies for chemical analyses have indicated small micromechanical bonding in RMGI. [23] According to the present results, these structural differences in the coronal and radicular dentins are inadequate to cause significant differences in the bond strength of RMGI. The results of experiments for validation of synergistic effects are one of the limitations of this study. Also, access to NPs in studies has some limitations.

Conclusion

Incorporation of 3 wt.% Ag2O and TiO2 NPs into RMGI created good antibacterial properties, without any significant effects on the shear bond strength. Also, comparison of antibacterial effects between RMGI containing NPs and conventional RMGI showed an increase in antibacterial activity against S. mutans over time. Besides, the shear bond strength was favorable in both groups. The results revealed that RMGI containing TiO2 NPs caused the least color change compared to the control group. Future studies need to investigate the possibility of using TiO2 and Ag2O NPs in combination with modified glass ionomer resin to induce antimicrobial properties and increase the shear bond strength.

Conflict of Interest

No Conflict of Interest Declared ■

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