Journal of Dental Biomaterials
Shiraz University of Medical Sciences, Shiraz, Iran

Journal of Dental Biomaterials. 2018;5(1)

Original Article

Evaluation of Antibacterial Properties of Dental Adhesives Containing Metal Nanoparticles

Shafiei F [a*]; Ashnagar A[b] ; Ghavami-Lahiji M [c] ; Najafi F[d] ; Amin Marashi SM[e]

[a]Assistant professor, Department of Dental Biomaterials, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[b]Pharm-D, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c]Ph.D. candidate for Dental Biomaterials, Research Center for Science and Technology in Medicine, Department of Dental Biomaterials, School of Dentistry,Tehran University of Medical Sciences, Tehran, Iran
[d]Assistant professor, Department of Resin and Additives, Institute for Color Science and Technology, Tehran, Iran
[e]Assistant professor, Department of Microbiology and Immunology, Alborz University of Medical Sciences, Karaj, Iran

ARTICLE INFO

Article History:
Received: 21 September 2017
Accepted: 26 November 2017

Key words:
Dental adhesive
Nanoparticle
Antibacterial
Silver
Titanium dioxide

Corresponding Author:
Farhad Shafiei
Department of Dental Biomaterials, School of Dentistry, Tehran University of Medical Sciences, North Kargar Avenue, Tehran 14174, Iran
Email: faradent@yahoo.com
Tel: +98-9121598469

Abstract

Statement of problem: Secondary dental caries is a common clinical finding in composite restoration. The development of a bactericidal dental adhesive provides a promising method to reduce the risk of secondary caries.

Objectives: This study aimed to assess the antibacterial activity of silver (Ag) and titanium dioxide (TiO_2) nanoparticles incorporated into an experimental dentin bonding agent formulation.

Materials and Methods: Ag and TiO_2 nanoparticles at 0.05, 0.1, 0.2, 0.5, and 1 wt% concentrations were incorporated into the adhesives. The suspensions were sonicated to ensure homogenous dispersion of nanoparticles in the adhesive system. Formulation was composed of acetone, 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane (Bis-GMA), 1,6-bis-[2-methacryloyloxyethyl carbonyl amino]-2,4,4-trimethylhexane (UDMA), trimethylolpropane trimethacrylate (TMPTMA), 2-hydroxyethyl methacrylate (HEMA), and photoinitiator, with polyvinylpyrrolidone (PVP) as the stabilizer. We counted the colony-forming units (CFU%) of two cariogenic bacteria, *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)*, that were exposed to the powdered light cured adhesive specimens. The effects of various concentrations of each nanoparticle were compared by one-way ANOVA, followed by the post hoc Bonferroni test.

Results: All samples exhibited definite antibacterial activity ($P<0.05$) compared to the control specimens. The Ag nanoparticle samples showed higher antibacterial properties compared to the TiO_2 nanoparticle samples. Increasing the concentration of nanoparticles resulted in significant differences in bactericidal properties, with the exception of 0.2 to 0.5 wt% Ag nanoparticle specimens exposed to *S. mutans* and the 0.2 to 0.5 wt% TiO_2 nanoparticle specimens exposed to *L. acidophilus*.

Conclusions: These metal-based nanoparticles exhibited dose-dependent bactericidal activities. The Ag nanoparticles had higher antibacterial activity compared to the TiO_2 nanoparticles. Incorporation of these nanoparticles into dental adhesives is a promising way to reduce the risk of secondary caries. However, further clinical evaluations should be performed.

Cite this article as: Shafiei F, Ashnagar A, Ghavami-Lahiji M, Najafi F, Amin Marashi SM. Evaluation of Antibacterial Properties of Dental Adhesives Containing Metal Nanoparticles J Dent Biomater, 2018;5(1):510-519.

Introduction

The basic mechanism behind tooth decay is demineralization attributed to acid produced by bacteria [1-3]. Carbohydrates metabolize to acid by cariogenic bacteria that exist in plaque such as *Streptococcus mutans (S. mutans) and Lactobacillus acidophilus (L. acidophilus)*. Thus, development of bonding agents and other restorative and preventive materials that have the capability to reduce bacterial activity at the tooth/composite interface can effectively decrease secondary caries [4-6].While the clinical performance of resin-based materials has greatly improved in terms of restoration aesthetics, durability, and bonding strength; recently, there is increased focus on the acquisition of antibacterial properties. Incorporation of soluble antimicrobial agents, such as chlorhexidine, in the resin matrix have been investigated [7]. The results have shown clear inhibition of bacteria, but the release kinetics is difficult to control and long-term effect is not expected. One of the advantages of adding a releasing agent is that the antibacterial effect can produce an impact beyond the area of the resinous material. However, frequently mechanical properties of the resin material are reduced [7]. Another strategy is to employ an antibacterial agent, which is immobilized in the resin matrix and not released. In this case, the antibacterial effect is limited to bacteria that directly contact the material. Antibacterial agents incorporated in adhesives [8, 9] and filler composites [10] have shown good bacterial inhibition. Among various metals, silver (Ag) has attracted significant attention due to its antibacterial effects [11-14]. One of the advantages of Ag over general antibiotics is that Ag has wide-spectrum antibacterial activity with very high efficiency and relatively low cytotoxicity [12]. Ag nanoparticles placed in a polymer matrix produce a large reservoir of Ag ions that can be released over time and provide a long-lasting antibacterial effect [15]. Titanium dioxide (TiO_2) is a bactericide and a biocompatible material with potential use in many applications. Bioactivity of this material can also be useful in closure of the gaps in the interface and remineralization of the adjacent tooth [16, 17]. We took into consideration the aforementioned problems and the increased need to develop an efficient and biocompatible dental adhesive to inhibit secondary caries. First, we aimed to synthesize a dental bonding incorporated with metal Ag and TiO_2 nanoparticles, and subsequently assessed the antibacterial activities of the light-cured dental bonding by exposing them to *S. mutans and L. acidophilus*.

Materials and Methods

Adhesive preparation

Table 1 lists the information about the materials and their use. In order to prepare 10 g base formulation of dentine bonding agent, we used a magnetic stirrer to mix 1.4 g of 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl] propane (Bis-GMA), 2.6 g of 2-hydroxyethyl methacrylate (HEMA), 0.8 g trimethylolpropane trimethacrylate (TMPTMA), 1.2 g of 1,6-bis-[2-methacryloyloxyethyl carbonyl amino]-2,4,4-trimethylhexane (UDMA), and 4 g of acetone. All weights were obtained by a laboratory scale (Sartorius AG, Germany) that had an accuracy of four decimal places. The mixture was mixed until homogenous. Then, we divided the base formulation into different containers and added the antibacterial nanoparticles at the following weight percentages: 0.05, 0.1, 0.2, 0.5, and 1 wt% to the mixture. We used Ag and TiO_2 spherical shaped nanoparticles that had a diameter of 20 nm and 99.9% purity. We added 0.5 wt% polyvinylpyrrolidone (PVP) to the mixture to avoid agglomeration of the nanoparticles. Direct sonication by a probe is the preferred method to disperse nanoparticles. Thus, in this study, we used ultrasound vibrations from an ultrasonic probe (UP400S - Hielscher Ultrasonics GmbH, Germany) with each mixture for 3 minutes, at 0.5 cycle with an amplitude of 60%. Then, 0.5 wt% photoinitiator (IRGACURE 819) and 0.1 wt% p-Methoxyphenol (PMP) were added to each bottle. After the addition of the photoinitiator, the mixing was continued with a magnetic stirrer in the dark at 40ºC for 30 minutes. The samples were stored in a light resistant glass bottles to avoid pre-term light curing from the environment.

Uncured adhesives were used for particle size analysis. The 1 at% Ag and TiO_2 nanoparticle adhesives were sonicated using an ultrasonic probe for 30 seconds before insertion into the DLS (Zetasizer Nano, Malvern Instruments, UK)

cuvettes. The results confirmed the presence of nano-sized particles in the resin. The nanoparticle-adhesives had an average polydispersity index (PDI) of 0.279 for Ag and 0.173 for TiO_2. This clearly indicated that the nanoparticle-adhesives were almost homogenous.

Shear bond strength test

We conducted a shear strength test to confirm the bonding properties of the newly synthesized adhesives. Commercial dental bonding (3M ESPE™ Single Bond™) was used as the control sample. A total of 18 dentinal samples were etched for 20 seconds with 37% commercial phosphoric acid, rinsed with distilled water, and slightly dried. Then, the prepared 1wt% Ag nanoparticle and TiO_2 nanoparticle dental adhesives were applied to the samples with a micro brush. After 15 seconds, a slight stream of air spray was used to ensure development of the resin tags. The adhesives were light cured for 40 seconds by a 400 mW/cm^2 intensity light curing unit (Coltolux 75, Coltene, Whaledent, NJ, USA). Then, we placed a plastic tube (diameter: 4 mm, height: 4mm) on the dentine surface and filled with dental resin composite (Shade A2, 3M ESPE™ Filtek™ Z250 universal restorative). The samples

Table 1: Materials used in the study

Materials	Manufacturer	Utilization
Nano silver (Ag)	US Research Nanomaterials	Filler
Nano Titanium dioxide (TiO_2)	Sigma- Aldrich	Filler
Acetone	Merck	Solvent
2,2-Bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl] propane (Bis-GMA)	Degussa	Adhesive monomer (di-methacrylate)
2-Hydroxyethyl methacrylate (HEMA)	Sigma- Aldrich	Hydrophilic monomer
1,6-bis-[2-methacryloyloxyethyl carbonyl amino]-2,4,4-trimethylhexane (UDMA)	Degussa	Adhesive monomer (di-methacrylate)
Trimethylolpropane trimethacrylate (TMPTMA)	Sigma- Aldrich	Adhesive monomer (tri-functional monomer)
Phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide (IRGACURE 819)	Sigma- Aldrich	Photoinitiator
P-Methoxyphenol (PMP)	Merck	Inhibitor
Polyvinylpyrrolidone (PVP)	Sigma-Aldrich	Prevent agglomeration of nanoparticles

were cured for 40 seconds. After removing the plastic tube, 40 seconds were cured again to ensure complete polymerization. Then, the specimens were stored in distilled water for 24 hours at 37°C. Shear bond strength was measured by a Universal Testing Machine (UTM) (Santam, SMT-20, Iran). The chisel was attached to the upper arm of the UTM. The load was applied parallel to the dentin/resin composite interface with a load cell of 20 kg (Bongshin Loadcell Co., Ltd., South Korea) at a crosshead speed of 0.5 mm/min until the specimens debonded.

Antibacterial assessment

We used a cylindrical stainless steel mold (diameter: 9 mm, height: 2 mm) to prepare the cured samples. The mold was placed on a cover glass. Then, uncured adhesive was injected into the mold by a 10 ml sterile syringe with a 0.22 µm syringe filter to ensure that probable agglomerated particles would not be present at the samples. Another cover glass was carefully placed on the mold such that no bubble was made in the adhesive. The samples were put in a vacuum oven (Ehret GmbH, Germany) for 30 minutes for complete evaporation of the acetone. The samples were cured for 60 seconds, 30 seconds per side. Then, the specimens were removed from the mold and kept in sterile surgical covers. All cured samples were ground by a laboratory universal grinder (Mortar Grinder PULVERISETTE 2, Fritsch). Powdered adhesive specimens were sterilized under a UV lamp (Laminar Flow UV Cabinet, JTLV C2, Iran) for 180 minutes.We used two cariogenic bacteria in this study – *S. mutans* (ATCC 35668) and *L. acidophilus* (ATCC 314). These bacteria were prepared from the microbial collection at Pasteur Institute of Iran, Tehran, Iran. We prepared 2.0 McFarland turbidity of each bacteria in sterile laboratory tubes. Turbidity was confirmed by a spectrophotometric (Biophotometer Plus, Eppendorf) assessment of optical density. We used two, 96-well microtiter plates for bacterial cultivation. Positive control wells consisted of culture medium plus bacterial solution. The powdered antibacterial-adhesives were placed into the wells. All wells were poured with 200 µl blood culture (Baharafshan, Iran) using a micropipette (Eppendorf Research). Then, 6.66 µl of the bacterial suspension was inserted into the designated wells. The microtiter plates were incubated for 24 hours at 37ºC in CO_2 by placing both microtiter plates in an isolated jar that contained a lit candle. The flame extinguished when all of the oxygen was consumed. After 24 hours, 1 µl from each well was extracted and diluted in a sterile laboratory tube with 999 µl of physiologic serum. Then, 10 µl of each solution was removed with a micropipette and spread on the surface of the solid medium. We used sterilized chocolate agar medium to grow *S. mutans* and sterilized MRS agar medium for *L. acidophilus*. All plates were incubated for 24 hours at 37ºC in CO_2. We counted the bacteria by determining the colony-forming unit (CFU%), as an estimate of viable bacteria.

Statistical analysis

Colony counts of the bacteria were standardized from 0%-100% based on the colony count of the control group. The effect of nanoparticle type and concentration for each bacteria was analyzed by two-way ANOVA. We took into consideration the significant interaction of the aforementioned factors and performed a comparison of the effects of each nanoparticle at various concentrations by one-way ANOVA, followed by the post hoc Bonferroni test. Nanoparticles were compared at each concentration by the independent t-test. Without adjustment for α error, the significance level was set at $P<0.05$.

Results

Shear strength test

The shear bond strength value confirmed that the specimens had bonding properties similar to

Table 2: Shear bond strength of commercial and two fabricated dental adhesive. Bond strength values are presented as MPa

Adhesive	Bond strength Mean ± SD (MPa)
Commercial, Single Bond (3M ESPE)	7.57 ± 0.1
Silver (Ag)-containing adhesive	7.58 ± 0.08
Titanium dioxide (TiO_2)-containing adhesive	7.55 ± 0.09

commercial adhesives (Table 2). The shear strength values of the specimens were similar to the control group; therefore, the specimens were considered for the antibacterial test.

Colony count test

We analyzed the interaction between concentration and nanoparticle by two-way ANOVA. The results indicated a significant interaction in both bacteria (P<0.001) as seen in Table 3.The number of the bacterial colonies was calculated. As described earlier, the colony counts were standardized concerning the control colony count. At 24 hours, the CFU% was approximately 500 for *L. acidophilus* for the control group. The Ag-adhesive had a gradual reduction in CFU% with increasing concentrations of Ag, as follows: 372 (0.05%), 340 (0.1%), 303 (0.2%), 185 (0.5%), and 130 (1%) for *L. acidophilus*. CFU% counts were approximately 320 for *S. mutans* in the control group. The Ag-containing adhesive also showed a gradual reduction in CFU% with increasing concentrations of Ag, as follows: 207 (0.05%), 183 (0.1%), 108 (0.2%), 104 (0.5%), and 51 (1%) for *S. mutans*. Mean and standard deviation (SD) of CFU% data is shown in Table 4. One-way ANOVA followed by Bonferroni analysis among different concentrations showed statistically significant differences (P<0.05), with the exception of two concentrations. There was no statistically significant difference between the 0.2 wt% and 0.5 wt% concentrations of the TiO_2 nanoparticle on *L. acidophilus* (P=0.068). In addition, the difference between the 0.2 wt% and 0.5 wt% concentrations of the Ag nanoparticle on *S. mutans* was not statistically significant (P=1.00). We used the t-test to compare the effects of each concentration on CFU% between both nanoparticles. The results indicated a statistically significant difference between the two types of nanoparticle-adhesives at each concentration. Figure 1 shows the behavior of these nanoparticles concerning CFU% for *L. acidophilus* and *S. mutans*.

Discussion

Antimicrobial surfaces present a major challenge, particularly in dentistry, where bacterial biofilms tend to accumulate and propagate on solid surfaces. Resin composite restorations are more susceptible to secondary caries due to the increased tendency to colonize bacteria on their surfaces. Polymerization shrinkage of the resin composites makes the bonding interface the weakest area. Thus, the role of a dental adhesive is important. One way to address this problem is to design materials with antibacterial properties. Dental bonding agents, as an important and delicate player in the integrity of a composite restoration, can be suitable hosts for antibacterial materials. The current study has aimed to fabricate antibacterial adhesives and evaluate the antibacterial properties of various concentrations of two nanoparticles, TiO_2 and Ag, incorporated into an adhesive.

Table 3: Two-way ANOVA (5x2) to assess the interactions of the nanoparticles and concentrations

Bacteria	Source	F	P-value
Lactobacillus acidophilus (L. acidophilus)	Concentration	2143.13	<0.001
	Nanoparticle	986.60	<0.001
	Concentration* Nanoparticle	222.97	<0.001
Streptococcus mutans (S. mutans)	Concentration	1060.90	<0.001
	Nanoparticle	782.48	<0.001
	Concentration* Nanoparticle	30.99	<0.001

* simultaneous effect of two variables (concentration and nanoparticle) on the colony count of bacteria

Table 4: Colony counts of *L. acidophilus* and *S. mutans* in Ag and TiO_2 nanoparticle containing specimens

Bacteria	concentration	CFU% of Ag-containing adhesive Mean±SD	CFU% of TiO_2-containing adhesive Mean±SD
L. acidophilus	0.05	73.80±0.71	80.02±1.19
	0.1	68.40±0.5	70.21±0.7
	0.2	59.76±0.9	63.97±1.01
	0.5	36.92±0.87	66.86±1.05
	1	26.18±0.9	37.20±1.17
S. mutans	0.05	64.28±1.35	92.029±1.29
	0.1	57.54±1.75	66.98±1.25
	0.2	33.33±1.54	52.46±1.11
	0.5	32.80±1.66	44.16±1.66
	1	16.14±2.34	30.62±1.73

CFU= Colony-forming unit, Ag= Silver, TiO_2= Titanium dioxide, *L. acidophilus*= *Lactobacillus acidophilus*, *S. mutans*= *Streptococcus mutans*

Nanotechnology has been employed in many fields in recent years. Currently, nanoparticles are used for different physical, biomedical, and pharmaceutical applications. Metal-based nanoparticles have promising antibacterial properties. Among these materials, the Ag nanoparticle and TiO_2 nanoparticle have been investigated in numerous studies.

It has been reported that Ag is highly toxic to the majority of microorganisms [18, 19]. The nanoparticle form of Ag exhibits an increased bactericidal effect because of enhanced surface area exposure to the microorganisms [19]. However, the mechanism of action of Ag on microorganisms is not fully understood. It has been suggested that loss of ability to replicate DNA and/or changes in the bacterial cell wall occur after the application of Ag nanoparticles [20]. In addition, the photocatalysis properties of TiO_2 have been employed in many fields. Produced free radicals (HO• and O_2 •−) from TiO_2 following UV exposure, are known as reactive oxygen species (ROS). ROS are strong oxidants that have the capability to induce oxidative damage in the cell walls of microorganisms [21]. Studies have shown that TiO_2 has photocatalysis and bactericidal properties; however, the use for this capability has been less studied in dental adhesives. It has shown good antibacterial property in resin composites; however, mechanical properties might decrease. Shirai *et al*. reported that after completion of UV radiation, the antimicrobial property of TiO_2 continued. This property could be employed as an adjunct treatment to eliminate residual bacteria after debridement [21]. The bioactivity of TiO_2 added to an adhesive was proven by Welch *et al*. with the formation of hydroxyl apatite at the surface. The advantages of this feature included closure of gaps between resin material and the tooth, as well as remineralization of the adjacent tooth [16]. Sun *et al*. reported that the mechanical properties and degree of conversion of the adhesive improved by the addition of the TiO_2 nanoparticle [22]. Dentinal tubules are reported to enhance bond strength with resinous materials due to formation of resin tags [16]. Dentinal tubules have diameters of approximately 1-2.5 µm [16, 23]. Therefore, we did not anticipate that our nanoparticle-containing adhesive would interfere with the bonding system. The nanoparticles were smaller than the dentinal tubules. The results of a preliminary study revealed that shear bond strength of the nanoparticle-containing adhesive was comparable to commercial counterparts. This finding agreed

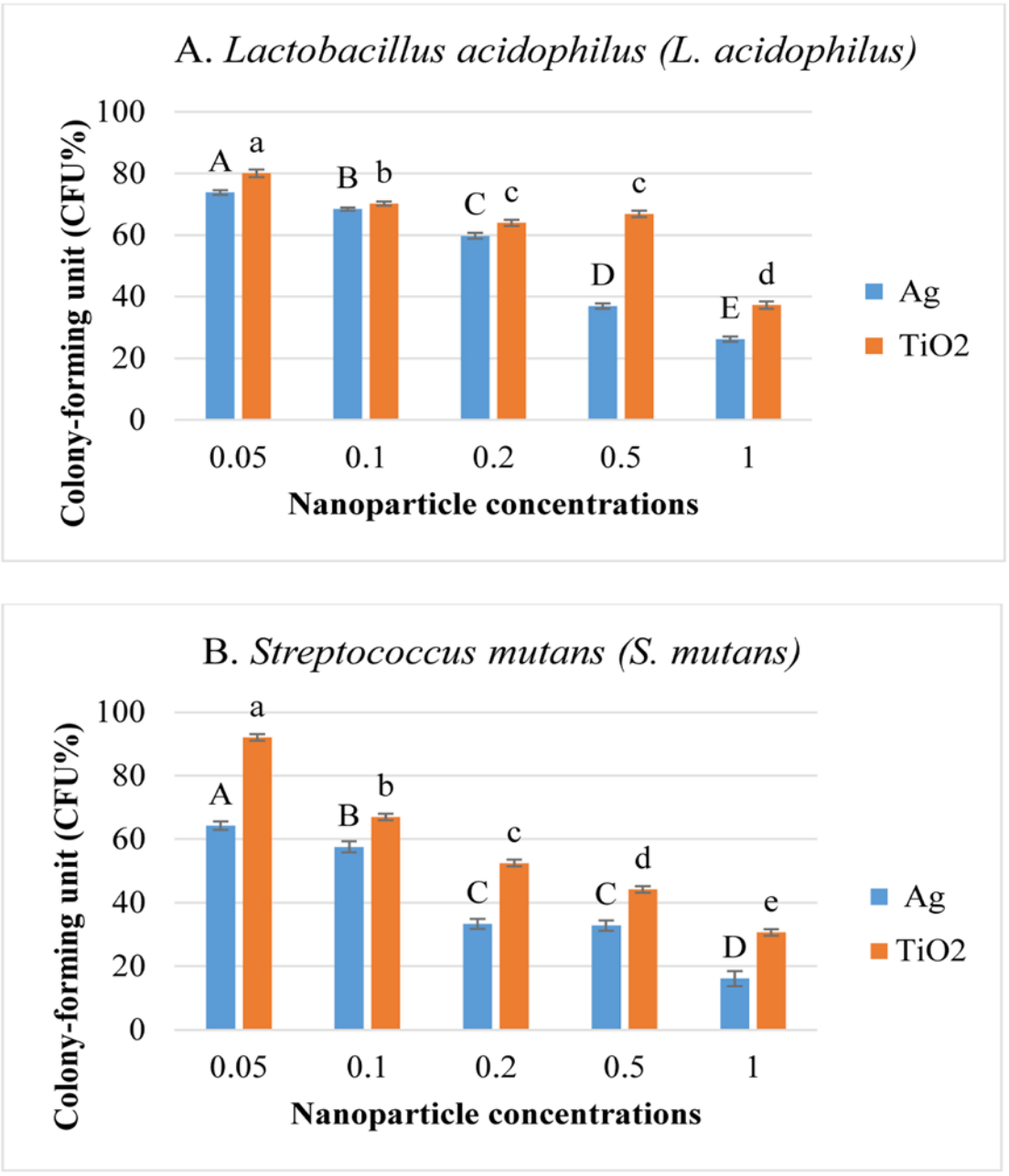

Figure 1: The effect of nanoparticle concentration on colony-forming unit (CFU%) in both *Lactobacillus acidophilus (L. acidophilus)* (A) and *Streptococcus mutans (S. mutans)* (B). Values marked by different capital and lowercase letters are significantly different in the silver (Ag) and titanium dioxide (TiO_2) groups ($P<0.05$)

with other studies [16, 24, 25] that added different nanoparticles to commercial adhesives and reported no reductions in shear bond strength. However, we synthesized antibacterial adhesives and the nanoparticle in our study was added to our experimental base formulation *et al*. [26] reported significant improvement in mechanical properties of synthetic dental adhesives, especially with incorporation of 0.1% and 0.2% diamond nanoparticles. No decrease in bond strength data might be explained by usage of the tri-functional monomer TMPTMA in our adhesive formulation. According to Silva, replacement of triethylene glycol dimethacrylate (TEGDMA) with a cross-linker monomer (TMPTMA) improved the chemical-mechanical properties of adhesives [27]. Here, we used Ag and TiO_2 nanoparticles to develop an intrinsic bactericidal methacrylate based dental adhesive. Various concentrations of each of the incorporated nanoparticles were made to identify the effect of concentration on bactericidal properties of Ag and TiO_2. The results indicated that adhesive containing nanoparticles exhibited definite antibacterial properties. This bactericidal

property was dose-dependent, which agreed with results reported by Degrazia *et al*. [28]. Each dosage had a statistically significant difference in CFU% compared to other concentrations, with the exception of the 0.2 wt% and 0.5 wt% Ag-containing samples in *S. mutans* and the 0.2 wt% and 0.5 wt% TiO_2-containing samples in *L. acidophilus*. This similarity should be noted when choosing the desired concentration because of the potential impact on mechanical properties while there would be no significant changes in bactericidal properties. We observed that the Ag nanoparticle specimens had a noticeably sharp drop in colonies from the 0.2 wt% to 0.5 wt% in *L. acidophilus* and from the 0.1 wt% to 0.2 wt% in *S. mutans* (Figure 1, Table 4). The TiO_2 nanoparticle specimens had a sharp drop in colonies from the 0.5 wt% to 1 wt% in *L. acidophilus*, whereas we observed that the CFUs of *S. mutans* followed an approximately regular pattern. The 1 wt% showed the highest antibacterial activity in both nanoparticles. The Ag particles had stronger bactericidal action against *S. mutans* and *L. acidophilus* compared to the TiO_2 particles. This difference was more noticeable with *S. mutans*, especially at the higher dosages. Cheng *et al*. [29] incorporated Ag nanoparticles into an amorphous calcium nanocomposite. They used 0.028 wt% nanoAg. [29]. The Ag containing specimens reduced approximately 15.2 CFU% compared to neat nanocomposite. This CFU% reduction was lower than our 0.05% Ag nanoparticle specimens that had a 35 CFU% reduction in *S. mutans*. This might be attributed to the higher amount of Ag used in our study. In addition, the methodology and antibacterial test differed between studies. Cheng *et al*. placed the bacteria on a large resin surface, whereas we immersed adhesive powder in the bacterial suspension. Welch *et al*. incorporated nano TiO_2 into a commercially available bonding agent to achieve a bioactive and bactericidal dental adhesive [16]. They reported that the antibacterial properties of the nano TiO_2 depended on UV irradiation time and were not concentration dependent. However, our results demonstrated that the bactericidal properties of the TiO_2 nanoparticle were concentration dependent. Welch *et al*. used *Staphylococcus epidermidis,* which is a part of the human skin's normal flora. The choice of this bacteria to assess bactericidal properties of a dental adhesive, which is faced with challenges from cariogenic bacteria seems irrelevant. In this study, we performed the CFU test to obtain preliminary information on the efficacy of the nanomaterial antibacterial agent. Considering the promising findings, further studies can be done with various microbial tests on these adhesives. We suggest assessing the effect of addition of these nanoparticles on the mechanical behavior of dental adhesive. Higher concentrations of these materials should be tested to obtain the highest bactericidal activity. Other nanoparticles such as zinc oxide (ZnO) might have antibacterial properties, supported by strong mechanical improvement.

Conclusions

We tentatively incorporated metal-based nanoparticles into a synthesized etch and rinse dental adhesive to assess antibacterial properties of the newly developed material on cariogenic bacteria. We found that these metal-based nanoparticle exhibited bactericidal activities in a dose-dependent manner without affecting shear bond strength. The Ag nanoparticles showed higher antibacterial activity compared to the TiO_2 nanoparticles. Incorporation of such materials into dental adhesives is a promising way to reduce the risk of secondary caries. However, further clinical evaluation should be performed.

Acknowledgements

This study was financially supported by a grant for scientific research (No. 92-03-69-23934) from Tehran University of Medical Sciences.

Conflict of Interest: None declared.

References

1. Featherstone JD. The continuum of dental caries—evidence for a dynamic disease process. J Dent Res. 2004;83:C39-C42.
2. Deng DM, Ten Cate JM. Demineralization of dentin by Streptococcus mutans biofilms grown in the constant depth film fermentor. Caries Res. 2003;38:54-61.
3. Totiam P, Gonzalez-Cabezas C, Fontana MR, *et*

al. A new in vitro model to study the relationship of gap size and secondary caries. Caries Res. 2007;41:467-73.
4. Chen C, Cheng L, Weir MD, *et al*. Primer containing dimethylaminododecyl methacrylate kills bacteria impregnated in human dentin blocks. Int J Oral Sci. 2016;8:239-245.
5. Ge Y, Wang S, Zhou X, *et al*. The use of quaternary ammonium to combat dental caries. Materials (Basel). 2015;8:3532-49.
6. Wang L, Li C, Weir MD, *et al*. Novel multifunctional dental bonding agent for class-V restorations to inhibit periodontal biofilms. RSC Advances. 2017;7:29004-14.
7. Imazato S, Kinomoto Y, Tarumi H, *et al*. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. Dent Mater. 2003;19:313-9.
8. Ahn SJ, Lee SJ, Kook JK, *et al*. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009;25:206-13.
9. Imazato S, Kuramoto A, Takahashi Y, *et al*. In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond. Dent Mater. 2006;22:527-32.
10. Yoshida K, Tanagawa M, Atsuta M. Characterization and inhibitory effect of antibacterial dental resin composites incorporating silver-supported materials. J Biomed Mater Res. 1999;47:516-22.
11. Brunetto PS, Fromm KM. Antimicrobial coatings for implant surfaces. CHIMIA International Journal for Chemistry. 2008;62:249-52.
12. Slenters TV, Hauser-Gerspach I, Daniels AU, *et al*. Silver coordination compounds as light-stable, nano-structured and anti-bacterial coatings for dental implant and restorative materials. J Mater Chem. 2008;18:5359-62.
13. Lee HY, Park HK, Lee YM, *et al*. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. Chem Commun (Camb). 2007;28:2959-61.
14. Monteiro DR, Gorup LF, Takamiya AS, *et al*. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents. 2009;34:103-10.
15. Damm C, Münstedt H, Rösch A. Long-term antimicrobial polyamide 6/silver-nanocomposites. J Mater Sci. 2007;42:6067-73.
16. Welch K, Cai Y, Engqvist H, *et al*. Dental adhesives with bioactive and on-demand bactericidal properties. Dent Mater. 2010;26:491-9.
17. Heravi F, Ramezani M, Poosti M, *et al*. In vitro cytotoxicity assessment of an orthodontic composite containing titanium-dioxide nano-particles. J Dent Res Dent Clin Dent Prospects. 2013;7:192-198.
18. Morones JR, Elechiguerra JL, Camacho A, *et al*. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16:2346-53.
19. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012;2:32.
20. Feng QL, Wu J, Chen GQ, *et al*. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res. 2000;52:662-8.
21. Shirai R, Miura T, Yoshida A, *et al*. Antimicrobial effect of titanium dioxide after ultraviolet irradiation against periodontal pathogen. Dent Mater J. 2016;35:511-6.
22. Sun J, Forster AM, Johnson PM, *et al*. Improving performance of dental resins by adding titanium dioxide nanoparticles. Dent Mater. 2011;27:972-82.
23. Parkinson CR, Sasov A. High-resolution non-destructive 3D interrogation of dentin using X-ray nanotomography. Dent Mater. 2008;24:773-7.
24. Melo MA, Cheng L, Zhang K, *et al*. Novel dental adhesives containing nanoparticles of silver and amorphous calcium phosphate. Dent Mater. 2013;29:199-210.
25. Zhang K, Melo MA, Cheng L, *et al*. Effect of quaternary ammonium and silver nanoparticle-containing adhesives on dentin bond strength and dental plaque microcosm biofilms. Dent Mater. 2012;28:842-52.
26. Ebadi Z, Atai M, Ebrahimi M. Mechanical and Anti-bacterial Properties of Dental Adhesive Containing Diamond Nanoparticles. Iran J Polym Sci Technol. 2011;24:121-31.
27. da SILVA EM, Miragaya L, Noronha-Filho JD, *et al*. Characterization of an experimental

resin composite organic matrix based on a tri-functional methacrylate monomer. Dent Mater J. 2016;35:159-65.

28. Degrazia FW, LEITUNE VCB, Garcia IM, *et al*. Effect of silver nanoparticles on the physicochemical and antimicrobial properties of an orthodontic adhesive. J Appl Oral Sci. 2016;24:404-10.
29. Cheng L, Weir MD, Xu HH, *et al*. Antibacterial amorphous calcium phosphate nanocomposites with a quaternary ammonium dimethacrylate and silver nanoparticles. Dent Mater. 2012;28:561-72.