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Evaluation of Bond Strength and Microleakage of a Novel Metal-titanate Antibacterial Agent

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Clinical Relevance

The novel antibacterial nanoparticulate metal-titanate complexes under investigation will allow clinicians to tackle the composite longevity problem at its weakest interface.

SUMMARY

Objectives: To evaluate the effect on both bond strength and microleakage of incorporation of a novel antibacterial nanoparticulate metal-titanate complex (nMT) into a dental adhesive system.

Materials and Methods: Eighty extracted human molars were prepared to determine whether incorporation of nMT into bonding agents can affect shear bond strength (SBS) and adhesive strength fatigue. SBS was measured with a universal testing machine, and the peak force at failure was recorded. An electromechanical fatigue machine was used for cyclic loading treatment of specimens. Differences in the SBS values among groups were identified using analysis of variance and Tukey post hoc analyses (α=0.05). Twenty standard Class V cavities were restored to examine microleakage when the primer/bonding resin was modified with 10 wt% nMT. Microleakage at the enamel and dentin margins was calculated as a percentage of the full length of the cavity. Results of the microleakage experiment were analyzed with paired and independent sample *t*-tests (α=0.05).

Results: The mean (± standard deviation) shear bond strength values of before fatigue and after fatigue ranged from 21.9 (2.5) MPa to 23.9 (3.8) MPa and from 17.1 (2.5) MPa to 17.7 (2.5) MPa respectively. No statistically significant differences in failure force were observed among groups (p=0.70). Microleakage under all conditions was significantly greater in the dentin margins than in the enamel margins (p<0.05). There was no evidence that microleakage differed between the experimental

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groups with modified primer and bonding resin.

Conclusions: Incorporating nMT into a dental adhesive system will not compromise the resin composite's tooth bonding and sealing ability.

INTRODUCTION

Biofilms are medically important because microbes in the biofilm state are more pathogenic than when they are planktonic (nonadherent and free floating). The National Institutes of Health reported[1] that bacteria growing as a biofilm cause 80% of infections in the body. Biofilms at the margin of an existing dental restoration give rise to secondary caries, which are the main complication necessitating replacement of composite fillings.[2,3] Currently there is a lack of research data both on 1) the effect of the cariogenic biofilm community structure and its metabolic processes on the tooth/resin composite interface and 2) the effect of the tooth/resin composite on biofilm formation and population distribution.

Monosodium titanate (MST) is an inorganic, particulate compound of titanium oxide ($NaTi_2O_5H$) with an amorphous core and crystalline surface. MST has a spherical morphology with an approximate diameter of 1-10 μm. Our team has developed microparticulate metal-titanate complexes as a new class of antibacterial agents.[4-7] The microparticulate gold (III)–loaded titanate complexes inhibit growth of oral bacteria at micromolar concentrations. We predict that nanoparticulate metal-titanate complexes (nMTs) in turn will be even more effective at inhibiting oral bacteria growth as such complexes have a significantly greater surface-to-volume ratio, resulting in more effective ion-exchange characteristics. This approach is innovative as nMTs are ceramic in nature and act like resin filler. In addition, because these complexes are not organic, degradation is not an issue, and thus they can have long-term effectiveness and also may be less likely than organic antibacterial agents to contribute to bacterial resistance.[8]

Dental composite restorations are becoming the material of choice for posterior restorations; thus, their longevity is important to patients, dentists, federal health agencies, and insurance companies. A survey of 24 prospective studies on the clinical performance of posterior resin composites published between 1996 and 2002 indicated that the primary reasons for composite failure were secondary caries, restoration fracture, and marginal defects.[2] Secondary caries was the principal cause of composite failure necessitating replacement of fillings; these caries are lesions at the margin of an existing restoration and usually occur in areas of biofilm stagnation.[9] For this reason, the cervical margins of restorations are commonly affected. In the oral cavity, mixed microbial biofilms can accumulate on hard and soft tissues and are involved in the pathogenesis of caries and periodontitis. A biofilm is an accumulation of bacteria, fungi, or protozoa on solid surfaces. In dentistry, two popular approaches to preventing biofilm formation are 1) to design a biomaterial that slowly releases an agent that is lethal to the approaching bacterial cells and 2) to develop a nonadhesive surface by modifying the surface chemistry of restorative materials.[10] Various chemical agents can affect bacterial adhesion indirectly by disrupting bacterial cell metabolism. Numerous materials have been impregnated with various antibiotics only to have most of the agent released over a very short time, thus providing no long-term effect.[11] Recent studies have shown that sublethal doses of antibiotics can induce bacterial resistance and actually enhance biofilm formation. The potential negative consequences of bacterial resistance to antibiotics are dire because they put all of society at risk.[12]

Metal-based antibacterials are an attractive alternative. Metal ions have chemical properties that inhibit bacterial growth.[13] The unique binding, coordination, and redox properties make development of bacterial resistance less likely and predict effectiveness across a broad bacterial spectrum. For example, Ag(I) and Hg have a long history as antibacterials.[14] However, fears of systemic toxicity have limited Ag(I) and Hg use in recent years.[15] Other metal ions, such as Au(III), Pd(II), and Pt(IV), have binding properties, coordination chemistries, and redox properties that suggest they also would be effective antibacterials. Unfortunately, development of new metal-based antibacterials has been severely impeded because of previous controversies and fears. Yet, given the increasing resistance of bacteria to organic antibacterials, metal-based antibacterials are a promising alternative. If systemic toxicity could be limited and therapeutic indices were optimized, metal ions and their associated compounds could emerge as a new powerful class of antibacterial agents.

The goal of this investigation was to evaluate the bond strength and microleakage of a kind of novel

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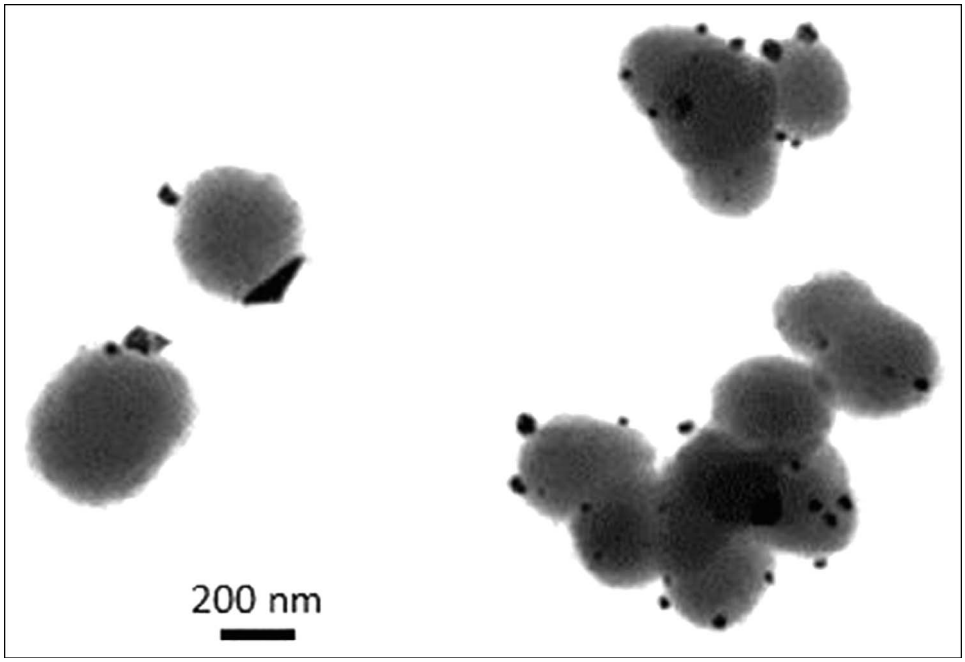

Figure 1. *Transmision electron microscopy (TEM) image of nanoparticulate gold-loaded monosodium titanate complexes.*

antibacterial nMT. This kind of nMT has been shown previously to have antimicrobial activity.[6]

METHODS AND MATERIALS

Experimental Materials

MSTs are particulate, micron-sized ion exchangers that may be useful solid-phase platforms for delivery of metal ions to inhibit bacterial growth.[4-7] The synthesis of micro- and nano-titanate particles based on a unique low-temperature (<80°C) sol-gel process has been reported.[16] The process produces titanates with superior ion-exchange characteristics compared to those produced using hydrothermal materials. Previous work[6] has shown that nanoparticulate titanate complexes with loaded gold and cisplatin have antimicrobial activities. An example of nanoparticulate gold-loaded MST complexes (nMTs) is illustrated in Figure 1. A 10% wt/wt nMT powder was added to Primer (All-Bond 2®, Bisco Inc, Schaumburg, IL, USA) and bonding resin (D/E resin, Bisco) and mixed in a dark room.

Shear Bond Strength (SBS) Test

The SBS test was conducted in three phases as the metal-titanate complexes were processed and available. In the first phase, we examined whether incorporation of MST affects the bond strength. In the second phase, we compared the effects of adding nMT. In the third phase, we examined the effect of cyclic loading on bond strength.

In the first and second phases, 80 extracted human molars were prepared with a coarse diamond bur at axial surfaces to create an approximately 8-mm-diameter enamel or dentin flat surface that was parallel with the long axis of each tooth. The surfaces were further wet polished up to 600 grit. Two surfaces from each tooth were selected for the bonding test, and the surfaces were etched with 32% H_3PO_4 (Uni-Etch, Bisco) for 15 seconds. They were thoroughly rinsed and the excess water removed with a brief burst of air. The modification of an adhesive system and bonding treatment are described in Table 1. Filtek Supreme Plus (3M ESPE, St Paul, MN, USA) was injected into a

Table 1: *List of Experimental Groups for Shear Bond Strength Test in This Study*

Groups	Bonding Treatment
Enamel (control)	Etching, priming, and bonding[a]
Enamel + 1	Etching, priming, and bonding with (D/E resin+10 wt% MST)
Enamel + 2	Etching, priming, and bonding with (D/E resin+10 wt% nMT)
Dentin (control)	Etching, priming, and bonding[a]
Dentin + 1	Etching, priming with (primer+10 wt% MST), and bonding
Dentin + 2	Etching, priming with (primer+10 wt% nMT), and bonding

Abbreviations: MST, monosodium titanate; nMT, nanoparticulate gold-loaded monosodium titanate complex.
[a] According to the manufacturer's instruction for the Universal Dental Adhesive System: All-Bond 2®.

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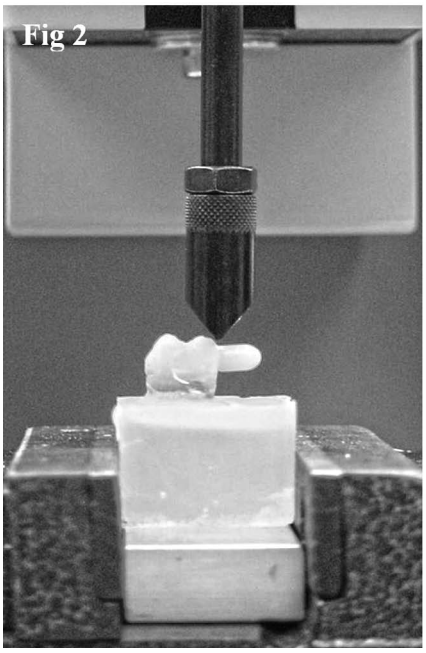

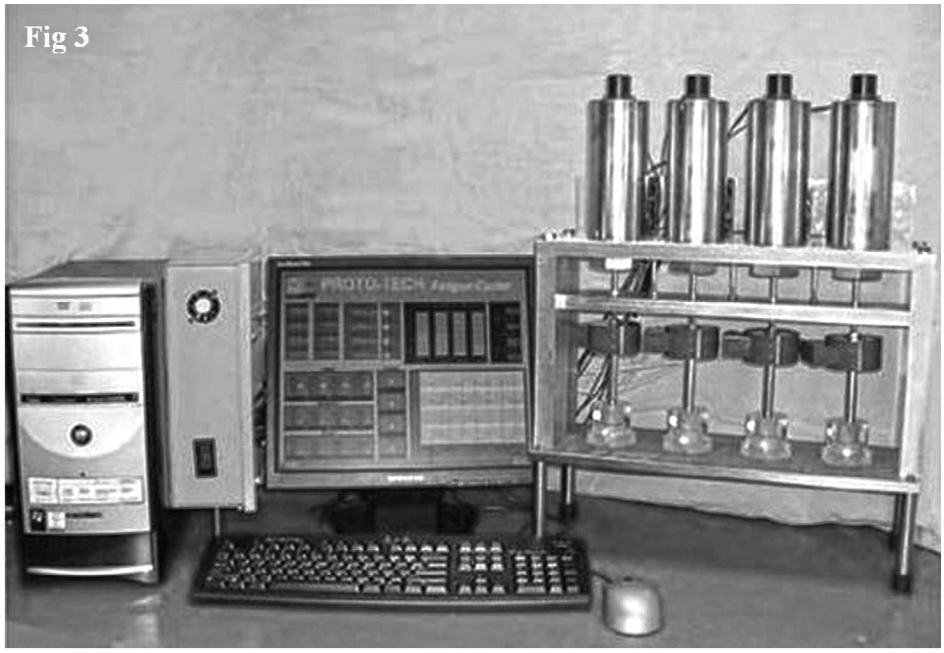

Figure 2. *Set up for shear bond strength testing.*

Figure 3. *A four-station fatigue cycler (Proto-tech, Portland, OR, USA) with DASYLab software (DASYLab, Norton, PA, USA).*

cellular gel capsule (4.4 mm in internal diameter, 12 mm in length) and attached perpendicularly on the etched enamel or dentin surface. The capsule was light-cured for 30 seconds on each surface (for a total of 120 seconds) using a LED light-curing unit (Elipar Frelight II, Dentsply, Konstanz, Germany) and stored in a water bath at 37°C for 24 hours. SBS was then measured with a universal testing machine (n=16, cross-head speed=5 mm/min), and the bond strength at failure was recorded (Figure 2).

In the third phase, a four-station electromechanical fatigue machine (Fatigue Cycler, Proto-tech, Portland, OR, USA) was used for cyclic loading treatment of specimens before the SBS testing. Specimens were mounted into a custom fixture inside a 37°C water chamber. The load for each station was adjusted with computerized software (DASYLab, Norton, PA, USA). The lower load limit was set at zero, and the maximum load applied was 70 N (which was approximately 30% of the average of the SBS values determined from the first and second phases, above). The load was applied at a rate of 1.2 Hz using a sine wave for 40,000 cycles (Figure 3). After cyclic loading, all specimens were stored in distilled water at 37°C for 24 hours and then the SBSs of postcyclic loading specimens were tested as described above. Differences in the SBS values among groups were identified using analysis of variance and Tukey post hoc analyses ($\alpha \leq 0.05$).

Microleakage Examination

Forty standard Class V cavities (4 × 2 × 2.5 mm) were prepared at the cemento-enamel junction (CEJ) on the buccal and lingual surfaces of 20 freshly extracted human molars. The cavities were prepared using diamond burs in a high-speed handpiece with water coolant. A single operator (SLD) performed all the cavity preparations and restorations. The specimens were randomly assigned to two study groups: group 1, primer (All-Bond 2®, Bisco) modified with 10 wt% nMT and group 2, bonding resin (D/E resin, Bisco) modified with 10 wt% nMT. Lingual cavities were treated by unaltered primer/adhesive resin as controls.

Cavities were then restored with resin composite (Filtek Supreme Plus) in the traditional incremental placement manner. The restorations were polished with aluminum oxide discs (Sof-Lex, 3M ESPE) and then stored in a water bath at 37°C for 24 hours to ensure adequate polymerization. The specimens were subjected to 500 cycles of thermocycling treatment between 5°C and 55°C with 30-second dwell time and a three-second transfer. Two coats of nail polish were then applied to the entire tooth surface within 1.0 mm of the restoration margins.

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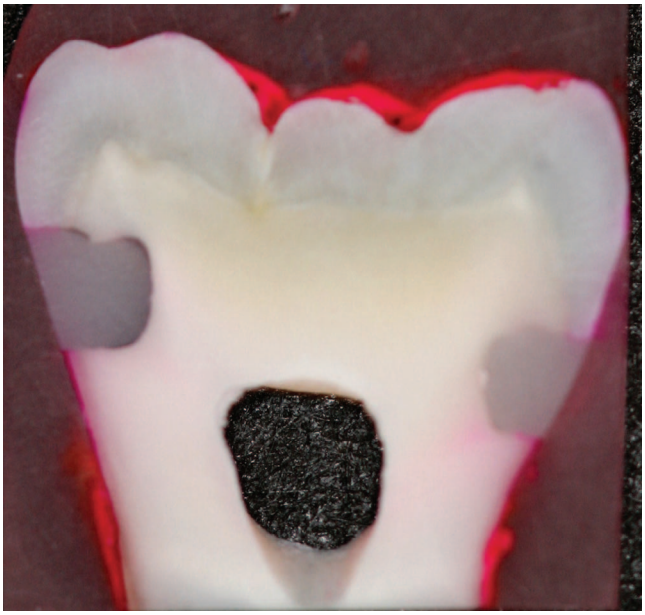

Figure 4. *Restored Class V cavities at the CEJ on the buccal and lingual surfaces to be evaluated for microleakage.*

The specimens were incubated separately in individual screw-capped tubes in a water bath at 37°C for 24 hours before immersion in a 0.2% Rhodamine B solution (Invitrogen, Carlsbad, CA, USA) for 24 hours. The specimens were then rinsed with distilled water for 10 minutes, dried, and embedded in a cold curing epoxy resin for two hours at 60°C. Blocks of resin were left at room temperature for six hours to achieve complete polymerization. Finally, two buccolingual sections were made through each restoration with a low-speed diamond saw (IsoMet, Buehler Ltd, Lake Bluff, IL, USA). Each tooth was sectioned into three pieces, producing four cross-sectional faces for evaluation. Sections were assessed for dye penetration with an optical microscope (Nikon Eclips E600, Tokyo, Japan) at ×20 magnification at the occlusal and cervical margins. A total of 160 scores for dentin margins and enamel margins were recorded. Microleakage at the enamel and dentin margins was calculated as a percentage of the full length of the cavity (Figure 4). The results of the microleakage examination were analyzed with paired and independent sample *t*-tests (α=0.05).

Operator Reliability Evaluation

To ensure consistency and reliability of the observations, 20 sections were chosen randomly to assess interoperator reliability. A second evaluator read each section under the same conditions. Correlation coefficients for both enamel and dentin percentages between the two evaluators (DCNC and SLD) were as follows: enamel%, $r = 0.915$; dentin%, $r = 0.936$. No statistically significant differences were observed between the two operators' evaluations (paired *t*-test, $p>0.05$). Following the interoperator reliability assessment, selected sections were also evaluated by a confocal laser scanning electron microscope (Zeiss510, Carl Zeiss Ltd, Thornwood, NY, USA) (Figure 5).

RESULTS

The mean (± standard deviation) SBS values of before fatigue and after fatigue ranged from 21.9 (2.5) MPa to 23.9 (3.8) MPa and from 17.1 (2.5) MPa to 17.7 (2.5) MPa, respectively. No statistically significant differences in failure force were observed among groups (p=0.70) (Figure 6). The results indicated that adding 10 wt% nMT to the adhesive system (either primer or bonding resin) did not affect the bond strength of the resin composite to either enamel or dentin.

The results of the microleakage experiment were analyzed with paired and independent sample *t*-tests (α=0.05). No significant differences were observed between test groups and control restorations (Table 2). No significant differences were observed between groups 1 and 2 in either dentin or enamel margins. However, strong evidence of differential leakage susceptibility was observed between enamel and dentin margins ($\alpha<0.01$). Microleakage under all conditions was significantly greater in the dentin margins than in the enamel margins ($p<0.05$). We found no evidence that microleakage differed between the experimental groups with modified primer and bonding resin.

DISCUSSION

Antimicrobial antibiotics, nanoparticles (NPs), polymers, and peptides have been developed to prevent and/or reduce bacterial growth and adhesion.[17-20] Metal-based antibacterials are a desirable choice because bacterial resistance to conventional antibiotics is a common problem. As metal complexes are inorganic, they can have long-term effectiveness as well as potentially reduced contribution to bacterial resistance.[8] Many recent studies have revealed that nanoparticles, especially silver NP (AgNP), have various properties that can be exploited for numerous biomedical applications. In one study,[21] the antibacterial activity of AgNP-modified hydrogel coatings was tested evaluating *in vitro* inhibition growth of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. The composite with AgNP was strongly antibacterial and greatly

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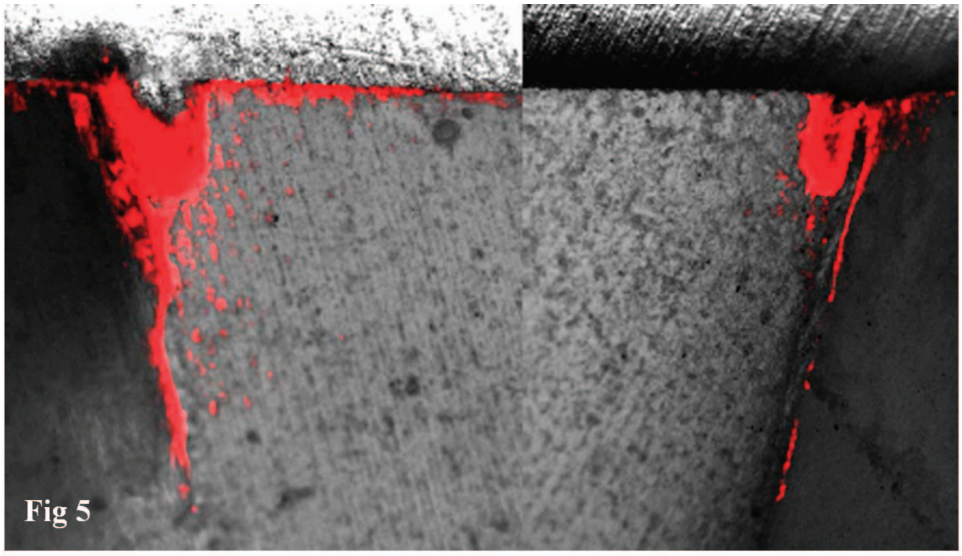

Figure 5. *Penetration of the 0.2% Rhodamine dye as indicated by confocal microscopy in the control enamel group compared to the treated enamel group. Note that the degree of penetration is very similar.*

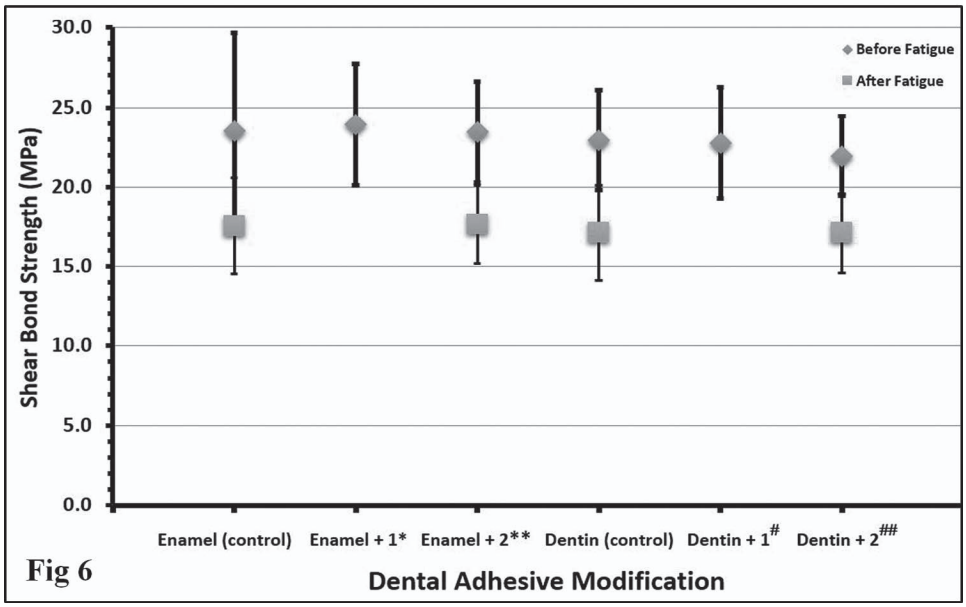

Figure 6. *Results of shear bond strength test. No statistically significant differences were determined between before-fatigue and after-fatigue groups,* p $>$ *0.05.* * *Bonding with D/E resin + 10 wt% MST;* # *bonding with D/E resin + 10 wt% nMT.* ** *Priming with D/E resin + 10 wt% MST;* ## *priming with D/E resin + 10 wt% nMT.*

reduced the titer counts, metabolic activity, and acid production of *Streptococcus mutans* biofilms.[22] Chlorhexidine (CHX) is also widely used as an antimicrobial agent, including for disinfection before placement of restorations. Hiraishi and others[23] reported that CHX had been shown to be released at relatively high rates from various methacrylate polymers. No inhibition effect on *Streptococcus*

Table 2: *Comparison of Experimental and Control Sections*

	Control		Experimental		Difference (Experimental−Control)				*p*-Value*
	Mean	SD	Mean	SD	Mean	SD	95% Confidence Interval		
Primer (n=10)									
Enamel	.77	.46	.80	.47	.03	.37	−.24	.29	.83
Dentin	2.18	.92	2.20	.89	.02	.47	−.31	.36	.88
Resin (n=10)									
Enamel	.67	.25	.72	.53	.04	.37	−.22	.30	.72
Dentin	2.60	.89	2.09	1.19	−.52	1.04	−1.26	.22	.15

* *Paired* t*-test on leakage measurements (average over three slices from each tooth).*

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mutans was further detected from resin disks after two weeks of water storage. Matrix metalloproteinase (MMP) may be partially responsible for hybrid layer degradation. Loss of hybrid layer integrity compromised resin-dentin bond stability. CHX acted as an MMP inhibitor, so it had beneficial effects on the preservation of dentin bond strength.[23]

Our research is focused on gold NP (AuNP) since we discovered that gold-loaded microparticulate metal-titanate complexes (μMTs) were effective as a new class of antibacterials. Gold-loaded μMTs inhibit growth of oral bacteria at micromolar concentrations. Other studies[24] also demonstrated antifungal and antiviral actions of AuNP.

Compared to microparticles, NPs offer many advantages. Nanotechnology modulates metals into their nano-size, which drastically changes the chemical, physical, and optical properties of metals. Inorganic nanoparticles and their nanocomposites are applied as effective antibacterial agents. Nanoparticulate metal oxides have unusual crystal morphologies, with an increased number of edges and corners of NPs, which in turn generates a large NP surface area for interaction with bacteria. Theoretically, nMTs will be even more effective because they have a significantly greater surface-to-volume ratio, which is expected to lead to more effective ion-exchange characteristics. More importantly, they are able to act as drug carriers or to concentrate drugs on their surfaces, which results in polyvalent effects that enhance drug efficacy.[25-27] NPs themselves can specifically attack biological targets after modification with targeting molecules.[28,29] nMT also has the advantage of acting as a bioactive molecule to carry other drugs.

Oral applications of nanoparticles have recently been considered.[30] The potential of NPs to control oral biofilm formation is related to their biocidal and antiadhesive capabilities. NPs have been incorporated into dental materials to improve antimicrobial activity. The presence of antibacterials in both the bonding systems and the filling material theoretically would inhibit or slow both the initiation and progression of caries adjacent to restorations. Problems can arise as a result of release of the antibacterial agent from the composite. Such problems may include toxic effects, influence on mechanical properties, and loss of effectiveness.[31] If systemic toxicity could be limited and therapeutic indices were optimized, metal ions and their associated compounds could emerge as a new powerful class of antibacterial agents. Martínez-Gutierrez and others[32-3] and Kasraei and others[34] suggested that NPs may also exert significant cytotoxicity on macrophages in association with a proinflammatory response and cellular apoptosis. Their findings showed that silver nanoparticles were cytotoxic in murine macrophages and in fibroblasts at concentrations of 10 and 50 μg/mL, respectively. Instead of incorporating our nMTs into the body of the restorative materials, our strategy was to add the materials to the primer and adhesive layer. Such a strategy will minimize the quantity of materials needed. Moreover, amorphous peroxititanates (APT) might be used to bind a variety of metal compounds with high-affinity forming complexes to control the delivery of metal-based drugs to the target tissue, avoiding systemic toxicity. Wataha and others[4] demonstrated that metal-APT complexes facilitate metal ion delivery (such as gold and platinum) to monocytes as well as fibroblasts. Composite resins containing 1% silver nanoparticles exhibited antibacterial activity against *Streptococcus mutans* and *Lactobacillus*.[34] In addition, the presence of antibacterials in the bonding systems (ie, at the critical interface) would theoretically affect the initiation and progression of caries. Antibacterial adhesives could inhibit the invading bacteria along tooth-restoration margins.

Our attempt to incorporate the nMT into either the primer or the adhesive resin carries with it advantages and disadvantages. Aside from the pure chemical makeup point of view, putting the nMT into the primer will be closer to the tooth substrate and may have a more direct antibacterial effect. However, the nMT will be subsequently covered by bonding resin and composite resin restoration, and, thus, its long-term release may be hampered. Incorporating the nMT into the resin separates the active ingredient further from the targeted tooth surface. Both conditions are still at the critical exposed interface. Zhang and others[35] reported that adding dental resins containing 12-methacryloyloxydodecylpyridinium bromide (MDPB) with AgNP into both primer and adhesive achieved the strongest antibiofilm efficacy.

Our results showed that 10 wt% nMT, when added to the bonding agent, did not affect bond strength or microleakage for either enamel or dentin. Although cyclic loading did lower the after-fatigue SBS, when the groups are compared across the same conditions, we did not find any significant differences. Therefore, incorporating nMT into dental adhesive systems will not compromise resin-tooth bonding and sealing ability. Hence, the incorporation of antibacterial agents (ie, nMTs) into dentin bonding agents

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may become an indispensable method for inhibiting residual bacteria in the cavity and secondary caries.

CONCLUSIONS

Within the limitations of this study, the addition of nMT to All-Bond 2® dental adhesive system did not affect the SBS and microleakage between composite and either human dentin or enamel. Incorporation of this novel material into a dental adhesive system does not appear to compromise either bonding or sealing ability and thus is a promising antibacterial agent with clinical significance.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Washington, Seattle, Washington.

Conflict of Interest

Author DCNC is one of the holders of the US patent No. US-2012-0156145-A1, "Use of titanium-based materials as bactericides," related to this manuscript. The other authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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