Original Article

A novel antimicrobial orthodontic band cement with in situ–generated silver nanoparticles

Danna Mota Moreira[a]; James Oei[b]; Henry Ralph Rawls[c]; Jeffrey Wagner[d]; Lianrui Chu[e]; Yiming Li[f]; Wu Zhang[g]; Kyumin Whang[c]

ABSTRACT

Objective: To develop an antimicrobial orthodontic band cement for the prevention of white spot lesions using a novel process that generates silver nanoparticles (AgNP) in situ.

Materials and Methods: Twenty-seven groups of AgNP-loaded Opal Band Cement (OBC) and two control groups were formulated with varying concentrations of additional benzoyl peroxide (0.5, 1.0, 1.5, or 2.0 wt%) and 2,2-(*p*-Tolylimino) diethanol (0.5 or 1 wt%). Rockwell$_{15T}$ hardness and near-infrared FTIR were used to assess degree of cure, three-point bending was used to determine modulus and ultimate transverse strength (UTS), and Ag^+ ion release was measured for up to 4 months in vitro using atomic absorption spectroscopy. Antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* was tested in vitro by counting colony-forming units for up to 28 days. Biocompatibility was evaluated following ISO specifications 7405 (2008), 10993-3 (2003), 10993-5 (2009), and 10993-10 (2010).

Results: Most of the experimental groups had hardness, modulus, and UTS values similar to those of the control group. Ag^+ ion release was observed for all AgNP-loaded groups for up to 4 months. Increase in Ag loading increased Ag^+ ion release and in vitro antimicrobial effect. The biocompatibility of the optimal AgNP-loaded OBC was comparable to that of negative controls.

Conclusion: A novel antimicrobial orthodontic band cement was developed that has comparable mechanical properties to controls, controlled and sustained Ag^+ ion release, significant bacterial inhibition in vitro, and excellent biocompatibility. (*Angle Orthod.* 2015;85:175–183.)

KEY WORDS: Antimicrobial agents/inhibitors; Cements; Dental materials; Silver nanoparticles

INTRODUCTION

Enamel demineralization (white spot lesion) is attributed to prolonged plaque accumulation around orthodontic attachments, with a rapid shift in the bacterial flora to higher levels of acidogenic bacteria such as *Streptococcus mutans* and *Lactobacilli*.[1] Despite advances to improve the practice of orthodontics, white spot lesions are still a significant risk associated with fixed appliance therapy.[2–4]

Overall management of white spot lesions includes methods of preventing demineralization and encouraging remineralization of existing lesions. However, the effectiveness of these methods has been limited by challenges, such as the efficacy of fluoride-containing products, localizing the fluoride to areas where it is needed, and, most importantly, the lack of predictability related to patient compliance.[4,5] However, even if the patient is compliant, orthodontic attachments make the mechanical removal of plaque difficult, causing an increased cariogenic challenge around orthodontic brackets and underneath bands. Thus, novel methods

[a] Postdoctoral Research Fellow, Comprehensive Dentistry, University of Texas Health Science Center, San Antonio, Tex.

[b] Research Assistant, Comprehensive Dentistry, University of Texas Health Science Center, San Antonio, Tex.

[c] Professor, Comprehensive Dentistry, University of Texas Health Science Center, San Antonio, Tex.

[d] Research & Development Senior Manager, Ultradent Products Inc, South Jordan, Utah.

[e] Professor, Developmental Dentistry, University of Texas Health Science Center, San Antonio, Tex.

[f] Professor and Director, Center for Dental Research, Loma Linda University, School of Dentistry, Loma Linda, Calif.

[g] Professor, Center for Dental Research, Loma Linda University School of Dentistry, Loma Linda, Calif.

Corresponding author: Dr Kyumin Whang, Comprehensive Dentistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, MSC 7914, San Antonio, TX 78229-3900
(e-mail: whang@uthscsa.edu)

Accepted: June 2014. Submitted: February 2014.
Published Online: August 6, 2014

of preventing white spot lesions around orthodontic appliances and bands are needed.

Studies[6] have shown that the addition of antimicrobial agents to dental resins and cements may prevent or reduce the formation of white spot lesions. Incorporation of zinc oxide into Fuji Ortho LC was shown[7] to add antimicrobial properties and holds potential for preventing decalcification associated with orthodontic treatment. The addition of chlorhexidine into glass ionomer cements was shown[8] to increase bacterial control around orthodontic appliances such as bands. Incorporating bioactive glass into a composite resin was shown[5] to buffer the acidic oral environment, release calcium ions, remove PO_4 (decreasing the resources for bacterial metabolism), and have antimicrobial effects resulting in the reduction of white spot lesions. Bonding agents with materials that have the highest antibacterial activities, such as benzalkonium chloride, chlorhexidine, or MDPB, were shown to reduce white spot lesion formation.[9] Finally, an antibacterial monomer-containing, self-etching adhesive was shown to reduce enamel demineralization around orthodontic brackets in vivo even after 30 days.[10] Thus, adding antimicrobial agents into an orthodontic band cement may be an effective method of preventing or reducing white spot lesion formation.

Another widely investigated biocompatible, broad-spectrum antimicrobial agent is silver (Ag). In particular, Ag in nanoparticulate form (AgNP) has been widely investigated because it can release Ag^+ ions more effectively as a result of its high surface area–to-volume ratio and can therefore result in better antimicrobial activity. However, AgNPs are difficult to incorporate and disperse, which can lead to weakening of the resin. In addition, the generation of AgNPs often involves the use of harsh chemicals or conditions, making it less useful for dental and medical applications.[11,12]

Recently we have developed a novel method of generating AgNPs in situ in acrylic resins using the resin's own curing process without the use of harsh chemicals or conditions.[11,13] The purpose of this work was to develop a novel antimicrobial orthodontic band cement for the prevention of white spot lesions using this technology.

MATERIALS AND METHODS

Synthesis of AgNP-Loaded Band Cement

The procedure for generating AgNP in situ has been described previously.[11,13] This method was adapted for a commercially available orthodontic band cement, Opal Band Cement (OBC; Ultradent Products Inc, South Jordan, UT), as follows. Ag benzoate (AgBz; 0.1, 0.5, or 1.0 wt% of monomer; Sigma Aldrich, St Louis, MO) was dissolved in dimethylaminoethyl methacrylate (DMAEMA; 2 wt%; EssTech, Essington, PA). Twenty-seven groups of AgNP-loaded OBC and two control groups were formulated with varying concentrations of additional benzoyl peroxide (BPS; B; 0, 0.5, 1.0, 1.5, or 2.0 wt%) and 2,2-(*p*-Tolylimino) diethanol (P-TIDE; A; 0, 0.5, or 1 wt%). The initiator system was adjusted because the formation of AgNPs uses the resin's polymerization process and interferes with the curing process and decreases mechanical properties.

Controls include OBC and the same cement formulated in house (UTOBC). UTOBC was formulated as a separate control because resins fabricated in house have different mechanical properties from those fabricated at UPI as a result of the differences in industrial and laboratory methods of incorporating filler particles. Thus, UTOBC is the appropriate control for the experimental groups.

The cement was poured into a mold (3/8-inch diameter $\times$ 1/16 inch thick) between two glass slides and light-cured on each side for 40 seconds using a VALO LED curing light (UPI). $Rockwell_{15T}$ hardness and near-infrared FTIR (NIR) were used to assess degree of cure, three-point bending was used to determine mechanical properties, and Ag^+ ion release was measured for up to 4 months in vitro using atomic absorption spectroscopy (AAS). Antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* was tested in vitro by counting colony-forming units for up to 28 days. Biocompatibility was evaluated following the International Standards Organization (ISO) specifications 7405 (2008), 10993-3 (2003), 10993-5 (2009), and 10993-10 (2010).[14–17]

$Rockwell_{15T}$ Hardness

The $Rockwell_{15T}$ hardness (Wilson Rockwell 4JR Hardness Tester, Wilson Hardness, Norwood, MA) of cured specimens ($n = 5$) was measured with a 15T 1/16-inch ballpoint indenter with a 15-kg force. Three measurements were made on different areas of the surface of each of the specimens to verify that it was cured evenly.

NIR Degree of Conversion

NIR degree of conversion (DoC) was determined for each group ($n = 5$) in the near-IR region (Nicolet 6700 FTIR Fourier Transform Infrared Spectrometer, Thermo Fisher Scientific, Waltham, MA) using the aliphatic C=C peak at 6165 cm^{-1}. DoC was calculated according to the following formula, where *Abs* is the height of absorption peak of the cured and uncured specimens:

$$\mathrm{DoC} = \left(1 - \frac{[Abs\ cured]}{[Abs\ uncured]}\right) \times 100.$$

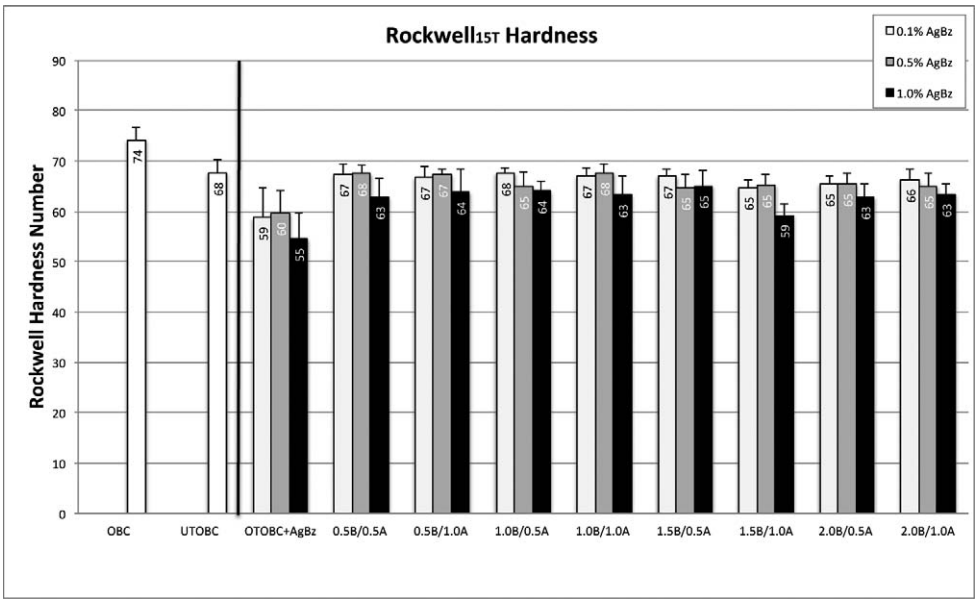

Figure 1. Rockwell$_{15T}$ hardness. OBC was harder than UTOBC. UTOBC with AgBz had lower hardness than UTOBC as a result of the in situ generation of AgNPs interfering with the polymerization. Most of the experimental groups had hardness values similar to that of UTOBC.

Three Point Bending Flexural Test

Modulus and ultimate transverse strength (UTS) of cured specimens (20 × 4 × 3 mm; n = 15) were determined using an Instron/MTS 1125 ReNew universal mechanical test instrument at a crosshead speed of 1 mm/min.

In Vitro Ag^+ Ion Release

Specimens (n = 10) were incubated at 37.5°C in 10 mL of sterile deionized (DI) water, and Ag^+ ion release was measured at certain intervals (1, 2, and 4 days; 1, 2, and 4 weeks; 2 and 4 months) using AAS (3030 Atomic Absorption Spectrometer, Perkin-Elmer). DI water was replaced at each time point to maintain sink conditions.

In Vitro Antibacterial Activity

The best-performing groups from the mechanical and Ag^+ ion release tests (0.5% BPO/0.5% P-TIDE groups at each AgBz concentration) were tested for in vitro antibacterial activity against *S mutans* (ATCC 25175, American Type Culture Collection [ATCC], Rockville, Md) and *L acidophilus* (ATCC 4356) by counting colony-forming units (CFUs) for up to 28 days. After culturing the bacteria overnight, bacterial density was adjusted to 0.2 at OD_{620nm}. The bacterial suspension was then diluted with broth to 5×10^7 (bacterial number at this density is about 400–800/mL), and 0.5 mL of that bacterial suspension was added to tubes containing resin specimens (n = 5). Tubes were incubated in a Coy anaerobic chamber (5% CO_2, 10% H_2, 85% N_2) at 24°C for 48 hours. A volume of 50 μL of solution from each specimen was homogeneously distributed onto the trypticase soy broth agar plate with a turntable. After inoculation, CFUs were counted to determine the inhibitory effect of AgNP-loaded resins.

Biocompatibility

The best-performing group from the antimicrobial tests, 0.5% BPO/0.5% P-TIDE with 0.5 wt% AgBz group (0.5% AgNP-OBC), was tested for biocompatibility following ISO specifications. Cytotoxicity (n = 4) was evaluated using the agar diffusion method using L929 mouse fibroblasts (ATCC CCL 1) following ISO specifications 7405 (2008) and 10993-5 (2009). Mutagenic potential (n = 2) was evaluated using the Ames *Salmonella*/microsome mutagenicity test following ISO specifications 7405 (2008) and 10993-3 (2003). Irritation potential was evaluated using the intracutaneous reactivity test in three young male New Zealand white rabbits (HsdOkd:NZW; Harlan Sprague Dawley, Ind) following ISO specifications 7405 (2008) and 10993-10 (2010). The delayed-type hypersensitivity potential was evaluated using the maximum sensitization test in young adult female Harley guinea pigs (Crl:(HA)BR; Charles River Laboratories, Massachusetts) following ISO specifications 7405 (2008) and 10993-10 (2010) (n = 10 for the experimental group and n = 5 for the control group). Irritation potential and the delayed-type hypersensitivity potential tests were reviewed and approved by the Loma Linda University Institutional Animal Care and Use Committee. The selection of the animal

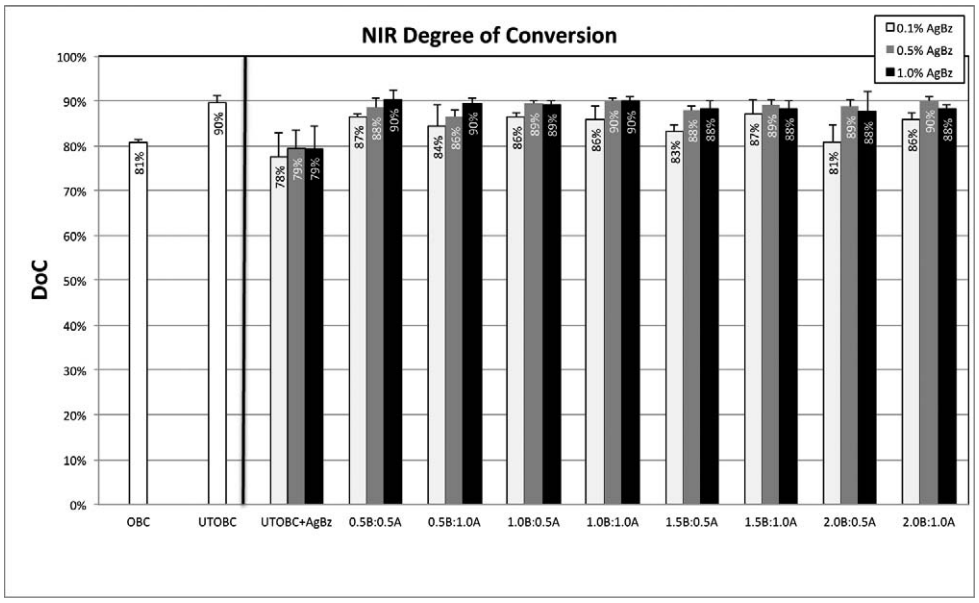

Figure 2. NIR degree of conversion (DoC). UTOBC had higher DoC than did OBC. Addition of AgBz lowered DoC, but additional BPO and P-TIDE raised the DoC to similar levels of UTOBC.

species and number of both tests follow pertinent ISO guidelines (2010).

Statistical Analysis

The results of the Rockwell$_{15T}$ hardness, NIR, three point bending test, in vitro Ag^{+} ion release, and CFUs were analyzed for statistical significance using analysis of variance (ANOVA) with Neuman-Keuls post hoc test to determine differences between groups at a $P < .05$ level. Biocompatibility evaluation was performed according to the criteria established by the ISO specifications previously mentioned. Means and standard deviations of revertants were calculated for each dose of the test agent for mutagenic potential, and one-way ANOVA and the Student-Newman-Keuls method were used to examine the significance of differences in means among doses. The animal growth data collected from the study on the delayed-type hypersensitivity potential were determined using the Student's *t*-test.

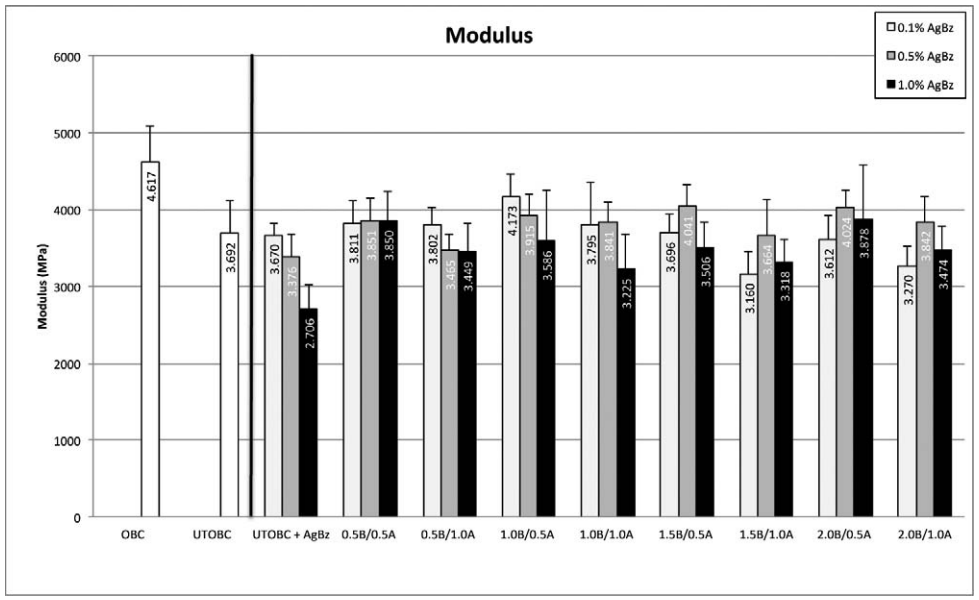

Figure 3. Modulus. OBC had the highest modulus. Increase in AgBz decreased modulus. However, additional BPO and P-TIDE increased modulus to comparable levels to that of UTOBC.

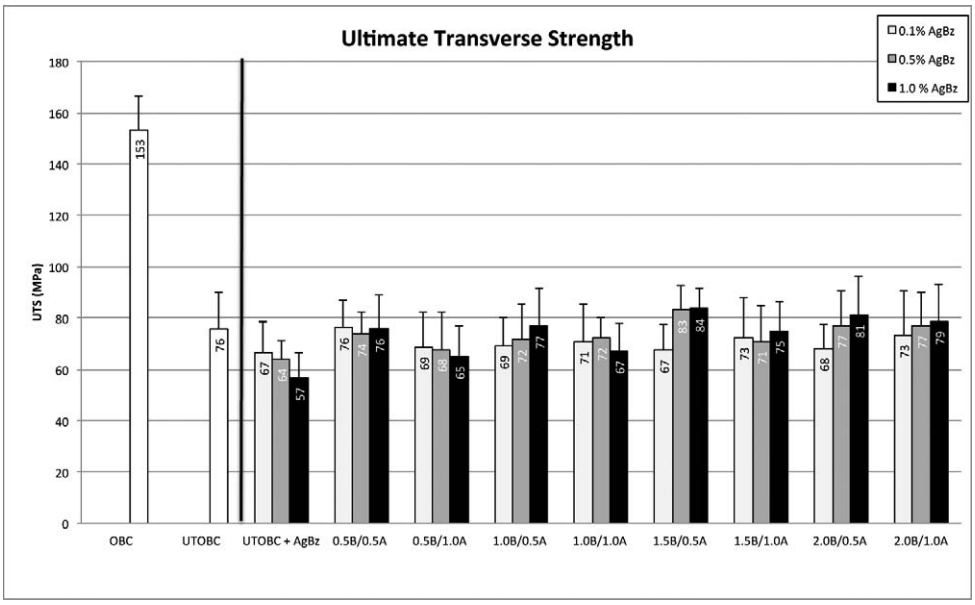

Figure 4. Ultimate transverse strength (UTS). OBC had the highest UTS. The incorporation of AgBz decreased UTS. Additional BPO and P-TIDE increased UTS to comparable levels to that of UTOBC.

RESULTS

Rockwell$_{15T}$ Hardness

Figure 1 shows the Rockwell$_{15T}$ hardness of the resins. OBC had significantly higher hardness than UTOBC. When AgBz was added to UTOBC, hardness dropped significantly, as expected, since the Ag^+ ions are formed via the polymerization process into Ag atoms, clusters, and AgNPs, which interferes with the curing of the material and decreases mechanical properties. However, adjusting the BPO and P-TIDE concentrations increased the hardness to acceptable levels. In general, most of the experimental groups had hardness values similar to those of the UTOBC group. Only a few 1.0 wt% AgBz groups had slightly lower hardness values.

NIR Degree of Monomer-Polymer Conversion (NIR DoC)

NIR DoC results generally confirmed the Rockwell$_{15T}$ hardness results. Figure 2 shows that UTOBC

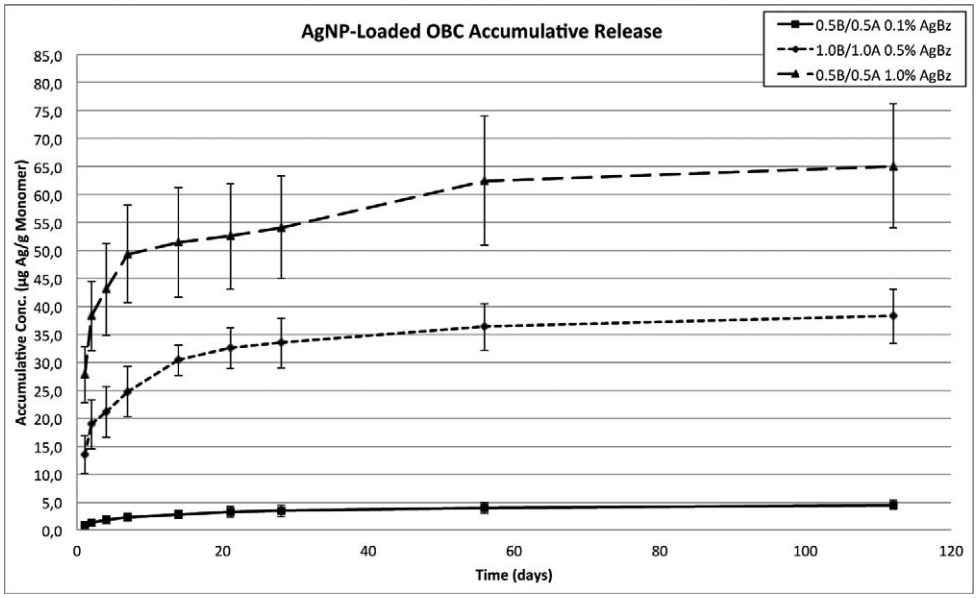

Figure 5. Cumulative Ag^+ ion release of representative specimens for up to 4 months. Ag^+ ion release was observed for all AgNP-loaded groups. The higher the Ag loading, the higher the Ag^+ ion release.

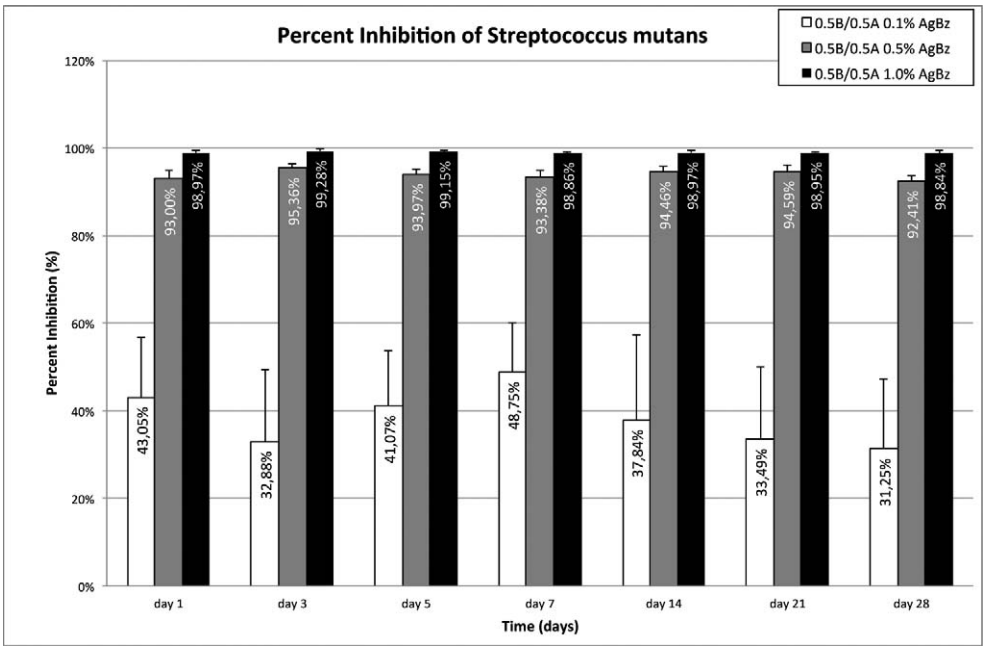

Figure 6. Percent *Streptococcus mutans* inhibition for up to 28 days. The higher the AgBz concentration, the greater the antimicrobial effect. The 0.5 and 1 wt% AgBz samples had over 90% inhibition of *S mutans*.

had a significantly higher DoC than did OBC. When AgBz was incorporated into UTOBC, DoC decreased significantly as a result of the generation of AgNP, interfering with the polymerization. However, adjusting the BPO and P-TIDE concentrations increased DoC to levels comparable to that of UTOBC. In general, most of the experimental groups had similar DoC to UTOBC. Only 1.5% BPO/0.5% P-TIDE 0.1% AgBz and 2.0% BPO/0.5% P-TIDE 0.1% AgBz groups had significantly lower DoC than all the other groups.

Three Point Bending Flexural Test

Figures 3 and 4 show the modulus and UTS of the resins. OBC had the highest modulus and UTS, and the incorporation of AgBz decreased the mechanical properties. Again, when additional BPO and P-TIDE

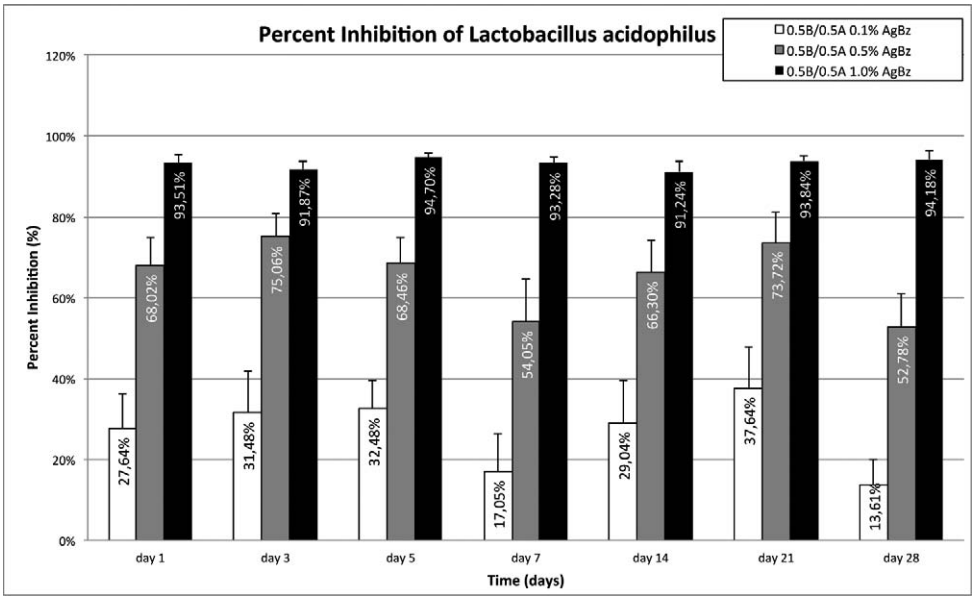

Figure 7. Percent *Lactobacillus acidophilus* inhibition for up to 28 days. Most of the AgNP-loaded groups significantly inhibited *L acidophilus*.

Table 1. List of Abbreviations

Abbreviation	Meaning
0.5% AgNP-OBC	0.5% BPO/0.5% P-TIDE with 0.5 wt% AgBz group
AAS	Atomic absorption spectroscopy
AgNP	Silver nanoparticles
AgBz	Silver benzoate
BPO	Benzoyl peroxide
CFUs	Colony-forming units
CII	Cumulative irritation index
DoC	NIR degree of conversion
DMAEMA	Dimethylaminoethyl methacrylate
NIR	Near-infrared Fourier transform infrared spectrometer
OBC	Opal Band Cement
P-TIDE	2,2-(*p*-Tolylimino) diethanol
UTOBC	Opal Band Cement fabricated in house
UTS	Ultimate transverse strength

were added, modulus and UTS increased to comparable levels to that of UTOBC.

In Vitro Ag^+ Ion Release

Figure 5 shows representative release profiles for each AgBz loading. Ag^+ ion release was observed for all AgNP-loaded groups, and in general, the higher the Ag loading, the higher the Ag^+ ion release.

In Vitro Antibacterial Activity

Figures 6 and 7 show the in vitro antibacterial activity against *S mutans* and *L acidophilus* up to 28 days. Additional BPO and P-TIDE had no effect on bacterial inhibition, but the higher the AgBz concentration, the greater the antimicrobial effect, with the 0.5 and 1 wt% AgBz samples having over 90% inhibition of *S mutans* for up to 28 days. AgNP-loaded resins were more effective against *S mutans* than *L acidophilus*. In general, most of the AgNP-loaded groups had significantly lower CFUs of *L acidophilus* than did UTOBC. Only the 0.1% AgBz group at day 28 did not have significantly lower *L acidophilus* CFUs than did UTOBC.

Biocompatibility

Cytotoxicity. Cytotoxicity was examined by measuring the zones of decolorization and evaluating cell lysis after 24 and 48 hours. No cell decolorization and lysis were observed in 0.5% AgNP-OBC. The observations were comparable to those of the negative controls.

Mutagenic potential. Data obtained from the Ames *Salmonella* test with and without S9 microsomal activation are summarized in Tables 2 and 3. Numbers of spontaneous revertants in the negative controls and induced revertants in the positive controls using the diagnostic mutagens were within the expected ranges. The data from the positive controls are all significantly higher than those of the corresponding negative controls ($P \leq .01$).

Table 2 presents the results of the experiments without S9. The extracts of 0.5% AgNP-OBC, including the highest dose tested, were not toxic to the four tester strains. No dose-related increases in the number of revertants were observed in any of the four strains tested, and the mean numbers of revertants for various doses of 0.5% AgNP-OBC extracts were comparable to those of the negative controls in all four strains.

The results obtained from the experiment with the S9 activation are presented in Table 3. The data are comparable to those obtained from the tests without S9. S9 is a rat liver microsomal preparation that contains various enzymes. It has been found to increase the sensitivity and overall performance of the Ames test, and, therefore, experiments conducted both with and without S9 are required for the Ames *Salmonella* mutagenicity test.[15]

Irritation potential. Mild erythema was observed on two of the five injection sites for saline extract and one of the five injection sites for cottonseed oil extract of the 0.5% AgNP-OBC in rabbit 1 after 24 hours. There was mild erythema in one of the five injection sites for saline control in rabbit 2 after 24 hours. Rabbit 3 did

Table 2. Evaluation of Mutagenic Potential of 0.5% Silver Nanoparticles–Opal Band Cement (AgNP-OBC) Using the Ames *Salmonella*/Microsome Mutagenicity Test (Without S9 Activation)

		Revertants/Plate[a]			
Test Agent[b]	Dose (Extracts), %	TA97a	TA98	TA100	TA102
Negative control	–	67 ± 3*	25 ± 8*	92 ± 6*	156 ± 11*
0.5% AgNP-OBC	10	57 ± 0*	29 ± 5*	99 ± 9*	160 ± 30*
	20	55 ± 0*	25 ± 10*	80 ± 6*	195 ± 1*
	33	52 ± 16*	34 ± 1*	107 ± 3*	185 ± 23*
	100	71 ± 27*	31 ± 3*	78 ± 9*	186 ± 18*
Positive control	–	1141 ± 42	1634 ± 36	2121 ± 218	1994 ± 55

[a] Mean ± standard deviation (n = 2).

[b] 0.5% AgNP-OBC indicates extracts of 0.5% AgNP-OBC; negative control, sterile Milli-Q pyrogen-free water; positive control, TA97a and TA98, 4-nitro-*o*-phenylenediamine (20 μg/plate); TA100, sodium azide (5 μg/plate); and TA102, t-butyl hydroperoxide (100 μg/plate).

* Values are not significantly different, as determined using the Student-Newman-Keuls method.

Table 3. Evaluation of Mutagenic Potential of 0.5% Silver Nanoparticles–Opal Band Cement AgNP-OBC Using the Ames *Salmonella*/Microsome Mutagenicity Test (with S9 Activation)

		Revertants/Plate[a]			
Test Agent[b]	Dose (Extracts), %	TA97a	TA98	TA100	TA102
Negative control	–	84 ± 7*	37 ± 16*	103 ± 4*	205 ± 36*
0.5% AgNP-OBC	10	78 ± 13*	37 ± 9*	95 ± 17*	233 ± 7*
	20	107 ± 7*	41 ± 1*	112 ± 14*	191 ± 26*
	33	105 ± 3*	43 ± 23*	104 ± 21*	190 ± 7*
	100	103 ± 15*	45 ± 9*	113 ± 45*	226 ± 11*
Positive control	–	1678 ± 67	3188 ± 195	1855 ± 19	1473 ± 52

[a] Mean ± standard deviation (n = 2).

[b] 0.5% AgNP-OBC indicates extracts of 0.5% AgNP-OBC; negative control, Sterile Milli-Q pyrogen-free water; positive control, TA97a, TA98, and TA100 2-aminofluorene (15 μg/plate); and TA102, 1,8-dihydroxyanthraquinone (50 μg/plate).

* Values are not significantly different, as determined using the Student-Newman-Keuls method.

not show any sign of erythema or edema in any of the injection sites. The observed erythema disappeared after 48 hours for rabbits 1 and 2, and no sign of erythema was detected at any of the injection sites of the three animals. No sign of edema formation was detected at any of the injection sites of the three animals.

The Cumulative Irritation Index (CII) value for the cottonseed oil extracts and saline extracts of 0.5% AgNP-OBC was 0.04, indicating negligible irritation potential of the test material according to the criteria of the ISO guidelines.

Delayed-type hypersensitivity potential. The animal growth data, as measured by animal body weight, are presented in Table 4. The values of the two groups in the different time periods are comparable, indicating no adverse effects of 0.5% AgNP-OBC on animal growth. No skin erythema or edema was detected in the negative control animals or in the animals that received the extracts of 0.5% AgNP-OBC at 24, 48, and 72 hours.

DISCUSSION

Enamel demineralization is the most frequent complication with fixed appliance therapy. This demineralization can become noticeable around brackets and bands within 1 month of band placement.[2,4,5] Fluoride-releasing orthodontic bonding agents, such as glass ionomer cements and resin-modified glass ionomer cements, were developed in part to prevent enamel demineralization.[5]

Despite the availability of various fluoride-delivery protocols for orthodontic patients, fluoride-releasing bonding materials showed almost no demineralization-inhibiting effect.[18] For that reason, several studies have looked at the use of antimicrobial agents to prevent or reduce the occurrence of white spot lesions and caries. Antimicrobial agents that have been shown to be effective include benzalkonium chloride, zinc oxide, chlorhexidine, and bioactive glass.[6–10] Silver and its nanoparticulate form (AgNPs) have also shown great antimicrobial potential.

In the present study, we began to explore the use of AgNPs to prevent or reduce the occurrence of white spot lesions in an orthodontic application. We adapted a novel method of generating AgNPs in situ in acrylic resins for use with commercially available orthodontic band cement, OBC. The initiator system was adjusted to produce comparable mechanical properties, such as $Rockwell_{15T}$ hardness, degree of conversion, modulus, and UTS to the control resin (UTOBC).

The in vitro Ag^+ ion release results showed that all AgNP-loaded groups released Ag^+ ions for up to 4 months in vitro, with the resins with higher Ag loading releasing more Ag^+ ions. This correlated well with in vitro antibacterial activity, with the higher AgBz loading having the higher inhibitory effect. However,

Table 4. Body Weight of Guinea Pigs During Experimental Period

	Body Weight (Mean ± standard deviation)[a]				
Group[b]	Initial	7th Day	14th Day	28th Day	31st Day
AgNP-OBC	298.6 ± 18.5*	315 ± 18.5*	348.2 ± 18.4*	404.2 ± 27.5*	422.6 ± 26.3*
Control	297.6 ± 16.5*	323.2 ± 18.9*	354.8 ± 21.7*	405.6 ± 30.2*	424.0 ± 32.5*

[a] The seventh day for intradermal induction, 14th day for topical induction, 28th day for topical challenge, and 31st day for the conclusion of the study.

[b] AgNP-OBC indicates silver nanoparticles–Opal Band Cement, 0.5% AgNP-OBC; negative control, phosphate-buffered saline. Sample size was 10 for AgNP-OBC group and 5 for the negative control.

* Values are not significantly different, as determined using the Student's *t*-test.

there was a difference in inhibitory effect for different bacterial species. AgNP-loaded resins were more effective against *S mutans* than *L acidophilus*.

The 0.5% BPO/0.5% P-TIDE with 0.5% AgBz group was chosen for biocompatibility evaluation because it has the best balance of mechanical properties and antimicrobial effect. The biocompatibility evaluation showed that 0.5% AgNP-OBC was not cytotoxic or mutagenic. Furthermore, 0.5% AgNP-OBC had negligible irritation potential, as evaluated using the intracutaneous reactivity test, and did not have any delayed-type hypersensitivity potential, as evaluated using the maximum sensitization test.

CONCLUSIONS

- We have developed a novel antimicrobial orthodontic band cement with in situ–generated AgNPs for the prevention of white spot lesions.
- These resins have comparable mechanical properties to controls, controlled and sustained Ag^+ ion release, significant bacterial inhibition in vitro, and excellent biocompatibility.

ACKNOWLEDGMENT

This project was partially funded by Ultradent Products Inc. All materials used in this work were provided by UPI.

REFERENCES

1. Lundstrom F, Krasse B. *Streptococcus mutans* and *lactobacilli* frequency in orthodontic patients: the effect of chlorhexidine treatments. *Eur J Orthod*. 1987;9:109–116.
2. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthod*. 2011;81:206–210.
3. Maxfield BJ, Hamdan AM, Tüfekçi E, Shroff B, Best AM, Lindauer SJ. Development of white spot lesions during orthodontic treatment: perceptions of patients, parents, orthodontists, and general dentists. *Am J Orthod Dentofacial Orthop*. 2012;141:337–344.
4. Robertson MA, Kau CH, English JD, Lee RP, Powers J, Nguyen JT. MI Paste Plus to prevent demineralization in orthodontic patients: a prospective randomized controlled trial. *Am J Orthod Dentofacial Orthop*. 2011;140:660–668.
5. Brown ML, Davis HB, Tufekci E, Crowe JJ, Covell DA, Mitchell JC. Ion release from a novel orthodontic resin bonding agent for the reduction and/or prevention of white spot lesions. An in vitro study. *Angle Orthod*. 2011;81:1014–1020.
6. Saito K, Hayakawa T, Kawabata R, Meguro D, Kasai K. In vitro antibacterial and cytotoxicity assessments of an orthodontic bonding agent containing benzalkonium chloride. *Angle Orthod*. 2009;79:331–337.
7. Spencer CG, Campbell PM, Buschang PH, Cai J, Honeyman AL. Antimicrobial effects of zinc oxide in an orthodontic bonding agent. *Angle Orthod*. 2009;79:317–322.
8. Farret MM, de Lima EM, Mota EG, Oshima HM, Barth V, de Oliveira SD. Can we add chlorhexidine into glass ionomer cements for band cementation? *Angle Orthod*. 2011;81:496–502.
9. Passariello C, Sannino G, Petti S, Gigola P. Intensity and duration of in-vitro antibacterial activity of different adhesives used in orthodontics. *Eur J Oral Sci*. 2014;122:154–160.
10. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Sagdic D. Effect of antibacterial monomer-containing adhesive on enamel demineralization around orthodontic brackets: an in-vivo study. *Am J Orthod Dentofacial Orthop*. 2011;139:650–656.
11. Fan C, Chu L, Rawls HR, Norling BK, Cardenas HL, Whang K. Development of an antimicrobial resin—a pilot study. *Dent Mater*. 2011;27:322–328.
12. Nam KY. In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. *J Adv Prosthodont*. 2011;3:20–24.
13. Oei JD, Zhao WW, Chu L, et al. Antimicrobial acrylic materials with in situ generated silver nanoparticles. *J Biomed Mater Res Part B*. 2012;100B:409–415.
14. International Organization for Standardization (ISO). Dentistry—Evaluation of biocompatibility of material devices used in Dentistry, 2nd ed. 2008; ISO: 7405.
15. International Organization for Standardization (ISO). Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity, 2nd ed. 2003; ISO: 10993-3.
16. International Organization for Standardization (ISO). Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity, 3rd ed. 2009; ISO: 10993-5.
17. International Organization for Standardization (ISO). Biological evaluation of medical devices—Part 10: Tests for irritation and sensitization, 3rd ed. 2010; ISO: 10993-10.
18. Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuipers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. A systematic review. *Caries Res*. 2004;38:413–420.