RESEARCH REPORTS
Biomaterials & Bioengineering

L. Cheng[1,2], K. Zhang[1,3], M.A.S. Melo[1,4], M.D. Weir[1], X. Zhou[2]*, and H.H.K. Xu[1]*[,5-7]

[1]Biomaterials & Tissue Engineering Division, Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, Baltimore, 650 West Baltimore Street, Baltimore, MD 21201, USA; [2]State Key Laboratory of Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, China; [3]Department of Orthodontics, School of Stomatology, Capital Medical University, Beijing, China; [4]Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceara, Fortaleza, CE, Brazil; [5]Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD, USA; [6]Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA; and [7]Department of Mechanical Engineering, University of Maryland, Baltimore County, MD, USA; *corresponding authors, hxu@umaryland.edu and zhouxd@scu.edu.cn

J Dent Res 91(6):598-604, 2012

Anti-biofilm Dentin Primer with Quaternary Ammonium and Silver Nanoparticles

ABSTRACT

Antibacterial bonding agents could combat recurrent caries at the tooth-composite margins. The objectives of this study were to develop novel antibacterial dentin primers containing quaternary ammonium dimethacrylate (QADM) and nanoparticles of silver (NAg), and to investigate the effects on dentin bond strength and dental plaque microcosm biofilms for the first time. Scotchbond Multi-Purpose ("SBMP") bonding agent was used. QADM and NAg were incorporated into SBMP primer, yielding 4 primers: SBMP primer (control), control + 10% QADM (mass), control + 0.05% NAg, and control + 10% QADM + 0.05% NAg. Human saliva was collected to grow microcosm biofilms. The NAg particle size (mean ± SD; n = 100) was 2.7 ± 0.6 nm. Dentin shear bond strengths (n = 10) with human third molars were approximately 30 MPa for all groups ($p > 0.1$). QADM-NAg-containing primer increased the bacteria inhibition zone by 9-fold, compared with control primer ($p < 0.05$). QADM-NAg-containing primer reduced lactic acid production and colony-forming units of total micro-organisms, total streptococci, and mutans streptococci by an order of magnitude. In conclusion, novel QADM-NAg-containing primers were strongly antibacterial without compromising dentin bond strength, and hence are promising to inhibit biofilms and secondary caries. The processing method of incorporating QADM and NAg together into the same primer produced the strongest antibacterial effect, which could have a wide applicability to other bonding systems.

KEY WORDS: antibacterial dentin primer, quaternary ammonium salt, dental biomaterials, bond strength, dental plaque microcosm biofilm, caries inhibition.

DOI: 10.1177/0022034512444128

Received January 31, 2012; Last revision February 28, 2012; Accepted March 12, 2012

INTRODUCTION

Composites are increasingly used due to their esthetics, direct-filling capability, and improved performance (Bayne *et al.,* 1998; Lim *et al.,* 2002; Xu *et al.,* 2006; Drummond, 2008; Ferracane, 2011). Nonetheless, half of restorations fail within 10 years, replacing them consumes 50 to 70% of dentists' time (Deligeorgi *et al.,* 2001), and the main cause of failure is secondary caries (Sakaguchi, 2005). Composites accumulate more biofilms/plaques than other restoratives (Beyth *et al.,* 2007), and biofilm acids can cause caries (Featherstone, 2004; ten Cate, 2006).

To address this problem, investigators developed novel antibacterial composites (Imazato, 2003; Beyth *et al.,* 2006; Namba *et al.,* 2009; Fan *et al.,* 2011). Quaternary ammonium salt (QAS) monomers, such as 12-methacryloyloxydodecylpyridinium bromide (MDPB), were effective in providing antibacterial properties (Imazato, 2003). While previous QAS monomers were monomethacrylates, a quaternary ammonium dimethacrylate (QADM) was recently synthesized (Antonucci *et al.,* 2011; Cheng *et al.,* 2012). An advantage of quaternary ammonium methacrylates is that they are copolymerized with other monomers after curing and hence provide long-lasting antibacterial effects (Imazato, 2003). In addition, Ag is another important antibacterial agent (Slenters *et al.,* 2008), which has shown a long-term antimicrobial effect (Damm *et al.,* 2007) and can result in less bacterial resistance than antibiotics (Percival *et al.,* 2005). Recent studies developed nanoparticles of silver (NAg) (Cheng YJ *et al.,* 2011; Cheng L *et al.,* 2012). However, QADM and NAg have not been incorporated into bonding agents.

Bonding agents enable composite to adhere to tooth structure (Spencer and Wang, 2002; Spencer *et al.,* 2010; Pashley *et al.,* 2011). It is desirable for bonding agents to be antibacterial, to inhibit recurrent caries at the tooth-composite margins (Imazato, 2003; Imazato *et al.,* 2007; Li *et al.,* 2009). Dentin primer has direct contact with tooth structure; hence it would be beneficial to use antibacterial primers. A literature search revealed only a few

reports on antibacterial primers. MDPB (Imazato *et al.,* 1997, 1998, 2007) and chlorhexidine (Hiraishi *et al.,* 2010) were used to develop antibacterial primers. There has been no report on primers containing QADM or NAg.

The objectives of this study were to develop antibacterial primers incorporating QADM and NAg and to investigate biofilm viability and acid production using a dental plaque microcosm model. It was hypothesized that: (1) incorporating QADM or NAg into primer would not compromise dentin shear bond strength; (2) QADM-NAg-containing primer, in uncured or cured state, would be strongly antibacterial; and (3) combining QADM and NAg in primer would yield antibacterial properties stronger than those that would be achieved by the addition of each agent alone.

MATERIALS & METHODS

Scotchbond Multi-Purpose (referred to as "SBMP") (3M, St. Paul, MN, USA) was used as the parent bonding agent to test the effect of QDMA-NAg incorporation. The purpose was to develop a model system, and the method of QADM-NAg incorporation could be applied to other bonding agents. SBMP etchant contained 37% phosphoric-acid. SBMP primer contained 35 to 45% 2-hydroxyethyl-methacrylate (HEMA), 10 to 20% copolymer of acrylic/itaconic acids, and 40 to 50% water. SBMP adhesive contained 60 to 70% BisGMA and 30 to 40% HEMA. QADM and NAg were incorporated into SBMP primer. SBMP etchant and adhesive were not modified.

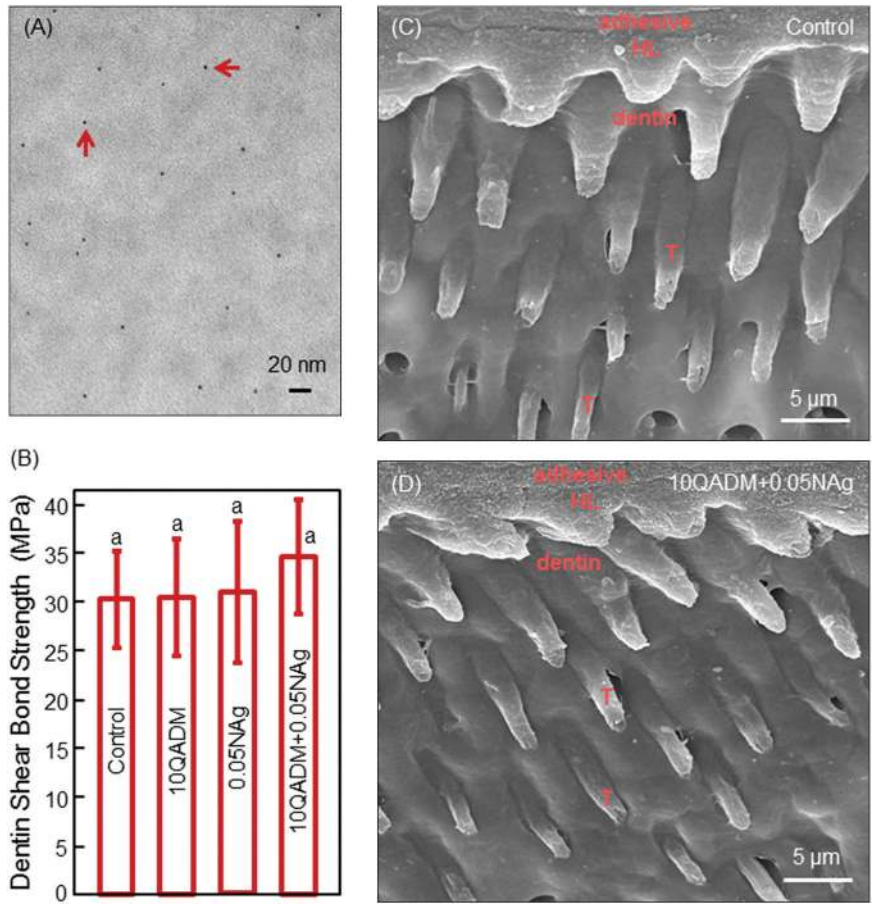

Figure 1. Effect of NAg and QADM incorporation into primer on dentin bonding. **(A)** TEM image of nanoparticles of silver (NAg). NAg particle sizes ranged from 2 to 5 nm, with mean ± SD (n = 100) of 2.7 ± 0.6 nm. NAg particles were well-dispersed in the resin, without agglomeration. In **(B),** dentin shear bond strength was between 30 and 35 MPa. Ten teeth were tested for each group (n = 10). Bars with the same letter indicate similar values ($p > 0.1$). **(C, D)** SEM of adhesive-dentin interface. T, resin tags formed by adhesive filling into dentinal tubules. HL, hybrid layer between the adhesive and the underlying mineralized dentin. A mixture of long and short resin tags was observed. Some tags were shorter because, during sample preparation, the sectioned surface was not exactly parallel to the long axis of dentinal tubules; thus some tubules were intersected by the cutting and were shortened. Numerous and similar resin tags were found for all 4 groups.

Bis(2-methacryloyloxy-ethyl) dimethylammonium bromide (QADM) was recently synthesized (Antonucci *et al.,* 2011). Ten mmol of 2-(*N,N*-dimethylamino)ethyl methacrylate (Sigma-Aldrich, St. Louis, MO, USA) and 10 mmol of 2-bromoethyl methacrylate (Monomer-Polymer Labs, Trevose, PA, USA) were combined in ethanol and stirred at 60°C for 24 hrs. The solvent was then evaporated, yielding the QADM (Cheng *et al.,* 2012). QADM was mixed with the SBMP primer at QADM/(primer + QADM) = 10wt%. This was selected because preliminary study showed that 10% of QADM in the primer provided antibacterial activity without compromising the dentin bond strength, while 20% QADM in primer decreased the bond strength.

Silver 2-ethylhexanoate (Strem, Newburyport, MA, USA) of 0.08 g was dissolved into 1 g of 2-(tert-butylamino)ethyl methacrylate (TBAEMA, Sigma). TBAEMA could facilitate Ag-salt dissolution in resin (Cheng YJ *et al.,* 2011). This Ag solution was mixed into SBMP primer at 0.05wt% of silver 2-ethylhexanoate, because preliminary study indicated that this concentration had no adverse effect on the dentin bond strength and color of the primer.

Hence, 4 primers were tested: (1) SBMP primer (control); (2) control primer + 10% QADM (termed "10QADM"); (3) control primer + 0.05% NAg (termed "0.05NAg"); and (4) control primer + 10% QADM + 0.05% NAg (termed "10QADM + 0.05NAg").

We used a transmission electron microscope (TEM, Tecnai-T12, FEI, Hillsboro, OR, USA) to examine NAg in resin. Following a previous study (Cheng YJ *et al.,* 2011), a thin sheet of mica was partially split, and the Ag-resin monomer was placed in the gap. The resin in the mica was pressed to form a film and photo-cured. The resin film was used for TEM examination.

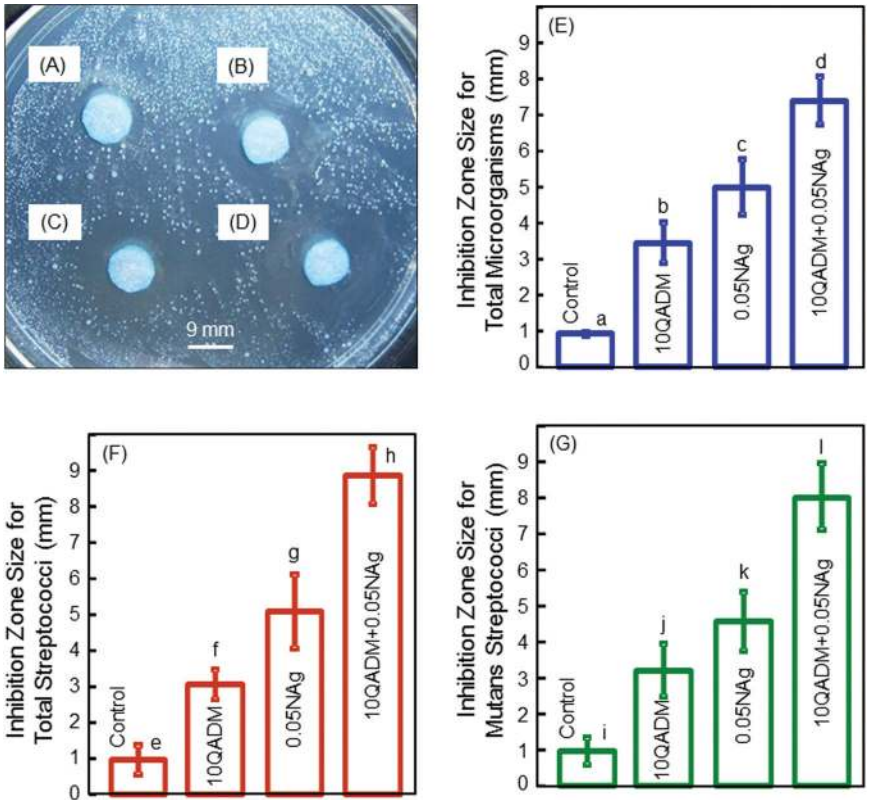

Figure 2. Antibacterial activity of uncured primers in the agar disk diffusion test. **(A-D)** Control primer, 10QADM, 0.05NAg, and 10QADM+0.05NAg, respectively. Note a small inhibition zone for control, and much wider inhibition zones for primers with QADM and NAg. This example is for mutans streptococci. Total micro-organisms and total streptococci had similar results. **(E-G)** Inhibition zone data for total micro-organisms, total streptococci, and mutans streptococci, respectively. Each value is mean ± SD (n = 6). Bars with dissimilar letters indicate values that are significantly different ($p < 0.05$).

Extracted caries-free human third molars were ground on 320-grit SiC paper until occlusal enamel was removed. The dentin was etched with 37% phosphoric acid for 15 sec (Antonucci *et al.,* 2009). A primer was applied, and the solvent was removed with an air stream. The adhesive was applied and light-cured for 10 sec (Optilux-VCL401, Demetron, Danbury, CT, USA).

A stainless-steel iris having a central opening (diameter = 4 mm, thickness = 1.5 mm) was held against the adhesive-treated dentin (Antonucci *et al.,* 2009). The opening was filled with a composite (TPH, Caulk/Dentsply, Milford, DE, USA) and light-cured for 60 sec. Specimens were stored in water at 37°C for 24 hrs. Dentin shear bond strength, S_D, was measured (Antonucci *et al.,* 2009). A chisel was aligned parallel to the composite-dentin interface and loaded *via* a Universal Testing Machine (MTS, Eden Prairie, MN, USA) at 0.5 mm/min until the composite-dentin bond failed. $S_D = 4P/(\pi d^2)$, where P is load-at-failure, and d is composite diameter.

The bonded tooth was cut through the center longitudinally, and the sections were polished with SiC papers up to 4,000 grit. Following a previous study (Imazato *et al.,* 2007), the polished surface was treated with 50% phosphoric acid and 10% NaOCl. Specimens were gold-coated and examined *via* scanning electron microscopy (SEM, Quanta-200, FEI).

Saliva was collected from a healthy donor having natural dentition without active caries and not having used antibiotics within the preceding 3 mos. The donor did not brush teeth for 24 hrs and abstained from food/drink intake for 2 hrs prior to donating saliva (Cheng L *et al.,* 2011).

Uncured primers were tested by an agar diffusion test (ADT). Saliva was added to growth medium containing mucin (concentration = 2.5 g/L), bacteriological peptone (concentration = 2.0 g/L), tryptone (concentration = 2.0 g/L), yeast extract (concentration = 1.0 g/L), NaCl (concentration = 0.35 g/L), KCl (concentration = 0.2 g/L), $CaCl_2$ (concentration = 0.2 g/L), and cysteine hydrochloride (concentration = 0.1 g/L) (pH = 7.0) (McBain, 2009). The inoculum was incubated (37°C, 5% CO_2) for 24 hrs. Three types of media were used. First, tryptic soy blood agar plates were used to determine total micro-organisms (Cheng L *et al.,* 2011). Second, mitis salivarius agar (MSA) plates, containing 15% sucrose, were used to determine total streptococci. Third, MSA plus 0.2 units of bacitracin/mL was used to determine mutans streptococci.

Bacteria suspension of 0.4 mL was poured onto each agar plate (diameter = 90 mm, thickness = 4 mm). Then, a 30-μL quantity of primer was impregnated into a sterile paper disk (diameter = 9 mm, thickness = 1.5 mm) (Imazato *et al.,* 2006). The primer-impregnated paper disk (referred to as "disk") was placed on a plate with bacteria and incubated for 48 hrs. Bacteria inhibition zone size = (Outer diameter of inhibition zone – disk diameter)/2.

Cured specimens were fabricated as schematically shown in Fig. 3A, following a previous study (Imazato *et al.,* 1998). A polyethylene mold (inner diameter = 9 mm, thickness = 2 mm) was situated on a glass slide. Primer was brushed on the glass slide. After the specimen was dried with a stream of air, SBMP adhesive was applied and cured for 20 sec with Optilux. Then, composite TPH was placed to fill the mold and light-cured for 1 min. Specimens were agitated in water to remove uncured monomers (Imazato *et al.,* 1998).

Each specimen was placed into a well of 24-well plates, and 1.5 mL inoculum was added. A 2-day incubation formed mature biofilms (Cheng L *et al.,* 2011). Specimens were then live-/dead-stained (Molecular Probes, Eugene, OR, USA) and examined by epifluorescence microscopy (TE2000-S, Nikon, Melville,

NY, USA). The area of green staining (live bacteria) was computed with the use of NIS Elements imaging software (Nikon). The area fraction of live bacteria = green staining area/total area of the image.

For acid production, specimens with 2-day biofilms were transferred to 24-well plates containing buffered-peptone water (BPW) plus 0.2% sucrose, and incubated for 3 hrs to allow biofilms to produce acid. Lactate concentrations were determined by an enzymatic method (Cheng L *et al.,* 2011). A microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA) was used to measure the 340-nm absorbance. Standard curves were prepared by a lactic acid standard (Supelco, Bellefonte, PA, USA).

For colony-forming units (CFU), specimens with 2-day biofilms were transferred into tubes with 2-mL cysteine-peptone water, and biofilms were harvested by sonication/vortexing (Fisher, Pittsburgh, PA, USA). Three types of agar plates were used: Tryptic soy blood plates to determine total micro-organisms; MSA plus 15% sucrose to determine total streptococci; and MSA plus 0.2 units of bacitracin/mL to determine mutans streptococci.

One-way and two-way analyses of variance (ANOVA) were performed to detect the significant effects of variables. Tukey's multiple comparison was used at $p = 0.05$.

RESULTS

TEM examination showed NAg sizes ranging from 2 to 5 nm (Fig. 1A). Measurement of 100 particles (mean ± SD) yielded 2.7 ± 0.6 nm. Dentin shear bond strength (Fig. 1B) ranged from 30 to 35 MPa, which was not compromised by the addition of QADM-NAg into primer ($p > 0.1$) (Figs. 1C, 1D). SEM examination of dentin-adhesive interfaces revealed numerous resin tags "T". There was no noticeable difference among the 4 groups.

Uncured QADM-NAg primers had a strong antibacterial activity (Fig. 2). Control primer had minimal inhibition zones (Fig. 2A). Primers 10QADM, 0.05NAg, and 10QADM+0.05NAg had much larger inhibition zones (Figs. 2B-2D). Inhibition zone sizes are plotted in Figs. 2E-2G for total micro-organisms, total streptococci, and mutans streptococci. Inhibition zone sizes for 10QADM+0.05NAg were 9-fold those of control ($p < 0.05$).

Biofilms were grown on cured primers (Fig. 3A). Live bacteria were stained green, and dead bacteria were stained red (Figs. 3B-3E). Yellow/orange colors appeared when live and dead bacteria were in close proximity. Control primer had full coverage of primarily live bacteria. 10QADM, 0.05NAg, and 10QADM+0.05NAg had much more red/yellow/orange staining, indicating a strong antibacterial activity. Adding QADM or NAg substantially reduced the live bacteria area fraction (Fig. 3F). Incorporation of QADM and NAg together had the strongest antibacterial activity among all groups.

The 10QADM, 0.05NAg, and 10QADM+0.05NAg reduced the CFU counts (Fig. 4A-4C). CFU on 10QADM+0.05NAg were an order of magnitude less than those on control primer. Lactic acid from biofilms (Fig. 4D) was reduced on 10QADM, 0.05NAg, and 10QADM + 0.05NAg, compared with control ($p < 0.05$). Adding QADM-NAg to primer imparted potent antibacterial effects. The strongest effects were achieved by combining QADM and NAg in the primer.

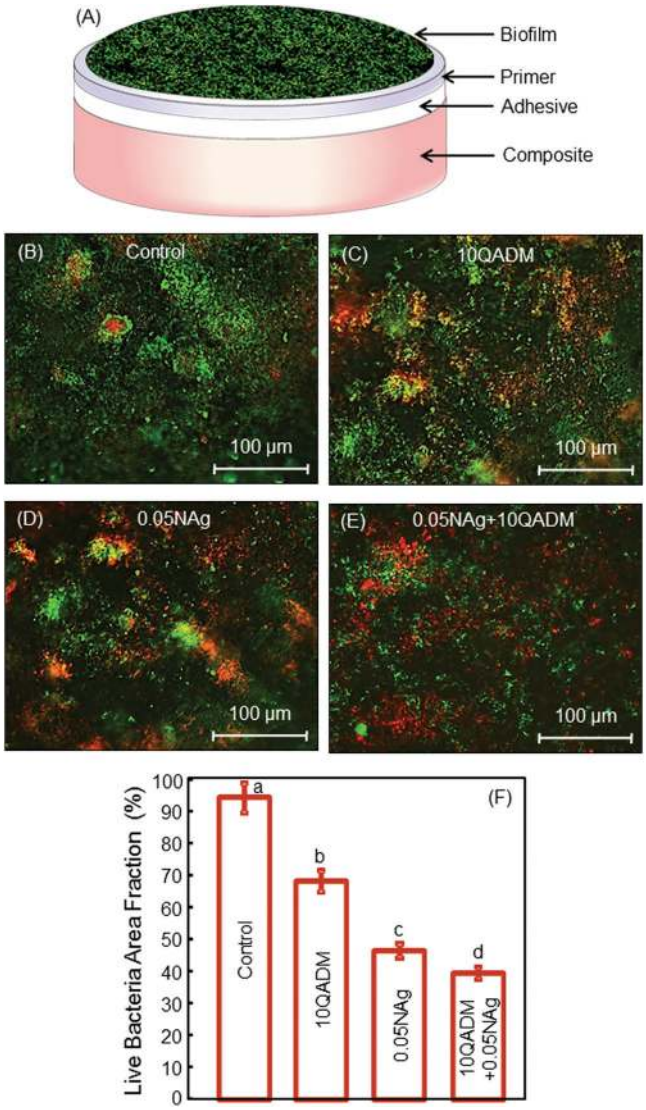

Figure 3. Cured primers with biofilms for live/dead staining. **(A)** Schematic of biofilm on the primer surface of a layered specimen. **(B-E)** Representative live/dead staining images. Live bacteria were stained green and dead bacteria were stained red. Live and dead bacteria close to (or on the top of) each other stained yellow/orange. Control primer was covered with primarily live bacteria. 10QADM, 0.05NAg, and 10QADM+0.05NAg primers had much more staining of compromised bacteria. **(F)** Live bacteria area fraction = area of green staining/total area of the image. Six specimens were used for each primer (n = 6). Three randomly chosen fields of view for each specimen yielded 18 images *per* primer. Values with dissimilar letters are significantly different ($p < 0.05$).

DISCUSSION

This study investigated, for the first time, the effects of QADM-NAg incorporation into primer on shear bond strength and microcosm biofilm response. While residual bacteria in tooth

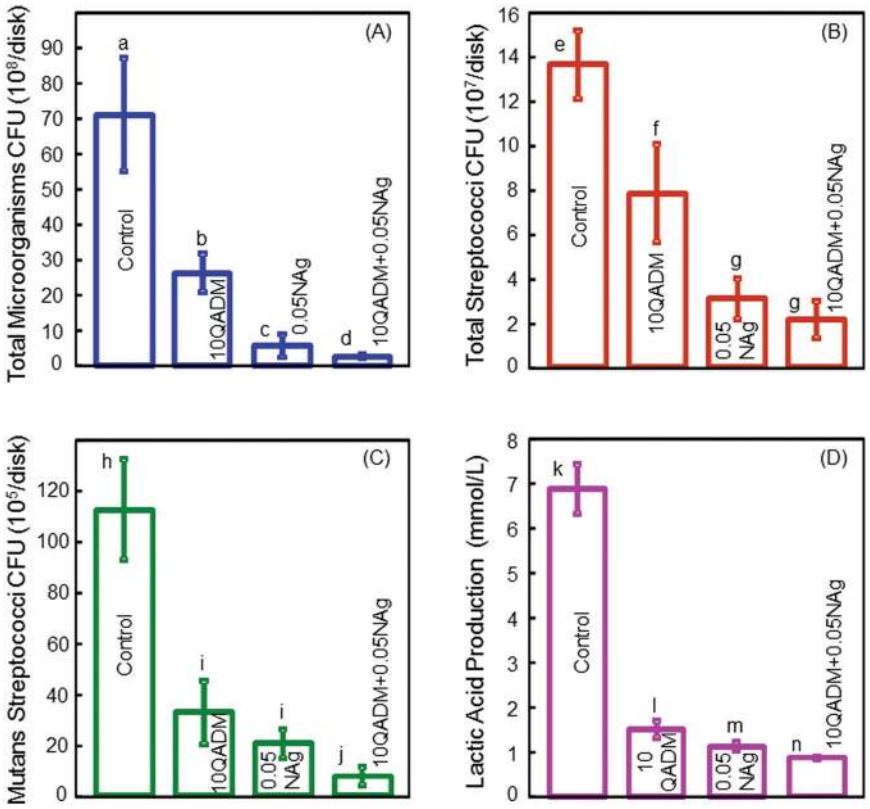

Figure 4. Biofilm CFU counts and lactic acid production on specimens with cured primers. **(A)** CFU of total micro-organisms. **(B)** CFU of total streptococci. **(C)** CFU of mutans streptococci. **(D)** Lactic acid production. Each value is mean ± SD (n = 6). In each plot, values with dissimilar letters are significantly different ($p < 0.05$). Adding QADM or NAg to the primer imparted a potent antibacterial effect. The strongest antibacterial effect was achieved with QADM and NAg together in the same primer.

cavity can lead to caries and pulp damage, primer comes into direct contact with the dentin and therefore could be an important vehicle for the delivery of antimicrobial agents. The new QADM-NAg-containing primer in the uncured state increased the microcosm inhibition zone by 9-fold, compared with commercial control primer. Control primer contained HEMA and copolymer of acrylic/itaconic acids, which could copolymerize with QADM and the Ag solution of 2-(tert-butylamino)ethyl methacrylate. The cured QADM-NAg-containing primer reduced biofilm CFU and lactic acid by an order of magnitude, compared with control. These strong antibacterial properties were achieved without compromising dentin bond strength. Furthermore, the method of combining QADM and NAg in the primer yielded the strongest antibacterial properties.

In previous studies, investigators used QAS monomers in one group of antibacterial resins (Imazato, 2003). The antimicrobial mechanism is that when the negatively charged bacterial cell comes into contact with the positively charged sites of QAS resin, the electrical balance of the cell membrane could be disturbed, and the bacterium could explode under its own osmotic pressure (Beyth *et al.,* 2006; Namba *et al.,* 2009). Uncured MDPB primer was antibacterial (Imazato *et al.,* 1997), and its antibacterial effect was maintained after being photo-cured (Imazato *et al.,* 1998). Antibacterial methacryloxylethyl-cetyl-dimethyl ammonium chloride and cetylpyridinium chloride were also investigated (Li *et al.,* 2009; Namba *et al.,* 2009; Xie *et al.,* 2011). The QADM of the present study has unique features. First, its synthesis was straightforward, because the reaction products were generated at quantitative amounts and required no further purification (Antonucci *et al.,* 2011). Second, while previous studies used QAS monomethacrylates, QADM is a dimethacrylate with reactive groups on both ends of the molecule. Hence, QADM could be incorporated into resin with a less negative impact on mechanical properties. Third, QADM is miscible with other dimethacrylates, with minimal monomer leachability due to reactive groups on both ends of the molecule, compared with monomethacrylates.

Another group of antibacterial resins incorporated Ag (Yamamoto *et al.,* 1996; Yoshida *et al.,* 1999; Fan *et al.,* 2011; Cheng YJ *et al.,* 2011). The antimicrobial mechanism was suggested to be Ag ions inactivating vital enzymes of bacteria, causing DNA to lose its replication ability, leading to cell death (Rai *et al.,* 2009). Because QADM and NAg act *via* different mechanisms, there may be a synergistic antibacterial effect that is more than simply being additive. Further study is needed to investigate how QADM-NAg synergy influences the development, metabolism, and bacterial composition of dental plaque biofilm. While QADM relies on contact inhibition, further study is needed to determine if NAg is capable of long-distance killing of bacteria due to Ag ion release. A previous study showed that Ag-containing resins had long-term antibacterial effects (Yoshida *et al.,* 1999). In the present study, the Ag salt was reduced to NAg *in situ*, avoiding the need for prefabricated nanoparticles to be mixed with resin, which could cause agglomeration. The small size of NAg could allow them to flow with primer into dentinal tubules to kill residual bacteria. The specific surface area $A = 6/(\rho d)$, where density $\rho = 10.5$ g/cm^3 for Ag, and particle size $d = 2.7$ nm. Hence, $A = 212$ m^2/g for NAg. In comparison, Ag particles with 1 μm size would have $A = 0.57$ m^2/g. The high surface area of NAg provided potent antibacterial effects at a low filler level to avoid negative influence on color and mechanical properties. Indeed, the QADM-NAg-containing primer had esthetics/color similar to those of control primer. Another issue is the cytotoxicity of the antibacterial agents. A previous study showed that MDPB had a smaller negative effect on the proliferation, differentiation, and mineralization of odontoblast-like cells than did BisGMA (Nishida

et al., 2010). Silver is known to have low toxicity and good biocompatibility with human cells (Slenters *et al.,* 2008). While a similarly good biocompatibility is expected for the QADM-NAg-containing primer, further study is needed to determine its cytotoxicity.

Single-species bacteria models have been used to test antibacterial resins (Hiraishi *et al.,* 2010; Antonucci *et al.,* 2011; Cheng YJ *et al.,* 2011). The present study used a microcosm model to test QADM-NAg-containing primers. Dental biofilm models can be divided into 3 categories: single-species, defined consortium, and microcosm (McBain, 2009). Plaque is a complicated ecosystem, with about 1,000 bacterial species (ten Cate, 2006). Hence, microcosm models could maintain the complexity/heterogeneity *in vivo* (McBain, 2009). Oral streptococci contain many groups, including the mutans streptococci group, which contains *S. mutans* and *S. sobrinus*, the major pathogens of caries. The QADM-NAg primer reduced total micro-organisms, total streptococci, and mutans streptococci by 10-fold. *In vivo* studies are needed to further investigate the antibacterial efficacy of the novel QADM-NAg primer.

In summary, this study formulated antibacterial primers by incorporating QADM and NAg, and investigated their effects on plaque microcosm biofilms for the first time. The results showed that: (1) QADM-NAg-containing primer did not decrease dentin bond strength; (2) QADM-NAg-containing primer had much larger bacteria inhibition zones, and greatly reduced biofilm viability, CFU, and lactic acid, compared with control; and (3) adding QADM and NAg together to primer produced the strongest antibacterial effects. QADM and NAg could have wide applications in other bonding systems. The novel antibacterial QADM-NAg-containing primer may be promising to inhibit biofilms and secondary caries.

ACKNOWLEDGMENTS

We thank Drs. J.M. Antonucci, N.J. Lin, S. Lin-Gibson, G.E. Schumacher, A.A. Giuseppetti, A.F. Fouad, Y.X. Bai, and L.K.A. Rodrigues for discussions. We acknowledge Esstech (Essington, PA, USA) for donating the materials, and the Core Imaging Facility of the University of Maryland for technical support. This study was supported by China National Science Foundation grant 81100745 (LC), the School of Stomatology of Capital Medical University in China (KZ), a scholarship from the Coordination for Improvement of Higher Education CAPES/Fulbright 0523/11-9 (MASM), NIH R01DE17974 and R01DE14190 (HX), and seed fund (HX) from the University of Maryland School of Dentistry. The author(s) declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

Antonucci JM, O'Donnell JN, Schumacher GE, Skrtic D (2009). Amorphous calcium phosphate composites and their effect on composite-adhesive-dentin bonding. *J Adhes Sci Technol* 23:1133-1147.

Antonucci JM, Zeiger DN, Tang K, Lin-Gibson S, Fowler BO, Lin NJ (2011). Synthesis and characterization of dimethacrylates containing quaternary ammonium functionalities for dental applications. *Dent Mater* 28:219-228.

Bayne SC, Thompson JY, Swift EJ Jr, Stamatiades P, Wilkerson M (1998). A characterization of first-generation flowable composites. *J Am Dent Assoc* 129:567-577.

Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI (2006). Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans. Biomaterials* 27:3995-4002.

Beyth N, Domb AJ, Weiss EI (2007). An *in vitro* quantitative antibacterial analysis of amalgam and composite resins. *J Dent* 35:201-206.

Cheng L, Exterkate RA, Zhou X, Li J, ten Cate JM (2011). Effect of galla chinensis on growth and metabolism of microcosm biofilms. *Caries Res* 45:87-92.

Cheng L, Weir MD, Xu HH, Antonucci JM, Kraigsley AM, Lin NJ, *et al.* (2012). Antibacterial amorphous calcium phosphate nanocomposite with quaternary ammonium salt and silver nanoparticles. *Dent Mater* doi: 10.1016/j.dental.2012.01.005 (in press).

Cheng YJ, Zeiger DN, Howarter JA, Zhang X, Lin NJ, Antonucci JM, *et al.* (2011). *In situ* formation of silver nanoparticles in photocrosslinking polymers. *J Biomed Mater Res B* 97:124-131.

Damm C, Münstedt H, Rösch A (2007). Long-term antimicrobial polyamide 6/silver nanocomposites. *J Mater Sci* 42:6067-6073.

Deligeorgi V, Mjör IA, Wilson NH (2001). An overview of reasons for the placement and replacement of restorations. *Prim Dent Care* 8:5-11.

Drummond JL (2008). Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res* 87:710-719.

Fan C, Chu L, Rawls HR, Norling BK, Cardenas HL, Whang K (2011). Development of an antimicrobial resin - A pilot study. *Dent Mater* 27:322-328.

Featherstone JD (2004). The continuum of dental caries - Evidence for a dynamic disease process. *J Dent Res* 83(Spec Iss C):39-42.

Ferracane JL (2011). Resin composite - State of the art. *Dent Mater* 27:29-38.

Hiraishi N, Yiu CK, King NM, Tay FR (2010). Effect of chlorhexidine incorporation into a self-etching primer on dentine bond strength of a luting cement. *J Dent* 38:496-502.

Imazato S (2003). Review: antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 19:449-457.

Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell RR, McCabe JF (1997). Incorporation of antibacterial monomer MDPB into dentin primer. *J Dent Res* 76:768-772.

Imazato S, Ehara A, Torii M, Ebisu S (1998). Antibacterial activity of dentine primer containing MDPB after curing. *J Dent* 26:267-271.

Imazato S, Tay FR, Kaneshiro AV, Takahashi Y, Ebisu S (2007). An in vivo evaluation of bonding ability of comprehensive antibacterial adhesive system incorporating MDPB. *Dent Mater* 23:170-176.

Li F, Chen J, Chai Z, Zhang L, Xiao Y, Fang M, *et al.* (2009). Effects of a dental adhesive incorporating antibacterial monomer on the growth, adherence and membrane integrity of *Streptococcus mutans. J Dent* 37:289-296.

Lim BS, Ferracane JL, Sakaguchi RL, Condon JR (2002). Reduction of polymerization contraction stress for dental composites by two-step light-activation. *Dent Mater* 18:436-444.

McBain AJ (2009). *In vitro* biofilm models: an overview. *Adv Appl Microbiol* 69:99-132.

Namba N, Yoshida Y, Nagaoka N, Takashima S, Matsuura-Yoshimoto K, Maeda H, *et al*. (2009). Antibacterial effect of bactericide immobilized in resin matrix. *Dent Mater* 25:424-430.

Nishida M, Imazato S, Takahashi Y, Ebisu S, Ishimoto T, Nakano T, *et al.* (2010). The influence of the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide on the proliferation, differentiation and mineralization of odontoblast-like cells. *Biomaterials* 31:1518-1532.

Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, *et al.* (2011). State of the art etch-and-rinse adhesives. *Dent Mater* 27:1-16.

Percival SL, Bowler PG, Russell D (2005). Bacterial resistance to silver in wound care. *J Hospital Infection* 60:1-7.

Rai M, Yadav A, Gade A (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 27:76-83.

Sakaguchi RL (2005). Review of the current status and challenges for dental posterior restorative composites: clinical, chemistry, and physical behavior considerations. *Dent Mater* 21:3-6.

Slenters TV, Hauser-Gerspach I, Daniels AU, Fromm KM (2008). Silver coordination compounds as light-stable, nano-structured and anti-bacterial coatings for dental implant and restorative materials. *J Mater Chem* 18:5359-5362.

Spencer P, Wang Y (2002). Adhesive phase separation at the dentin interface under wet bonding conditions. *J Biomed Mater Res* 62:447-456.

Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, *et al.* (2010). Adhesive/dentin interface: The weak link in the composite restoration. *Ann Biomed Eng* 38:1989-2003.

ten Cate JM (2006). Biofilms, a new approach to the microbiology of dental plaque. *Odontology* 94:1-9.

Xie D, Weng Y, Guo X, Zhao J, Gregory RL, Zheng C (2011). Preparation and evaluation of a novel glass-ionomer cement with antibacterial functions. *Dent Mater* 27:487-496.

Xu X, Ling L, Wang R, Burgess JO (2006). Formation and characterization of a novel fluoride-releasing dental composite. *Dent Mater* 22:1014-1023.

Yamamoto K, Ohashi S, Aono M, Kokubo T, Yamada I, Yamauchi J (1996). Antibacterial activity of silver ions implanted in SiO_2 on oral streptococci. *Dent Mater* 12:227-229.

Yoshida K, Tanagawa M, Atsuta M (1999). Characterization and inhibitory effect of antibacterial dental resin composites incorporating silver-supported materials. *J Biomed Mater Res* 47:516-522.