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Effects of dual antibacterial agents MDPB and nano-silver in primer on microcosm biofilm, cytotoxicity and dentin bond properties

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Abstract

Objectives—The objective of this study was to investigate the effects of dentin primer containing dual antibacterial agents, namely, 12-methacryloyloxydodecylpyridinium bromide (MDPB) and nanoparticles of silver (NAg), on dentin bond strength, dental plaque microcosm biofilm response, and fibroblast cytotoxicity for the first time.

Methods—Scotchbond Multi-Purpose (SBMP) was used as the parent bonding agent. Four primers were tested: SBMP primer control (referred to as “P”), P+5%MDPB, P+0.05%NAg, and P+5%MDPB+0.05%NAg. Dentin shear bond strengths were measured using extracted human teeth. Biofilms from the mixed saliva of 10 donors were cultured to investigate metabolic activity, colony-forming units (CFU), and lactic acid production. Human fibroblast cytotoxicity of the four primers was tested in vitro.

Results—Incorporating MDPB and NAg into primer did not reduce dentin bond strength compared to control ($p>0.1$). SEM revealed well-bonded adhesive-dentin interfaces with numerous resin tags. MDPB or NAg each greatly reduced biofilm viability and acid production,

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compared to control. Dual agents MDPB+NAg had a much stronger effect than either agent alone ($p<0.05$), increasing inhibition zone size and reducing metabolic activity, CFU and lactic acid by an order of magnitude, compared to control. There was no difference in cytotoxicity between commercial control and antibacterial primers ($p>0.1$).

Conclusions—The method of using dual agents MDPB+NAg in the primer yielded potent antibacterial properties. Hence, this method may be promising to combat residual bacteria in tooth cavity and invading bacteria at the margins. The dual agents MDPB+NAg may have wide applicability to other adhesives, composites, sealants and cements to inhibit biofilms and caries.

Keywords

Antibacterial primer; dentin bond strength; silver nanoparticles; MDPB; human saliva microcosm biofilm; caries inhibition

1. Introduction

Many studies clearly indicate the prevalence of dental caries worldwide.^{1–3} The basic mechanism of caries is demineralization of enamel and dentin via acids generated by bacterial biofilms (dental plaque).^{4,5} Oral biofilms produce acids from carbohydrates to cause tooth decay.⁶ Therefore, it is beneficial to develop a new generation of bioactive dental resins that can effectively inhibit bacteria and tooth decay.^{7–12} To inhibit caries, antibacterial resins and composites containing quaternary ammonium salts (QAS) were developed.^{7–15} Novel resins and composites with 12-methacryloyloxydodecylpyridinium bromide (MDPB) were demonstrated to effectively hinder bacteria and biofilm growth.^{7,8,16} Other studies synthesized new antibacterial resins employing antibacterial agents such as methacryloxyethyl cetyl dimethyl ammonium chloride (DMAE-CB) and cetylpyridinium chloride (CPC), as well as other compositions.^{9,11–15,17–19}

Adhesives are useful to bond the composite restoration to the tooth structure and maintain a functional interface.^{20–24} Many studies have contributed to improvements in bond strength, interface durability, and chemical compositions of bonding agents.^{25–30} Incorporating antibacterial agents into adhesives would be beneficial to combat biofilms and recurrent caries at the tooth-composite interfaces.^{7,18,19} While residual bacteria could exist in the prepared tooth cavity, microleakage may occur during service and bacteria can invade the tooth-restoration margins. Therefore, antibacterial adhesives could be used to hinder bacteria invasion and growth.^{7,16} Indeed, previous studies demonstrated that MDPB-containing adhesives markedly decreased the growth of *Streptococcus mutans* (*S. mutans*) and other species of oral bacteria.^{7,16}

Besides the adhesive, it is also desirable to develop antibacterial primers.^{31–33} Primer directly contacts the tooth structure and flows into dentinal tubules, and hence can serve as a carrier for antibacterial agents. Recently, an MDPB-containing primer was reported as “The world’s first antibacterial adhesive system”.⁸ MDPB-containing primer possessed potent antibacterial effects,^{31,32} and inactivated residual bacteria in cavities both in vitro and in vivo.^{34,35} Another study reported a primer containing chlorhexidine with an effective antimicrobial activity.³³ A quaternary ammonium dimethacrylate (QADM)-containing primer was also reported.¹⁴ There have been only a few reports on the development of antibacterial primers and more efforts are warranted. A recent study combined QADM and nanoparticles of silver (NAg) into a primer and obtained stronger antibacterial potency than using a single antibacterial agent.¹⁴ However, previous studies on MDPB used only one antibacterial agent in the bonding agent, without combining with a second antibacterial agent such as NAg in the bonding agent to enhance the antibacterial activity. The rationale

for using dual antibacterial agents is that while MDPB is known to be antibacterial, adding NAg may further increase the antibacterial potency of the primer, without compromising the dentin bond strength or the cytotoxicity.

Therefore, the objectives of this study were to incorporate both MDPB and NAg into a primer, and to investigate their combined effects on dental plaque microcosm biofilm viability, metabolic activity and lactic acid production for the first time. It was hypothesized that: (1) Incorporating MDPB or NAg individually into a primer would achieve substantial antibacterial effects; (2) Combining MDPB and NAg together in the primer would achieve greater anti-biofilm potency than MDPB or NAg alone; (3) Antibacterial functions via the dual agents could be obtained without adversely affecting dentin bond strength or human fibroblast cytotoxicity, compared to commercial non-antibacterial control.

2. Materials and methods

2.1. Preparation of antibacterial primers containing MDPB and NAg

Scotchbond Multi-Purpose (referred as “SBMP”) (3M, St. Paul, MN) was used as the parent bonding system to test the effect of MDPB and NAg incorporation. According to the manufacturer, SBMP etchant contains 37% phosphoric acid. SBMP primer contains 35–45% of 2-hydroxyethylmethacrylate (HEMA), 10–20% of a copolymer of acrylic and itaconic acids, and 40–50% of water. SBMP adhesive contains 60–70% of bisphenol A diglycidyl methacrylate (BisGMA), 30–40% of HEMA, tertiary amines and photo-initiator. The present study incorporated MDPB and NAg into the SBMP primer. The SBMP etchant and adhesive were used without modification.

MDPB, a polymerizable bactericide, was synthesized by combining a quaternary ammonium dodecylpyridinium bromide and a methacryloyl group.^{7,8} The MDPB powder was provided by Kuraray Medical Inc. (Tokyo, Japan). MDPB was dissolved into the SBMP primer at a MDPB/(primer + MDPB) mass fraction of 5%, following a previous study.³²

Silver (Ag) is known to have antibacterial, antifungal and antiviral capabilities.^{36,37} Recently, resins containing nanoparticles of silver (NAg) were synthesized with antibacterial functions.^{12,38–40} Briefly, silver 2-ethylhexanoate (Strem, Newburyport, MA) of 0.1 g was dissolved into 1 g of 2-(tert-butylamino)ethyl methacrylate (TBAEMA, Sigma).³⁸ TBAEMA was used to improve the solubility by forming Ag-N coordination bonds with Ag ions, thereby facilitating the Ag salt to dissolve in the resin solution.³⁸ This Ag solution was mixed with SBMP primer at a silver 2-ethylhexanoate/(primer + silver 2-ethylhexanoate) mass fraction of 0.05%, following a previous study.¹⁴

Therefore, four primers were tested in the present study: (1) SBMP control primer; (2) control primer + 5% MDPB (termed “P+MDPB”); (3) control primer + 0.05% NAg (termed “P+NAg”); (4) control primer + 5% MDPB + 0.05% NAg (termed “P+MDPB+NAg”).

2.2. Dentin Shear Bond testing

The use of extracted human teeth was approved by the University of Maryland. Caries-free human third molars were used for dentin bonding. After removing the tooth crown by sawing (Isomet, Buehler, Lake Bluff, IL), the tooth was ground perpendicular to the longitudinal axis of the tooth on 320 grit SiC paper until occlusal enamel was completely removed. The dentin surface was etched with etchant for 15 s and rinsed with water.⁴¹ A primer was applied, and the solvent was evaporated with an air stream. Then, the unmodified SBMP adhesive was applied and light-cured for 10 s (Optilux VCL401, Demetron, Danbury, CT). A stainless-steel iris, having a central opening with a diameter of 4 mm and a thickness of 1.5 mm, was held against the adhesive-treated dentin surface.⁴¹ The

central opening was filled with a composite (TPH, Dentsply, Milford, DE) and light-cured for 60 s. The bonded specimens were stored in water at 37 °C for 24 h. The dentin shear bond strength, S_D , was measured as previously described.^{14,40,41} A chisel was held parallel to the composite-dentin interface and loaded via a Universal Testing Machine (MTS, Eden Prairie, MN) at 0.5 mm/min until the composite-dentin bond failed. S_D was calculated as: $S_D = 4P/(\pi d^2)$, where P is the load at failure, and d is the diameter of the composite.⁴¹ Ten teeth were tested for each group (n = 10).

The adhesive-tooth interface was examined via scanning electron microscopy (SEM). The bonded tooth was cut through the center in the longitudinal direction via the diamond saw (Isomet). The sectioned surface was polished with increasingly finer SiC paper up to 4000 grit. Three specimens were prepared for each group. Following a previous study,¹⁶ the polished surface was treated with 50% phosphoric acid for 30 s, then with 10% NaOCl for 2 min. After being thoroughly rinsed with water, the specimens were air dried and sputter-coated with gold. The dentin-adhesive interfaces were examined via SEM (Quanta 200, FEI, Hillsboro, OR).^{14,40}

2.3. Dental plaque microcosm biofilm model

Saliva is ideal for growing biofilms to maintain much of the complexity and heterogeneity in vivo.⁴² In the present study, whole human saliva was used as an inoculum to provide multi-species biofilms consisting of organisms found in the oral cavity. To represent the diverse bacterial populations, saliva from ten healthy individuals was collected and combined for the experiments, following a previous study.⁴³ The ten healthy individuals had natural dentitions without active caries or periopathology, and without using antibiotics within the last 3 months.^{43,44} They did not brush teeth for 24 h and abstained from food/drink intake for 2 h prior to donating saliva.^{14,40} Stimulated saliva was collected during parafilm chewing and kept on ice. An equal volume of saliva from each of the ten donors was combined, and diluted to 70% saliva and 30% glycerol.^{43,44} Aliquots of 1 mL were stored at -80 °C for subsequent use. The human saliva microcosm biofilm model was approved by the University of Maryland.

2.4. Agar disk diffusion test (ADT)

Un-cured primers were tested by ADT. The saliva-glycerol stock was added, with 1:50 final dilution, to a growth medium as inoculum.^{14,40} The growth medium contained mucin (type II, porcine, gastric) at a concentration of 2.5 g/L; bacteriological peptone, 2.0 g/L; tryptone, 2.0 g/L; yeast extract, 1.0 g/L; NaCl, 0.35 g/L; KCl, 0.2 g/L; CaCl₂, 0.2 g/L; cysteine hydrochloride, 0.1 g/L; haemin, 0.001 g/L; and vitamin K₁, 0.0002 g/L, at pH 7.⁴⁵ The inoculum was incubated at 37 °C and 5% CO₂ for 24 h. Three types of agar plates were prepared. First, tryptic soy blood agar culture plates were used to determine total microorganisms.¹⁴ Second, mitis salivarius agar (MSA) culture plates, containing 15% (by mass) sucrose, were used to determine total streptococci.⁴⁶ This is because MSA contains selective agents crystal violet, potassium tellurite and trypan blue, which inhibit most gram-negative bacilli and gram-positive bacteria except streptococci, thus enabling streptococci to grow.⁴⁶ Third, cariogenic mutans streptococci are known to be resistant to bacitracin, and this property was used to isolate mutans streptococci from the oral microflora. Hence, MSA agar plates with 0.2 units of bacitracin per mL were used to determine mutans streptococci.^{14,40,47}

Bacteria suspension of 0.4 mL was poured onto each agar plate with a diameter of 90 mm.¹⁴ Then, 20 µL of primer was impregnated into a sterile paper disk with a diameter of 6 mm and a thickness of 1.5 mm, following previous studies.^{14,32} The primer-impregnated paper disk was placed on a plate with bacteria and incubated for 48 h. The bacteria inhibition zone

size was measured as: (Outer diameter of inhibition zone - paper disk diameter)/2.³² Six specimens were measured for each group (n = 6).

2.5. Fabrication of specimens for biofilm experiments

Cured tri-layer disk specimens were fabricated as schematically shown in Fig. 3A, following a previous study.³¹ The four primer groups were tested. Each primer was placed into a polyethylene mold (inner diameter = 9 mm, thickness = 2 mm) situated on a glass slide. After drying with a stream of air, 10 μ L of SBMP adhesive was applied on top of the primer and cured for 20 s with Optilux. Then, a composite (TPH) was placed on the adhesive to fill the mold and cured for 1 min (Triad 2000, Dentsply, Milford, DE). The disks were well cured, and after the cured samples were removed from the molds, there was no primer left on the glass slide as confirmed with optical microscopy (Nikon TMS, Tokyo, Japan). The cured specimens were agitated in water for 1 h to remove any uncured monomers, following a previous study.³¹ The agitation was done in 200 mL of distilled water with a stir bar via a magnetic stirrer at a speed of 100 rpm (Bellco Glass, Vineland, NJ). The specimens were then dried and sterilized with ethylene oxide sterilizer (Anprolene AN 74i, Andersen, Haw River, NC).^{14,40}

2.6. Live/dead bacterial staining assay

The saliva-glycerol stock was added, with 1:50 final dilution, to the growth medium. Each cured disk was placed into a well of a 24-well plate with the primer surface facing up, and 1.5 mL of inoculum was added to each well. They were incubated in 5% CO₂ at 37 °C for 8 h. The disks were transferred into a new 24-well plate with 1.5 mL fresh medium, and then incubated with 5% CO₂ at 37 °C. After 16 h, each specimen was transferred into a new 24-well plate with 1.5 mL fresh medium, and incubated in 5% CO₂ at 37 °C for 24 h.^{14,40,47} This totaled 2 days of incubation, which was shown previously to be sufficient to form microcosm biofilms.^{14,40,47} Biofilms on specimens were rinsed with phosphate-buffered saline (PBS), live/dead stained (Molecular Probes, Eugene, OR), and examined with an inverted epifluorescence microscope (Eclipse TE2000-S, Nikon, Melville, NY).^{14,40,47}

2.7. MTT assay of metabolic activity of biofilms

MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a colorimetric assay that measures the enzymatic reduction of MTT, a yellow tetrazole, to formazan.^{11,12} Disk were inoculated with microcosm bacteria and incubated for 2 d to form biofilms, as described above. The disk with biofilm was transferred to a new 24-well plate, then 1 mL of MTT dye (0.5 mg/mL MTT in PBS) was added to each well and incubated at 37 °C in 5% CO₂ for 1 h. During this process, the metabolically active bacteria reduced the MTT to purple formazan. After 1 h, the disks were transferred to a new 24-well plate, 1 mL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals, and the plate was incubated for 20 min with gentle mixing at room temperature in the dark. After mixing via pipetting, 200 μ L of the DMSO solution from each well was transferred to a 96-well plate, and the absorbance at 540 nm was measured via a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA). A higher absorbance is related to a higher formazan concentration, which indicates a higher metabolic activity in the biofilm on the disk.¹²

2.8. Lactic acid production

Disks with 2-day biofilms were rinsed with cysteine peptone water (CPW) to remove the loose bacteria. They were transferred to 24-well plates containing buffered-peptone water (BPW) plus 0.2% sucrose, and incubated for 3 h to allow biofilms to produce acid. The BPW solutions were stored and lactate concentrations were determined using an enzymatic

method.^{14,40,47} The 340-nm absorbance of BPW was measured with the microplate reader. Standard curves were prepared using a standard lactic acid (Supelco, Bellefonte, PA).¹²

2.9. Biofilm colony-forming unit (CFU) counts

Three types of agar plates were prepared as described in Section 2.4: Tryptic soy blood agar culture plates were used to determine total microorganisms, mitis salivarius agar (MSA) culture plates containing 15% sucrose to determine total streptococci, and MSA agar plates with 0.2 units of bacitracin per mL to determine mutans streptococci.^{14,40,47} The disks with 2-day biofilms were transferred into tubes with 2 mL CPW, and the biofilms were harvested by sonication (3510R-MTH, Branson, Danbury, CT) for 5 minutes, followed by vortexing at 2400 rpm for 30 seconds using a vortex mixer (Fisher Scientific, Pittsburgh, PA).¹⁴ The bacterial suspensions were serially diluted, spread onto the agar plates, and incubated for 3 d at 5% CO₂ and 37 °C. The number of colonies that grew were counted and used, along with the dilution factor, to calculate the total CFU on each disk.

2.10. Cytotoxicity of bonding agent eluent with human gingival fibroblasts

To prepare specimens for cytotoxicity testing, the cover of a sterile 96-well plate was used, and 10 µL of a primer was placed in the bottom of the dent.¹⁸ The primer was dried with a stream of air, then 20 µL of unmodified SBMP adhesive was applied and photo-polymerized for 20 s, while being covered with a Mylar strip. This yielded a cured primer/adhesive disk of approximately 8 mm in diameter and 0.5 mm in thickness. After sterilizing with ethylene oxide, six specimens of the same group were immersed in 10 mL of fibroblast medium (FM, ScienCell, San Diego, CA) and agitated for 24 h at 37 °C to obtain eluent from the disks.⁴⁸ The original extract solution, which was highly concentrated with resin eluent, was then serially diluted to concentrations relevant to conditions in vivo. Three solutions were thus prepared, at dilutions of 32-fold (1 mL of original extract + 32 mL of fresh FM), 64-fold, and 128-fold, respectively. This is because the amount of saliva flow for an average person is approximately 1000 to 1500 mL per day,⁴⁹ hence the 128-fold dilution corresponded to a total solution of 1280 mL for the six disks. The 32-fold and 64-fold corresponded to a total solution of 320 mL and 640 mL respectively, which would be equivalent to 1/4 and 1/2 of the normal saliva amount per day, to take into account of patients with reduced saliva production. The total resin volume for the 6 disks was 151 mm³. Therefore, the resin volume divided by the total solution volume for the three solutions was 0.12, 0.24, and 0.47 mm³/mL, respectively. Fresh FM without any resin eluent (resin/solution volume ratio = 0 mm³/mL) served as control for the fibroblast cytotoxicity test.

Human gingival fibroblasts (HGF, ScienCell, San Diego, USA) were cultured in FM supplemented with 2% fetal bovine serum, 100 IU/mL penicillin and 100 IU/mL streptomycin. The HGF protocol was approved by the University of Maryland. A seeding density of 4000 cells/well was used in 96-well plates.⁵⁰ After 24 h of incubation at 37 °C with 5% CO₂ in air, the culture medium was removed and replaced with 100 µL of one of the four solutions described above. The cells were cultured in these solutions for another 48 h, then 20 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) solution at a concentration of 5 mg/mL was added to each well. After incubating in a darkroom for 4 h, the unreacted dye was removed and 150 µL/well of dimethylsulfoxide (DMSO, Sigma) was added.⁵⁰ The solution absorbance was measured via a microplate reader (SpectraMax) at 492 nm.⁵⁰ The absorbance for FM without any resin eluent was set as 100%. Fibroblast viability with eluents = Absorbance with eluents/absorbance of control.⁵⁰

2.11. Statistical analysis

One-way and two-way analyses of variance (ANOVA) were performed to detect the significant effects of variables. Tukey's multiple comparison test was used to compare the data at a p value of 0.05. Standard deviations (sd) serve as an estimate for standard uncertainties associated with the measurements.

3. Results

The ADT results for un-cured primers are shown in Fig. 1. The commercial control primer produced minimal inhibition zones. Incorporating NAg or MDPB into the primer greatly increased the inhibition zone size, with examples for mutans streptococci shown in (A). Adding dual agents MDPB and NAg into the primer achieved the largest inhibition zone size. The quantitative inhibition zone sizes are plotted in (B–D) for total microorganisms, total streptococci, and mutans streptococci, respectively. In general, the inhibition zone size for the MDPB-containing primer was approximately 5-fold that of the control primer. Adding NAg into the MDPB-containing primer doubled the inhibition zone size to be about 10-fold that of control primer ($p < 0.05$).

The dentin bonding results are shown in Fig. 2: (A) Dentin shear bond strength (mean $\pm$ sd; $n = 10$), and (B) representative SEM image of the adhesive-dentin bond. In (A), all the bond strengths were not significantly different from each other ($p > 0.1$). Therefore, incorporating MDPB and NAg into the primer did not compromise the dentin bond strength. A representative example of the dentin-adhesive interface is shown in (B) for the control; all four groups had similar dentin-adhesive interfacial features with numerous resin tags.

Fig. 3 shows the results of the live/dead assay: (A) Schematic of the biofilm experiment; (B–E) representative images of biofilms adherent on layered disks with SBMP control primer, P+NAg, P+MDPB, and P+MDPB+NAg, respectively. The control primer was fully covered by primarily live bacteria. In contrast, specimens with primer containing 0.05% NAg or 5% MDPB had substantial amounts of dead bacteria. The primer containing dual agents (5% MDPB and 0.05% NAg) had noticeably more staining of compromised bacteria than other groups.

Fig. 4 plots: (A) the metabolic activity, and (B) lactic acid production (mean $\pm$ sd; $n = 6$). In (A), biofilms on control primer had a relatively high metabolic activity. Incorporation of MDPB or NAg each greatly decreased the metabolic activity of biofilms ($p < 0.05$). Adding dual agents (MDPB and NAg) together in the same primer further significantly reduced the metabolic activity, compared to MDPB or NAg alone ($p < 0.05$). A similar trend is observed in lactic acid production in (B). Biofilms on the control primer produced the most amount of lactic acid. Adding MDPB or NAg each decreased the acid production substantially, compared to the control ($p < 0.05$). The primer with 5% MDPB and 0.05% NAg together had the least lactic acid ($p < 0.05$). These results demonstrate that adding MDPB or NAg into the primer imparted a potent antibacterial effect, and the strongest effect was achieved by combining MDPB and NAg in the same primer.

The biofilm CFU counts are plotted in Fig. 5 for: (A) Total microorganisms, (B) total streptococci, and (C) mutans streptococci (mean $\pm$ sd; $n = 6$). Adding MDPB or NAg each decreased the biofilm CFU, compared to the commercial primer control ($p < 0.01$). The incorporation of dual agents (MDPB and NAg) in the primer had a significantly stronger antibacterial effect than using MDPB or NAg alone ($p < 0.05$).

The results on human fibroblast cytotoxicity of bonding agent eluents are plotted in Fig. 6. The commercial FM without any resin eluent addition served as control. Two-way ANOVA

showed no significant effects of material type or resin volume/solution volume ratio ($p > 0.1$), with no significant interactions between the two variables ($p > 0.1$). Fibroblast viability for all the primer groups was around 100% and matched that of the FM control ($p > 0.1$). This was true at all the three resin/solution volume ratios tested. These data show that, compared to the commercial non-antibacterial primer, incorporating MDPB and NAg into the primer had no adverse effect on the fibroblast cytotoxicity ($p > 0.1$).

4. Discussion

MDPB has been extensively investigated as an antibacterial monomer and shown to be promising for dental applications including use in composite, primer and adhesive.^{7,8} In previous studies, each antibacterial resin contained only one type of antibacterial agent (MDPB). In the present study, dual antibacterial agents MDPB and NAg were combined for the first time in the primer. The results showed that MDPB indeed imparted a strong antibacterial function to a commercial SBMP primer, greatly reducing the microcosm biofilm viability, metabolic activity, CFU and lactic acid production. These results confirmed the previous studies reporting MDPB with strong antibacterial properties.^{7,8} Furthermore, the present study showed that the antibacterial potency of MDPB-containing primer can be further increased by the incorporation of a second antibacterial agent, NAg. The dual antibacterial agent method was supported by two benefits: (1) The use of dual antibacterial agents in the primer reduced the biofilm activity by more than half, compared to that using MDPB alone; (2) the use of dual antibacterial agents in the primer did not adversely affect the dentin shear bond strength and fibroblast cytotoxicity.

It is important to develop antibacterial primers in combating secondary caries. Caries is a dietary carbohydrate-modified bacterial infectious disease caused by acid production by biofilms.⁴⁻⁶ Therefore, antibacterial bonding agents are promising to inhibit biofilms and secondary caries at the tooth-restoration interface. Primer directly contacts the tooth structure at the interface, and hence can serve as a carrier to deliver antibacterial agents. There are often residual bacteria present in the prepared tooth cavity.^{18,51} With the increased interest in Minimal Intervention Dentistry and preservation of tooth structure,⁵² more carious tissues with active bacteria could remain in the prepared tooth cavity. For patients of certain ethnicity and poverty levels with a high incidence of untreated caries, the atraumatic restorative treatment (ART) method could be especially useful as it can be readily performed without requiring electricity and anesthesia.^{53,54} However, ART may not completely remove the carious tissues.^{53,54} Therefore, applying a strongly-antibacterial primer could be especially beneficial for these applications, as the un-cured primer with MDPB + NAg (Fig. 1) could kill the bacteria remaining in the prepared tooth cavity.

In addition, once cured, the bonded interface could combat the future invading bacteria in vivo. While a complete sealing of the tooth-restoration interface is an important goal, it is often difficult to achieve. Polymerization shrinkage combined with chewing and wear stresses could create microcracks and form microgaps at the margins. Previous studies indeed showed the existence of microgaps at the tooth-restoration interfaces,^{55,56} which could harbor the invading bacteria to secrete acids and cause secondary caries. Therefore, it is important for the bonding agent in the cured state at the tooth-restoration interface to remain antibacterial. In the present study, when the primer contained MDPB and NAg, the cured primer/adhesive samples effectively inhibited the dental plaque microcosm biofilm growth. Therefore, the primer containing MDPB and NAg is promising to kill not only the residual bacteria in the prepared tooth cavity, but also the invading bacteria along the margins during service. While antibacterial primers have great potential to help inhibit biofilms and caries, to date, there have been only a few reports on them.^{8,14,33} The present study represents the first report in which MDPB and NAg were combined in the primer. The

results indicate that the MDPB-NAg primer would contribute significantly to inhibiting biofilm growth, acid production, and secondary caries.

The use of dual antibacterial agents, and specifically, the combination of quaternary ammonium monomer with NAg, may be a promising approach for developing strong antibacterial biomaterials. Ag has been shown to possess antibacterial, antifungal, and antiviral functions,^{36,37} and has been known to be an effective antibacterial agent against a wide range of micro-organisms.^{57–59} Regarding its antimicrobial mechanism, it was suggested that the Ag ions could inactivate the vital enzymes of bacteria to cause the bacterial DNA to lose its replication ability, leading to cell death.^{57,59} Ag has several properties worth noting: Low toxicity and good biocompatibility with human cells;⁵⁸ long-term antibacterial effect due to sustained silver ion release;⁶⁰ and less bacterial resistance than antibiotics.⁶¹ An additional advantage for NAg is the small particle size and the associated high specific surface area, which yielded a strong antibacterial potency at a low NAg filler level in the resin. A low NAg filler level was advantageous because it did not adversely affect the physical properties of the primer. Furthermore, while the addition of NAg and MDPB to primer imparted a strong antibacterial activity, it is important that such addition does not compromise the biocompatibility of the bonding agent. This was indeed verified via the human gingival fibroblast cytotoxicity test, which showed not only that adding MDPB and NAg had no adverse effect on cytotoxicity compared to the commercial primer, but also that all the solutions with resin eluents had fibroblast viability similar to that using fibroblast medium without any resin eluents. Therefore, it is possible to use NAg in the MDPB resins to obtain additional strong antibacterial functions without compromising physical properties such as dentin bond strength and cytotoxicity.

MDPB has been shown to possess potent antibacterial activity against various oral bacteria including facultative and obligate anaerobe in coronal lesions, as well as bacterial species isolated from root caries such as actinomyces and *Candida albicans*.⁸ For application in composites, MDPB copolymerized with other monomers of the composite, yielding a strong antimicrobial effect against bacteria.⁷ For use in primer, MDPB imparted a potent antibacterial effect to a dentin primer, without compromising the dentin bond strength.⁶² In another study, the MDPB primer was applied to cavities in dog teeth infected with *S. mutans* and exhibited in vivo antibacterial effects.³⁵ The MDPB-containing bonding agent after curing also demonstrated a strong antibacterial effect against the growth of *S. mutans*, without influencing the bond strength or curing characteristics.⁵¹ In addition, a composite restoration containing MDPB was shown to inhibit the progression of artificial secondary root caries lesions using extracted human teeth with Class V cavities.⁶³ For all these various applications and restorations using MDPB, the antibacterial efficacy could be substantially increased via the addition of a small amount of NAg. Regarding antibacterial mechanisms, MDPB is covalently bonded with the polymer structure and immobilized in the resin, exerting contact-inhibition against adherent bacteria.^{7,8,16} Ag has long-term antibacterial effect due to the sustained silver ion release,⁶⁰ which could not only kill bacteria on the surface, but also bacteria away from the surface. Therefore, the effects of releasing (NAg) and non-releasing (MDPB) antimicrobials could be synergistic and complimentary to each other, to enhance the resin's antibacterial efficacy. The present study showed that incorporating 0.05% of NAg into the MDPB primer doubled the inhibition zone size, and cut the biofilm lactic acid and CFU by half, compared to those using MDPB alone. In addition, several other studies developed antibacterial dental materials using novel compositions.^{9,13,18} It is likely that the incorporation of NAg into these new antibacterial formulations will also greatly enhance the antibacterial potency, without compromising physical and mechanical properties. However, it should be noted that clinical conditions in vivo could significantly influence the antibacterial efficacy of the restoration. For example, fluctuating shear could occur due to saliva flow, drinking beverages, and tooth brushing.

Fluctuating shear that occurs supragingivally could impact the efficacy of various antibacterial agents that might be released from materials placed in the oral cavity. Due to the hydrodynamic conditions in vivo, the antibacterial agents released from the material may be flushed away and the antibacterial effect may thus be limited. Another factor is that the fluids in vivo may dilute and decrease the local concentration of silver ions, and thereby adversely impact the antibacterial efficacy of silver-based materials in the oral cavity. Therefore, further studies are needed to investigate the antibacterial and anti-caries efficacy of the MDPB-NAg primer under in vivo conditions. Further studies should also investigate the promise of using the MDPB-NAg combination in various adhesive systems, composites, and glass ionomer cements to achieve strong antibacterial and caries-inhibiting capabilities.

5. Conclusions

The present study investigated the effects of dual antibacterial agents MDPB and NAg in dentin primer on dental plaque microcosm biofilms for the first time. The hypotheses were verified that incorporating MDPB or NAg individually into the primer achieved substantial antibacterial effects; MDPB + NAg in the primer achieved greater anti-biofilm potency than MDPB or NAg alone; and antibacterial functions via the dual agents were obtained without compromising dentin bond strength or biocompatibility. Therefore, the dual agents of MDPB and NAg may have wide applicability to other bonding systems, composites, sealants and cements. The novel antibacterial MDPB-NAg primer may be promising to inhibit oral biofilms and secondary caries.

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References

1. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. *American Journal of Dentistry*. 2009; 22:3–8. [PubMed: 19281105]
2. Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, et al. Trends in oral health status: United States, 1988–1994 and 1999–2004. *Vital and Health Statistics*. 2007; 11:1–92. [PubMed: 17633507]
3. Dye BA, Thornton-Evans G. Trends in oral health by poverty status as measured by Healthy People 2010 objectives. *Public Health Reports*. 2010; 125:817–830. [PubMed: 21121227]
4. Deng DM, ten Cate JM. Demineralization of dentin by *Streptococcus mutans* biofilms grown in the constant depth film fermentor. *Caries Research*. 2004; 38:54–61. [PubMed: 14684978]
5. Totiam P, Gonzalez-Cabezas C, Fontana MR, Zero DT. A new *in vitro* model to study the relationship of gap size and secondary caries. *Caries Research*. 2007; 41:467–473. [PubMed: 17827964]
6. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiological Reviews*. 1986; 50:353–380. [PubMed: 3540569]
7. Imazato S. Review: Antibacterial properties of resin composites and dentin bonding systems. *Dental Materials*. 2003; 19:449–457. [PubMed: 12837391]
8. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dental Materials Journal*. 2009; 28:11–19. [PubMed: 19280964]

9. Xie D, Weng Y, Guo X, Zhao J, Gregory RL, Zheng C. Preparation and evaluation of a novel glass-ionomer cement with antibacterial functions. *Dental Materials*. 2011; 27:487–496. [PubMed: 21388668]
10. Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, Breschi L, Nishitani Y, Tay FR, Pashley DH. The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs. *Journal of Dental Research*. 2011; 90:535–540. [PubMed: 21212315]
11. Antonucci JM, Zeiger DN, Tang K, Lin-Gibson S, Fowler BO, Lin NJ. Synthesis and characterization of dimethacrylates containing quaternary ammonium functionalities for dental applications. *Dental Materials*. 2012; 28:219–228. [PubMed: 22035983]
12. Cheng L, Weir MD, Xu HHK, Antonucci JM, Kraigsley AM, Lin NJ, Lin-Gibson S, Zhou XD. Antibacterial amorphous calcium phosphate nanocomposite with quaternary ammonium salt and silver nanoparticles. *Dental Materials*. 2012; 28:561–572. [PubMed: 22305716]
13. Xu X, Wang Y, Liao S, Wen ZT, Fan Y. Synthesis and characterization of antibacterial dental monomers and composites. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2012; 100:1511–1162.
14. Cheng L, Zhang K, Melo MA, Weir MD, Zhou X, Xu HH. Anti-biofilm dentin primer with quaternary ammonium and silver nanoparticles. *Journal of Dental Research*. 2012; 91:598–604. [PubMed: 22492276]
15. Weng Y, Howard L, Guo X, Chong VJ, Gregory RL, Xie D. A novel antibacterial resin composite for improved dental restoratives. *Journal of Materials Science: Materials in Medicine*. 2012; 23:1553–1561. [PubMed: 22466818]
16. Imazato S, Tay FR, Kaneshiro AV, Takahashi Y, Ebisu S. An in vivo evaluation of bonding ability of comprehensive antibacterial adhesive system incorporating MDPB. *Dental Materials*. 2007; 23:170–176. [PubMed: 16469372]
17. Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. *Biomaterials*. 2006; 27:3995–4002. [PubMed: 16564083]
18. Li F, Chai ZG, Sun MN, Wang F, Ma S, Zhang L, Fang M, Chen JH. Anti-biofilm effect of dental adhesive with cationic monomer. *Journal of Dentistry*. 2009; 88:372–376.
19. Namba N, Yoshida Y, Nagaoka N, Takashima S, Matsuura-Yoshimoto K, Maeda H, Van Meerbeek B, Suzuki K, Takashida S. Antibacterial effect of bactericide immobilized in resin matrix. *Dental Materials*. 2009; 25:424–430. [PubMed: 19019421]
20. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. *Journal of Biomedical Materials Research*. 2002; 62:447–456. [PubMed: 12209931]
21. Ikemura K, Tay FR, Endo T, Pashley DH. A review of chemical-approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers. *Dental Materials Journal*. 2008; 27:315–329. [PubMed: 18717159]
22. Park J, Ye Q, Topp E, Misra A, Kieweg SL, Spencer P. Water sorption and dynamic mechanical properties of dentin adhesives with a urethane-based multifunctional methacrylate monomer. *Dental Materials*. 2009; 25:1569–1575. [PubMed: 19709724]
23. Ritter AV, Swift EJ Jr, Heymann HO, Sturdevant JR, Wilder AD Jr. An eight-year clinical evaluation of filled and unfilled one-bottle dental adhesives. *Journal of the American Dental Association*. 2009; 140:28–37. [PubMed: 19119164]
24. Garcia-Godoy F, Kramer N, Feilzer AJ, Frankenberger R. Long-term degradation of enamel and dentin bonds: 6-year results in vitro vs. in vivo. *Dental Materials*. 2010; 26:1113–1118. [PubMed: 20800270]
25. Tay FR, Carvalho RM, Yiu CK, King NM, Zhang Y, Agee K, et al. Mechanical disruption of dentin collagen fibrils during resin-dentin bond testing. *Journal of Adhesive Dentistry*. 2000; 2:175–192. [PubMed: 11317391]
26. Marshall SJ, Bayne SC, Baier R, Tomsia AP, Marshall GW. A review of adhesion science. *Dental Materials*. 2010; 26:e11–e16. [PubMed: 20018362]

27. Park J, Eslick J, Ye Q, Misra A, Spencer P. The influence of chemical structure on the properties in methacrylate-based dentin adhesives. *Dental Materials*. 2011; 27:1086–1093. [PubMed: 21816460]
28. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. *Dental Materials*. 2011; 27:1–16. [PubMed: 21112620]
29. Roeder L, Pereira RNR, Yamamoto T, Ilie N, Armstrong S, Ferracane J. Spotlight on bond strength testing-Unraveling the complexities. *Dental Materials*. 2011; 27:1197–1203. [PubMed: 21944280]
30. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J. State of the art of self-etch adhesives. *Dental Materials*. 2011; 27:17–28. [PubMed: 21109301]
31. Imazato S, Ehara A, Torii M, Ebisu S. Antibacterial activity of dentine primer containing MDPB after curing. *Journal of Dentistry*. 1998; 26:267–271. [PubMed: 9594480]
32. Imazato S, Kuramoto A, Takahashi Y, Ebisu S, Peters MC. In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond. *Dental Materials*. 2006; 22:527–532. [PubMed: 16198404]
33. Hiraishi N, Yiu CK, King NM, Tay FR. Effect of chlorhexidine incorporation into a self-etching primer on dentine bond strength of a luting cement. *Journal of Dentistry*. 2010; 38:496–502. [PubMed: 20298738]
34. Imazato S, Torii Y, Takatsuka T, Inoue K, Ebi N, Ebisu S. Bactericidal effect of dentin primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin. *Journal of Oral Rehabilitation*. 2001; 28:314–319. [PubMed: 11350583]
35. Imazato S, Kaneko T, Takahashi Y, Noiri Y, Ebisu S. In vivo antibacterial effects of dentin primer incorporating MDPB. *Operative Dentistry*. 2004; 29:369–375. [PubMed: 15279474]
36. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *International Journal of Antimicrobial Agents*. 2009; 34:103–110. [PubMed: 19339161]
37. Allaker RP. The use of nanoparticles to control oral biofilm formation. *Journal of Dental Research*. 2010; 89:1175–1186. [PubMed: 20739694]
38. Cheng YJ, Zeiger DN, Howarter JA, Zhang X, Lin NJ, Antonucci JM, Lin-Gibson S. In situ formation of silver nanoparticles in photocrosslinking polymers. *Journal of Biomedical Materials Research Part B*. 2011; 97:124–131.
39. Fan C, Chu L, Rawls HR, Norling BK, Cardenas HL, Whang K. Development of an antimicrobial resin—a pilot study. *Dental Materials*. 2011; 27:322–328. [PubMed: 21112619]
40. Zhang K, Melo MAS, Cheng L, Weir MD, Bai YX, Xu HHK. Effect of quaternary ammonium and silver nanoparticle-containing adhesives on dentin bond strength and dental plaque microcosm biofilms. *Dental Materials*. 2012; 28:842–852. [PubMed: 22592165]
41. Antonucci JM, O'Donnell JN, Schumacher GE, Skrtic D. Amorphous Calcium Phosphate Composites and Their Effect on Composite-Adhesive-Dentin Bonding. *Journal of Adhesion Science and Technology*. 2009; 23:1133–1147. [PubMed: 19696914]
42. McBain AJ. In vitro biofilm models: an overview. *Advances in Applied Microbiology*. 2009; 69:99–132. [PubMed: 19729092]
43. Pratten J, Wilson M, Spratt DA. Characterization of in vitro oral bacterial biofilms by traditional and molecular methods. *Oral Microbiology and Immunology*. 2003; 18:45–49. [PubMed: 12588458]
44. Badawi H, Evans RD, Wilson M, Ready D, Noar JH, Pratten J. The effect of orthodontic bonding materials on dental plaque accumulation and composition in vitro. *Biomaterials*. 2003; 24:3345–3350. [PubMed: 12763461]
45. McBain AJ, Sissons C, Ledder RG, Sreenivasan PK, De Vizio W, Gilbert P. Development and characterization of a simple perfused oral microcosm. *Journal of Applied Microbiology*. 2005; 98:624–634. [PubMed: 15715865]
46. Shewmaker PL, Gertz RE Jr, Kim CY, Fijter S, DiOrio M, Moore MR, Beall BW. *Streptococcus salivarius* meningitis case strain traced to oral flora of anesthesiologist. *Journal of Clinical Microbiology*. 2010; 48:2589–2591. [PubMed: 20504987]

47. Cheng L, Weir MD, Zhang K, Wu E, Xu SM, Zhou XD, Xu HHK. Dental plaque microcosm biofilm behavior on calcium phosphate nanocomposite with quaternary ammonium. *Dental Materials*. 2012; 28:853–862. [PubMed: 22578992]
48. International Standard Organization (ISO). 10993-5:2009(E). Biological evaluation of medical devices-Part 5: Tests for in vitro cytotoxicity. 3rd edition. International Standardization Organization; 2009.
49. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *Journal of Prosthetic Dentistry*. 2001; 85:162–169. [PubMed: 11208206]
50. Huang L, Xiao YH, Xing XD, Li F, Ma S, Qi LL, Chen JH. Antibacterial activity and cytotoxicity of two novel cross-linking antibacterial monomers on oral pathogens. *Archives of Oral Biology*. 2011; 56:367–373. [PubMed: 21074143]
51. Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. *Dental Materials*. 2003; 19:313–319. [PubMed: 12686296]
52. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry--a review. FDI Commission Project 1-97. *International Dental Journal*. 2000; 50:1–12. [PubMed: 10945174]
53. Mandari GJ, Frencken JE, van't Hof MA. Six-year success rates of occlusal amalgam and glass-ionomer restorations placed using three minimally intervention approaches. *Caries Research*. 2002; 37:246–253. [PubMed: 12771499]
54. Frencken JE, van't Hof MA, van Amerongen WE, Holmgren CJ. Effectiveness of single-surface ART restorations in the permanent dentition: a meta-analysis. *Journal of Dental Research*. 2004; 83:120–123. [PubMed: 14742648]
55. Duarte SJ, Lolato AL, de Freitas CR, Dinelli W. SEM analysis of internal adaptation of adhesive restorations after contamination with saliva. *The Journal of Adhesive Dentistry*. 2005; 7:51–56. [PubMed: 15892364]
56. Loguercio AD, Reis A, Bortoli G, Patzlaft R, Kenshima S, Rodrigues Filho LE, Accorinte Mde L, van Dijken JW. Influence of adhesive systems on interfacial dentin gap formation in vitro. *Operative Dentistry*. 2006; 31:431–441. [PubMed: 16924983]
57. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. *Nanotechnology*. 2005; 16:2346–53. [PubMed: 20818017]
58. Slenters TV, Hauser-Gerspach I, Daniels AU, Fromm KM. Silver coordination compounds as light-stable, nano-structured and anti-bacterial coatings for dental implant and restorative materials. *Journal of Materials Chemistry*. 2008; 18:5359–5362.
59. Rai M, Yada A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*. 2009; 27:76–83. [PubMed: 18854209]
60. Damm C, Munsted H, Rosch A. Long-term antimicrobial polyamide 6/silver nanocomposites. *Journal of Materials Science*. 2007; 42:6067–6073.
61. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. *Journal of Hospital Infection*. 2005; 60:1–7. [PubMed: 15823649]
62. Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell RR, McCabe JF. Incorporation of antibacterial monomer MDPB into dentin primer. *Journal of Dental Research*. 1997; 76:768–772. [PubMed: 9109826]
63. Thome T, Mayer MP, Imazato S, Geraldo-Martins VR, Marques MM. In vitro analysis of inhibitory effects of the antibacterial monomer MDPB-containing restorations on the progression of secondary root caries. *Journal of Dentistry*. 2009; 37:705–711. [PubMed: 19540033]

Figure 1.

Un-cured primers in agar disk diffusion test. (A) Typical examples of inhibition zones of the four different primers against mutans streptococci. (B–D) Inhibition zone sizes for total microorganisms, total streptococci, and mutans streptococci, respectively. Each value is mean $\pm$ sd (n = 6). In each plot, dissimilar letters indicate values that are significantly different from each other ($p < 0.05$). NAg or MDPB alone each substantially increased the inhibition zone size. The use of dual agents MDPB and NAg together in the primer yielded the largest inhibition zones.

Figure 2. Bonding to dentin using extracted human molars: (A) Dentin shear bond strength (mean $\pm$ sd; $n = 10$), and (B) representative SEM image of the adhesive-dentin interfacial features. The four groups used different primers, but the same unmodified SBMP adhesive. In (A), all strength values were similar ($p > 0.1$). In (B), an example is shown for the control. All groups had similar dentin-adhesive interfacial features. Numerous resin tags were observed which were formed by the adhesive resin filling into the dentinal tubules.

Figure 3.

Live/dead bacterial staining assay of biofilms adherent on the layered disks: (A) Schematic of biofilm experimental setup; (B–E) representative images of biofilms for control primer, P +NAG, P+MDPB, and P+MDPB+NAG. Live bacteria were stained green, and dead/compromised bacteria were stained red. Live and dead bacteria in close proximity of each other yielded yellow/orange colors.

Figure 4.

MTT metabolic activity and lactic acid production of biofilms adherent on the layered disks (mean $\pm$ sd; n = 6). (A) MDPB or NAg alone each greatly decreased the metabolic activity. Dual agents (MDPB + NAg) yielded the lowest metabolic activity. (B) Lactic acid production of biofilms was substantially reduced by MDPB or NAg alone. The greatest reduction was achieved by using MDPB and NAg together in primer. In each plot, dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

Figure 5.

Colony-forming unit counts (CFU) of biofilms adherent on the layered disks (mean $\pm$ sd; n = 6). (A) Total microorganisms, (B) total streptococci, and (C) mutans streptococci. In each plot, dissimilar letters indicate values that are significantly different from each other ($p < 0.05$). Adding MDPB or NAG into the primer imparted a potent antibacterial effect, and the strongest effect was achieved by combining MDPB and NAG in the same primer.

Figure 6.

Human gingival fibroblast cytotoxicity (mean $\pm$ sd; $n = 6$). The culture medium consisted of fibroblast medium (FM) plus eluents from the cured primer/adhesive disks. Primer/adhesive volume (in unit mm³) divided by the total solution volume (mL) was 0.12, 0.24, and 0.47 mm³/mL, respectively. The 0.12 mm³/mL ratio corresponded to 6 disks in 1280 mL of solution, which compared to 1000 to 1500 mL/day of saliva for an average person. Even at higher concentrations of 0.24 and 0.47 mm³/mL, the resin eluent still had no adverse effect on the fibroblast viability. FM without resin eluents was used as control which had resin/solution volume ratio of 0. Fibroblast viability was measured by the MTT absorbance and normalized by FM control. All groups had cell viability matching that without resin eluents ($p > 0.1$).