Combating dental caries via restorative materials containing antibacterial and remineralizing nanoparticles

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ABSTRACT

Dental caries at the margins of restorations has been the main reason for restoration failure. Restorative dental materials such as resin composite and adhesive systems are in contact with tooth and can be the ideal vehicle for delivering anticaries agents. Based on nanotechnology, nanoparticles of silver (NAg) were introduced into restorative materials to achieve antimicrobial properties. Another strategy to combat caries lesions around restorations is to drive the shift of demineralization to remineralization process via calcium (Ca) or phosphate (P) ion release. This was achieved via resins filled with nanoparticles of amorphous capcium phosphate (NACP). In the present contribution we report the development of restorative materials including primer, adhesive and composites that containing NAg and NACP. The antibacterial, remineralizing and mechanical properties of these new materials indicate that novel nano-sized agents can fight bacteria and reduce the demineralization in restored tooth cavities.

Keywords: nanoparticles, dental materials, nanotechnology, dental caries.

1 INTRODUCTION

Dental caries is a widespread public health problem, despite much effort in health promotion and disease prevention. Tooth caries is caused by acidic attack from cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus*. They are present in aggregates of microorganism cells attached to each other and to a tooth surface to form oral biofilm or dental plaque. Decreases in pH (<5.5) of oral biofilm due to the action of bacterial acids can lead to desolution of calcium and phosphate from the tooth causing demineralization. Resin-based composite and adhesive systems are popular direct restorative options in dentistry. Caries at the margins of restorations has been the main reason for restoration failure. The development of direct-filling restorative dental materials with antibacterial and remineralizing properties is a promising approach to addressing the caries problem. Restorative dental materials such as resin composite and adhesive systems are in contact with tooth and can be the ideal vehicle for delivering anticaries agents. Tremendous efforts have been made in developing approaches that not only show high antibacterial efficacy, but also maintain the load-bearing properties of dental materials. Nanotechnology is a promising approach to developing the next generation of dental materials, to not only replace the missing tooth volume as traditional restorations, but also inhibit oral biofilms and remineralize tooth caries. Based on this approach, nanoparticles of silver (NAg) were introduced into restorative dental materials to achieve antimicrobial properties. NAg possess great efficacy against cariogenic bacteria with high surface area-to-volume ratio thereby requiring only a low concentration for efficacy. Dental materials containing NAg were recently reported [1-4]. NAg incorporation into composite containing nanoparticles of amorphous calcium phosphate (NACP) yielded a potent antibacterial activity while using a low NAg filler level [1,2]. Modified adhesive system components with the addition of NAg as primer and adhesive also achieved great antibacterial effects on oral biofilms, without adversely affecting the dentin bond strength [3-6]. The small size of NAg could allow them to flow with the primer into dentinal tubules to kill residual bacteria inside the dental structure.

Another strategy to combat caries lesions around restorations, also based on nanotechnology, is to drive the shift of demineralization to remineralization via calcium (Ca) or phosphate (P) ion release from NACP. Previous studies have reported successful incorporation of this remineralizing agent in adhesive and composites [5-8]. In addition to these agents, Antibacterial quaternary ammonium methacrylates (QAMs) were synthesized and incorporated into dental resins. Recently, a new quaternary ammonium monomer dimethylaminododecyl methacrylate (DMADDM) was synthesized and showed strong antibacterial effect [9]. Here we report the development of dental materials including primer, adhesive and composites containing the above cited agents. The antibacterial, remineralizing and mechanical properties of these new materials indicate that novel nano-sized agents can fight bacteria and reduce the demineralization in restored tooth cavities.

2 MATERIALS AND METHODS

A set of antibacterial and remineralizing agents were syntetized and incorported in dental primer and adhesive components. The new antibacterial monomer DMADDM with an alkyl chain length of 12 was synthesized using a modified Menschutkin reaction method. This method, in which a tertiary amine group is reacted with an organo-halide, is desirable because the reaction products were generated at quantitative amounts and required no further purification [9].

NAg incorporation into dental materials

The incorporation of NAg into dental resins was shown to be promising to achieve a strong antibacterial activity [2,3]. NAg could be formed in the resin in situ, without the need to mix nanoparticles with resin, thus avoiding the agglomeration issue. Silver 2-ethylhexanoate powder (Strem, New Buryport, MA, USA) could be dissolved in 2-(tert-butylamino) ethyl methacrylate (TBAEMA, Sigma-Aldrich, St. Louis, MO) [1,2]. This Ag solution was then mixed into a resin at 0.05-0.10% mass fraction of silver 2-ethylhexanoate. TBAEMA was selected since it contains reactive methacrylate groups and therefore can be chemically incorporated into a dental resin upon photopolymerization. This method produced NAg with a mean particle size of approximately 2.7 nm that were well dispersed in the cured resin matrix [2].

NACP incorporation into dental materials

To synthesize NACP, calcium carbonate ($CaCO_3$, Fisher, Fair Lawn, NJ) and dicalcium phosphate anhydrous ($CaHPO_4$, Baker, Phillipsburg, NJ) were dissolved into an acetic acid solution to obtain final calcium and phosphate ionic concentrations of 8 mmol/L and 5.333 mmol/L, respectively [1,2]. This solution was sprayed into a heated chamber to remove the liquid and volatile acids. Then an electrostatic precipitator (AirQuality, Minneapolis, MN) was used to collect the dried NACP powder which had a mean particle size of approximately 116 nm [7]. The NACP was incorporated into dental adhesive in a range from 0-40% [5]. Typical NACPand NAg incorporated in dental adhesives are shown in Fig. 1.

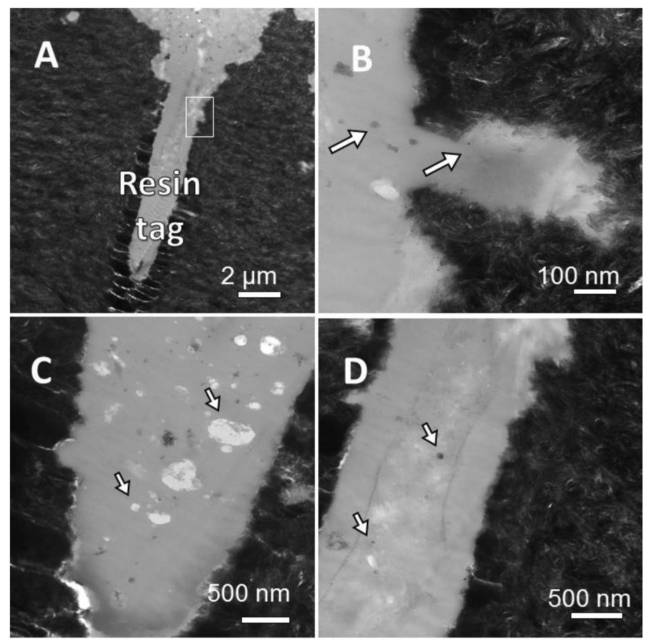

Fig. 1 TEM photomicrographs illustrating the infiltration of experimental adhesive system in demineralized dentin matrix. (**A**) Apparent long resin tags formation through the dentinal tubule were observed. Deposition of many NAg and NACP particles appeared along the tag (white arrow). (**B**) Higher magnification of selected area (square delimitation) of section of (A), highlighting adequate resin infiltration. (**C**) Higher magnification of end of tag; evident presence of nanosilver and NACP particles (**D**) Higher magnification showing the mixture of studied nanoparticles with the presence of variety of sizes.

Antibacterial and Mechanical properties

Using a dental plaque biofilm model, colony-forming unit (CFU) counts for total microorganisms, total streptococci, and mutans streptococci were assessed after biofilm growing over the 9 mm disks. [3,4].

Mechanical properties were measured by shear bond test to evaluate the bonding strenght of new materials do human dentin.

3 RESULTS

Mechanical properties of dental primer and adhesives containing nano-sized antibacterial and remineralizing agents are plotted in Fig. 2. These strengths matched/exceeded the values of the primer and adhesive components without antibacterial agent incorporated.

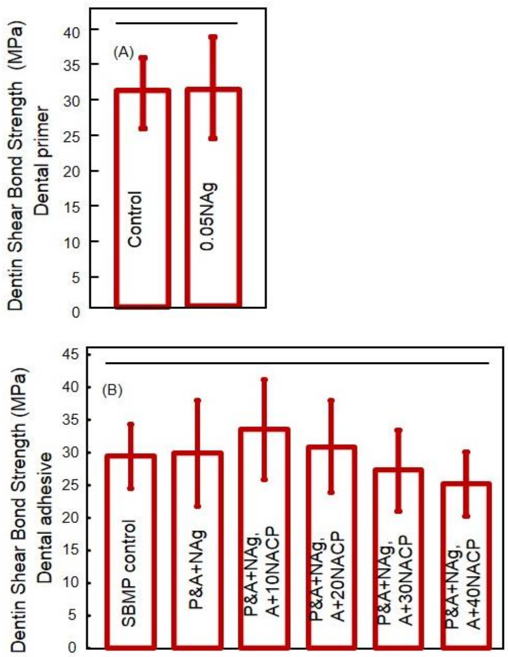

Fig. 2. Dentin shear bond strength. (A) Primer containing 0.05% NAg, and (B) adhesive containing NAg and NACP (0-40%) Adding NAg and NACP into the bonding agent did not significantly decrease the dentin bond strength. The horizontal line indicates values not significantly different from each other ($p > 0.1$). (Adapted from Ref. [4,8] with permission).

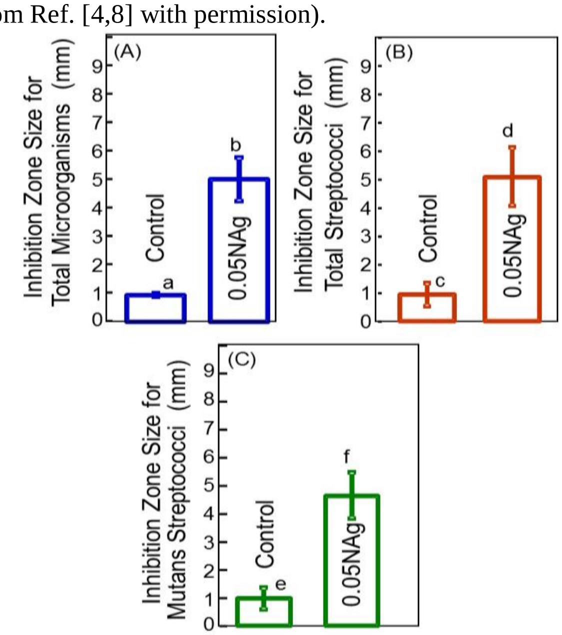

Fig. 3. Antibacterial activity of un-curd primers in agar disk diffusion test. Inhibition zone data for total microorganisms, total streptococci, and mutans streptococci, respectively. Each value is mean ± sd ($n = 6$). Bars with dissimilar letters indicate values that are significantly different ($p < 0.05$). (Adapted from Ref. [3] with permission).

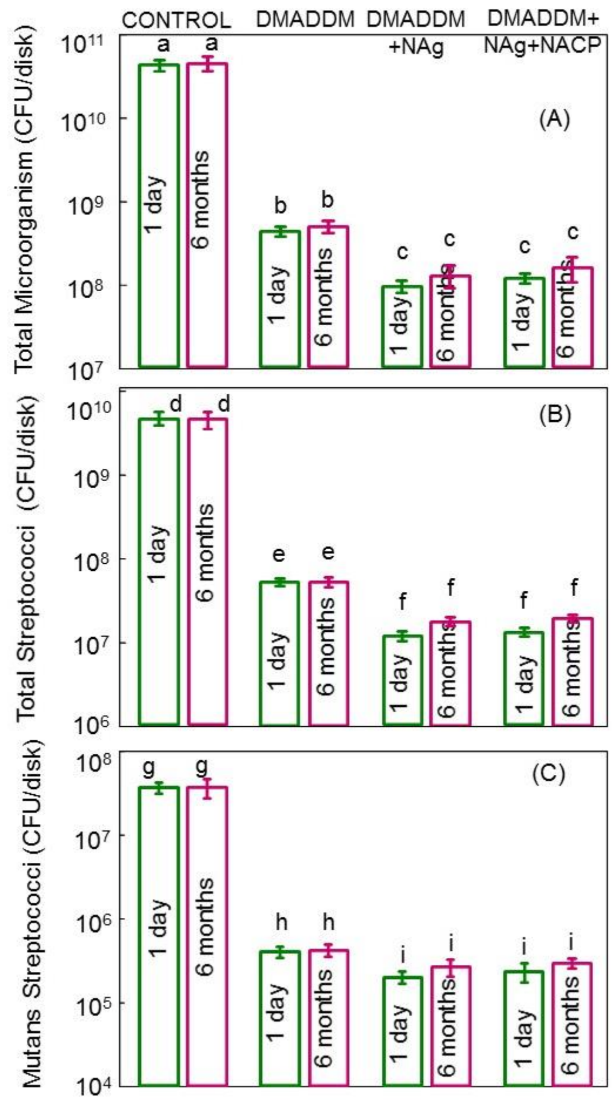

Fig. 4. Dental plaque biofilm colony-forming units (CFU) formed on the surface of dental adhesive containing different antibacterial/remineralizing agents. (A) Total microorganisms, (B) total streptococci, and (C) mutans streptococci. Each value is (mean ± sd; $n = 6$). After 6 months, the combining agents reduced the biofilm CFU of the commercial SBMP control by more than two orders of magnitude. In each plot, four groups were tested as listed at the top of the figure: Control; control + DMADDM; control + DMADDM + NAg; control + DMADDM + NAg + NACP. Values with dissimilar letters are significantly

different ($p < 0.05$). (Adapted from Ref. [9] with permission).

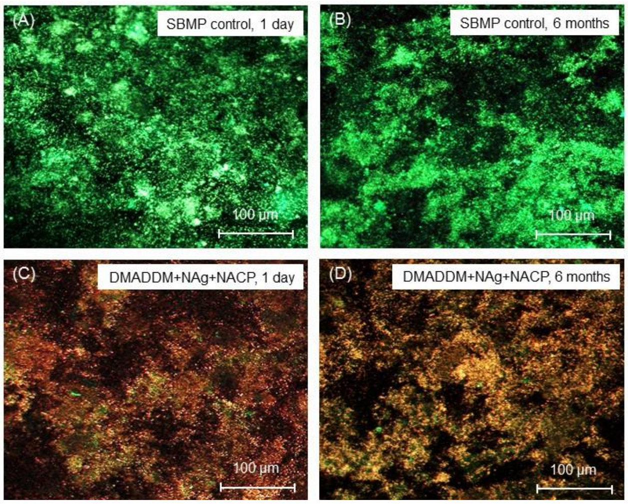

Fig 5. Fluorescence images of biofilm grown on the adhesive surface to investigate the antibacterial activity of the adhesive containing DMADDM + NAg + NACP at 1 d and 6 months, respectively. Live bacteria were stained green, and dead bacteria were stained red. Live and dead bacteria in close vicinity and on the top of each other appeared yellow or orange. (Adapted from Ref. [9] with permission).

4 CONCLUDING REMARKS

- The current data showed that adding the NAg in primer and the combining of NAg, DMADDM and NACP into adhesive reached stronger antibacterial properties. Dental plaque microcosm biofilm viability and CFU were significantly reduced with with the incorporation of nanotecnology-based agents, NAg and NACP.
- Antibacterial adhesives would be important to combat bacteria and offer protection against secondary caries and pulpal damage.
- Because of the small size of NAg and the high specific surface area, a low NAg filler level could be used in the resin to achieve a potent antibacterial efficacy. It is beneficial to use a low NAg filler level in the resin so that the NAg did not adversely affect the resin color and physical/mechanical properties. In the current study, the presence of NAg in adhesive and primer did not compromise the dentin bond strength.
- The new formulations have the potential to kill residual bacteria in the tooth cavity and inhibit the invading bacteria along the tooth-restoration margins, with NACP for Ca and P ions to remineralize tooth lesions. The novel combination of antibacterial and remineralizing agents is promising for incorporation into a wide range of dental adhesives to inhibit caries.

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