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Nanotechnology strategies for antibacterial and remineralizing composites and adhesives to tackle dental caries

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Abstract

Dental caries is the most widespread disease and an economic burden. Nanotechnology is promising to inhibit caries by controlling biofilm acids and enhancing remineralization. Nanoparticles of silver were incorporated into composites/adhesives, along with quaternary ammonium methacrylates (QAMs), to combat biofilms. Nanoparticles of amorphous calcium phosphate (NACP) released calcium/phosphate ions, remineralized tooth-lesions and neutralized acids. By combining NAg/QAM/NACP, a new class of composites and adhesives with antibacterial and remineralization double benefits was developed. Various other nanoparticles including metal and oxide nanoparticles such as ZnO and TiO₂, as well as polyethylenimine nanoparticles and their antibacterial capabilities in dental resins were also reviewed. These nanoparticles are promising for incorporation into dental composites/cements/sealants/bases/liners/adhesives. Therefore, nanotechnology has potential to significantly improve restorative and preventive dentistry.

Keywords

amorphous calcium phosphate nanoparticles; dental caries; oral biofilms; quaternary ammonium methacrylate; silver nanoparticles; tooth lesion remineralization

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Dental caries is a dietary carbohydrate-modified bacterial infectious disease, and is one of the most common bacterial infections in humans [1]. It is a threat to oral and systemic health, and creates a heavy financial burden worldwide [1,2]. The basic mechanism of dental caries is demineralization through the attack by acids generated by bacteria in dental plaque biofilms [3–5]. Acidogenic bacteria growth and biofilm formation with exposure to fermentable carbohydrates are responsible for caries development [6,7]. Microbial communities within the oral cavity are polymicrobial and exist primarily as biofilms on various surfaces including the teeth, dental materials and mucosa. A salivary- or dietary-derived proteinaceous layer, called acquired pellicle, is formed on the surface, and then the initial colonizers adhere to the acquired pellicle, which can in turn influence the subsequent sequence of microbial colonization [8]. Oral biofilms are made up of a community of microbial species embedded in a matrix of bacterial components, salivary proteins and peptides, as well as food debris [8]. The mode of biofilm growth is clearly distinguished from planktonic growth; for example, biofilms can survive antimicrobial agents up to 1000-times the concentration required to kill planktonic microorganisms.

Once biofilm acids have caused tooth decay, the treatment involves removing the carious tissues and filling the tooth cavity with a restorative material. Approximately, 200 million tooth cavity restorations are placed annually in the USA. Composites are increasingly used for tooth cavity restorations because of their excellent esthetics and improved performance [9,10]. The composite restoration is bonded to the tooth structure via an adhesive [11,12]. However, composites in vivo tend to accumulate more biofilms than other restorative materials [13–15]. Plaques adjacent to the restoration margins could result in secondary caries and compromise the restoration's longevity. Indeed, secondary caries at the restoration-tooth margins is a primary reason for restoration failure [16]. As a result, more than half of all restorations fail within 10 years [17,18], and 50–70% of all the restorations placed are replacements of the failed restorations [16]. Replacement dentistry is a major economic burden, considering that the annual cost for tooth cavity restorations in the USA was approximately US\$46 billion [19]. Because caries at the restoration margins is a main reason for restoration failures [20,21], it would be highly desirable for the composite and bonding agent to possess antibacterial and remineralization capabilities.

Recent studies indicate that nanotechnology could provide novel strategies in prevention and treatment of dental caries, specifically in the control and management of dental plaque biofilms and remineralization of initial dental caries [22]. Nanoparticles are generally considered to be of sizes in the neighborhood of 100 nm or smaller, and the exploitation of their unique attributes to combat infection has increased markedly over the past decade [23]. As the particle sizes are reduced from micrometers to nanometers, the resultant properties can change dramatically. For example, hardness, active surface area, chemical reactivity and biological activity can all be altered [24]. The application of nanoparticles in dentistry can be categorized into two directions: preventive dentistry and restoration dentistry [25]. This review article will focus on recent reports on antibacterial and remineralizing agents including silver nanoparticles and calcium phosphate nanoparticles in dental composite and adhesives. The antibacterial and remineralizing approaches are promising to impact both preventive and restoration dentistry to inhibit dental caries. Therefore, this review will cover

recent developments in dental nanocomposites and nanostructured bonding agents with antibacterial and remineralization capabilities [26,27].

Synthesis of nanoparticles of silver for antibiofilm dental resins

Silver has antibacterial, antifungal and antiviral properties [23,28]. The antimicrobial mechanism appears to be that Ag ions could inactivate the vital enzymes of bacteria, causing the DNA in the bacteria to lose its replication ability, leading to cell death [29]. Ag-containing silica particles were used as fillers to synthesize antibacterial dental composites [30,31]. More recently, nanoparticles of silver (NAg) demonstrated a high antibacterial efficacy in dental materials [32–37]. NAg had a high surface-area-to-mass ratio; hence, a small amount of NAg was sufficient for the composite to be strongly antibacterial. A small amount of NAg was desirable as it did not compromise the color, esthetics and mechanical properties of the composite [33]. Studies showed that NAg imparted a strong antibacterial activity to dental resins, greatly reducing biofilm growth and lactic acid production, without negatively affecting the other physical and mechanical properties of the resins [33–39].

There are several challenges for adding NAg into a resin. First, nanoparticles could readily form agglomerates. Second, Ag salt does not dissolve well in the hydrophobic dental monomers such as bisphenol glycidyl dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA). A recent study developed a unique approach to preparing nanocomposite with well-dispersed NAg in the resin matrix without nanoparticle agglomeration [33]. In this method, NAg were formed in situ in the resin without the need to mix nanoparticles with the resin. Briefly, Ag 2-ethylhexanoate salt was dissolved in the 2-(tertbutylamino)ethyl methacrylate (TBAEMA) monomer, which was then mixed with the BisGMA-TEGDMA monomers. TBAEMA contains reactive methacrylate groups and therefore can be chemically incorporated into the polymer network. TBAEMA can form Ag–N coordination bonds with Ag ions, thereby facilitating the Ag salt to dissolve in the resin monomer [33,38]. This method reduced the Ag salt to Ag nanoparticles in the resin, and the nanoparticles became part of the resin upon photopolymerization. A representative transmission electron microscopy (TEM) image in Figure 1 shows the dispersion of NAg in resin matrix without agglomeration. The NAg sizes were measured to be (2.7 ± 0.6) nm [38]. Resins containing NAg greatly reduced bacteria growth, biofilm colony-forming units (CFU) and the viability and metabolic activity of biofilms [33,38,39].

NAg could be used alone in the resin for antibacterial activity, or together with other bioactive agents to obtain desirable properties. For example, a recent study combined NAg with nanoparticles of amorphous calcium phosphate (NACP) in a dental composite to obtain the dual benefits of antibacterial and remineralization capabilities [39]. These desirable properties were achieved without adversely affecting the load-bearing properties of the composite to resist chewing forces in vivo. The flexural strength and elastic modulus of the NACP nanocomposite containing NAg matched those of a commercial dental composite [39]. NAg can also be combined with a quaternary ammonium methacrylate (QAM) in resin to enhance the antibacterial potency. The antimicrobial mechanism of quaternary ammonium salts appears to be that they can cause bacteria lysis by binding to the cell membrane and causing cytoplasmic leakage [40]. When the negatively charged bacterial cell

contacts the positively charged (N^+) sites of the QAM resin, the electric balance of the cell membrane could be disturbed and the bacterium could explode under its own osmotic pressure [41]. QAM resins appeared to be able to inhibit 3D biofilms [42]. It was suggested that a stress condition in the bacteria could trigger a built-in suicide program in the biofilm, which was also termed the programmed cell death [43,44]. Being challenged by bactericidal agents may serve as a trigger for programmed cell death in the surrounding bacteria [43,44]. This may be responsible for the QAM resin to kill not only the bacteria contacting the resin surface but also through the bacteria in the 3D biofilm away from the resin surface [42].

It is beneficial to combine QAM with NAg in dental resins. For example, methacryloyloxydodecyl pyridinium bromide (MDPB) showed effective antibacterial activity in composites and adhesives [45–47]. Recently, NAg and MDPB were combined in a dental primer [46]. The results showed that using 5% MDPB + 0.05% NAg in the primer achieved greater antibiofilm potency than using MDPB or NAg alone [46]. In another study, MDPB and NAg were added into both primer and adhesive [47]. To avoid compromising the dentin bond strength, mass fractions of 2.5% MDPB and 0.1% NAg were incorporated into primer and adhesives based on previous studies [45,48,49]. The dual use of NAg and MDPB showed that the dental resin can cause contact-killing of bacteria via MDPB, and long-distance killing via the release of Ag ions. Hence, NAg and MDPB in the resin were complimentary to each other to inhibit dental plaque biofilms.

Various QAM compositions were synthesized including methacryloxyethyl cetyl dimethyl ammonium chloride (DMAE-CB) [50], quaternary ammonium polyethylenimine (PEI) [40], quaternary ammonium dimethacrylate (QADM) [38,51] and dimethylaminododecyl methacrylate (DMADDM) [52,53]. Bis(2-methacryloyloxyethyl) dimethylammonium bromide (IDMA-1) was synthesized as a QADM for use in dental resins [38,51,54,55]. Its synthesis was carried out using a modified Menschutkin reaction, where a tertiary amine group was reacted with an organohalide. A benefit of this reaction is that the reaction products are generated at virtually quantitative amounts and require minimal purification [56]. Most antibacterial monomers such as MDPB, DMAE-CB and DMADDM are monomethacrylates. On the other hand, QADM is a dimethacrylate, and as a result, is expected to have minimal monomer leach out due to having reactive groups on both ends of the molecule for polymerization and covalent bonding with the resin matrix, compared with monomethacrylates [38,56]. While QADM was relatively weak in antibacterial activity, significantly higher mass fractions of QADM could be incorporated into dental resins to increase its antibacterial function, without compromising the mechanical properties of the resin [38,51,54,56]. Furthermore, all these QAMs could be potentially combined with NAg to substantially increase the antibacterial potency, which requires further study.

Since mechanical properties are important for load-bearing dental restorative materials, previous studies investigated the effects of silver nanoparticles on the mechanical properties of dental resins. Incorporation of small amounts of NAg (e.g., 0.02% mass fraction) did not significantly reduce the flexural strength of a composite [33]. However, greater amounts of silver could degrade the composite mechanical properties. In another study, the hardness of the light-cured resins containing 0.1% by mass of Ag benzoate nanoparticles decreased significantly, compared with the control group [34].

Another issue with silver incorporation into dental restorative materials is the color and esthetics of the material. When the Ag benzoate concentration in the resin was increased, the color of the resin became darker [34]. In another study incorporating NAg with 2.7 nm particle size in a dental composite, it was found that when the NAg mass fraction was increased to 0.175%, the composite had a brownish color that was accompanied by a precipitous loss in mechanical strength [39]. Therefore, under the conditions of that study [39], to maintain esthetics and mechanical strength, the NAg mass fraction of higher than 0.042% should not be used. The composite containing 0.042% of NAg had no noticeable difference in color, compared with that without NAg. The composite containing 0.042% NAg greatly reduced biofilm metabolic activity, CFU and lactic acid production, compared with a commercial composite control [39].

Use of nanoparticles in primers & adhesives

The concept of dental caries treatment has changed with the development of new composites and bonding agents. Complete removal of carious dentin during cavity preparation is no longer supported [57]. There are often residual bacteria remaining in the prepared tooth cavities and it is virtually impossible to remove all bacteria [58]. In addition, to protect pulp vitality and preserve more tooth tissues, minimally invasive techniques are recommended for the treatment of deep caries lesions [57,59,60]. However, the cavity likely will contain more residual bacteria with the preservation of more affected tissue [61]. Therefore, it would be highly beneficial to develop antibacterial primers and adhesives to kill the residual bacteria in the affected dentin in the tooth cavity. Furthermore, while a complete sealing of the tooth-restoration interface is an important goal, it is difficult to achieve. Indeed, many studies revealed microgaps and microleakage at the tooth-restoration interfaces, which could allow for bacteria invasion [62,63]. These microgaps could further deteriorate due to fatigue stresses and compromise the durability of the bonded interface. Therefore, an antibacterial and remineralizing bonding agent could inhibit the invading bacteria and hinder secondary caries at the margins.

Adding NAg and QADM into a commercial primer (Scotchbond™ Multi-Purpose [SBMP], 3M, MN, USA) achieved a strong antibacterial activity (Figure 2). Control primer had a minimal bacterial inhibition zone (Figure 2A). The modified primers were SBMP + 10% QADM, or + 0.05% NAg, or + 10% QADM + 0.05% NAg. They had much-larger bacterial inhibition zones (Figure 2B–D). As quantified in Figure 2E, the inhibition zone size for SBMP primer containing 10% QADM + 0.05% NAg were ninefold that of the SBMP control primer ($p < 0.05$) [51]. Similarly, the lactic acid production by biofilms on the resins was also greatly reduced via the incorporation of NAg and QADM (Figure 2F).

Tooth cavity disinfection via a primer containing NAg and QADM was investigated via bacteria-impregnated dentin in vitro using extracted human teeth [64]. Figure 3A shows dentinal tubules ‘T’ before *Streptococcus mutans* impregnation. The dentin after *S. mutans* impregnation is shown in Figure 3B. The cross section of dentin is shown in Figure 3C, revealing *S. mutans* inside dentinal tubules. This is shown more clearly at a higher magnification (Figure 3D). Therefore, *S. mutans* were successfully impregnated into the interior of the dentin blocks [64]. After *S. mutans* impregnation, either a control primer or an

antibacterial primer was applied to the dentin. Then, the bacteria in dentin was harvested by a sonication method [64]. Figure 3E plots the *S. mutans* CFU harvested from the dentin block for different groups. The CFU was the highest in the control dentin blocks without applying a primer. Primers with QADM and NAg reduced the *S. mutans* CFU in dentin blocks, compared with control SBMP. The CFU from dentin treated with the primer containing 10% QADM + 0.1% NAg was about 5% of the CFU of SBMP control primer. Dentin treated with primer containing 10% QADM + 0.1% NAg reduced the CFU by three orders of magnitude, compared with the CFU in control dentin without primer. These results demonstrate that antibacterial primers were able to kill the bacteria residing inside the dentinal tubules of dentin blocks [64]. Furthermore, studies showed that bonding agents containing NAg and QADM continued to have a strong antibacterial activity after being photocured, thereby exerting a long-lasting effect against residual bacteria in the dentinal tubules as well as new invading bacteria along the margins due to microleakage [49,51,65].

There were several preliminary investigations on the biocompatibility of the new antibacterial and remineralizing dental resins. An in vitro study showed that dental resins containing QADM and NAg had acceptable biocompatibility, with resin eluent cytotoxicity against fibroblast cells that was similar to that using control culture medium without any resin [66]. In an animal study, dental adhesives and composites containing NACP as well as DMADDM + NACP showed better biocompatibility with significantly more tertiary dentin formation in vivo, compared with control group without NACP or DMADDM + NACP [67]. However, cytotoxicity is an issue that needs to be addressed, and further studies should investigate the cytotoxicity and biocompatibility of various antibacterial and bioactive dental resins in vitro and in vivo.

Durability of antibacterial activity of nanostructured dental resins

To study the durability of the antibacterial properties, bonding agents containing NAg, NACP and DMADDM were examined in long-term water aging. Typical dentin-adhesive interfaces are shown in Figure 4A showing the bonding agent filling dentinal tubules and forming resin tags. The hybrid layer between the adhesive and the underlying mineralized dentin was formed via bonding agent infiltrating into the demineralized collagen layer. At a higher magnification (Figure 4B), numerous NACPs were seen inside a resin tag. At an even higher magnification, both NACP and NAg were visible in the resin inside the dentinal tubules (Figure 4C). Due to their small particle size, both NAg and NACP successfully flowed with the adhesive into the dentinal tubules, which could help inhibit residual bacteria inside dentin and remineralize remnants of lesions in the prepared cavity.

Dentin shear bond strengths are plotted in Figure 4D. The commercial bonding agent SBMP lost 35% of its dentin bond strength in 6 months of water aging. However, the antibacterial bonding agent containing DMADDM, NAg and NACP showed no loss in dentine bond strength in water aging. Furthermore, as shown in a previous study [68], the biofilm viability, metabolic activity and lactic acid production were substantially reduced via the new bonding agent. Compared with the control, bonding agent-containing DMADDM and NAg decreased the biofilm CFU by more than two orders of magnitude. There was no significant decrease in the antibacterial potency after water-aging for 6 months [68].

The long-lasting antibacterial activity of the bonding agent is consistent with that of a nanocomposite. A NACP–QADM nanocomposite was water-aged for 6 months [54]. The antibiofilm effect did not decrease after 6 months of water immersion, compared with that at 1 day. Figure 5 shows biofilms on composites cultured for 2 days. Dense biofilms were observed to completely cover the surfaces of two commercial composites (CompositeR [Renaamel, Cosmedent, IL, USA] and CompositeF [Heliomolar, Ivoclar, NY, USA]) [54]. However, the NACP–QADM nanocomposite had much less biofilms, and in many areas the resin surface ('R' in Figure 5C & F) was bare without biofilm coverage. Higher magnification (Figure 5D & E) showed that the *S. mutans* grew in chains. The chains twisted in three dimensions and were long in the biofilm architecture on the commercial composites. In contrast, the *S. mutans* chains were much shorter on NACP–QADM nanocomposite, where the chain appeared to be disintegrated and many bacteria were ruptured into small debris (Figure 5F). The antibacterial activity of the nanocomposite was maintained and not lost after 180 days of water-aging [54].

Enamel lesion remineralization via NACP nanocomposite

NACP were synthesized using a spray-drying technique [69]. Figure 6A and B shows typical TEM images of NACP. In Figure 6A, the small particles had sizes of the order of 10 nm, and the large particles had sizes of about 100–300 nm. An example of a large particle is shown in Figure 6B, which appeared to contain several small particles with sizes of the order of 10 nm. The small particles likely fused to form the larger particles in the spray-drying chamber before they were completely dried. The measurement in TEM of 50 randomly selected particles yielded an average particle size of 112 nm [70].

The NACP nanocomposite was 'smart' and could dramatically increase the calcium and phosphate ion release at a cariogenic low pH, when these ions would be most needed to inhibit caries [69]. In addition, NACP nanocomposite could neutralize a lactic acid solution of pH 4 by rapidly increasing the pH to nearly 6 [71], which could avoid caries formation. Another study with quantitative microradiography [72] showed that, after a cyclic demineralization/remineralization regimen for 30 days, the NACP nanocomposite successfully remineralized preexisting enamel lesions (Figure 6C). As plotted in Figure 6D, NACP nanocomposite achieved an enamel remineralization that was fourfold that of a commercial fluoride-releasing composite control. The enamel lesion next to NACP nanocomposite had the highest remineralization of $21.8 \pm 3.7\%$, much higher than the $5.7 \pm 6.9\%$ for the fluoride-releasing composite. Enamel control without composite had further demineralization of $-26.1 \pm 16.2\%$ during the cyclic demineralization/remineralization regimen for 30 days [72]. Therefore, the novel NACP nanocomposite is promising for remineralization of demineralized tooth structures.

Human in situ study on NACP nanocomposite

In situ caries models involve the use of appliances or devices that create defined conditions in human mouth to simulate the natural process of dental caries. These models attempt to provide clinically relevant information in a relatively short period without causing irreversible tissue changes in the natural dentition [73]. A recent study investigated in situ

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caries inhibition via NACP nanocomposite in human participants [74]. 25 participants wore palatal devices containing bovine enamel slabs with cavities restored with NACP nanocomposite or control composite. In order to provide an accelerated cariogenic challenge, eight-times daily at 2 h intervals, the volunteer removed the appliance, dripped one drop of a 20% sucrose solution on each slab and then placed the appliance back into the mouth [74]. After 14 days, the adherent biofilms were collected for analyses. Transverse microradiography was used to determine the enamel mineral profiles at the margins. Significant secondary caries was created in the composite–enamel margins for control composites without NACP, due to biofilm acid attacks. However, around the NACP nanocomposite in a 0–250 μm region in enamel from the composite–enamel interface, the enamel mineral loss associated with NACP nanocomposite was only 1/3 of the enamel mineral loss around the control composite [74]. Enamel mineral loss (mean \pm SD; $n = 25$) around NACP nanocomposite was $13.8 \pm 9.3 \mu\text{m}$, much less than $33.5 \pm 19.0 \mu\text{m}$ of the control ($p = 0.001$) [74]. Biofilms on the NACP nanocomposite restorations in situ had calcium and phosphate ion concentrations that were much higher than those on the control composite. This provided a large ion reservoir in biofilms and plaques that could be released during acid attacks, thus preserving tooth enamel from demineralization in the human mouth [74]. Therefore, NACP nanocomposite substantially reduced caries formation in a human in situ model, and it is promising for use in caries-inhibiting dental restorations.

Other studies using nanotechnology to tackle oral biofilms & caries

Besides NAg and NACP, several other types of nanoparticles were also shown to have anticaries properties [75–80]. For remineralization, hydroxyapatite (HA; $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) nanoparticles were investigated [75,76]. HA is the structural prototype of the major mineral component of teeth and bones and also the final stable product in the precipitation of calcium and phosphate ions in neutral or basic solutions. A 10% suspension containing nano-HA particles (10–20 nm diameter; 60–80 nm length) promoted remineralization of the superficial layer of the initial caries lesions to a depth of 20–40 μm [75,76]. However, little remineralization could be obtained by nano-HA in the body of the lesion. In another study, carbonate hydroxyl apatite nanoparticles were shown to be effective in repairing micrometer-sized defects in tooth surfaces in vitro [77].

In addition, several types of metal and oxide nanoparticles have received attention for their antimicrobial activities. Besides silver nanoparticles, nanoparticles of copper, ZnO and TiO_2 were also shown to possess potent antibacterial functions [23,81]. A previous study incorporated 0–5% by mass of ZnO nanoparticles into flowable resin composites [81]. While the composites significantly inhibited the growth of *S. mutans*, the incorporation of ZnO nanoparticles in minute amounts of about 1% by mass did not adversely affect the mechanical properties of the composite [81]. Moreover, TiO_2 has well-known pathogenic effects [82]. Indeed, the use of TiO_2 nanoparticles in dental adhesive materials yielded a strong antibacterial effect [83].

Furthermore, novel PEI nanoparticles were developed and incorporated into dental resins [40,84,85]. PEI nanoparticles could be immobilized in resin-based materials to exert a strong antibacterial effect without the leachout of the nanoparticles, thus preserving the

antibacterial activity and mechanical properties for potential dental applications [40]. The antibacterial effects of using PEI nanoparticles at 0.5, 1 and 2% by mass in a provisional cement were studied in vitro against *S. mutans* and *Enterococcus faecalis* [86]. The results indicated that the minimum effective concentration should be 1% of PEI nanoparticles to ensure a proper antibacterial activity for the dental cement [86]. Consistent results were obtained in another study, where PEI nanoparticles incorporated at a low concentration of 1% by mass in a resin composite showed a strong antibiofilm activity, and exhibited a potent broad-spectrum antibacterial activity against human salivary bacteria [87].

Conclusion

The application of nanotechnology to combat dental caries, including the inhibition of biofilm formation and regulating the demineralization and remineralization balance, is a promising direction for the prevention and treatment of tooth decay. NAg and QAMs have received particular attention as a result of their strong antimicrobial activity and durability. Calcium phosphate nanoparticles have the capability of inhibiting demineralization and enhancing remineralization via releasing calcium and phosphate ions. Incorporation of nanoparticles into dental composites and adhesives could have double benefits: antibacterial capability and remineralization of tooth lesions. In view of recurrent caries at the tooth-restoration margins as the main factor for restoration failures, the novel nanocomposite and nanostructured adhesives with an effective cariesinhibiting capability are promising for a wide range of dental applications. Novel agents such as NAg, NACP, QAMs and others are promising for use in various dental composites, adhesives, cements and sealants to inhibit dental caries.

Future perspective

Current restorative materials such as composites are typically bioinert and replace the missing volume of the tooth cavity. It is beneficial for future restorative materials to not only replace the missing volume but also be bioactive and have beneficial therapeutic properties. Such desirable properties include antibacterial activity to inhibit biofilm acids, and remineralization capability to reverse tooth decay. Nanotechnology is playing a key role in the development of bioactive restorative materials. In addition, many studies on anticaries nanoparticles have been carried out under in vitro conditions, and there is a clear need for further in vivo and in situ studies. While single-species biofilms were often used for in vitro experiments, dental plaque is a complicated ecosystem with about 1000 bacterial species [75]. Even when dental microcosm biofilm models were used with saliva as the inoculum, different individuals may have different biofilm compositions and dietary habits. Therefore, dental composites and adhesives with dual benefits of antibacterial and remineralization capabilities need to be investigated in human in situ or in vivo models. More studies are needed to investigate the anticaries properties of novel dental materials containing a combination of NAg, QAMs and NACP, as well as other types of nanoparticles.

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- of considerable interest

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Executive summary

Synthesis of nanoparticles of silver for antibiofilm dental resins

- Nanoparticles of silver (NAg) with particle size as small as 2.7 nm had a high surface area-to-mass ratio; hence, a small amount of NAg was sufficient for dental resin to be strongly antibacterial. Resins containing NAg greatly reduced biofilm growth and lactic acid production.
- NAg could be used alone in resin, or together with other agents for desirable properties, including nanoparticles of amorphous calcium phosphate (NACP) for remineralization, or quaternary ammonium methacrylate to enhance the antibacterial potency.

Use of nanoparticles in primers & adhesives

- Incorporation of NAg into primer and adhesive achieved a strong antibacterial activity.
- Tooth cavity disinfection was achieved via a primer containing NAg by testing bacteria-impregnated dentin of extracted human teeth.
- Preliminary investigations indicated that dental resins containing NAg and quaternary ammonium methacrylate had an acceptable biocompatibility.

Durability of antibacterial activity of nanostructured dental resins

- The novel antibacterial bonding agent containing dimethylaminododecyl methacrylate, NAg and NACP showed no loss in dentine bond strength in water aging for 6 months. There was no significant decrease in antibacterial potency after water aging for 6 months.
- A NACP–quaternary ammonium dimethacrylate nanocomposite was water aged for 6 months. The antibacterial activity of the nanocomposite was maintained and not lost after water aging.

Enamel lesion remineralization via NACP nanocomposite

- NACP nanocomposite was ‘smart’ and greatly increased the calcium and phosphate ion release at a cariogenic low pH, when these ions were most needed to inhibit caries.
- NACP nanocomposite could neutralize a lactic acid solution of pH 4 by rapidly increasing the pH to nearly 6, which could avoid caries formation.
- NACP nanocomposite successfully remineralized the pre-existing enamel lesions.

Human in situ study on NACP nanocomposite

- Caries inhibition via NACP nanocomposite was investigated in human participants. The enamel mineral loss associated with NACP nanocomposite was only one-third of the enamel mineral loss around control composite without NACP.

Other studies using nanotechnology to tackle oral biofilms & caries

- For remineralization, hydroxyapatite nanoparticles promoted remineralization of caries lesions to a depth of 20–40 μm . Carbonate hydroxyl apatite nanoparticles were also effective in repairing micrometer-sized defects in tooth surfaces in vitro.
- For antibacterial activity, nanoparticles of copper, ZnO and TiO₂ were incorporated into dental composites and adhesives and shown to possess potent antibacterial functions. Quaternary ammonium polyethylenimine nanoparticles were developed and incorporated into dental resins, which exhibited strong antibacterial activities.

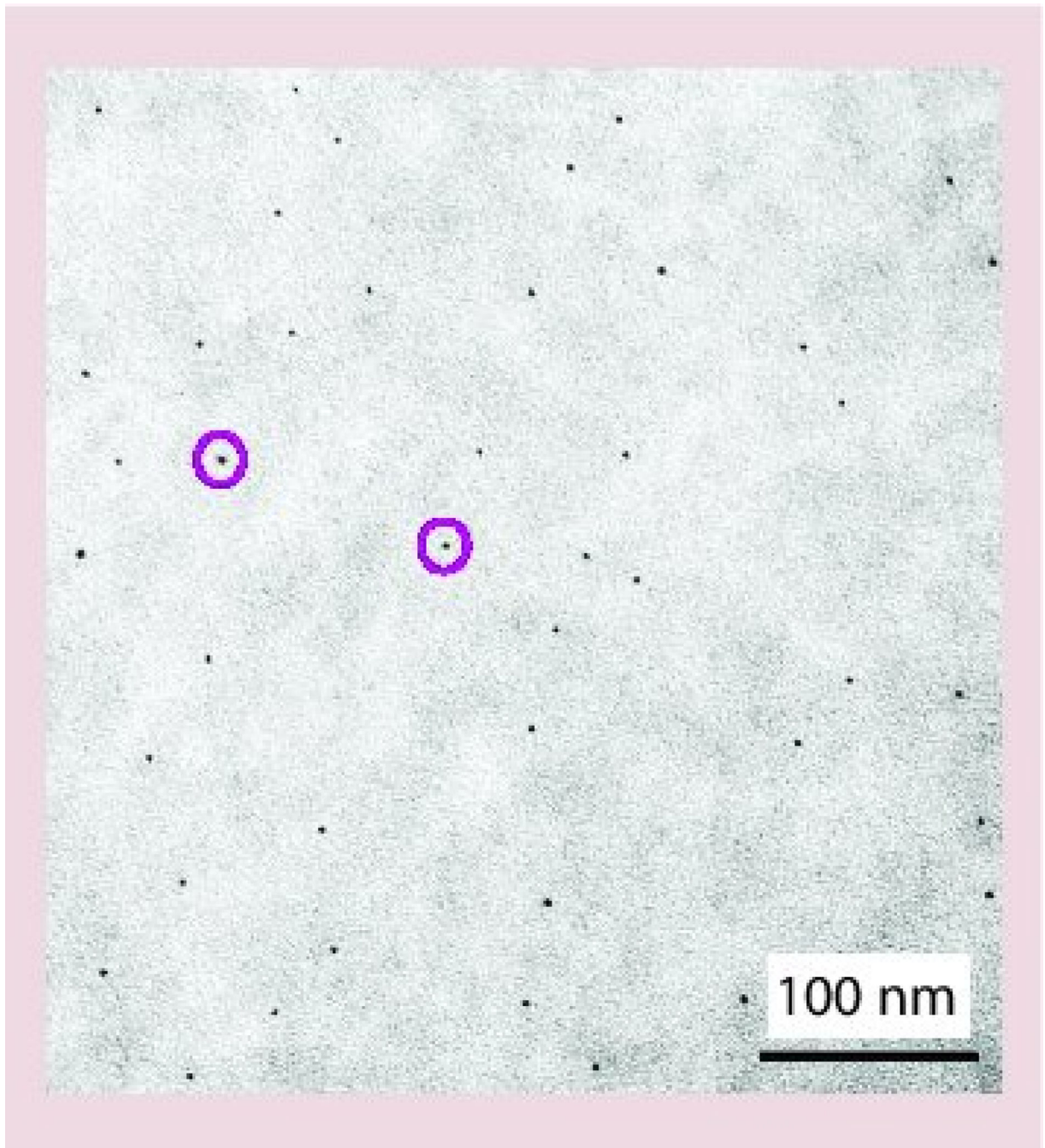


Figure 1.

Representative transmission electron microscopy image of nanoparticles of silver formed in situ in a dental resin matrix. The nanoparticles of silver appeared to be well dispersed in the resin without noticeable agglomerates. The nanoparticle of silver sizes were measured via high-magnification transmission electron microscopy to be 2.7 ± 0.6 nm [35]. Adapted with permission from [38].

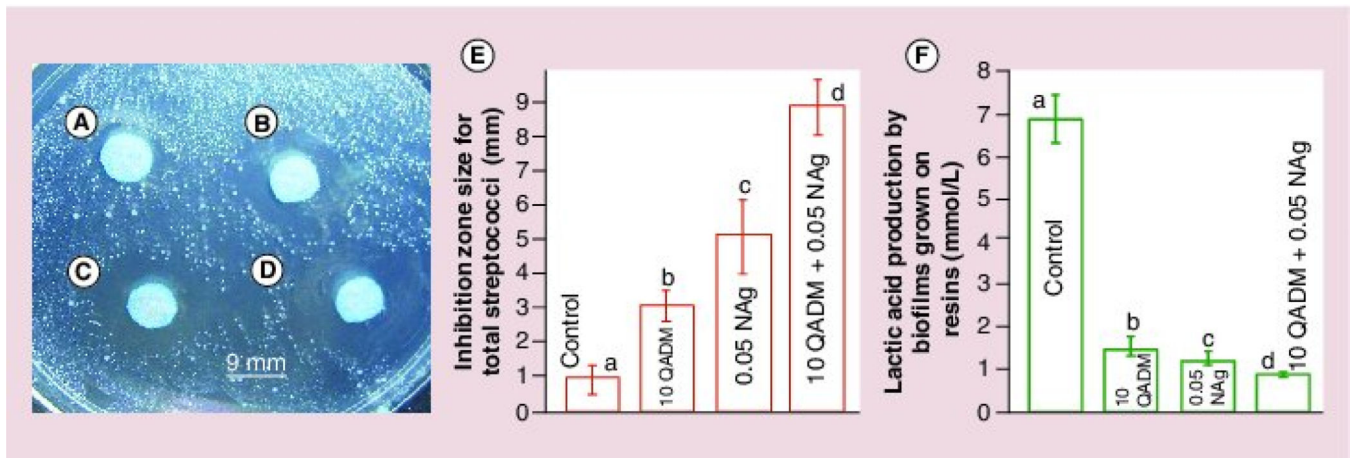
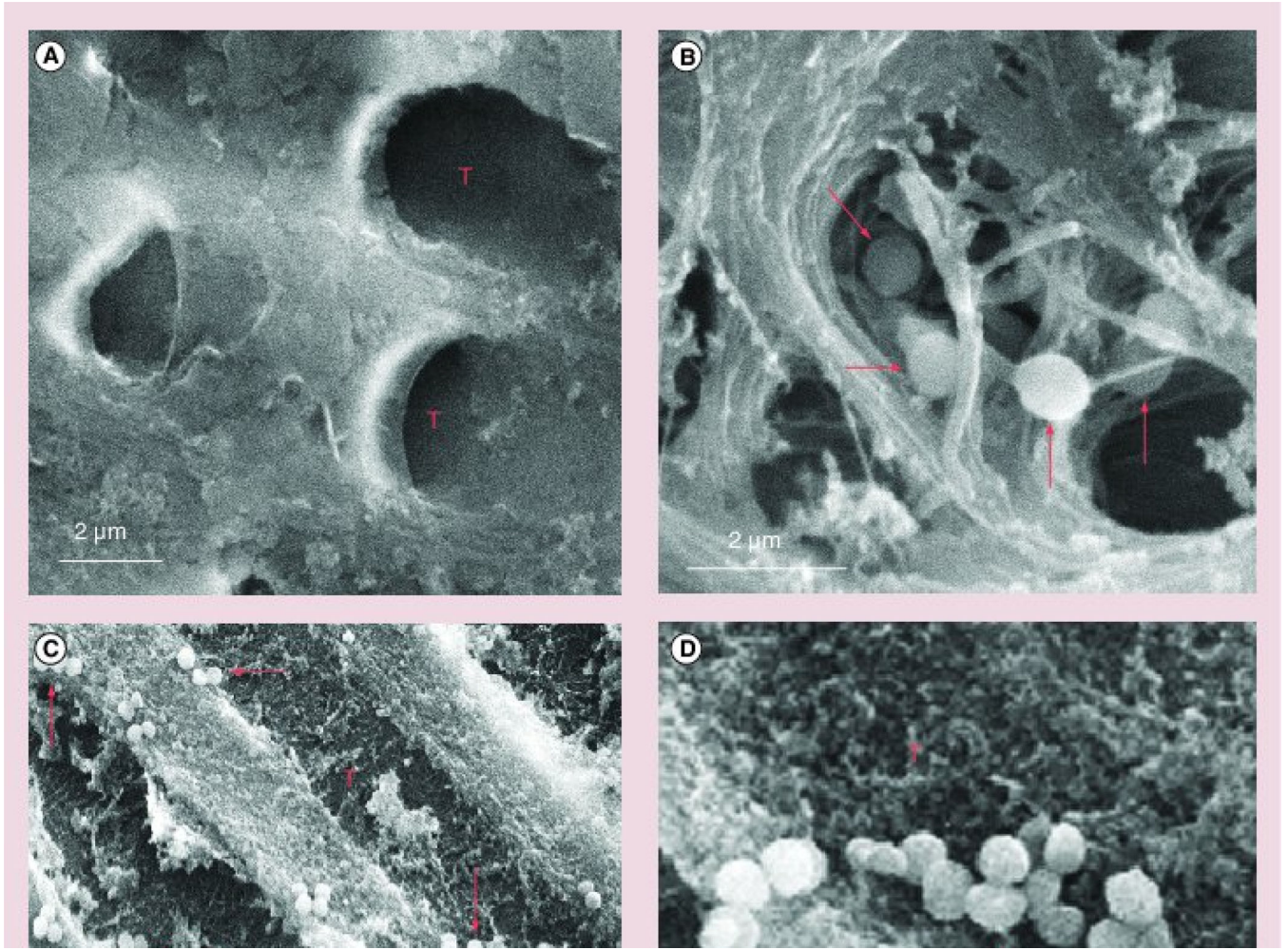


Figure 2.

Antibacterial activity of dental primer containing nanoparticles of silver and quaternary ammonium dimethacrylate. (A–D) Uncured primer in the agar disk diffusion test: (A) control primer (Scotchbond Multi-Purpose [SBMP], 3M, MN, USA); (B) control primer + 10% QADM; (C) control primer + 0.05% NAg; and (D) control primer + 10% QADM + 0.05% NAg. Note a small inhibition zone for control primer, and much wider inhibition zones for primers with QADM and NAg. (E) Inhibition zone size (mean \pm standard deviation; $n = 6$). (F) Lactic acid production by biofilms cultured for 2 days on various cured resins. Values with dissimilar letters are significantly different from each other ($p < 0.05$). NAg: Nanoparticles of silver; QADM: Quaternary ammonium dimethacrylate. Adapted with permission from [54].



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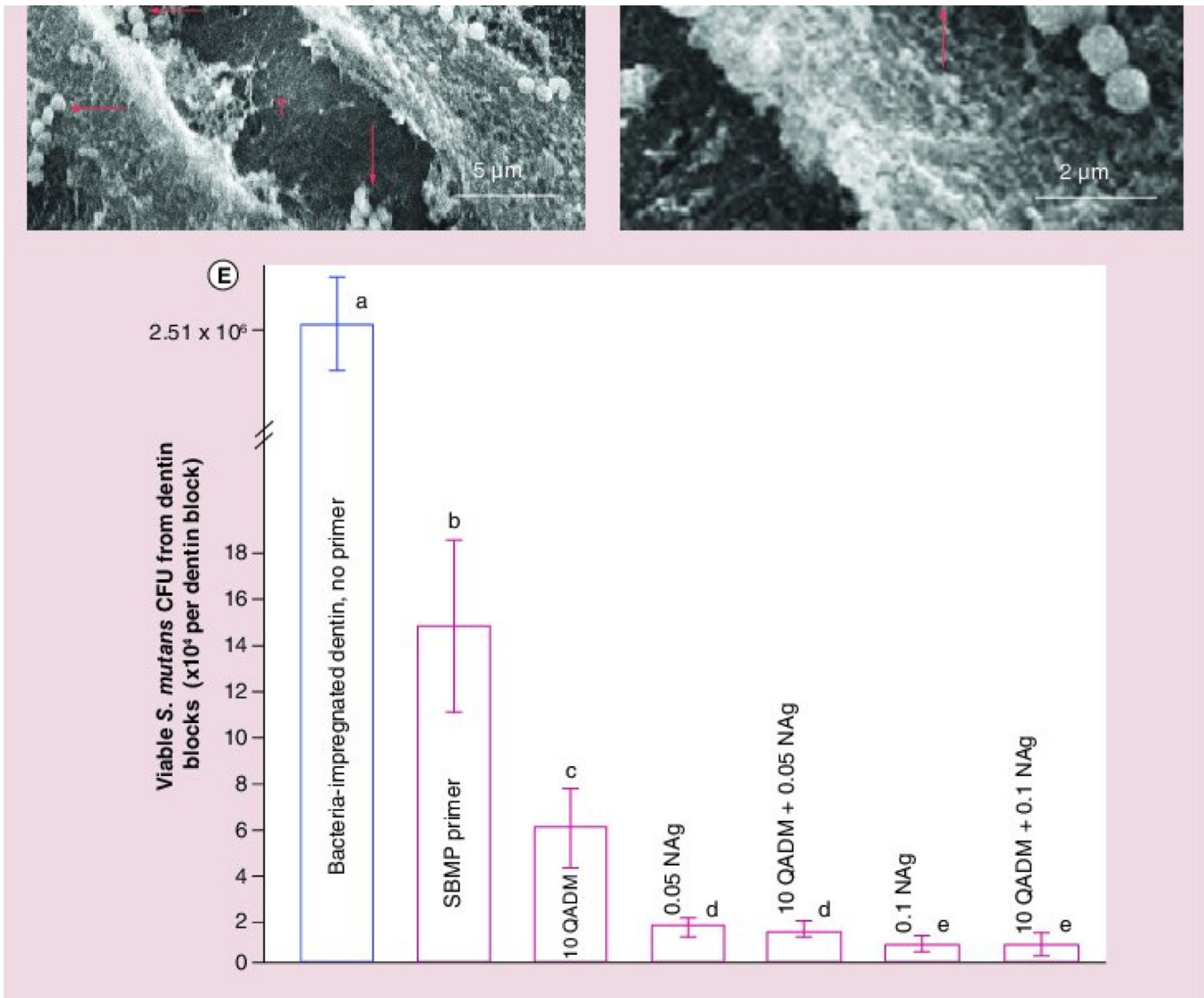


Figure 3.

Killing bacteria inside dentinal tubules in dentin. (A) Dentinal tubules before *Streptococcus mutans* impregnation, (B) *S. mutans* impregnation into tubules on the external surface of dentin with the tubules perpendicular to the surface, (C) *S. mutans* in tubules parallel to the surface on the cross section of dentin by opening the dentin block after bacteria impregnation and (D) higher magnification of the cross section as in (C). 'T' indicates dentinal tubules. Scotchbond Multi-Purpose (SBMP; MN, USA) is a control primer. Arrows indicate *S. mutans* in tubules. (E) *S. mutans* CFU in dentin blocks harvested by sonication (mean \pm sd; n = 6). The CFU of the bacteria-impregnated dentin without primer was 2.51×10^6 (CFU/dentin block). The other groups had the units of 10^4 CFU/dentin block. CFU in dentin treated with 10% QADM + 0.1% NAg was three orders of magnitude less than the CFU of control dentin.

CFU: Colony-forming units; NAg: Nanoparticles of silver; QADM: Quaternary ammonium dimethacrylate.

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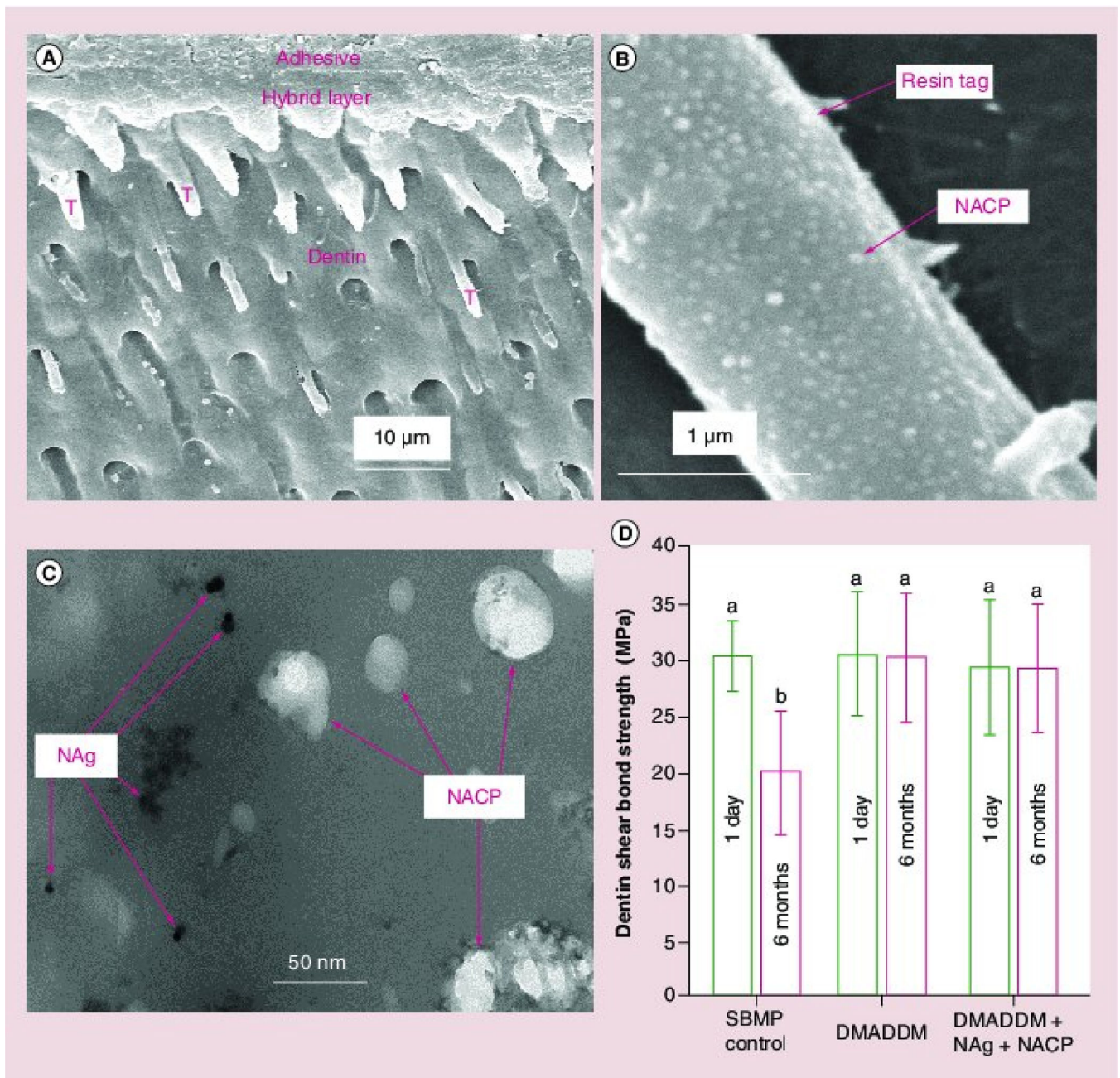


Figure 4. Durability of dentin bonding agent containing nanoparticles of silver, dimethylaminododecyl methacrylate and nanoparticles of amorphous calcium phosphate. (A) Dentin–adhesive interface showing resin tags “T”; (B) higher magnification of a resin tag showing numerous NACPs inside a dentinal tubule; (C) higher magnification showing both NAg and NACP in dentinal tubule and (D) dentin shear bond strength (mean \pm SD; $n = 10$). Values with dissimilar letters are significantly different from each other ($p < 0.05$). There was a 35% loss in bond strength for commercial bonding agent in 6 months’ water-

aging. There was no bond strength loss for antibacterial bonding agents incorporating DMADDM, NAg and NACP.

DMADDM: Dimethylaminododecyl methacrylate; NACP: Nanoparticles of amorphous calcium phosphate; NAg: Nanoparticles of silver.

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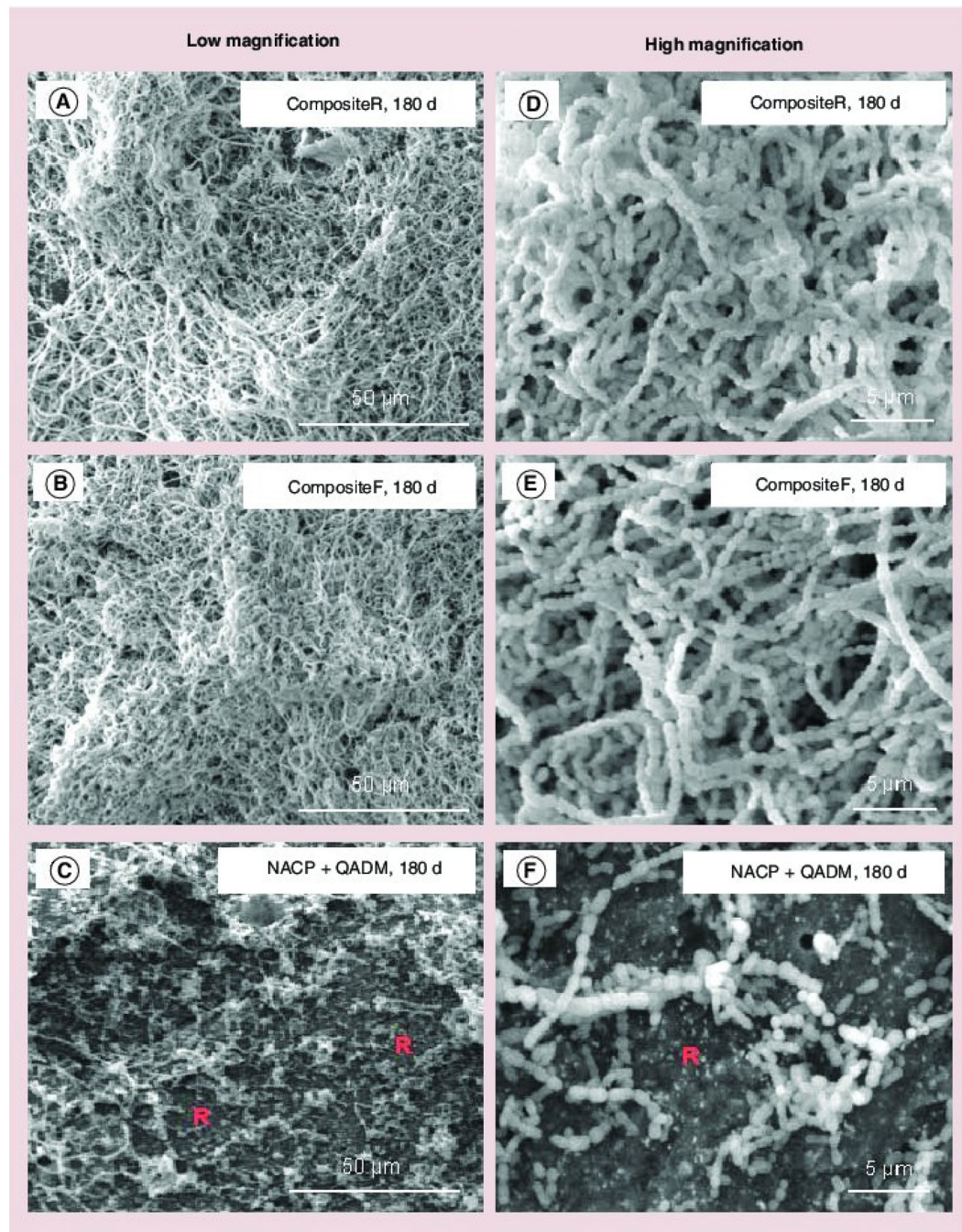


Figure 5. SEM images of biofilms grown for 2 days on composites. (A–C) Lower magnification and (D–F) higher magnification. Three composites were tested: CompositeR (Renamel, Cosmedent, IL, USA); CompositeF (Heliomolar, Ivoclar, NY, USA) and composite containing NACP and QADM. For each composite, water-aging for 1–180 days made no difference in biofilm appearance. The images shown here are for composites aged for 180 days, to demonstrate the long-term antibacterial activity of the NACP-QADM nanocomposite. Commercial composites had dense biofilms. NACP-QADM had much less

biofilm coverage. In (C & F), 'R' indicates the resin composite surface not covered by biofilms. (F) The *Streptococcus mutans* chains were much shorter on NACP-QADM nanocomposite.

d: Days; NACP: Nanoparticles of amorphous calcium phosphate; QADM: Quaternary ammonium dimethacrylate.

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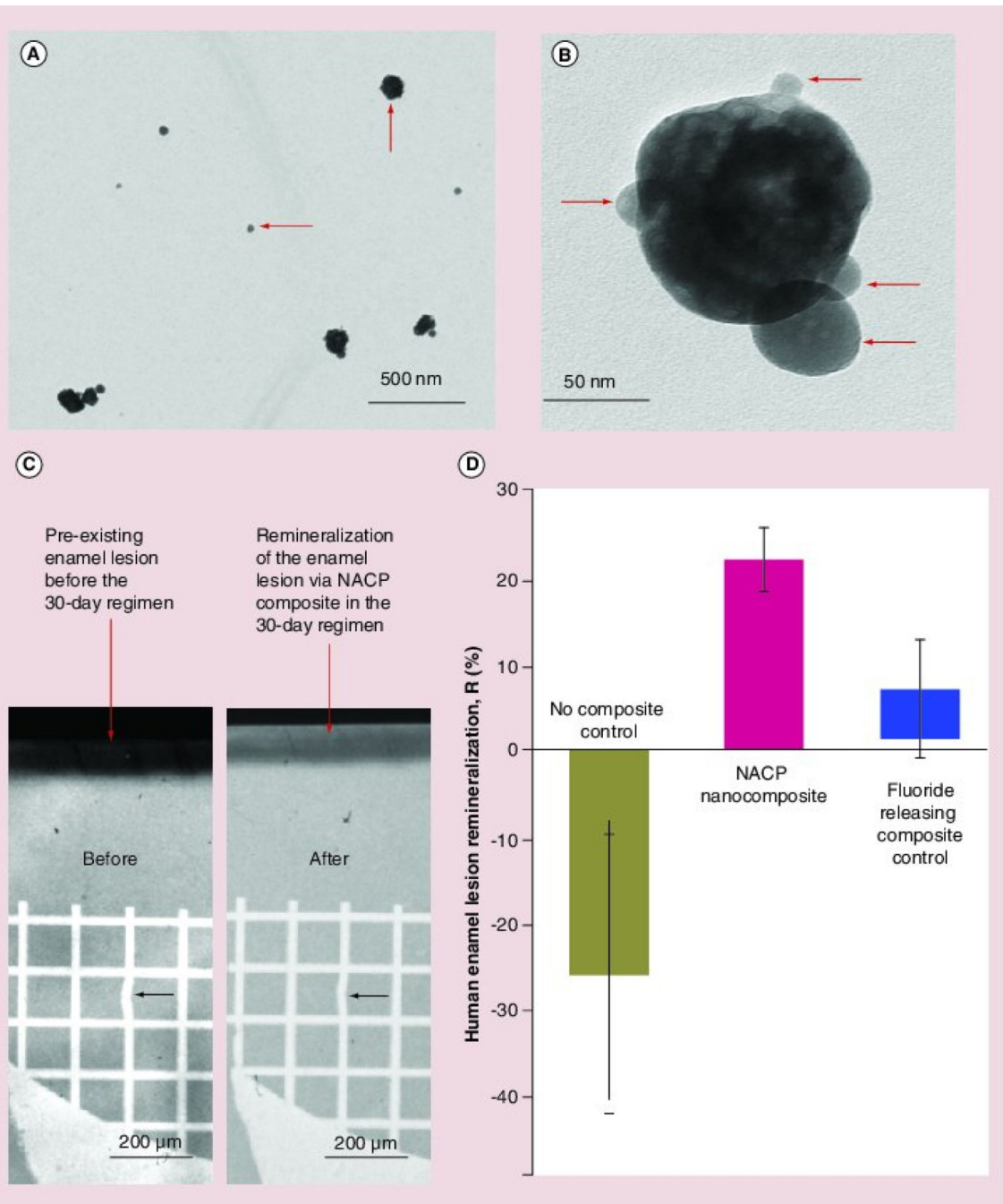


Figure 6. Remineralization of enamel lesions via nanoparticles of amorphous calcium phosphate nanocomposite. (A & B) NACP made by a spray-drying technique. Arrows in (A) indicate NACP at a lower magnification. At a higher magnification in (B), arrows indicate the small NACP with particle sizes of the order of 10 nm, which adhered together to form a larger particle. (C) Enamel lesion before the 30-day cyclic demineralization/remineralization regimen, and successful remineralization of the lesion after the 30-day regimen. (D) Percentage of remineralization (mean \pm standard deviation; n = 6) of human enamel lesions

in the 30-day cyclic demineralization/remineralization regimen. These three values are different from each other ($p < 0.05$).

NACP: Nanoparticles of amorphous calcium phosphate.

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