Original Article

The effect of N-acetylcysteine on the antibacterial capability and biocompatibility of nano silver–containing orthodontic cement

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ABSTRACT

Objectives: To determine whether the incorporation of N-acetylcysteine (NAC) improves the antibacterial ability and biocompatibility of nano silver (NAg)–containing orthodontic cement.

Materials and Methods: NAg was synthesized using a sodium citrate reduction method. NAg particles were characterized using transmission electron microscopy and ultraviolet-visible absorption spectra. NAg and NAC were incorporated into a resin-modified glass ionomer cement. Enamel shear bond strength (SBS), antibacterial capability, and cytotoxicity were evaluated.

Results: Incorporating 0.15% NAg and 20% NAC had no adverse effect on the SBS of orthodontic cement ($P > .1$). Adding NAC into NAg-containing cement greatly reduced the biofilm metabolic activity and lactic acid production ($P < .05$) and lowered the colony unit–forming counts by approximately 1 log ($P < .05$). The cell viability against NAg-containing cement was improved by NAC ($P < .05$).

Conclusions: The incorporation of NAC into NAg-containing cement achieved stronger antibacterial capability and better biocompatibility, without compromising the enamel SBS. The combined use of NAC and NAg is promising to combat caries in orthodontic practice. (*Angle Orthod.* 2021;91:515–521.)

KEY WORDS: N-acetylcysteine; Nano silver; Orthodontic cement; Biocompatibility

INTRODUCTION

White spot lesions (WSLs) are defined as a subsurface enamel porosity that manifests as chalky opacities around brackets on the tooth surface.[1,2] WSLs are one of the most common and evident adverse effects of fixed orthodontic treatment. The incidence of WSLs ranges from 23.4% to 49.6%.[3] The high prevalence of WSLs causes esthetic concerns and decreases patient satisfaction with orthodontic treatment.[4] New approaches to prevent the development of WSLs have always been desired.

The etiology of WSL formation during orthodontic treatment is well documented. Briefly, the orthodontic appliances provide more surface area for bacterial adhesion and decrease the efficacy of oral hygiene maintenance. Thus, biofilms are more likely to develop at the tooth surface adjacent to fixed appliances. In addition, the placement of orthodontic appliances could increase the percentage of cariogenic bacteria in oral biofilms.[5] The biofilm could metabolize carbohydrates into organic acids, thereby leading to enamel demineralization, which manifests as incipient WSLs.[6] Therefore, approaches that suppress the metabolism of bacteria would be helpful in protecting enamel from demineralization.

Nanotechnology has been applied to improve the performance of dental materials.[7] The small size of nanoparticles enables these materials to be more effective in inhibiting bacterial adherence and biofilm

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formation with stronger antibacterial capability than traditional particles.[8] Silver possesses long-lasting antibacterial properties and produces less bacterial resistance than antibiotics.[9] The incorporation of nano-sized silver particles (NAg) into dental materials could effectively suppress the growth of oral bacteria.[10] When compared with traditional silver particles, NAg could exert stronger antibacterial abilities at a lower level fill, thus avoiding significantly compromising the mechanical and color properties of dental materials.[11] However, adding NAg alone into dental materials has not seemed sufficient to prevent WSLs.[12] In addition, the use of NAg in dental composites has raised concerns regarding cytotoxicity.[13] Thus, the antibacterial ability and biocompatibility of NAg-containing cement needs improvement to combat WSLs.

N-acetylcysteine (NAC) has therapeutic effects in a wide range of diseases and has been approved for clinical use by the US Food and Drug Administration since 1963.[14] NAC possesses antimicrobial abilities and reduces biofilm formation of oral bacteria.[14] In addition, NAC is a direct antioxidant and diminishes the cumulative effects of oxidative stress on cells, thereby preventing the cell damage caused by dental composites.[15,16] Therefore, NAC is a potential candidate for improving the antibacterial properties and biocompatibility of NAg-containing dental materials. However, there has been no report to date on the combined use of NAg and NAC to modify orthodontic cement.

The objectives of this study were to develop an orthodontic cement containing NAg and NAC for the first time and evaluate its antibacterial behavior and biocompatibility. The following hypotheses were tested: (1) the incorporation of NAg and NAC into orthodontic cement would not compromise the bonding strength, (2) the simultaneous use of NAg and NAC would achieve stronger antibacterial activity than using NAg alone, and (3) using NAC would decrease the cytotoxicity of NAg-containing orthodontic cement.

MATERIALS AND METHODS

Synthesis of NAg

The NAg was commercially purchased (Shanghai So-Fe Biomedicine Co, Ltd, Shanghai, China). In accordance with the manufacturer's instructions, a conventional sodium citrate reduction method was used to synthesize NAg.[17] The NAg was characterized by transmission electron microscopy (TEM; JEOL Ltd, Tokyo, Japan) and ultraviolet-visible absorption spectra (UV-vis; Allsheng Instruments Co Ltd, Hangzhou, China).

Incorporation of NAg Into Resin Modified Glass Ionomer

A commercial, resin modified glass ionomer cement (GC Ortho LC, Fuji, Aichi-ken, Japan), denoted as GC, was used as the parental system. NAg was added into GC at a mass fraction of 0.15%. Mass fractions greater than 0.15% were not used to avoid compromising the mechanical properties of the parental system based on preliminary experiments. The unmodified GC and another orthodontic cement (Transbond XT, 3M, Irvine, Calif) were used as controls.

Incorporation of NAC Into Resin Modified Glass Ionomer

NAC was obtained commercially (Sigma-Aldrich, St Louis, Mo). Four mass fractions of NAC were incorporated into NAg-containing GC: 5%, 10%, 20%, and 30%. Mass fractions greater than 30% were not used because of reductions in enamel bonding strength.

Enamel Shear Bond Strength Evaluation

Forty-two human maxillary premolars were collected with the informed consent of patients. Each premolar was vertically embedded into self-curing acrylic resin (Fuji, Aichi-ken, Japan) which allowed the buccal axis of premolar crowns to be parallel to the mechanical load in an shear bond strength (SBS) test. After acid etching with 37% phosphoric acid gel (XihuBiom, Hangzhou, China) for 30 seconds and rinsing with water for 10 seconds, metal orthodontic brackets (Shinye, Hangzhou, China) were bonded to the buccal center of each tooth using orthodontic paste. According to the manufacturers' instructions, a paired primer was used with Transbond XT, whereas other cements were used without any primer. The following groups were tested:

- Transbond XT (denoted as TB control)
- GC control
- GC + 0.15% NAg (denoted as GC+NAg)
- GC + 0.15% NAg + 5% NAC (denoted as GC+NAg+5%NAC)
- GC + 0.15% NAg + 10% NAC (denoted as GC+NAg+10%NAC)
- GC + 0.15% NAg + 20% NAC (denoted as GC+NAg+20%NAC)
- GC + 0.15% NAg + 30% NAC (denoted as GC+NAg+30%NAC)

For each SBS test, a chisel was connected to a universal testing machine and placed above the bracket base. The testing machine applied a mechanical load to the bracket base via the chisel until the bracket was debonded. The SBS was calculated by

dividing the peak load by the surface area of the bracket base.

Specimen Preparation

The bonding strength decreased significantly in the SBS test when the mass fraction of NAC exceeded 20%. Thus, the following four groups were included in the subsequent experiments: TB control, GC control, GC+NAg, and GC+NAg+20%NAC.

The cover of a 96-well plate was used as the mold to prepare cement specimens. Cement pastes were placed in each well of the plate cover and light cured for 1 minute. The cement disks were approximately 8 mm in diameter and 0.6 mm in thickness. The cured disks were immersed in distilled water and stirred using a magnetic bar at 200 rpm for 1 hour to remove the initial burst of uncured monomers.[6]

Biofilm Formation

Streptococcus mutans ATCC 700610 was used to evaluate the antibacterial ability of orthodontic cement, because it is the primary bacteria causative of dental caries. The *S mutans* stock was added into brain-heart infusion (BHI) broth and incubated at 37°C anaerobically overnight. The *S mutans* suspension was diluted to approximately 10^7 colony-forming units (CFU)/mL to prepare the inoculation.

Each cement disk was placed in a well of a 24-well plate and immersed by 1.5 mL of inoculation medium. The cement disks were incubated at 37°C in 5% CO_2 for 24 hours. The disks were then transferred to a new 24-well plate containing 1.5 mL fresh BHI broth in each well and incubated at 37°C for another 24 hours. A total of 48 hours of incubation was able to form a mature biofilm on cement disks.[18]

Biofilm Metabolic Activity Assay

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a colorimetric assay that measures the enzymatic reduction of MTT. The MTT assay was conducted to evaluate the metabolic activity of biofilms grown on the cement disks.[6] Briefly, each disk with 2-day biofilm was immersed by 1 mL of MTT solution and incubated at 37°C for 1 hour. The specimens were then transferred to a new 24-well plate containing 1 mL of dimethylsulfoxide (DMSO) in each well and incubated in the dark for 20 minutes. After that, the absorbance of DMSO solution at 540 nm was determined.

Lactic Acid Production Assay

Each disk with 2-day biofilm was transferred into a new 24-well plate and immersed by 1.5 mL of buffered peptone water (BPW) plus 0.2% sucrose. The disk was then incubated at 37°C anaerobically for 3 hours to allow the biofilm to produce lactic acid. Subsequently, BPW was collected and transferred to a 96-well plate to measure the absorbance at 340 nm. The standard curve was established using lactic acid standard solutions.

Biofilm CFU Counts

The 2-day biofilm was harvested in phosphate-buffered saline by scraping from the cement disks followed by sonication and vortexing. The bacterial suspensions were serially diluted and spread onto BHI agar plates. The plates were incubated at 37°C for 48 hours. The CFU counts were determined by counting the colony number on the BHI plates.

Cytotoxicity Analysis

Each cement disk was placed in a 48-well plate and immersed by 500 μL of Dulbecco's Modified Eagle Medium (DMEM), yielding the mass ratio of cement surface area to solution volume to be approximately 1.8 cm^2/mL, following the recommendation of the International Standards Organization (ISO).[19] The disks were incubated at 37°C in 5% CO_2. The culture medium was replaced daily. The extracts were collected on days 1, 4, and 7 and stored at −20°C for cytotoxicity analysis.

The extracts plus 10% fetal calf serum, 100 IU/mL penicillin, and 100 IU/mL streptomycin were used to culture human gingival fibroblasts (HGFs). DMEM without extracts served as the control. HGFs were incubated at 37°C in 5% CO_2 for 24 hours. After that, the MTT assay was performed to investigate cell density. The optical ratios of the experimental groups to the control group were used as cell viabilities.

Statistical Analysis

Statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, Ill). The normal distribution of all data was identified by Shapiro-Wilk analysis. One-way analyses of variance and Fisher least significant difference post hoc test were applied to detect significant differences in bonding strength, antibacterial behavior, and biocompatibility. The statistical significance level was set at $P = .05$.

RESULTS

A representative TEM image of NAg is shown in Figure 1A. The synthesized NAg was composed of fine spherical particles with an average size of approximately 15 nm. The UV-vis spectrum of NAg showed the absorption peak was at 410 nm (Figure 1B), which

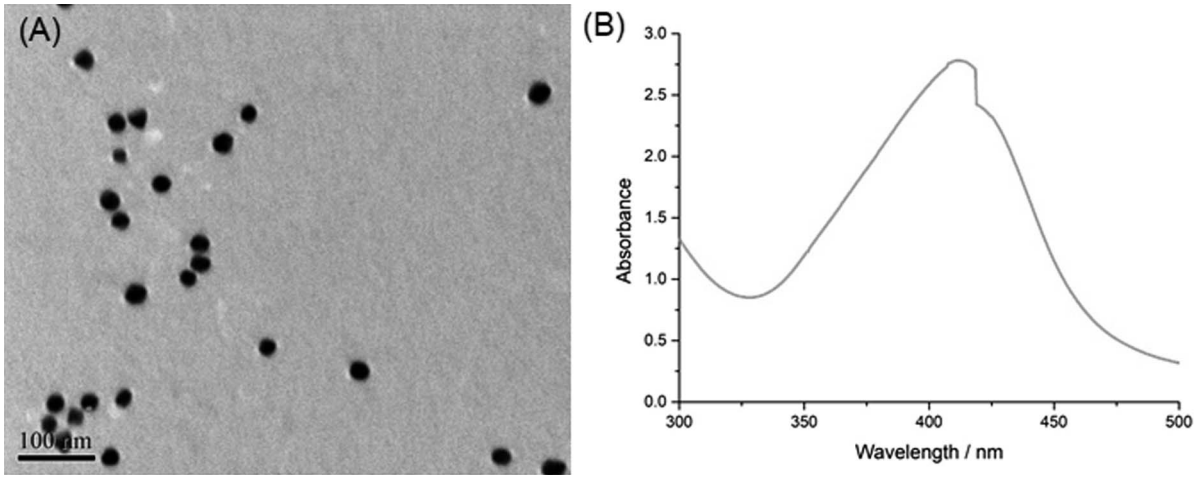

Figure 1. Characterization of NAg. (A) Typical TEM image of NAg. (B) UV-vis spectra of NAg.

was approximately identical to that reported in the literature.[17]

The enamel bonding strengths of the orthodontic cements are shown in Figure 2. TB control had the highest SBS results. The incorporation of 0.15% NAg into GC control had no adverse effects on SBS ($P > .1$). The SBS was not compromised until the mass fraction of NAC reached 30% ($P < .05$).

The metabolic activity and lactic acid production of biofilms are plotted in Figure 3. TB control had the highest metabolic activity, followed by GC control ($P < .05$). The incorporation of NAg significantly reduced the metabolic activities and lactic acid production ($P < .05$), which was further decreased by NAC ($P < .05$).

The biofilm CFU counts are shown in Figure 4. The CFU counts in TB control were close to 10^8 CFU/disk, which was slightly higher than that of GC control ($P < .05$). The CFU counts of GC+NAg were decreased to 10^7 CFU/disk ($P < .05$). The addition of NAC yielded the lowest CFU counts ($P < .05$).

The cell viabilities against the extracts of orthodontic cements are displayed in Figure 5. All eluents of orthodontic cements reduced cell viabilities as compared with the negative control ($P < .05$). The cell viabilities of GC+NAg+20%NAC were 73.8%, 77.6%, and 78.7% for days 1, 4, and 7, respectively, which were lower than TB control and GC control ($P < .05$) but higher than GC+NAg ($P < .05$).

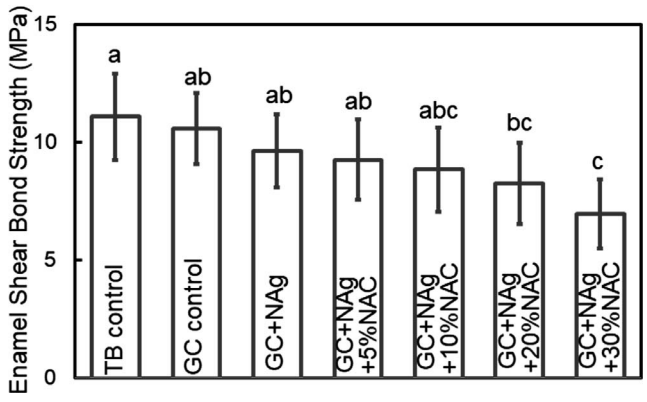

Figure 2. Enamel shear bonding strength (SBS) of orthodontic cements. Adding 0.15% NAg did not adversely affect the bond strength. The SBS of NAg-containing cement was not compromised until the mass fraction of NAC reached 30%. The different letters indicate significant differences between groups ($P < .05$).

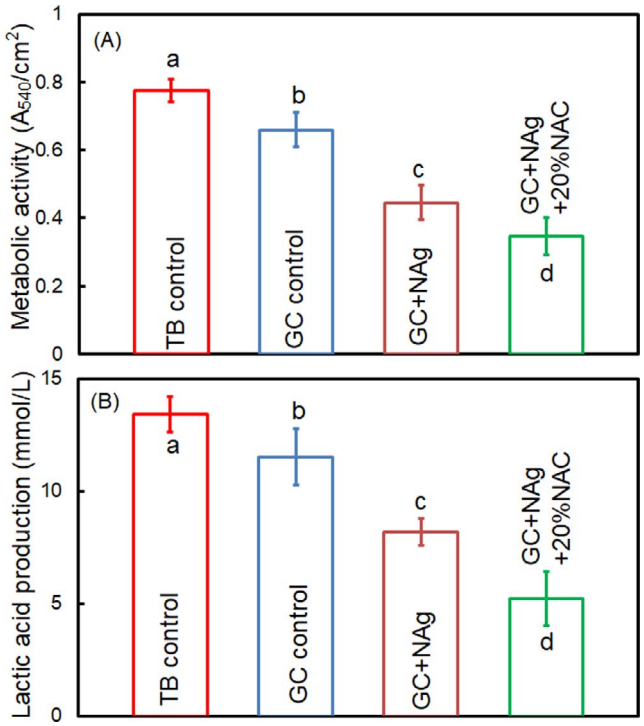

Figure 3. Quantitative antibacterial effects for biofilm grown on cement disks. (A) Metabolic activity. (B) Lactic acid production. The different letters indicate significant differences between groups ($P < .05$).

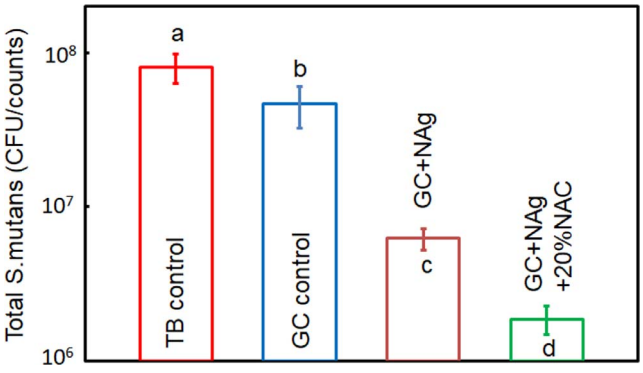

Figure 4. Colony-forming unit (CFU) counts of biofilms on cement disks. Incorporating NAg greatly reduced CFU counts, which were further decreased by NAC. The different letters indicate significant differences between groups ($P < .05$).

DISCUSSION

A major concern for adding bioactive agents into orthodontic cement is the reduction of bonding strength. The minimum SBS recommended for clinical use is approximately 7.8 MPa, which is high enough to prevent accidental debonding but not too high to hinder the removal of brackets after treatment.[20] In this study, the SBS of GC+NAg+20%NAC was 8.25 MPa. This value was slightly higher than the minimum recommended SBS, indicating that it was acceptable for orthodontic practice. However, future studies are still needed to improve bonding strength to guarantee acceptable behavior in clinical practice. In contrast to a recent study in which increased adhesion of sealer was observed after adding NAC,[21] the current study showed that incorporating NAC decreased the bonding strength. This difference could be attributed to two factors. First, enamel was used in this study, whereas the previous study used dentin. Second, a bracket-tooth model was used to test the SBS in this study, whereas the previous study used a push-out test to evaluate adhesion.

It is believed that the antibacterial action of the Ag ion is due to its ability to inactivate the vital enzymes of bacteria, which inhibits DNA replication, thus causing bacteria death.[22] Consistent with previous studies demonstrating the antibacterial capability of NAg against oral bacteria,[10] the antibacterial activity of GC against *S mutans* was enhanced by adding NAg in this study. Significant reductions in mechanical properties were observed when the mass fraction of NAg reached 0.20% in the pilot study. Thus, 0.15% NAg was used to achieve a balance between antibacterial capability and mechanical properties.

The efficacy of NAC in inhibiting biofilm metabolism is well documented,[14] although the mechanisms have not been fully elucidated.[14] Current evidence suggests the antibacterial ability of NAC is likely related to the inhibition of cysteine utilization, reaction with bacterial cell proteins, and disturbance of intracellular redox equilibrium.[23] In this study, adding NAC into NAg-containing cement reduced the metabolic activity, lactic acid production, and CFU counts of *S mutans* biofilm by 22.5%, 36.2%, and 70.1%, respectively, indicating the combined use of NAg and NAC is more effective in inhibiting bacterial metabolism than using NAg alone.

Leachable monomers from dental material are a likely cause of cellular stress and cell damage via the formation of reactive oxygen species (ROS).[24] Similarly, the cytotoxicity of NAg is also partially caused by ROS increment.[25] The balance of ROS production and

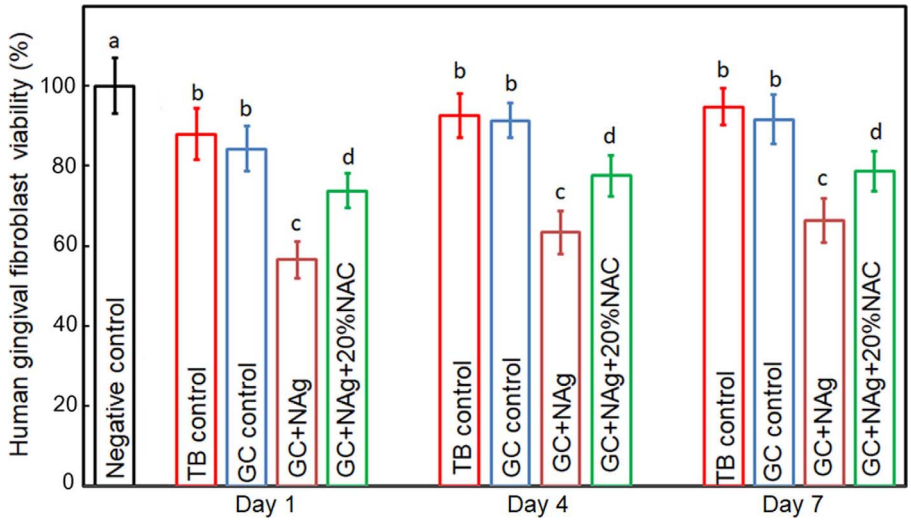

Figure 5. Cytotoxicity of orthodontic cements. The use of NAg reduced cell viability at all three time points (day 1, day 4, and day 7), which was partially reversed by the addition of NAC. The different letters indicate significant differences between groups ($P < .05$).

antioxidant systems is critical to cell death and survival.[26] High ROS levels regulate redox-sensitive transcription factors and cause acute injury to cellular proteins, lipids, and DNA, which lead to cell death.[27] NAC is a direct antioxidant that reacts rapidly with hydroxyl radicals, thus detoxifying ROS.[14] In this study, the cell viabilities against NAg-containing cement were increased to more than 70% of the negative control by adding NAC, which should be considered noncytotoxic according to the ISO.[19] This finding is consistent with previous studies demonstrating the ability of NAC to decrease cell damage caused by dental materials in various cell types, which is likely due to the reduction of ROS.[24]

CONCLUSIONS

- A novel orthodontic cement with antibacterial capabilities and acceptable biocompatibility was developed by incorporating NAg and NAC into an orthodontic cement for the first time.
- NAC can be used along with NAg to achieve favorable antibacterial capability and biocompatibility.
- Incorporating NAC and NAg into orthodontic cement might be an effective and safe approach to achieve antibacterial activity in orthodontic practice.

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