An evaluation of the antibacterial properties and shear bond strength of copper nanoparticles as a nanofiller in orthodontic adhesive

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Objectives: To evaluate the antibacterial properties and effects of an orthodontic adhesive containing copper nanoparticles (NPs) on the material’s shear bond strength.

Methods: Antimicrobial activity was analysed by a disk diffusion test against S. aureus, E. coli and S. mutans. The NPs were added to the orthodontic adhesive at 0.0100 wt%, 0.0075 wt%, and 0.0050 wt%. Sixty extracted bicuspids were divided into two groups and the enamel of all teeth was conditioned with phosphoric acid. A coat of moisture insensitive primer (MIP) was applied prior to the bonding of brackets with composite resin. Group I served as a control and the bonding procedure was performed according to the manufacturer’s instructions. Group II comprised the test teeth, into which 0.0100 wt% copper NPs were included in the MIP. Samples were tested and statistically analysed (p ≤ 0.05). The adhesive remnant index (ARI) was also assessed microscopically.

Results: The adhesive with copper NPs showed a bactericidal effect against the bacteria under study. A significantly higher bond strength was obtained with the orthodontic adhesive that included 0.0100 wt% of copper NPs (15.23 ± 6.8 MPa) in comparison with the control group (9.59 ± 4.3 MPa). The ARI scores indicated that the groups were significantly different and strengthened by the incorporation of NPs (p = 0.004).

Conclusion: The results of the present study suggested that an orthodontic adhesive, which included copper NPs, significantly increased material shear bond strength without adverse side effects on colour and appearance. The adhesive interface was strengthened by homogeneously dispersed copper NPs added as a nanofiller.

Received for publication: October 2014
Accepted: April 2015

Introduction
Despite the great scientific advances in adhesive materials used in orthodontics, further improvements are needed in order to prevent the undesirable formation of white spot lesions.1 The decalcification of enamel is common during fixed orthodontic treatment and is associated with the accumulation of dental plaque retained around appliances and the bonding composite.

Previous studies have shown that there is a significant increase in caries-causing bacteria when fixed orthodontic appliances are placed.² Usually, acid production by bacteria causes demineralisation of the enamel surface, which may lead to dental caries.³ A desirable property of contemporary orthodontic adhesives is an antibacterial effect. However, past evidence has demonstrated that the addition of antibacterial components such as chlorhexidine, varnish
and gel significantly decreased shear bond strength of the bonding material.\textsuperscript{4,5}

Currently, a major application of nanotechnology is an antibacterial effect produced by metal nanoparticles (NPs) of either gold,\textsuperscript{6} silver,\textsuperscript{7} zinc\textsuperscript{8} or copper.\textsuperscript{9} Copper is significantly more affordable than silver or gold and so it is economically attractive. The antibacterial properties of copper NPs have been widely studied.\textsuperscript{9-17} In earlier trials,\textsuperscript{18} copper NPs were prepared by a simple chemical method and their antibacterial activity was tested against \textit{Staphylococcus aureus}, \textit{Escherichia coli} and \textit{Streptococcus mutans}, with promising results that indicated a potential use in dental materials science. Therefore, the purpose of the present study was to evaluate the antibacterial properties and effects on material shear bond strength of an orthodontic adhesive that contained copper NPs.

**Materials and methods**

A conventional light cured orthodontic adhesive (Transbond MIP, 3M Unitek, CA, USA) was selected for use in this study due to its hydrophilic properties. Copper NPs, suspended in isopropyl alcohol, were added to the adhesive resin with a micropipette in concentrations of 0.0100 wt%, 0.0075 wt%, and 0.0050 wt%. The copper NPs were synthesised according to a previously published report.\textsuperscript{18}

**Antibacterial test**

The adhesive’s antibacterial activity was determined by a disk diffusion technique which conformed to the recommended standards of the National Committee for Clinical Laboratory Standards.\textsuperscript{19} Mueller-Hinton agar (MHA) plates were prepared and inoculated with 200 μl of bacterial culture. The culture was adjusted with sterile saline to achieve a turbidity equivalent to a 0.5 McFarland standard. Disks made of filter paper were impregnated with either 20 μl of chlorhexidine or bonding adhesive containing one of three different concentrations of copper NPs. The disks were firmly placed on the agar plates.

Antibacterial testing was performed against three culture strains: \textit{S. aureus}, \textit{E. coli} and \textit{S. mutans}. The antibacterial activity of the adhesive was determined in two batches for each strain using (a) unpolymerised adhesive and (b) polymerised adhesive that had been cured for 20 seconds with an LED (Ortholux, 3M Unitek, CA, USA). Two positive controls comprising chlorhexidine at 2% (Dentsply) and specific drugs – Cefotaxime (30 μg) against \textit{S. mutans} and \textit{S. aureus} and trimethoprim-sulfamethoxazole (1.25/3.75 μg) against \textit{E. coli} – were used. Adhesive without chlorhexidine or NPs was tested against the same bacteria and served as a control.

The inhibition of the antibacterial halos generated on the agar plates was measured by using reflected light over the agar plate. The measured distances were rounded to the nearest millimeter with the use of the ImageJ 1.47e software program (National Institutes of Health, MD, USA). The program was calibrated using a known distance and each determination was repeated three times.

**Shear bond strength (SBS)**

**Teeth**

Sixty freshly extracted, healthy (without caries and restoration-free) human premolars were cleaned with a rotary brush and stored in a 0.2% solution of distilled water and thymol at 4°C until required. Samples were cleaned with fluoride-free pumice paste using rubber prophylactic cups, and washed with water and air-dried. Each premolar was individually embedded in an acrylic mould with its labial surface parallel to the mould base. This ensured that the labial surface would be parallel to the applied force during the shear bond test. The teeth were randomly divided in two equal groups (N = 30).

**Brackets**

Stainless steel bicuspid brackets (0.018 inch, Alexander Discipline, Ormco Corp., CA, USA) were used. The average surface area of the bracket base was determined to be 14.21 mm\textsuperscript{2}, which was obtained by averaging 10 randomly-measured bracket bases.

**Bonding procedure**

Group I (control): The bonding surface was etched with 37% phosphoric acid gel for 15 seconds, rinsed with water for 30 seconds, and dried with oil- and moisture-free air until the enamel had a faintly frosty appearance. A thin coat of Transbond Moisture Insensitive Primer (MIP, 3M Unitek) was applied to the etched surface. The orthodontic brackets were bonded with Transbond Plus CC Adhesive (3M

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Unitek) and light cured (Ortholux, 3M Unitek) for 12 seconds.

Group II (experimental): The bonding procedure was performed following the procedures applied to group I; however, the MIP (3M Unitek) was combined with 0.0100 wt% copper NPs. The copper NPs were stored in solution, which required and justified the use of a moisture insensitive primer. Since the experimental adhesive incorporating 0.0100 wt% copper NPs was the concentration previously determined to show antibacterial effects, it was used to test the SBS.

Storage
A 0.017 x 0.025 inch stainless steel wire was ligated into each bracket slot to reduce deformation of the bracket during the debonding process. The teeth were stored in distilled water at 37°C for 24 hours.

SBS test
A universal testing machine (Autograph AGS-X, Shimadzu Corp., Tokyo, Japan) with a crosshead speed of 0.5 mm/min delivered an occluso-gingival shear load to the bracket using a chisel-edge plunger. The maximum load was recorded in megapascals (MPa). Descriptive statistics and the Student t-test were applied (SPSS 19, IBM Corp., IL, USA) to analyse the data (p < 0.05).

Adhesive remnant index (ARI)
After shear debonding, the enamel surfaces were microscopically examined under X10 magnification to determine the amount of residual adhesive. The quantity of adhesive was scored for each tooth using the adhesive remnant index (ARI) scale, which ranges from 0 to 3. Zero indicates no adhesive remaining on the tooth; 1 indicates less than half of the enamel bonding site is covered with adhesive; 2 indicates more than half of the enamel bonding site is covered with adhesive; and 3 indicates the enamel site is covered entirely with adhesive. The chi-square test was applied to evaluate the ARI.

Distribution of nanoparticles in the adhesive
An ultrastructural TEM and qualitative analysis was performed to determine the distribution and size of the copper NPs dispersed within the polymerised orthodontic adhesive.

Results
Antibacterial test
The antibacterial effects observed on the agar plates are shown in Figure 1 and Table I. The experimental adhesive containing 0.0100 wt% copper NPs was the only concentration that showed antibacterial properties and those results were comparable with the effects of chlorhexidine. The adhesives containing 0.0075 wt%, and 0.0050 wt% copper NPs displayed no antibacterial activity.

SBS
The SBS values (expressed in MPa) and descriptive...
The mean value of shear bond strength of the experimental group was significantly higher (15.2 ± 6.8 MPa) than the control group 9.5 ± 4.3 MPa (Student t-test \( p = 0.0001 \)). The addition of copper NPs to the moisture insensitive primer significantly increased the SBS. In addition, the copper NPs offered antibacterial effects around the orthodontic bracket, as well as in the interface between the enamel and adhesive.

**ARI**

The remnant scores indicating the amount of adhesive remaining after the SBS test are shown in Table III. There was a significant difference in the debonding pattern between the groups. ARI scores in the control group were mainly distributed in the 0 and 1 range. The remnant scores in group II were mainly distributed in the 1 and 2 range.

### Distribution of nanoparticles in adhesive

The distribution of copper NPs within the polymer was homogeneous as no aggregation of nanoparticles was observed. The distribution of nanoparticles in the adhesive is shown in Figure 2. The size of the NPs was less than 20 nm and their shape was almost spherical, which produced a greater surface area to volume ratio and rendered the NPs more reactive.
The formation of white spot lesions (WSL) is a common side effect related to orthodontic fixed appliances. Due to its rough surface, excess composite around a bracket base is a common site of plaque accumulation. Oral pH is an additional critical factor that promotes the development of enamel demineralisation. In order to prevent WSL, an adhesive with antibacterial properties would be desirable. In the present study, antibacterial testing showed that adhesive containing nanoparticles was effective against bacteria in a similar manner to the bactericidal effects of chlorhexidine. In comparison, the antibacterial activity of unpolymerised adhesive was higher than polymerised adhesive, possibly due to the diffusion capability of the adhesive on the agar plates.

Recently, composite resins with nanofillers have been developed and introduced in order to reduce shrinkage during polymerisation. Nanoparticles can provide higher dimensional stability and decrease surface roughness, which is an important factor related to bacterial adhesion. It was therefore considered that an orthodontic adhesive that incorporated copper NPs would deliver a reduction in WSL by controlling bacterial activity.

Camphorquinone is the initiator responsible for resin polymerisation. As polymerisation occurred, the incorporated NPs appeared to have little influence on the process. The time required to fully cure the adhesive with NPs was the same required for the control adhesive. The adhesive containing the NPs was light cured together with the unfilled composite resin for 12 seconds, as determined by the timer of the Ortholux (3M Unitek) curing device. However, experiments with higher concentrations of nanoparticles (>3%) have indicated that the colour (dark reddish brown) of the copper NPs affects the activation of the visible light photo-initiator.
While the adhesive was reinforced by homogeneously-incorporated copper NPs as a nanofiller, a previous study reported that varying the percentage weight of the nanofiller favorably influenced resin debonding characteristics. The earlier results also indicated that increasing the level of the nanofillers beyond a certain adhesive weight fraction reduced the interface strength. In addition, nanoparticles improved the coefficient of thermal expansion of the resin, and provided more dimensional stability. In the present research, the SBS was increased significantly by the addition of 0.0100 wt% copper NPs into the orthodontic adhesive. The values obtained were much higher than the requirements reported for clinical practice.

According to the ARI scores in the experimental group, the adhesive remnants were higher than those for the control group, which indicated that the adhesion between enamel and resin likely increased with the addition of copper NPs.

The tendency for NPs to coalesce into macro-size aggregates has been shown to affect the material properties. The addition of a nanofiller to the adhesive matrix may confer improved properties to the composites but an even dispersion of the nanoparticles is required. TEM images showed an even dispersion of copper NPs; however, further studies are necessary to determine the toxicity and biocompatibility of the NPs for intra-oral application.

Conclusions

Under the conditions of the present study, the following conclusions may be drawn:

- The distribution of copper NPs in the adhesive resin was homogeneous and without aggregation.
- The orthodontic adhesive containing 0.0100 wt% copper NPs as nanofiller expressed antibacterial activity.
- The SBS of orthodontic brackets significantly increased following the use of orthodontic adhesive containing 0.0100 wt% copper NPs.
- The ARI was significantly higher in the experimental group, which indicated that the bond strength between enamel and adhesive was higher.
- The colour appearance of the tooth was not affected by the addition of 0.0100 wt% copper NPs.

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