COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFECT OF Ag & CuO NANOPARTICLES INCORPORATION IN ORTHODONTIC ADHESIVE AND THEIR INFLUENCE ON SBS

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ABSTRACT

Objective: This research compared the antibacterial efficacy of silver and copper oxide nanoparticles incorporated into a commercial orthodontic adhesive and their effects on shear bond strength (SBS) of stainless steel orthodontic brackets bonded to enamel surface.

Methodology: Ninety extracted premolars were randomly allocated into three groups (n = 30). Orthodontic adhesive (Enlight, Ormco) was blended with silver (100 nm, 2% w/w) and copper oxide (50 nm, 1% w/w) nanoparticles to form nano-adhesives. Antibacterial activity of control and modified adhesives were assessed against Streptococcus mutans after 24 hs by agar diffusion test. The degree of conversion for all groups was determined using a Fourier transformed infra-red spectrometer. The control and nano-modified adhesives were then used to bond brackets to the teeth after acid etching using phosphoric acid for 30 seconds, then rinsing with water for 15 seconds. Shear bond strength was assessed using a Universal testing machine and the adhesive remnant index (ARI) scores were examined.

Results: The addition of nanoparticles significantly increased the antibacterial efficiency of the adhesive against S.mutans after 24 hs (P<0.0001). The findings of the study suggest that the degree of conversion, SBS and ARI scores were not significantly different from the control group after incorporation of either Ag or CuO NPs.

Conclusion: Both nano-adhesives showed significant antibacterial activity against Streptococcus mutans with no adverse effect neither on the degree of conversion nor SBS nor adhesive remenant index.

KEYWORDS: Silver nanoparticles; Copper oxide nanoparticles; Antibacterial; shear bond strength.

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INTRODUCTION

The oral cavity provides specific fundamental features for bacterial propagation that produce acids which are capable of demineralizing the tooth surface. [1] Biofilm plays a critical role in these microorganisms adherence to the enamel surface. [2] The organic acids induced by several bacteria, mostly Streptococcus mutans and Streptococcus sobrinus, that are recognized as the principal microorganisms in caiogenesis, caused enamel demineralization. [3]

During fixed orthodontic treatment, decalcification of tooth surfaces appears in about 50% to 75% of patients which enhances plaque accumulation and accordingly white spot lesions around brackets, that in turn increase the caries potential. [4]

An important goal of the clinician during orthodontic treatment is the prohibition of white spot lesions and caries. [5] The antibacterial effect is an eligible feature of contemporary orthodontic adhesives. Nevertheless, former evidence revealed that the incorporation of anti-bacterial products like chlorhexidine, gel and varnish reduced significantly the SBS of the bonding material. [6] Recently, an essential implementation of nanotechnology is the use of metal nanoparticles (NPs) of zinc, gold, copper or silver to produce antibacterial effect. [7]

The outstanding antimicrobial properties of silver nanoparticles against microorganisms have received great attention. Most AgNPs inner core is formed of metallic silver, while the outer surface consists of either sulfide or oxide layers. Ag$^+$ ions are emitted through degradation of the silver oxide surface layer. [8]

Silver nanoparticles have obvious potency in antibacterial therapy. So, they are considered as efficient strategy facing resistant bacteria. Furthermore, smaller size ameliorates wear resistance, which is beneficial in various dental fields. [9]

Copper is a prevalent metal in dentistry and medicinal studies because of its low toxicity and antibacterial characteristics. The antimicrobial efficacy is produced by either ions form obtained from copper NPs (1-100nm). [10] It has been reported that CuO NPs had powerful antimicrobial action on bacterial plaque growth and colonization. [11]

The purpose of the present work is to compare the antimicrobial effect of incorporation of both Ag NPs and CuO NPs in orthodontic adhesives and their impact on SBS of stainless steel orthodontic brackets attached to enamel surface.

MATERIALS AND METHODS

Nano-Adhesive Preparation

Silver nanoparticles <100 nm particle size, Sigma-Aldrich Co (St. Louis, MO, USA, Lot # MKBN3581V) were obtained and utilized in the present work. The CuO nano-particles were synthesized by laser ablation technique and characterized in Cairo National Research Center, the average particle size was ~55 nm. [12, 13]

Light cure orthodontic adhesive Enlight (Ormco, CA, USA) orthodontic adhesive was utilized to bond the brackets to the buccal surface of teeth.

Nano-powders were weighed on a balance accurate to 0.0001g. 2 % w/w ratio for silver and 1% w/w ratio for CuO were determined after an antibacterial pilot study to determine MIC. Adhesive was blended with nanoparticles after being placed in an Eppendorf tube, then wrapped in aluminum foil and mixed using an amalgamator at 3500 rpm for 5 min, until a homogenous color was obtained.

Antibacterial activity testing (agar well diffusion)

Agar plate inoculation was done through diffusing an adequate amount of microbial specimens across the entire plate surface. Using a plastic mold, the adhesive was made into a 6 mm diameter X 2mm thickness specimen. Using a Bre.

lux Power Unit; Bredent GmbH & Co., Senden, Germany; specimens were cured for 20 seconds according to the manufacturer's recommendations with 1400 mW/cm^2 irradiance, 10 mm tip diameter and a 430–480 nm wavelength. With a sterile tip, 6 mm diameter holes were punched aseptically. Incubation of the agar plates was then done under appropriate conditions depending on the examined strain. The sensitivity of the utilized antimicrobial agents was detected after 24 hours of incubation by measuring their inhibitory zones against the tested Streptococcus mutans. [14, 15]

Assessment of Degree of Conversion (DC)

A Fourier-transform infrared spectrometer coupled to an attenuated total reflectance accessory (Nicolet iS10 FTIR Spectrometer, Thermo Scientefic, Madison, Wl, USA) was utilized to measure the degree of conversion. Adhesive samples were withheld on the attenuated total reflectance diamond crystal before and after curing. Infrared spectra of both the cured and uncured adhesives were registered in absorbance mode in the 4000–400 cm^{-1} wave number range at 4 cm^{-1} resolution.

The spectrum of the unpolymerized resin was firstly assessed and the infrared spectrum was then registered. The adhesive was persistently contacting the sensor during spectra recording.

Calculation of the degree of conversion percentage was done from the aliphatic carbon-to-carbon double bond at 1638 cm^{-1} and normalized against the aromatic component group at 1608 cm^{-1} using the following formula:[16]

DC% = {1- [Caliphatic / Caromatic] / [Ualiphatic/ Uaromatic]} × 100

Where Caliphatic = absorption peak at 1638cm^{-1} of the polymerized specimen,

Caromatic = absorption peak at 1608cm^{-1} of the polymerized specimen

Ualiphatic = absorption peak at 1638cm^{-1} of the unpolymerized specimen and

Uaromatic = absorption peak at 1608cm^{-1} of the unpolymerized specimen.

Shear bond strength of teeth to brackets 'surfaces

Sample's preparation

The sample size was calculated using online sample size calculator (https://clincalc.com/stats/ samplesize.aspx) based on the mean of the antimicrobial activity of Vidal et al [17] with a level of alpha error of 5% and a study power of 95%. The minimal sample size entailed for this study was calculated to be 25 subjects for each group. To account for any probable drop out; a total sample of 30 subjects was decided to be enclosed in each group.

The premolar teeth had normal buccal surface morphology and were atraumatically extracted for orthodontic purpose. For shear bond strength test, 90 human premolars (3 groups; 30 each) were enrolled in the study. All teeth were cleaned with fluoride free pumice paste using rubber cups (Addler, India). Then, they were preserved in distilled water at room temperature, which was changed daily before use.

Measuring the base surface area of the brackets

The surface areas of bracket bases were scanned by an extra-oral 3D scanner (DOF Inc., ASD 180323002Q, Korea) taking into account the design of the mesh work. Then, the scanned bases were measured in mm^2 by a special program utilized for 3D digitization of the meshes (MeshLab 2020.03).

Bonding procedure

Enamel surfaces were polished with pumice. Then, rinsed with water and dried using air syringe. The enamel was etched by gently dabbing the 37% phosphoric acid solution (Ormco) for 30 sec, rinsed thoroughly with forceful air-water spray and dried until obtaining dull and frosty appearance. A thin, uniform layer of bond was placed on the conditioned enamel using micro-brush.

A small amount of Enlight adhesive was extruded on the bracket base. The bracket was positioned immediately on the tooth surface, adapted to its

correct position and firmly pressed. Then, removal of excess adhesive from the periphery of the bracket base was done. The adhesive was light-cured for 40 seconds using a dental light-curing unit (Bre. lux Power Unit; Bredent GmbH & Co., Senden, Germany) with 1200 mW/cm^2 intensity.

Shear bond strength measurement

For assessing the SBS, the samples were set in the lower jaw of a universal testing (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK) in a way that base of the bracket is parallel to the direction of the chisel shear force.

Samples were positioned to contact the bracket incisal wings and chisel blade pushing force, to obtain the maximal parallel direction of force from the blade to the labial surface of the crown. The specimens were stressed with a cross-head speed of 1 mm/min in an occlusogingival direction. [18]

The force needed for bracket displacement was calculated in newton (N) and shear bond strength was measured in mega pascal (MPa) according to the following formula;

$$SBS\ (MPa) = \frac{Force\ (N)}{Surface\ area\ of\ bracket\ (mm^2)}$$

The teeth were examined after debonding under a stereomicroscope (SMZ 800, Nikon, Japan) at 10× magnification to assess the amount of residual adhesive, according to the adhesive remnant index. The adhesive remnant index scores ranged from 0 to 3 as shown in Table 1: [19]

TABLE (1) The Ranges of Adhesive Remnant Index (ARI) Scores

Score	Criteria
0	No adhesive remained on the enamel.
1	Less than 50% of adhesive remained on the enamel.
2	More than 50% of adhesive remained on the enamel.
3	All adhesive remained on the enamel.

Statistical analysis

Data were entered and analyzed using IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Qualitative data were expressed as number. Quantitative data were expressed as mean ± standard deviation (SD) if normally distributed. Chi-Square test was used for comparison of qualitative data. One Way ANOVA was utilized for comparison of parametric quantitative data for more than two groups.

RESULTS

Antibacterial activity

The mean (SD) of the inhibitory zone diameter for the control adhesive at 24 h was 3.2 (1.1). For the nano-adhesive (AgNPs) group was 28.1 (2.3), while for the nano-adhesive (Cu-O) group, the inhibition zone diameter was 17.8 (2.7). Both nano-adhesive groups showed significantly higher inhibition zone diameter than the control group (P<0.0001), no significant difference as regard inhibition zone was detected between nano-adhesive groups (P= 0.108) (table 2).

TABLE (2): Means (standard deviations) of inhibition zones (mm) of the three groups of the study in agar plate against Streptococcus mutans.

Groups	Means (SD)	df	MS	P Value	F crit
G1 Control	3.2 (1.1)	2	471	<0.0001	5.25
G2 Silver	28.1[a] (2.3)				
G3 CuO	17.8[a] (2.7)				

One way ANOVA. Similar superscripted letters denote non-significant difference.

Degree of convergence

One-sample Kolmogorov-Smirnov tests showed a normal distribution in all groups. The descriptive

data for the degree of convergence of the three study groups are presented in Table 3. The greatest mean degree of convergence was observed in the nano-adhesive Cuo group and the lowest in the control group, All study groups showed insignificant difference (p=0.931).

TABLE (3) Means (standard deviations) of degree of convergence (%) of three groups of the study.

Groups	Means (SD)	df	MS	P Value	F crit
G1 Control	44.33ª (1.38)	2	10.97	0.9314	5.14
G2 Silver	46.19ª (17.37)				
G3 CuO	48.17ª (12.31)				

One way ANOVA. Similar superscripted letters denote non-significant difference.

Shear bond strength

One-sample Kolmogorov-Smirnov tests showed a normal distribution in all groups. The descriptive data for the shear bond strength of the three study groups are presented in Table 4. The greatest mean SBS was detected in the control group followed by the nano-adhesive Cuo group and the lowest was in the nano-adhesive Ag group. All study groups showed insignificant difference (p=0.29).

TABLE (4) Means (standard deviations) of shear bond strength (Mpa) of three groups of the study.

Groups	Means (SD)	df	MS	P Value	F cri
G1 Control	8.95 ª (3.37)	2	7.58	0.29	3.885
G2 Silver	6.70 ª (1.29)				
G3 CuO	7.25 ª (1.24)				

One way ANOVA. Similar superscripted letters denote non-significant difference.

TABLE (5) Adhesive Remnant index (ARI) scores of the study Groups

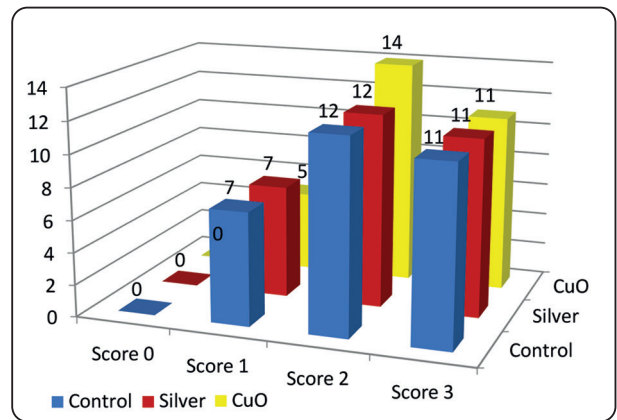|	Score	G1 Control	G2 Silver	G3 CuO	Total
ARI	0	0	0	0	0
	1	7	7	5	19
	2	12	12	14	38
	3	11	11	11	33
	Total	30	30	30	90

Chi square test, P = 0.959

Fig. (1): Adhesive Remnant index (ARI) scores among studied groups.

The results of stereo-microscopic analysis of the three groups are listed in Table 5 & figure 1. Both control and silver nano-adhesive groups showed ARI score 2 in 12 specimens of 30. The CuO nano-adhesive group showed ARI score 2 in 14 specimens out of 30. No significant difference was detected.

DISCUSSION

Orthodontic appliances promote bacterial plaque accumulation and enhance the development of white spot lesions. A prevalent goal in orthodontic treatment is to ameliorate the therapeutic effect by utilizing novel substances that reduce or inhibit dental caries and enhance remineralization simultaneously without compromising the shear bond strength. [20]

Therefore, several antimicrobial agents have been attempted to be added to orthodontic adhesives. One of the antimicrobial agents that have lately gained a lot of interest is metal nanoparticles.[21] Their unique antimicrobial characteristics are related to their high chemical activity, reduced size and associated large surface area / volume ratio. [22] The high surface area of the nanoparticles permits the presence of greater number of atoms at the surface which enables maximal contact with the environment. The reduced size of these nanoparticles allows easier permeation through cell membrane. The positive charges of metal ions are pivotal for the antimicrobial action as they allow electrostatic action between the positive charge of the nanoparticles and the negative charge of the cell membrane of the bacteria. [23] The addition of these metal nanoparticles in orthodontics should not have adverse effect on adhesive SBS or other mechanical properties. [24]

The objective of this study is to compare the effect of incorporation of AgNPs and CuO NPs into a commercial orthodontic adhesive against cariogenic bacteria along with their influence on SBS of stainless steel orthodontic brackets bonded to premolar teeth.

Silver based nanomaterials suppress the bacterial metabolism through the progressive release of Ag^+, Ag^{2+} and Ag^{3+}. These ions have an affinity for several groups, like carboxyl, phosphate, thiol and amino groups, which impedes their biological actions. Firstly, they influence the action of proteins and enzymes. [25]

Few in-vitro studies examined the effect of incorporation of silver nanoparticles into orthodontic adhesives. Jasso-Ruiz1 et al [26] found that addition of AgNPs to orthodontic brackets decreased significantly the adherence of cariogenic streptococcus compared to those without AgNPs.

Sodagar et al [27] reported that 5 and 10 % silver/ hydroxyapatite NPs incorporated in orthodontic adhesive discs induce bacterial growth inhibition zones while insertion of 1% silver/hydroxyapatite

NPs into orthodontic adhesive did not produce the same effect.

Bahador et al [28] revealed that addition of 0.05% AgNPs significantly enhanced the shear bond strength of Nano-Bond adhesive. Conversely, Reddy et al. [29] detected significant reduction in SBS after incorporating 1% AgNPs to the orthodontic adhesive (Transbond). This is in line with Degrazia et al [30] who reported that high concentration of AgNPs decreased the SBS as a result of NPs agglomeration inside the primer, which produces weakness and affects the curing process. On the other hand, Ahn et al. [31] found that orthodontic adhesives composed of 250 ppm and 500 ppm AgNPs displayed antimicrobial effects without affecting the shear bond strength.

Reynolds [32] demonstrated that the minimum adequate bond strength required for most orthodontic demands during routine clinical practice was 5.9–7.8 MPa.

In our study, we added 100 nm (2% w/w) AgNPs into the orthodontic adhesive and assessed the diameter of inhibitory zones at 24 hs. We observed significant antibacterial effect against the tested streptococcus mutans.

The degree of conversion is a substantial parameter in estimating the physical, mechanical and biological characteristics of photo-activated composite resins. It has been reported that composite characteristics tend to be improved by increasing the degree of conversion during photo-polymerization. A suboptimal degree of conversion may be responsible for the inherent interface instability and the decreased interfacial strength. [33] Among different methods for estimation of the degree of conversion of dental composites, Fourier Transform Infra-red Spectroscopy is one of the most widely employed methods due to the numerous sampling techniques and the availability of equipment. In the present study, the degree of conversion of the adhesive containing 100 nm (2% w/w) AgNPs showed non-significant difference

from the control group which reflects no adverse effect on the characteristics of the adhesive.

The findings of the study suggest that SBS revealed non significant decrease after incorporation of silver nanoparticles and it was still above the recommended shear bond strength of 5.9–7.8 MPa.

The Adhesive Remnant Index is one of the most considerably utilized systems in orthodontics, to investigate the amount of adhesive remaining on the tooth after removal of brackets. [34] The aim of this current study was to comparatively analyze the ARI of three adhesives used for bonding steel brackets. The ARI was evaluated under stereomicroscopy with 10× magnification for determination of the fracture mode of specimens. AgNPs group showed ARI score 2 (more than half of the adhesive remained on the tooth) in 12 out of 30 specimens similar to that in the control group which denotes no alteration in the fracture mode.

Copper is a well recognized anti-inflammatory and antimicrobial element which has a long history in medical applications. Few studies were accomplished to investigate the antibacterial mechanism of CuNPs. Three mechanisms were mainly suggested. Firstly, the permeability of the bacterial membrane is changed due to the accumulation of copper NPs. Then, they detach the membrane lipopolysaccharides, proteins and intracellular molecules and produce disturbance in the plasma membrane. Secondly, nanoparticles induce free radicals (in the form of ions or NPs) which cause oxidative damage in the cellular composition. Thirdly, the cell uptake of ions (created by nanoparticles) suppresses intracellular ATP production and DNA replication. [35]

Some studies advocated the addition of CuO NPs with polymers, the same as Ag. Argueta-Figueroa et al. [36] incorporated 0.0100%, 0.0075% and 0.0050% concentrations of nanoparticles and reported that composite containing copper NPs induced significant inhibitory action on the studied microbes. This was in consistency with Toodehzaeim et al [37] who found that adhesives containing 0.01, 0.5 and 1.0 wt. % copper oxide NPs displayed significant antimicrobial effects against Streptococcus mutans and the inhibition zone was not statistically increased by increasing the concentration of copper oxide nanoparticles.

Pourhajibagher et al [38] recorded that the addition of 1% Copper showed antimicrobial activity against Streptococcus mutans and did not affect shear bond strength. On the other hand, Behnaz et al [39] revealed that addition of different percentages of copper oxide (0.01, 0.5, and 1 wt%) not only affect adversely the SBS but also increased it.

In a study of Argueta-Figueroa et al, [36] they concluded that adding 0.0100 wt% Cu nanoparticles into the orthodontic adhesive significantly increased the shear bond strength. Regarding the ARI scores, the resin remnants were higher in the experimental group which revealed that the addition of copper NPs increased the adhesion between enamel and resin.

In the present work, we added 50 nm copper oxide (1% w/w) into the orthodontic adhesive. We find significant antibacterial effect after 24 h against Streptococcus mutans which is a commonly found anaerobic bacterium that presents in the human oral cavity and has a significant contribution to tooth decay. There was no significant decline in the inhibition zone compared to silver group.

The highest degree of convergence was observed in the nano-adhesive Cuo group compared to either silver or control groups. This increase, although non significant, may denote durable dentin bond strength and inherent bond stability.

The bond strength value revealed non significant increase compared to silver NPs group, while non significant decrease compared to the control group and it was still above the minimum requirement that offers clinically acceptable shear bond strength.

According to ARI scores, CuO nano-adhesive group exhibited ARI score 2 in 14 specimens out of 30. This increase, inspite of being non significantly greater than that in nano-silver group, could be related to higher adhesive bond strength.

CONCLUSION

Incorporation of either AgNPs or CuO NPs into orthodontic adhesive exhibited the same antibacterial activity against Streptococcus mutans with no adverse effect neither on the degree of conversion nor shear bond strength nor adhesive remanent index.

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