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Antiadherence and Antimicrobial Properties of Copper Oxide Nanoparticles against *Streptococcus mutans, Staphylococcus aureus,* and *Candida albicans* on Orthodontic Brackets: An in Vitro Study

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Abstract

Aim: This study aimed to investigate the antimicrobial efficacy and anti-adhesive properties of copper oxide nanoparticle (CuONP) coatings applied to metal orthodontic brackets.
Methods: Two hundred forty orthodontic brackets were divided into two groups (n=120 each) to assess antimicrobial activity and microbial adhesion. Each group was further subdivided into three subgroups (n=40) based on the test organism: *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Within each subgroup, 20 brackets were coated with CuONPs, and 20 served as uncoated controls. CuONPs were synthesized via chemical reduction and characterized using UV–Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and zeta potential analysis. The dip-coating technique was employed for bracket coating. Antimicrobial activity was assessed by the agar well diffusion method, and microbial adhesion was evaluated through colony-forming unit (CFU) counts. Statistical analysis was performed using the Wilcoxon signed-rank test, with a significance level of $P<0.05$.
Results: CuONP-coated brackets demonstrated strong antimicrobial activity, with inhibition zones measuring 9.55 ± 1.58 mm for *Staphylococcus aureus*, 5.97 ± 3.89 mm for *Streptococcus mutans*, and 4.37 ± 3.35 mm for *Candida albicans* ($P=0.001$). Coated brackets also exhibited a significant reduction in microbial adhesion, with CFU counts decreasing from 714.55 ± 160.90 to 201.10 ± 66.16 for *Staphylococcus aureus* and from 474.40 ± 93.13 to 171.20 ± 84.54 for *Candida albicans* ($P<0.001$).
Conclusion: CuONP-coated orthodontic brackets exhibited potent antimicrobial and anti-adhesive effects against key oral pathogens. These findings suggest that CuONP surface modification could serve as an effective strategy to reduce biofilm formation, enhance oral hygiene, and minimize the risk of white spot lesions during fixed orthodontic treatment.
Keywords: Candida Albicans, Copper Oxide Nanoparticles, Orthodontic Brackets, Staphylococcus Aureus, Streptococcus mutans

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Background

Fixed orthodontic appliances, including brackets, wires, and bands, pose challenges for maintaining oral hygiene due to their complex design and increased surface area, which promote plaque accumulation. Their irregular shapes hinder natural self-cleansing by saliva, lips, tongue, and cheeks, creating a favorable environment for microbial colonization. This condition increases the risk of early caries and white spot lesions, affecting both the esthetics and the integrity of the enamel (1).

Studies have shown that fixed appliances are associated with elevated levels of *Streptococcus mutans*, a key contributor to dental caries (2–4). *Streptococcus mutans*, a Gram-positive bacterium, thrives in biofilms on tooth surfaces and produces acids through carbohydrate metabolism, leading to enamel demineralization and white spot lesions. Orthodontic brackets exacerbate this by providing surfaces that are conducive to bacterial adhesion

and biofilm formation. Research also highlights the interaction between *Streptococcus mutans* and *Candida albicans*, wherein *Candida albicans* promotes *Streptococcus mutans* growth and biofilm accumulation (5). The co-existence of these organisms enhances cariogenicity and plays a significant role in caries development (6,7). Another notable biofilm-forming pathogen is *Staphylococcus aureus*. It produces polysaccharide intercellular adhesin (PIA), which contributes to biofilm stability and resistance to antimicrobial agents and host defenses, increasing the risk of infection and systemic complications (8).

Preventive strategies, such as fluoride application and antimicrobial mouthwashes, are used to reduce microbial colonization but often lack long-term efficacy and suffer from poor patient compliance (9). This has driven interest in nanotechnology, particularly metallic nanoparticles, as an alternative to traditional antimicrobial agents.

Nanoparticles such as carbon nanotubes, graphene, hydroxyapatite, zirconia, silica, and titania have been extensively applied in dentistry, as their large surface area, strong adhesion to dental tissues, compositional similarity to teeth and bone, tooth-like color, high mechanical strength, excellent biocompatibility, cost-effectiveness, and antibacterial potential collectively enable their use in a wide range of applications, including dental restorations, surface coatings, implant modifications, polishing agents, cavity repair, enamel remineralization, hypersensitivity reduction, caries prevention, and overall enhancement of restorative and preventive dental treatments (10–15). In addition, silver nanoparticles (AgNPs) and copper oxide nanoparticles (CuONPs) have been widely investigated in dentistry, incorporated into restorative materials, prosthetics, implants, composite resins, and cements due to their potent antimicrobial activity, biocompatibility and ability to reduce bacterial colonization, thereby supporting long-term oral health and improving the durability of dental restorations (16–18).

Despite these advantages, the application of copper oxide nanoparticles (CuONPs) in orthodontics remains underexplored. Orthodontic appliances, particularly brackets, are highly susceptible to microbial adhesion and biofilm accumulation, which can lead to white spot lesions, dental caries, and soft tissue complications during treatment. Modifying bracket surfaces with CuONPs could provide sustained antimicrobial effects, reducing bacterial colonization and plaque formation throughout therapy. This presents a novel approach for addressing biofilm-related complications in fixed appliance therapy. Although CuONPs have been studied in restorative and endodontic materials, currently, no published studies are available on the incorporation of CuONPs onto orthodontic brackets. This represents a significant gap in the literature. Therefore, the present study aims to address this gap by evaluating the antimicrobial efficacy and adherence characteristics of CuONPs when incorporated onto orthodontic brackets, focusing on key pathogens, including *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. By exploring this novel application, the study seeks to introduce an innovative strategy to enhance oral health during orthodontic treatment and reduce the risk of microbial complications.

Methods

The study protocol was approved by the Institutional Ethics Committee (Ref. No. IEC/Ortho/27/23), affiliated with Atal Bihari Vajpayee Medical University. In this in vitro study, a total of 240 orthodontic brackets (Signature ECO®, Orthodontic Supplies Ltd, Loughborough, Leicestershire, UK) were selected and divided into two main groups (n=120 each) to evaluate antimicrobial efficacy and microbial adhesion. CapitoliumNewsW01-2RegularEach group was further subdivided into three subgroups (n=40), based on the test organisms: *Streptococcus*

mutans, *Staphylococcus aureus*, and *Candida albicans*. In each subgroup, 20 brackets were coated with copper oxide nanoparticles (CuONPs), while the remaining 20 served as uncoated controls.

Synthesis of CuONPs

Copper oxide nanoparticles were synthesized by chemical reduction using copper (II) sulfate pentahydrate as the precursor and starch as the capping agent. An 0.1-M copper sulfate solution was added to 120 mL of a 1.2% starch solution and stirred vigorously for 30 minutes. Then, 50 mL of 0.2-M ascorbic acid was added while stirring continuously. This was followed by the slow addition of 30 mL of 1-M sodium hydroxide. The mixture was heated at 80°C for 2 hours. The resulting precipitate was filtered and washed three times with deionized water and then with ethanol to remove excess starch, followed by air-drying at room temperature. The dried CuONPs were stored in glass vials for further analysis.

Characterization of Synthesized CuONPs

Multiple techniques were used for characterization.

- **UV-Vis spectrophotometry(Ultraviolet–Visible Spectrophotometry):** DataStream-CE7000 Series spectrophotometer; wavelength range: 200–600 nm
- **SEM (Scanning Electronic Microscope):** FEI Quanta 250 FEG; 20 kV accelerating voltage, 10 mm working distance, magnification: ×5,000–200,000, secondary electron detector
- **XRD (X-ray diffraction):** Bruker D8 Advance diffractometer with Cu Kα radiation (λ=1.5406 Å); scan range 10°–90° 2θ; step size: 0.02°; scan speed: 1°/min
- **FTIR spectroscopy (Fourier Transform Infrared Spectroscopy):** Bruker Alpha FTIR spectrometer; samples prepared as KBr pellets; scanned in the range of 4000–400 cm^{-1}
- **Zeta potential:** Zetasizer Nano ZS device (Malvern Panalytical Company); samples diluted in deionized water at room temperature

Storage and Stability

In the present study, CuONPs were synthesized and preserved in sterile glass vials at 2–8°C until completion of detailed physicochemical characterization. Upon confirmation of their size and properties via characterization, the nanoparticles were subsequently coated onto orthodontic brackets. Zeta potential analysis was performed as one of the characterization methods for CuONPs to check the stability of the suspension. The stability of nanoparticles is crucial in biomedical applications, as it maintains the uniform distribution and consistent antimicrobial activity of the nanoparticles when applied to a metal surface (19).

Coating of CuONPs on Metal Orthodontic Brackets

The CuONPs were subsequently coated onto orthodontic brackets using the dip-coating method. A total of 120 metal brackets were sterilized by immersion in 10% glutaraldehyde solution for 10 minutes, then rinsed thoroughly with sterile distilled water and dried. The dip-coating process involved immersing brackets in a CuONP suspension for 10 minutes to ensure adhesion and uniform nanoparticle distribution. The coated brackets were preserved under controlled, humidity-free conditions to minimize contamination. The synthesis was not repeated, and the nanoparticle size and properties were considered consistent across batches, as confirmed by physicochemical characterization. To further verify and ensure successful coating on the bracket surfaces, SEM analysis was performed at a magnification of ×1,000 with a scale bar of 50 µm to check for the surface topography.

Antimicrobial Tests

Three microbial strains were used: *Streptococcus mutans* (ATCC® 25175-0266P™), *Staphylococcus aureus* (ATCC® 6538-0485P™), and *Candida albicans* (ATCC® 2091-0896P™), all procured from MTCC-CSIR (Chandigarh, India). Antimicrobial activity was assessed using the well diffusion method on nutrient agar plates.

Each strain was cultured in Müller–Hinton broth for 24 hours at 37°C. Microbial suspensions were evenly spread on agar plates. CuONP-coated brackets were placed on the inoculated plates, which were incubated at 37°C for 24 hours. Zones of inhibition (ZOI) were measured in millimetres to assess antimicrobial activity.

Adherence Testing

Microbial adhesion was evaluated using the colony-forming unit (CFU) method. Each bracket was placed in an Eppendorf tube with 1 mL of Müller–Hinton broth and 825 µL of CuONP suspension. Negative controls received 125 µL of deionized water instead of the nanoparticle

suspension. Next, 100 µL of standardized microbial suspensions (*Staphylococcus aureus*, *Candida albicans*) were added. The tubes were sealed and incubated at 37°C for 24 hours.

After incubation, the brackets were transferred to fresh tubes containing 1 mL of PBS and sonicated for 5 minutes to dislodge adherent cells. The suspension was plated on nutrient agar and incubated at 37°C for 48 hours. CFU/mL was determined to quantify adhesion. As *Streptococcus mutans* frequently produces dense and confluent growth at high bacterial loads, colony enumeration was impractical and therefore was not included in the present study.

Statistical Analysis

Data were entered into Microsoft Excel 2007 and analyzed using SPSS 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (means, standard deviations, frequencies, and percentages) were calculated. A significance level of $P<0.05$ was set.

The normality of the data was assessed using the Shapiro–Wilk test, and the homogeneity of variances was evaluated using Levene's test. Depending on the data distribution, intergroup comparisons were carried out using the Mann–Whitney U test. Intragroup comparisons were conducted using Wilcoxon's signed-rank test.

Results

Characterization of CuONPs

Table 1 presents the characteristics of CuONPs used in the present study. The UV–Vis absorption spectrum exhibited a characteristic peak at 379 nm, suggesting nanoparticle size and morphology conducive to vigorous plasmonic activity. The X-ray diffraction (XRD) pattern of the synthesized copper nanoparticles exhibited peaks at 2θ values of 43.64°, 50.3°, and 74.39°, corresponding to the (111), (200), and (220) planes of metallic copper, respectively. These reflections align with the standard JCPDS (Joint Committee on Powder Diffraction Standards) copper file No. 04–0836, confirming the presence of a face-centered cubic (FCC) crystal structure.

As illustrated in Figure 1, the SEM image revealed quasi-spherical Cu2O nanoparticles (20±5 nm). FTIR spectrum confirmed CuO formation, with peaks at 3432 cm^{-1} (O–H), 1631 cm^{-1} (H2O bending), 1072 and 1382 cm^{-1} (Cu–O), and 530 cm^{-1} (monoclinic CuO); minor peaks at 1400–1500 cm^{-1} indicated organic residues. The zeta potential was -28.5 mV. It is well established that nanoparticles with values between +30 mV and -30 mV are considered stable suspensions; hence, -28.5 mV indicates moderate colloidal stability. Such values limit agglomeration, thereby ensuring a stable solution for further applications (20).

SEM Images of Non-coated and CuONP-coated Orthodontic Brackets

SEM images of the non-coated orthodontic bracket and the CuONP-coated orthodontic bracket are illustrated in Figure 2.

Non-coated orthodontic brackets revealed smooth surfaces with visible machining lines indicating the original manufacturing marks. Additionally, minimal surface irregularities were present, and the texture suggested a relatively clean metallic finish with no nanostructured layer.

Table 1: Characteristics of CuONPs used in the study.

UV-Vis Spectrophotometry	FTIR	SEM	XRD	Zeta Potential
Absorption peak at 379 nm	Peaks at 3432, 1631, 1072, 1382, 530 cm^{-1}	Quasi-spherical; average size 20 ± 5 nm	Peaks at 43.4°, 50.3°, 74.39°; FCC; ~20 nm	–28.5 mV

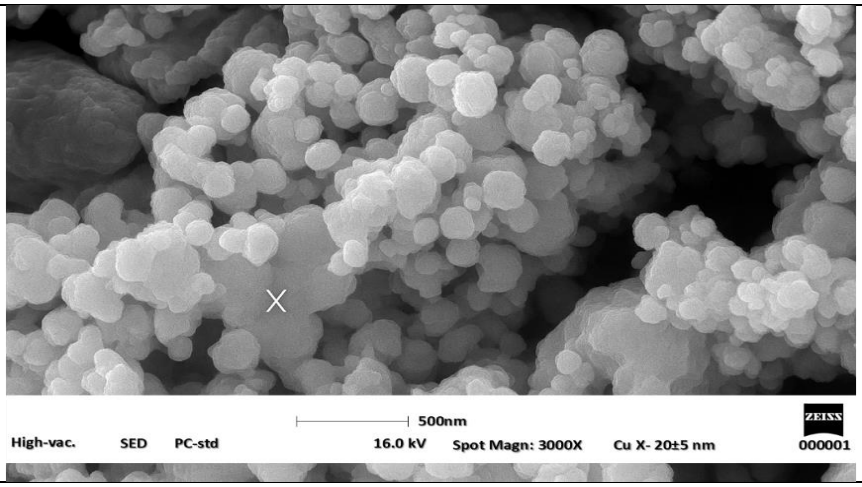


Figure 1: Representation SEM image of multiple fields for Cu2O nanoparticles

CuONP-coated brackets exhibited a rough, granular morphology with distinct clusters, suggesting successful deposition of CuONPs on the bracket surface. The coating obscured the underlying machining lines, confirming that the nanoparticle layer was continuous and adherent to the surface.

Antimicrobial Activity

Intragroup comparison:

The antimicrobial activity of the CuONP-coated brackets was confirmed by the presence of growth inhibition zones of *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* around the substrates, as illustrated in Figure 3. According to Table 2, *Staphylococcus aureus* showed the widest inhibition zone (9.55±1.58 mm; 95% CI: 8.81–10.29), followed by *Streptococcus mutans* (5.97±3.89 mm; 95% CI: 4.15–7.80) and *Candida albicans* (4.37±3.35 mm; 95% CI: 2.80–5.95). The calculated effect sizes indicated that CuONP coatings possess strong inhibitory potential and statistically significant inhibition zones against all tested microorganisms.(Figure 4)

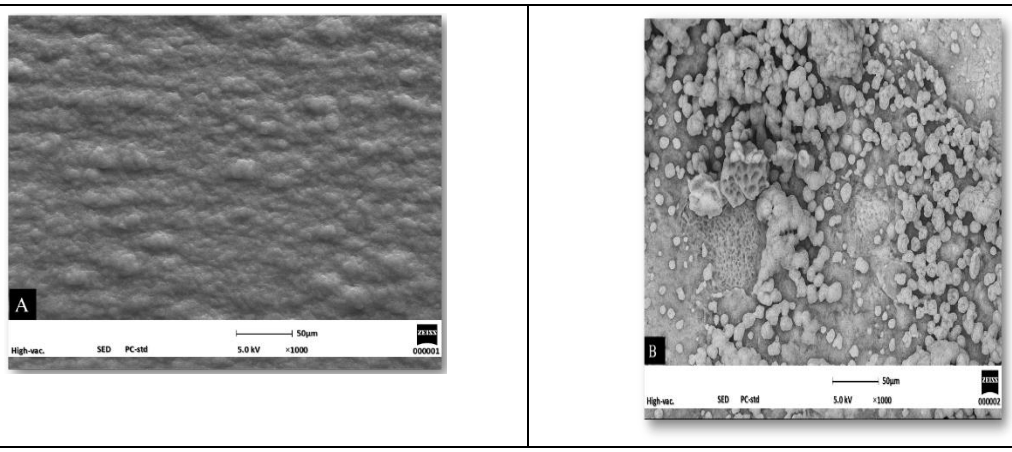


Figure 2: SEM image of A) Non-coated orthodontic bracket
B) CuONPs-coated orthodontic bracket

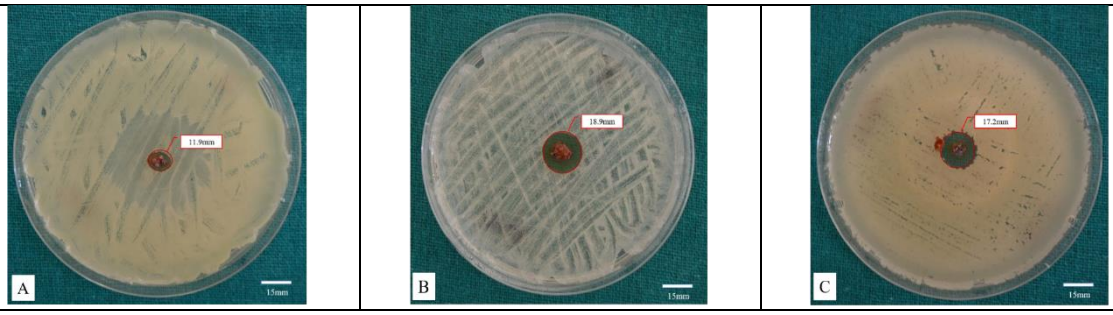


Figure 3 : Inhibition zone of growth against CuONP-coated brackets A) *Streptococcus mutans,* B) *Staphylococcus aureus* and C) *Candida albicans* (scale bar, 15mm)

Table 2: Intragroup comparison of mean ±SD, median and 95% confidence interval of inhibition zone for non-coated and CuONP-coated brackets on three different bacterial culture plates*

Microorganism	N	Mean ± Standard Deviation(mm)	Median (IQR) (mm)	Standard Error	95% Confidence Interval for Mean(mm)		**P-value	Effect size
					Lower Bound	Upper Bound		
Streptococcus mutans	20	5.97±3.89	5.0 (3.0–8.0)	0.870	4.15	7.80	0.001	1.53
Staphylococcus aureus	20	9.55 ±1.58	10.0 (9.0–11.0)	0.353	8.81	10.29	0.001	6.04
Candida albicans	20	4.37±3.35	4.0 (2.0–6.0)	0.750	2.80	5.95	0.001	1.30

* 0.00 was considered for the non-coated group which indicates no measurable inhibition zone
**Mann-Whitney U test

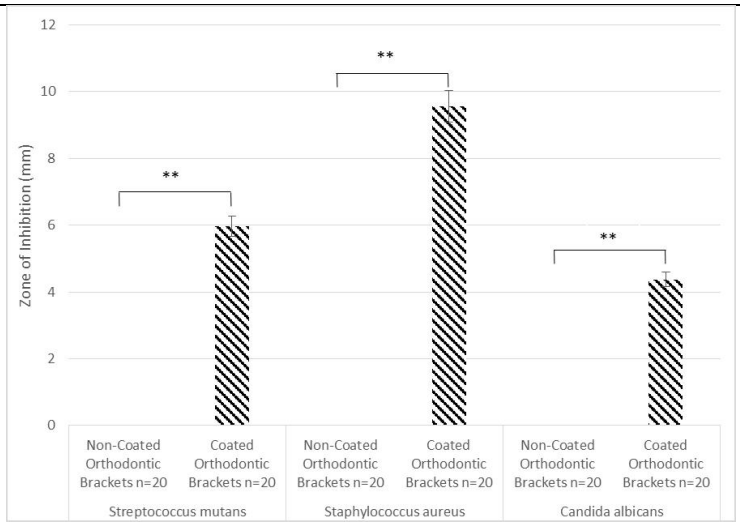


Figure 4: Comparison of inhibition zone for coated and non-coated orthodontic brackets against *Streptococcus mutans, Staphylococcus aureus and Candida albicans*.
(**p =0.001)

Intergroup comparison: As shown in Table 3, one-way ANOVA followed by post hoc Tukey tests demonstrated significant differences in the inhibition zones between the tested microorganisms. *Staphylococcus aureus* exhibited significantly larger inhibition zones compared to both *Streptococcus mutans* (mean difference = +3.575 mm, P=0.002, 95% CI: 1.21–5.94) and *Candida albicans* (mean difference = +5.175 mm, P<0.001, 95% CI: 2.81–7.54). In contrast, no statistically significant difference was observed between *Streptococcus mutans* and *Candida albicans* (mean difference = +1.600 mm, P=0.241, 95% CI: –0.76 to 3.96). (Figure 5)

Adhesion Testing

As illustrated in Figures (6,7), quantifying *Streptococcus mutans* adhesion was challenging due to its growth pattern. Instead of forming distinct colonies, *Streptococcus mutans* exhibited confluent growth, creating a lawn that hindered the counting of individual colonies.

As shown in Table 4 and illustrated in Figure 8, the anti-adhesive properties of non-coated and coated orthodontic brackets against *Staphylococcus aureus*, revealed significantly higher bacterial adhesion in non-coated brackets (714.55±160.90 CFU/mL, 95% CI: 643.29–785.81) than in coated ones (201.10±66.16 CFU/mL, 95% CI: 171.79–230.41) with a considerable effect size (Cohen's d=4.16). The difference was statistically significant (P=0.001). As shown in Table 5, a similar trend for *Candida albicans*, with non-coated brackets exhibiting higher fungal adhesion (474.40±93.13 CFU/mL, 95% CI: 433.17–515.63) compared to coated brackets (171.20±84.54 CFU/mL, 95% CI: 133.75–208.65), with a considerable effect size (Cohen's d=3.41). This difference was also statistically significant (P=0.001). (Figures 9,10)

Table 3: Intergroup comparisons of mean± SD, median and 95% confidence interval of inhibition zones for non-coated and CUONP-coated brackets among three different microorganisms

(I) Group	(J) Group	Mean Difference (I-J)	Standard Error	*P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Streptococcus mutans	Staphylococcus Aureus	-3.575	0.982	0.002	-5.94	-1.21
	Candida Albicans	1.600	0.982	0.241	-0.76	3.96
Staphylococcus Aureus	Streptococcus mutans	3.575	0.982	0.002	1.21	5.94
	Candida Albicans	5.175	0.982	< 0.001	2.81	7.54
Candida Albicans	Streptococcus mutans	-1.600	0.982	0.241	-3.96	0.76
	Staphylococcus Aureus	-5.175	0.982	< 0.001	-7.54	-2.81

* Tukey HSD test.

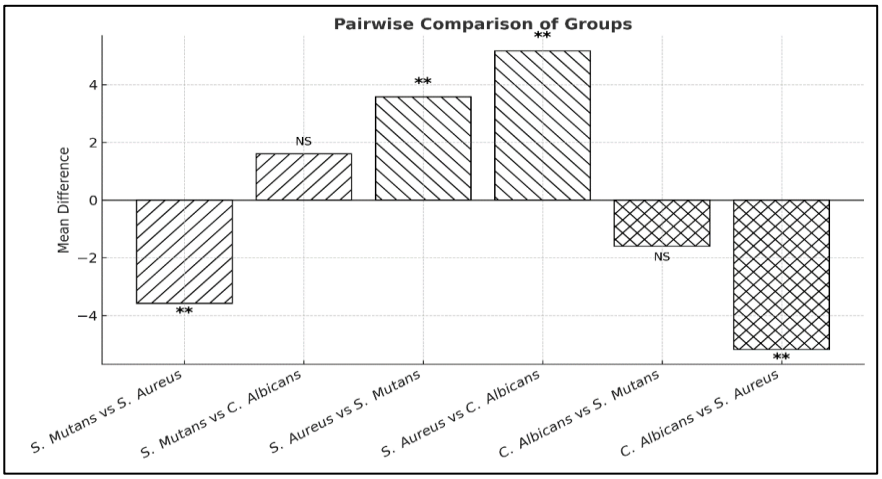


Figure 5: Mean differences between *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans*. (**p = 0.001, NS=Not Significant)

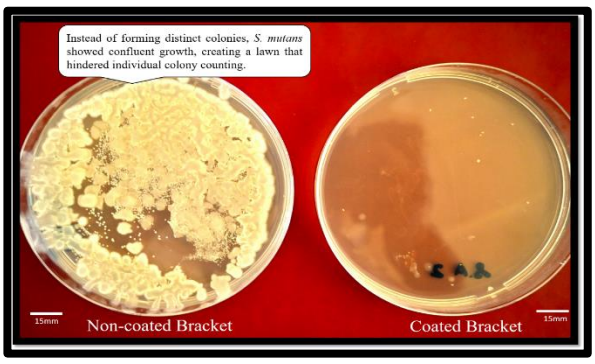


Figure 6: Agar plates showing bacterial colonies of *Streptococcus mutans* against non-coated and coated orthodontic brackets (scale bar, 15mm)

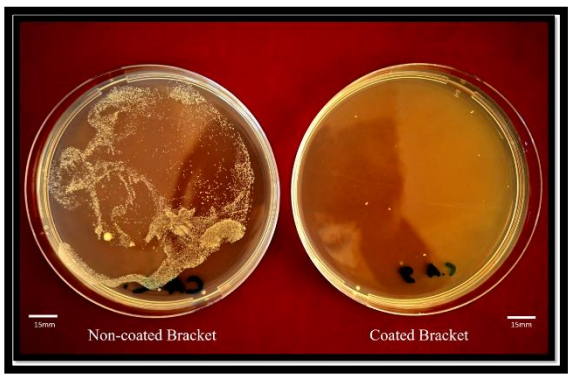


Figure 7 : Agar plates showing bacterial colonies of *Staphylococcus aureus* against non-coated and coated orthodontic brackets (scale bar, 15mm)

Table 4: Comparison of mean± SD, median and 95% confidence interval of inhibition zones for *Staphylococcus aureus* between non-coated and CUONP-coated brackets

	Mean ± Standard Deviation (CFU/mL)	Median (IQR)	Standard Error	95% Confidence Interval		*P value	Effect size
				Lower Bound	Upper Bound		
Non-Coated Orthodontic Brackets	714.552 ± 160.901	710.5(629.5–853.5)	35.978	643.29	785.81	<0.001	4.16
Coated Orthodontic Brackets	201.102 ± 66.157	196.5(138.0–268.25)	14.793	171.79	230.41		

*Mann–Whitney U test

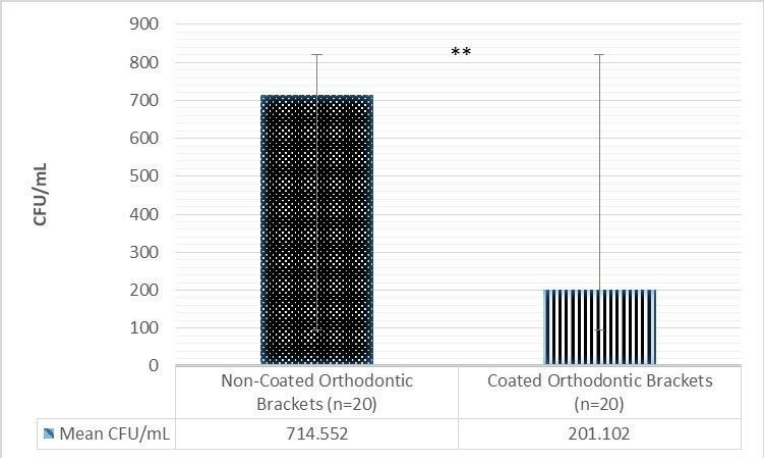


Figure 8: Comparison *Staphylococcus aureus* colonization (CFU/mL) between non-coated and coated orthodontic brackets

** p=0.001

Table 5: Comparison of mean± SD, median and 95% confidence interval of inhibition zones for *Candia Albicans* between non-coated and CUONP-coated brackets

	Mean ± Standard Deviation (CFU/mL)	Median (IQR)	Standard Error	95% Confidence Interval		*P-value	Effect size
				Lower Bound	Upper Bound		
Non-Coated Orthodontic Brackets	474.402 ± 93.133	483.5 (393.0–562.0)	20.825	433.17	515.63	<0.001	3.41
Coated Orthodontic Brackets	171.202 ± 84.543	206.5 (174.25–236.75)	18.904	133.75	208.65		

*Mann-Whitney U test

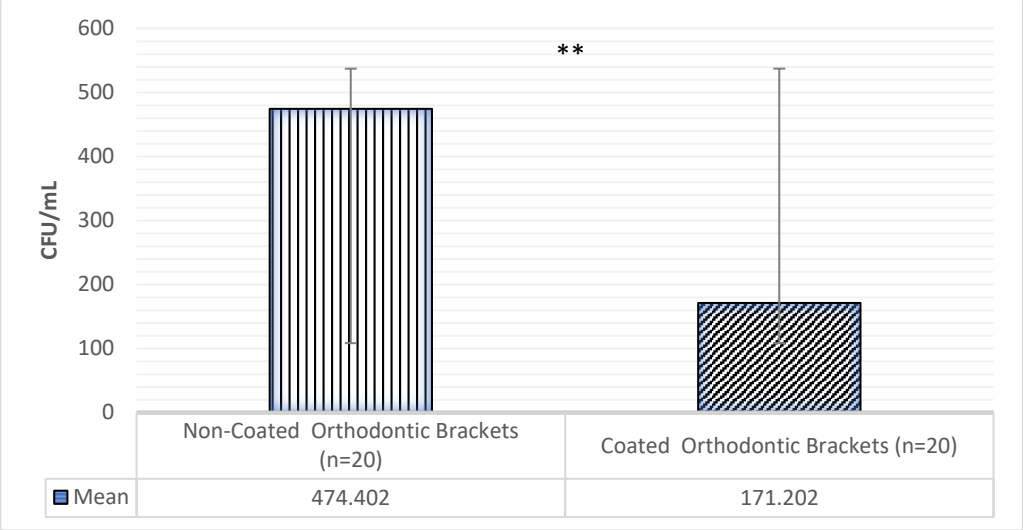


Figure 9: Comparison of mean colony counts of *Candida Albicans* between non-coated and coated orthodontic brackets. (**p = 0.001)

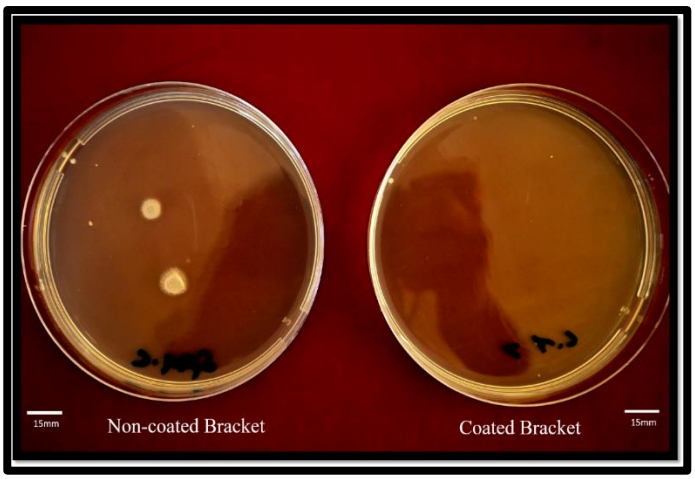


Figure 10: Agar plates showing bacterial colonies of *Candida Albicans* against non-coated and coated orthodontic brackets (scale bar, 15mm)

Discussion

The intricate design of orthodontic appliances promotes plaque retention and biofilm accumulation, hindering effective oral hygiene maintenance and predisposing the enamel surface to demineralization.

Expanding on these findings, researchers have explored strategies such as surface modifications of orthodontic appliances. Despite their proven benefits in dentistry, the incorporation of copper nanoparticles in orthodontics remains insufficiently explored. Various studies have demonstrated the effective antimicrobial properties of silver and zinc oxide nanoparticles when applied to orthodontic materials. Zeidan et al. (20) demonstrated that coatings of silver, zinc oxide, and their combination on orthodontic brackets significantly reduced bacterial colonization. Similarly, Bahrami et al. (21) evaluated silver- and zinc oxide-coated orthodontic bands and found substantial antibacterial effects along with acceptable cell viability.

Additionally, Tristán-López et al. (22) demonstrated that incorporating silver nanoparticles into orthodontic adhesives enhanced their antibacterial activity against oral pathogens. These findings highlight the potential for copper nanoparticles to provide comparable antimicrobial

benefits, although their application on orthodontic brackets remains relatively underexplored and warrants further investigation. The integration of such technologies could revolutionize orthodontic care by reducing the risk of white spot lesions and improving treatment outcomes. As research progresses in understanding microbial interactions with orthodontic appliances, copper nanoparticle coatings hold strong potential in addressing one of the persistent challenges in orthodontics.

As in the present study, the successful synthesis of copper oxide nanoparticles was confirmed through multiple characterization techniques.

UV–Vis spectrophotometry showed a distinct surface plasmon resonance (SPR) peak at 379 nm, which is characteristic of nanoscale copper particles. The appearance of such a peak occurs due to collective oscillations of conduction electrons in response to light, and its presence confirms nanoparticle formation within a short time frame (9 minutes) (23). X-ray diffraction (XRD) analysis exhibited sharp peaks at 2θ=43.4°, 50.3°, and 74.39°, corresponding to the 111, 200, and 220 crystallographic planes, respectively. These reflections were consistent with a face-centered cubic (FCC) crystalline structure of copper, indicating high crystallinity and phase purity (24). Scanning electron microscopy (SEM) revealed quasi-spherical Cu2O nanoparticles with an average size of approximately 20 nm, exhibiting uniform dispersion and minimal agglomeration. Quantitative image analysis indicated a narrow size distribution centered around 45 nm, supporting the reproducibility of the synthesis method (25). Fourier transform infrared spectroscopy (FTIR) confirmed the chemical composition by showing characteristic peaks at 3432 cm^{-1} (O–H stretching of adsorbed moisture), 1631 cm^{-1} (H2O bending), 1072 and 1382 cm^{-1} (Cu–O stretching vibrations), and 530 cm^{-1} (monoclinic CuO), validating the presence of copper oxide bonds (26). Zeta potential analysis revealed a surface charge of –28.5 mV, indicating moderate colloidal stability. Typically, nanoparticles with zeta potentials greater than ±30 mV are considered highly stable, while those between –25 mV and –30 mV have intermediate stability (27). The zeta potential at –28.5 mV lies just below the standard –30 mV threshold; hence, it is considered moderately stable.

After the successful synthesis and characterization of copper oxide nanoparticles (CuONPs), they were applied to metallic orthodontic brackets to assess their antimicrobial and adhesion properties. CuONPs were coated using the dip-coating method, and deposition was confirmed through scanning electron microscopy (SEM), as illustrated in Figure 2(B). SEM analysis revealed that uncoated brackets exhibited relatively smooth surfaces with distinct machining lines. In contrast, CuONP-coated brackets demonstrated a rough, granular morphology with well-distributed nanoparticle clusters, indicating successful and uniform surface deposition. A similar approach was reported by Syed et al. (28), who also employed the safe dip-coating method for orthodontic archwires to deposit nanoparticles, later confirming the coating through SEM analysis. This method enables controlled layering, ensuring optimal surface coverage while preserving the bracket structure and function. The primary objective was to evaluate its potential in reducing microbial adhesion, a key concern in orthodontics due to the risk of plaque accumulation and white spot lesions.

The results revealed significant differences in adhesion levels between microbial species. Statistical analyses showed a substantial reduction in microbial adhesion with CuONP-coated brackets. For *Staphylococcus aureus*, the mean adhesion count on non-coated brackets was 714.552±160.901, which decreased to 201.102±66.157 on coated brackets. The anti-adhesive effect was also evaluated against *Candida albicans*, which is known for its ability to form biofilms on appliances. Colony counts for *Candida albicans* declined, with non-coated brackets showing a mean count of 474.402±93.133, which significantly reduced to 171.202±84.543 on coated brackets. These findings highlight the effectiveness of CuONP coatings in mitigating microbial colonization and their potential in enhancing oral hygiene and reducing infection-related complications in orthodontics.

Similar findings were reported by Espinosa-Cristóbal et al. (29), who demonstrated that silver nanoparticles exhibit strong anti-adhesive activity against *Streptococcus mutans* on orthodontic brackets and wires. Their study showed that AgNPs significantly reduced bacterial adhesion even with orthodontic modules, confirming their effectiveness. Additionally, Jasso-Ruiz et al. (30) reported significantly greater *Streptococcus mutans* adhesion on brackets without silver nanoparticles than on those with silver nanoparticle coatings. Their study indicated that AgNP-coated groups had the lowest bacterial adhesion, further supporting their potential to inhibit bacteria. These findings align with the antimicrobial properties of CuONPs in the present study, reinforcing that nanoparticle coatings can reduce microbial adhesion.

The results also revealed a challenge in

quantifying *Streptococcus mutans* adhesion due to its growth pattern. Instead of forming distinct colonies, *Streptococcus mutans* showed confluent growth, creating a lawn that hindered individual colony counting. The difficulty in quantifying *Streptococcus mutans* by colony counting has been recognized in previous studies. The Dentocult SM strip system was specifically developed to provide categorical scores because *Streptococcus mutans* frequently produces dense, confluent growth at high bacterial loads, rendering colony enumeration impractical (31). Similarly, Yano et al. (32) demonstrated that *Streptococcus mutans* often escapes reliable quantification by standard plate counts due to overgrowth and uneven colony formation. In contrast, quantitative PCR (qPCR) provided more consistent detection. In addition, biofilm investigations by Childers et al. (33) confirmed that *Streptococcus mutans* inherently forms thick, confluent biofilms rather than discrete colonies, underscoring its robust adhesion properties and the limitations of CFU-based methods.

This suggests that *Streptococcus mutans* may have a different adhesion mechanism compared to other microbes, possibly forming a more robust biofilm structure. This inability to obtain precise counts underscores the need for alternative quantification methods, such as biofilm biomass analysis, fluorescence staining, or molecular techniques. Future studies should investigate the factors influencing *Streptococcus mutans* adhesion, including surface roughness, bracket composition, and environmental conditions, to gain a deeper understanding of colonization dynamics and develop more effective antimicrobial strategies.

The antimicrobial efficacy of CuONP-coated brackets was evaluated by measuring zones of inhibition against *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Coated brackets restricted microbial growth, forming clear inhibition zones. Among the tested microorganisms, *c* exhibited the largest inhibition zone, with a mean diameter of 9.55±1.58 mm, indicating its high susceptibility to CuONP-coated brackets. This pronounced antimicrobial effect is likely due to multiple mechanisms, including the disruption of the bacterial cell membrane, inhibition of key enzymatic pathways, and oxidative stress generated through reactive oxygen species (ROS) production upon nanoparticle interaction (34). Intergroup analysis using one-way ANOVA, followed by post hoc Tukey tests, further confirmed that *Staphylococcus aureus* produced significantly larger inhibition zones than both *Streptococcus mutans* (mean difference = +3.575 mm, P=0.002) and *Candida albicans* (mean difference = +5.175 mm, P<0.001), highlighting its greater vulnerability to copper oxide nanoparticles. This observation is consistent with previous reports that Gram-positive bacteria, particularly *Staphylococcus aureus*, are more susceptible to metal oxide nanoparticles due to the structural composition of their thick peptidoglycan cell walls, which facilitates nanoparticle adhesion and penetration, ultimately enhancing oxidative stress-mediated killing (35).

For *Streptococcus mutans*, the mean inhibition zone was (5.97±3.89 mm), indicating moderate susceptibility with notable variability. The inhibition zone was significantly smaller than that of *Staphylococcus aureus* but not significantly different from that of *Candida albicans* (P=0.241), indicating an intermediate antimicrobial response. Similarly, Sims et al. (36) demonstrated that *Streptococcus mutans* biofilms, particularly when in combination with *Candida albicans,* form a dense extracellular polymeric substance (EPS) matrix that restricts penetration of nanochitosan, limiting its antimicrobial action. This EPS-mediated resistance mechanism, as described by Sims et al. (36), is a well-documented barrier that hinders the diffusion of nanoparticles and reduces their bioavailability within mature biofilms, potentially explaining the reduced susceptibility observed in our results.

Candida albicans had the smallest inhibition zone (4.37±3.35 mm), indicating lower sensitivity, possibly due to its eukaryotic cell structure, which is more resistant to antimicrobials (37). The values were significantly lower than those of *Staphylococcus aureus* but not statistically different from those of *Streptococcus mutans*. The lower antifungal effect of CuONPs is consistent with earlier literature, which indicates that fungal cell walls, rich in chitin and glucans, can provide enhanced protection against nanoparticle-induced oxidative stress compared to bacterial cells. This observation is supported by Carmo et al. (38), who reported that CuONPs inhibited *the growth and biofilm formation of Candida albicans* only at higher concentrations.

By addressing microbial adhesion and offering antimicrobial activity, CuONP coatings represent a promising development in orthodontics. Their application may enhance biofilm management and reduce risks associated with fixed appliances. However, beyond antimicrobial efficacy, their biocompatibility and cytotoxic profile are equally critical for clinical translation. Several studies have documented the dose- and size-dependent cytotoxicity of copper nanoparticles (39–43). Consistently, oral cell model studies indicate that the impact of CuO nanoparticles is influenced by

their dose, size, and exposure time. Colloidal CuONP mouth rinses induced apoptosis in gingival fibroblasts at levels similar to those of chlorhexidine and higher than those of other metallic nanoparticles (44).

In contrast, when CuONPs are immobilized on orthodontic brackets, ISO 10993-5 assays on L929 fibroblasts report ≥70% cell viability, classifying them as non-cytotoxic by ISO standards. Copper ion release from these coatings remained well below toxic thresholds for up to 28 days, with hybrid CuO+ZnO coatings demonstrating the most favourable cell viability among the nano-coated groups (45). Collectively, these findings highlight that while colloidal CuONPs in solution may present cytotoxic concerns, their immobilization on orthodontic surfaces substantially enhances biocompatibility while preserving their antimicrobial potential.

The enhanced antimicrobial properties of nanoparticles could complement existing preventive measures, such as fluoride varnishes, sealants, and improved oral hygiene protocols. Importantly, patients with poor compliance in maintaining oral hygiene would benefit most from such passive antimicrobial protection. Future innovations may also explore extending CuONP coatings to other orthodontic components, such as archwires, molar bands, and aligner attachments, thereby further broadening their clinical utility.

The present study is limited to in vitro testing conditions, which do not fully replicate the complexities of the oral environment. Factors such as salivary flow, immune responses, dietary variations, pH fluctuations, and mechanical wear from mastication and oral hygiene practices may significantly influence the antimicrobial performance and long-term stability of CuONP coatings. Future research should focus on clinical trials to evaluate the durability and efficacy of CuONP-coated orthodontic brackets.

From a manufacturing perspective, the large-scale integration of CuONP coatings onto orthodontic brackets would require standardized coating methods, such as dip-coating, sol–gel, or plasma-assisted deposition, to ensure uniformity and cost-effectiveness. The dip-coating method used in this study is a simple and reproducible technique; however, industrial adaptation would require scalability, quality assurance, and material standardization. Manufacturers would also need to ensure that CuONP coatings do not compromise the bracket's mechanical properties, such as torque expression, slot precision, and bond strength, which are critical for treatment efficiency.

Another critical parameter is that nanoparticle-based medical devices must comply with stringent regulatory standards, including ISO 10993 for biocompatibility testing and approvals from agencies such as the Food and Drug Administration (FDA), which require extensive in vivo safety and efficacy data. Therefore, well-structured clinical trials are essential to validate the long-term effectiveness, safety, and clinical applicability of these devices before their routine use in orthodontics.

Overall, while challenges remain, CuONP-coated orthodontic brackets represent a promising advancement in bioactive orthodontics. With further optimization, their integration into mainstream orthodontic practice could reduce microbial colonization, enhance treatment outcomes, and significantly lower the risk of enamel demineralization and WSLs during fixed appliance therapy.

Limitations

The present study has several limitations. First, it was conducted under in vitro conditions that do not fully replicate the complex and dynamic environment of the oral cavity, where factors such as salivary flow, pH fluctuations, temperature changes, dietary habits, and mechanical forces from mastication and toothbrushing may affect the antimicrobial performance and long-term stability of CuONP coatings. Second, the study did not evaluate the cytotoxicity or biocompatibility of the CuONP-coated brackets on oral tissues, which is essential before clinical application. Third, the sample size was limited to laboratory-scale testing, and variability in nanoparticle distribution or coating thickness may occur in large-scale production. Fourth, only three microbial strains—*Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*—were tested, whereas the oral cavity harbors a more diverse microbiome that may influence biofilm formation and antimicrobial resistance. Fifth, the study focused on short-term antimicrobial effects, and the long-term durability, ion release behavior, and potential for nanoparticle leaching over time were not assessed. Finally, the mechanical properties of the coated brackets, such as bond strength, frictional resistance, and torque control, were not evaluated, which are critical for ensuring clinical efficiency and safety. Future in vivo studies and clinical trials are necessary to confirm the long-term efficacy, safety, and practicality of incorporating CuONP coatings into orthodontic practice.

Conclusion

The antimicrobial and anti-adhesive properties of CuONP-coated orthodontic brackets were significantly higher than those of uncoated brackets. The CuONP coatings showed greater inhibition against *Streptococcus mutans, Staphylococcus aureus,* and *Candida albicans*, with the largest inhibition zone observed against *Staphylococcus aureus*. Bacterial adhesion was significantly reduced on coated brackets compared to uncoated ones. Although *Streptococcus mutans* exhibited confluent growth, which limited discrete colony counting, it also suggested an enhanced biofilm-forming ability. These compelling results suggest a paradigm shift in the design of orthodontic appliances. CuONP coatings may mark the beginning of a revolutionary advancement in bioactive orthodontics by dramatically reducing microbial colonization and associated complications. This innovative approach holds transformative potential to elevate infection control standards and redefine preventive strategies in contemporary orthodontic practice.

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None.

Conflicts of Interest

The authors report no conflicts of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request

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