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Associate Professor, V S Dental College and Hospital, Bangalore, Karnataka, India A comparative evaluation of antimicrobial effect on stainless steel molar bands and bondable buccal tubes coated with three different nanoparticles - an *In vitro* study

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#### **Abstract**

Facial appearance is significantly influenced by dental and smile aesthetics, positioning orthodontic treatment as a vital tool in enhancing dentofacial attractiveness. Fixed orthodontic appliances have improved occlusal alignment and smile esthetics, evolving from full banding of teeth to the use of direct bonding techniques with acid-etching and resin adhesives. While molar bands are still common, newer technologies like bondable buccal tubes are gaining popularity. However, fixed appliances create an altered oral environment that promotes plaque accumulation, increases Streptococcus mutans and Lactobacillus counts, and lowers pH, all of which contribute to enamel demineralization and white spot lesions. Initial bacterial adhesion at the bracket adhesive enamel interface is critical in this process. Though conventional preventive methods such as oral hygiene instructions, fluoride treatments, and antimicrobial rinses are used, their success heavily relies on patient compliance. Consequently, there is growing interest in compliance-independent solutions.

Nanotechnology offers promising alternatives. Nanoparticles exhibit unique antibacterial properties due to their small size and large surface area. Silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), and calcium carbonate nanoparticles (CaCO<sub>3</sub> NPs) have shown significant antimicrobial activity and biocompatibility. Coating orthodontic components like brackets, bands, archwires, and buccal tubes with these nanoparticles has demonstrated reduced bacterial colonization and plaque formation, thereby potentially minimizing enamel damage during orthodontic treatment.

**Keywords:** Orthodontics, nanoparticles, antibacterial coatings, enamel demineralization, plaque control

## Introduction

A person's facial appearance is closely linked to dental and smile esthetics, making orthodontic treatment an important means of enhancing dentofacial attractiveness. Fixed orthodontic appliances have revolutionized treatment by improving occlusal relationships and smile esthetics. Initially, all teeth were banded, but the introduction of acid-etching and resin adhesives enabled direct bonding of brackets to incisors, canines, and premolars. Despite the increasing use of bondable buccal tubes, molar bands remain common in clinical practice.

However, fixed appliances alter the oral environment, promoting plaque accumulation, increased *Streptococcus mutans* and *Lactobacillus* counts, and lowering pH, thereby predisposing to enamel demineralization and white spot lesions. The initial adhesion of bacteria to the bracket-adhesive-enamel interface is a critical step in this process. Although oral hygiene instructions, fluoride-releasing materials, and antimicrobial rinses have been used, their effectiveness is limited and highly dependent on patient compliance. Hence, strategies requiring minimal cooperation are desirable.

Nanotechnology has emerged as a promising solution. Nanoparticles, due to their small size, high surface area, and unique physicochemical properties, possess enhanced antibacterial potential when coated onto orthodontic materials. Silver nanoparticles (AgNPs) are among the most studied, demonstrating broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, including *S. mutans*. Zinc oxide nanoparticles (ZnO NPs) also

Correspondence Author: Dr. Prajwal S V S Dental College and Hospital, Karnataka, India exhibit antimicrobial, UV filtering, and biofilm-inhibiting properties, with excellent biocompatibility. Similarly, calcium carbonate (CaCO<sub>3</sub>) nanoparticles are safe, bioresorbable, and pH-sensitive, making them useful in controlled release and biomedical applications. Applying such coatings to orthodontic components archwires, brackets, bands, and buccal tubes has been shown to reduce bacterial colonization and plaque accumulation, thereby minimizing gingival irritation and enamel decalcification. Although previous studies have evaluated individual nanoparticles, comparative data on silver, zinc oxide, and calcium carbonate coatings on orthodontic bands and buccal tubes remain scarce.

This study aimed to evaluate and compare the antibacterial properties of nano-Ag, nano-ZnO, and nano-CaCO<sub>3</sub> coated orthodontic bands and buccal tubes. Such an approach may provide a novel method of reducing microbial colonization around fixed appliances, thereby enhancing oral health outcomes during orthodontic treatment.

**Null Hypothesis:** There is a statistically significant difference in the antimicrobial activity of the molar bands and buccal tubes coated using three different nanoparticles.

**Sample size of estimation:** Sample size estimation was done using G POWER version 3.1.9. The effective size is assumed to be 0.723. With a conventional alpha level of 0.05 and a desired power of 80%, the total sample size will be 64. The sample size of estimation for this comparative study is 64(16 in each group). The design of sampling is convenience sampling.

Materials and methodology: This study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Vokkaligara Sangha Dental College and Hospital, Bengaluru in association with Dextrose Technologies Pvt Ltd, Bengaluru.

**Procurement of Materials:** Silver, Zinc oxide, and Calcium carbonate nanoparticles, thirty-two stainless steel molar bands and thirty-two buccal tubes were procured.

**Preparation of Nanoparticle Suspension:** Nanoparticle suspension were prepared by mixing 0.1gm of powder with 3 ml of acetone.

**Setup of ESAVD Equipment:** The electrostatic spray nozzle was assembled and connected to the power supply for electrostatic charging. Connect the syringe pump to the spray nozzle. Position the molar bands and buccal tubes on the heating substrate, ensuring they are spaced 3 cm from the nozzle. Set the syringe pump to deliver the nanoparticle solutions at a flow rate of 10 mL/hr.



Fig 1: Electrostatic spray-assisted vapor deposition, Hindhi Vac,

**Deposition Process:** Start the heating substrate to maintain the desired temperature for the deposition process. Begin electrostatic charging and initiate the spray process by activating the syringe pump. Spray each nanoparticle solution separately onto the molar bands and buccal tubes, ensuring an even coating. Monitor the deposition process to maintain consistent spray and coverage.

**Post-Deposition Treatment:** Allow the molar bands and buccal tubes to cool down after the deposition process. Inspect the coated samples for uniformity and adherence of the nanoparticle layer.

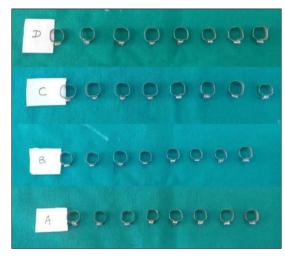


Fig 2: Molar Bands

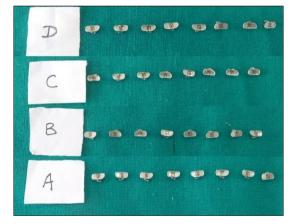


Fig 3: Bondable Buccal Tubes



Fig 4: SEM with monitor

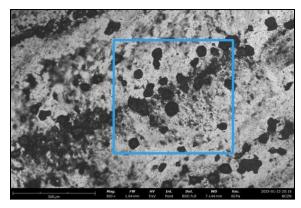


Fig 5: Molar band under SEM

Characterization Using SEM and EDS: Prepare the coated buccal tube and buccal cap samples for SEM analysis. Use SEM [Thermo scientific, Phenom ProX] to obtain high-resolution images of the surface morphology of the nanoparticle-coated samples. Perform Phenome ProX EDS analysis to determine the elemental composition and distribution of the nanoparticles on the surface.

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	О	Oxygen	31.240	12.100
13	Al	Aluminum	6.280	4.100
14	Si	Silicon	2.353	1.600
26	Fe	Iron	52.441	70.900
28	Ni	Nickel	4.716	6.700
29	Cu	Copper	2.275	3.500
30	Zn	Zinc	0.695	1.100

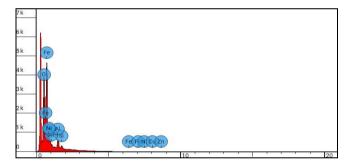


Fig 6: Energy Dispersive X-ray Spectroscopy

## Presence of Zn

**Adhesion Evaluation:** The adhesion of coated bands and tubes will be evaluated by immersing them in artificial saliva for 30 days. The bands will then be brushed with a soft toothbrush using distilled water twice daily for 30 days. FESEM will be used to confirm the presence of nanoparticles.

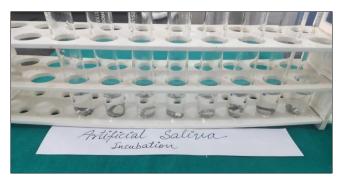


Fig 7: Incubation in artificial Saliva





Fig 8 and 9: Molar bands and buccal tubes brushed using a soft toothbrush

**Microbial Incubation:** *Streptococcus mutans* were incubated in BHI media, *Lactobacillus acidophilus* in MRS media under optimum conditions. *Candida albicans* was incubated in Sabouraud Dextrose broth (SDB) at 37°C for 24 - 48 hours.

Inoculation and Dilution: Molar bands and buccal tubes were introduced into tubes containing microbial suspensions standardized to a 0.5 McFarland concentration and incubated for 48 hours. After incubation, the bands and tubes were washed under aseptic conditions with 1 mL of normal saline. They were then placed in tubes containing 1 mL of sterile brain-heart infusion (BHI) broth and vortexed at high speed for 1 minute. The resulting suspension was serially diluted, and a 100 μL sample was spread plated onto respective agar media (SDA for fungi and BHI for bacteria).

**Incubation and CFU Calculation:** The plates were incubated under appropriate conditions. After 24-48 hours, the colony-forming units (CFU) per millilitre (CFU/mL) for each sample were calculated.







Fig 10: Incubation and CFU Calculation

## Results

Data was entered in the excel spreadsheet. The data was analyzed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics was used to describe the values of the colony count for each microbial strain in the three groups (colony forming units/mL). One-way variance analysis, repeated-measures analysis, and the post hoc Games-Howell and Tukey tests were used to analyze the data. The level of significance was set at p<0.05.

Table 1: Descriptive statistics

Component	Organism	Mean (x10 <sup>5</sup> )	Standard Deviation	Lower limit	Upper limit
	Streptococcus mutans	98.75	7.924	92.13	105.37
Control uncoated Molar band	Candida albicans	112.50	3.464	109.60	115.40
	Lactobacillus acidophilus	100.75	6.042	95.70	105.80
	Streptococcus mutans	62.75	5.651	58.03	67.47
Control uncoated Buccal Tube	Candida albicans	176.25	13.360	165.05	187.42
	Lactobacillus acidophilus	101.50	5.398	96.99	106.01
	Streptococcus mutans	32.50	10.351	23.85	41.15
Ag NP coated Molar band	Candida albicans	21.00	9.196	13.31	28.69
	Lactobacillus acidophilus	91.13	5.793	86.28	95.97
	Streptococcus mutans	45.00	14.142	33.18	56.82
Ag NP coated Buccal Tube	Candida albicans	37.38	6.232	32.16	42.59
-	Lactobacillus acidophilus	93.00	5.657	88.27	97.73
	Streptococcus mutans	96.25	7.778	89.75	102.75
ZnO coated Molar band	Candida albicans	40.13	5.817	35.26	44.99
	Lactobacillus acidophilus	96.50	7.211	90.47	102.53
	Streptococcus mutans	94.38	6.696	88.78	99.97
ZnO coated Buccal Tube	Candida albicans	60.38	6.567	54.88	65.87
	Lactobacillus acidophilus	97.00	10.515	88.21	105.79
	Streptococcus mutans	94.63	8.383	87.62	101.63
CaCO3 coated Molar band	Candida albicans	120.88	4.970	116.72	125.03
	Lactobacillus acidophilus	93.63	6.479	88.21	99.04
0.002	Streptococcus mutans	95.63	8.733	88.32	102.93
CaCO3 coated	Candida albicans	167.38	11.148	158.06	176.69
Buccal Tube	Lactobacillus acidophilus	94.13	8.526	87.00	101.25

Table 2: Comparison of MOLAR BAND among the different materials among the different organisms (One - way ANOVA)

Component	Organism	Mean (x10 <sup>5</sup> )	Standard Deviation	Lower limit	Upper limit	P value
Streptococcus mutans	Uncoated	98.75	7.924	92.13	105.37	0.02
	Ag NP coated	32.50	10.351	23.85	41.15	
	Zn O coated	96.25	7.778	89.75	102.75	
	CaCO3 coated	94.63	8.383	87.62	101.63	
Candida albicans	Uncoated	112.50	3.464	109.60	115.40	
	Ag NP coated	21.00	9.196	13.31	28.69	0.01
	Zn O coated	40.13	5.817	35.26	44.99	
	CaCO3 coated	120.88	4.970	116.72	125.03	
Lactobacillus acidophilus	Uncoated	100.75	6.042	95.70	105.80	
	Ag NP coated	91.13	5.793	86.28	95.97	0.04
	Zn O coated	96.50	7.211	90.47	102.53	0.04
	CaCO3 coated	93.63	6.479	88.21	99.04	

The "uncoated" variants serve as a baseline for comparison against the coated molar bands. A P-value of 0.02, 0.01, 0.04 for the uncoated Streptococcus mutans, Candida albicans, and Lactobacillus acidophilus suggests a statistically significant difference compared to the coated variants.

**Streptococcus mutans (Figure 1)**: The Ag NP (Silver Nanoparticles) coating notably reduces the mean count of Streptococcus mutans compared to the uncoated group. The mean drops from 98.75 to 32.50. ZnO (Zinc Oxide) and CaCO3 (Calcium Carbonate) coatings have a relatively

minor impact on Streptococcus mutans, with mean values of 96.25 and 94.63, respectively, compared to the uncoated mean of 98.75

Candida albicans (Figure 2): Similar to Streptococcus mutans, the Ag NP coating substantially reduces the mean count of Candida albicans (from 112.50 to 21.00). The ZnO coating also reduces the mean count of Candida albicans, but not as drastically as the Ag NP coating (from 112.50 to 40.13). Interestingly, the CaCO3 coating appears to increase the mean count of Candida albicans slightly, from 112.50 in the uncoated group to 120.88

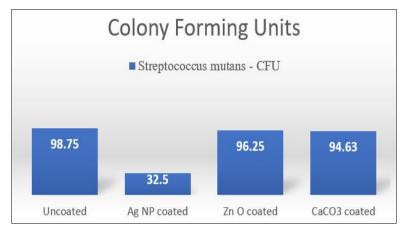


Fig 11: Distribution of Streptococcus mutans in Molar Band among different materials

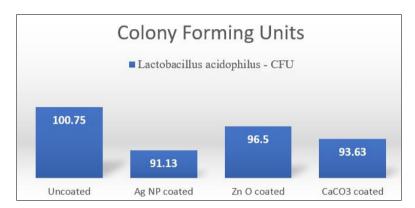


Fig 12: Distribution of Candida albicans in Molar Band among different materials

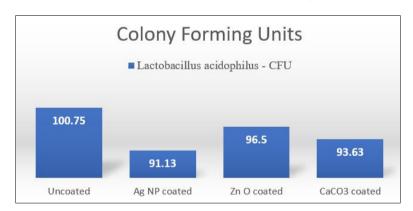


Fig 13: Distribution of Lactobacillus acidophilus in Molar Band among different materials

**Lactobacillus acidophilus (Figure 13):** The coatings appear to have a less pronounced effect on Lactobacillus acidophilus compared to the other two organisms. The mean

counts for all coated variants (Ag NP, Zn O, and CaCO3) are close to the uncoated mean of 100.

Table 3: Comparison of BUCCAL TUBE among the different materials among the different organisms (One - way ANOVA)

Component	Organism	Mean (x10 <sup>5</sup> )	<b>Standard Deviation</b>	Lower limit	Upper limit	P value
	Uncoated	62.75	5.651	58.03	67.47	
	Ag NP coated	45.00	14.142	33.18	56.82	0.02
Streptococcus mutans	Zn O coated	94.38	6.696	88.78	99.97	0.02
	CaCO3 coated	95.63	8.733	88.32	102.93	
	Uncoated	176.25	13.360	165.05	187.42	0.01
Candida albicans	Ag NP coated	37.38	6.232	32.16	42.59	
	Zn O coated	60.38	6.567	54.88	65.87	
	CaCO3 coated	167.38	11.148	158.06	176.69	
	Uncoated	101.50	5.398	96.99	106.01	
Lactobacillus acidophilus	Ag NP coated	93.00	5.657	88.27	97.73	0.04
	Zn O coated	97.00	10.515	88.21	105.79	0.04
	CaCO3 coated	94.13	8.526	87.00	101.25	

The "uncoated" variants serve as a control for comparison against the coated buccal tube variants. A P-value of 0.00\* for the uncoated *Streptococcus mutans*, *Candida albicans*,

and *Lactobacillus* acidophilus suggests a statistically significant difference compared to the coated variants.

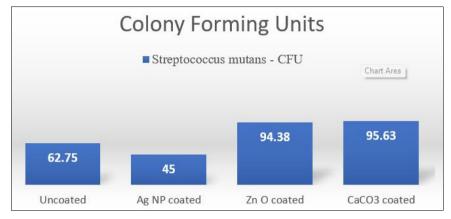


Fig 14: Distribution of Streptococcus mutans in Buccal Tube among different materials

**Streptococcus mutans** (**Figure 14**): The Ag NP (Silver Nanoparticles) coating reduces the mean count of *Streptococcus mutans* compared to the uncoated group (62.75 to 45.00). ZnO (Zinc Oxide) and CaCO3 (Calcium Carbonate) coatings increase the mean count of *Streptococcus mutans* compared to the uncoated group, with mean values of 94.38 and 95.63, respectively.

Candida albicans (Figure 15): Similar to *Streptococcus mutans*, the Ag NP coating reduces the mean count of *Candida albicans* (from 176.25 to 37.38). The Zn O coating also reduces the mean count of *Candida albicans*, but not as drastically as the Ag NP coating (from 176.25 to 60.38). The CaCO3 coating reduces the mean count of *Candida albicans* slightly, from 176.25 in the uncoated group to 167.38.

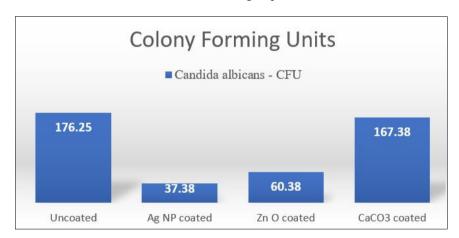


Fig 15: Distribution of Candida albicans in Buccal Tube among different materials

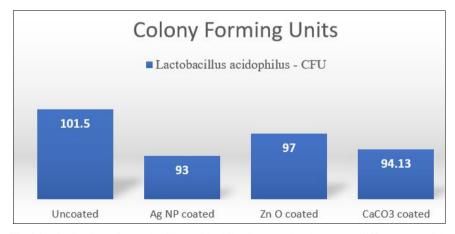


Fig 16: Distribution of Lactobacillus acidophilus in Buccal Tube among different materials

**Lactobacillus acidophilus (Figure 16):** The coatings appear to have little effect on *Lactobacillus acidophilus* compared to the other two organisms. The mean

counts for all coated variants (Ag NP, Zn O, and CaCO3) are close to the uncoated mean of 101.50.

**Table 4:** Pairwise comparison of antimicrobial effect of different materials among the four groups using the post hoc Tukey HSD & Games - Howell

Molar Band (Pair)	Mean Difference	p Value
Uncoated Vs Ag NP	66.25	0.00*
Uncoated vs Zn O	2.500	0.94
Uncoated vs CaCO3	4.125	0.778
Ag NP vs Zn O	-63.750	0.00*
Ag NP vs CaCO3	-62.125	0.00*
Zn O vs CaCO3	1.625	0.982

**Table 5:** Pairwise comparison of antimicrobial effect of different material among the four groups using the post hoc Tukey HSD & Games - Howell

Buccal Tube (Pair)	Mean Difference	p Value
Uncoated Vs Ag NP	17.75	0.004
Uncoated vs Zn O	-31.63	0.002
Uncoated vs CaCO3	-32.88	0.002
Ag NP vs Zn O	-49.38	0.001
Ag NP vs CaCO3	-50.625	0.002
Zn O vs CaCO3	-1.250	0.99

#### Discussion

Molar bands and buccal tubes are indispensable components of fixed orthodontic appliances, providing stability and control during treatment. However, their complex morphology creates retentive sites for food debris and bacterial colonization, making them major contributors to plaque accumulation, gingivitis, enamel demineralization, and caries during orthodontic therapy. Conventional preventive strategies such as fluoride application, antimicrobial rinses, and oral hygiene instructions are often insufficient and rely heavily on patient compliance. As a result, nanoparticle coatings on orthodontic appliances have gained attention as a passive, long-term method to inhibit bacterial adhesion and biofilm formation.

In the present study, three nanoparticles silver (AgNPs), zinc oxide (ZnNPs), and calcium carbonate (CaCO<sub>3</sub> NPs) were evaluated for their antibacterial potential when coated on orthodontic bands and buccal tubes. Each demonstrated distinct mechanisms of action and clinical advantages.

## Silver nanoparticles (AgNPs)

Displayed the most potent antibacterial activity. Their effectiveness is attributed to multiple mechanisms: disruption of bacterial cell membranes through interaction with thiol groups, inhibition of DNA replication, and generation of reactive oxygen species (ROS) leading to oxidative stress. The continuous release of silver ions ensures prolonged activity, making them suitable for the extended duration of orthodontic treatment. However, the cytotoxic potential of AgNPs toward human cells remains a concern, particularly at high concentrations or with prolonged exposure. Although the concentrations used in this study fall within biocompatible limits, further work is required to optimize release profiles that balance antibacterial efficacy with tissue safety.

Zinc oxide nanoparticles (ZnNPs): also showed significant antibacterial action, consistent with earlier studies. Their antimicrobial effect stems from ROS generation, membrane damage, and enzyme inhibition, disrupting bacterial metabolism and protein synthesis. A unique advantage of ZnNPs is their ability to promote

enamel remineralization by slowly releasing zinc ions, which facilitate calcium and phosphate deposition. This dual action antibacterial and remineralizing makes ZnNPs particularly useful for patients prone to white spot lesions. Compared with AgNPs, ZnNPs carry a lower toxicity risk, but high concentrations can still adversely affect oral tissues. Careful optimization of ion release and dosage is therefore required before widespread clinical adoption.

Calcium carbonate nanoparticles (CaCO<sub>3</sub> NPs) Demonstrated moderate antibacterial effects, particularly against *Streptococcus mutans*, the primary cariogenic bacterium. Their mechanism may involve altering local pH, reducing bacterial adhesion, and releasing calcium ions that support remineralization of adjacent tooth surfaces. Although less potent than AgNPs or ZnNPs, CaCO<sub>3</sub> NPs are inexpensive, biocompatible, and bioresorbable, making them a promising adjunctive material. Their ability to combine antibacterial action with remineralization benefits highlights their potential as a safe, multifunctional coating material.

When comparing the three nanoparticles, AgNPs were most effective, followed by ZnNPs and then CaCO<sub>3</sub> NPs. Each, however, has unique strengths: AgNPs provide broadspectrum antibacterial protection, ZnNPs offer both antimicrobial and remineralization benefits, and CaCO<sub>3</sub> NPs contribute biocompatibility and long-term mineral support. The clinical choice of coating may thus depend on patient risk profiles: patients with high caries risk may benefit most from ZnNPs or CaCO<sub>3</sub> NPs, whereas patients with recurrent infections may require the stronger antibacterial action of AgNPs.

## Clinical implications

Of nanoparticle coatings are considerable. They may reduce bacterial adhesion and plaque accumulation on orthodontic appliances, lowering the incidence of gingivitis, caries, and white spot lesions. Importantly, they act passively, minimizing dependence on patient compliance, which is often poor in adolescents. Additionally, coatings may improve appliance longevity by resisting bacterial corrosion and reducing maintenance needs.

## Limitations and future directions

Must be acknowledged. This study was conducted in vitro; the oral environment introduces variables such as saliva, dietary factors, and mechanical stresses that may alter nanoparticle performance. The durability of coatings under functional loads and their resistance to wear require further evaluation. Long-term safety also remains a concern, as nanoparticle release and systemic absorption could pose risks, especially in young patients. In vivo studies are therefore essential to confirm biocompatibility, antibacterial effects, and remineralization potential. Cost-effectiveness is another important factor, as additional manufacturing steps may increase appliance costs and affect clinical feasibility. Looking forward, the development of hybrid or multifunctional coatings may provide the most benefit. For example, combining AgNPs with CaCO3 NPs could deliver strong antibacterial protection while simultaneously promoting remineralization. Smart coatings with controlled, staged release profiles initial antibacterial action followed by long-term mineral support could provide comprehensive protection throughout orthodontic treatment.

## Conclusion

The use of molar bands and buccal tubes covered with silver, zinc, and calcium carbonate nanoparticles appears to be a promising strategy to improve the antibacterial effect of orthodontic appliances. Each of the nanoparticles listed above has its own merits: AgNPs produce a strong antimicrobial effect, ZnNPs present both antibacterial and remineralizing features, and CaCO<sub>3</sub> NPs proffer biocompatibility and moderate antibacterial efficacy. These coatings can help achieve better clinical ends by reducing the risk of oral disease associated with orthodontic treatment, however, further research must be conducted to ascertain the efficacy, durability, and safety of incorporation into clinical use. By looking ahead, we may be able to turn a page in orthodontics and offer a safer treatment with longerlasting results by overcoming the constraints of current orthodontic devices and fostering further inquiry regarding particle coatings.

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