RESEARCH PAPER

Evaluation of tensile strength and antibacterial efficacy of nano-coated orthodontic elastomeric ligatures: An In vitro study

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ABSTRACT

Aim::To evaluate and compare the antibacterial efficacy and mechanical properties of orthodontic elastomeric ligatures coated with silver and zinc oxide nanomaterials.

Materials and Methods:

A total of 135 grey elastomeric ligatures were divided into three groups (n=45 each): uncoated (control), silver nanoparticles (AgNP) coated, and zinc oxide nanoparticles (ZnONP) coated. Nanoparticles were synthesized via chemical reduction and applied to the ligatures. Their surface distribution was verified using scanning electron microscopy (SEM). Antibacterial activity against Streptococcus mutans was evaluated using the disc diffusion method. Mechanical properties including peak load, ductility, and ultimate tensile strength were tested at 2, 4, and 8 weeks using a universal testing machine. Data were analyzed with Tukey's post hoc test ($p < 0.05$).

Results::ZnONP coated modules showed the strongest antibacterial activity, followed by AgNP-coated ones. The control group had the least effect. Importantly, the nanoparticle coatings did not compromise the ligature's mechanical performance over time.

Conclusion::Coating orthodontic elastomeric ligatures with AgNPs or ZnONPs significantly improves their antibacterial action without affecting the mechanical properties. ZnONPs, in particular, showed superior and sustained antibacterial activity against Streptococcus mutans.....

Keywords: Elastomeric ligatures, silver nanoparticles, zinc oxide nanoparticles,Antibacterial, Mechanical properties, White spot lesion

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INTRODUCTION

White spot lesions (WSLs) are a common side effect of fixed orthodontic appliances, often caused by poor oral hygiene and plaque buildup around brackets and bands [1]. These early signs of enamel demineralisation can appear as soon as four weeks after appliance placement [2]. While some remineralisation may occur post-treatment, full restoration of enamel is rare [3]. Fixed appliances can also disrupt the oral microbiome, increasing levels of cariogenic bacteria like Streptococcus mutans and raising the risk of tooth decay [4].

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WSL prevalence in orthodontic patients ranges from 25% to 73%, largely due to inconsistent diagnostic criteria [5]. The most significant risk factor remains inadequate oral hygiene, yet this is often compromised by discomfort and poor compliance in the early phases of treatment.

To address these challenges, research has focused on orthodontic materials with built-in antibacterial properties. Among them, elastomeric ligatures offer a unique opportunity. Positioned close to enamel surfaces and routinely replaced, they can serve as a vehicle for sustained, localised antimicrobial delivery, minimising the need for patient compliance. These ligatures are still widely used due to their ease of application, cost-effectiveness, and hygienic advantages [6].

Nanoparticles (less than 100 nm in size) have gained attention for their enhanced surface reactivity and antimicrobial capabilities [7]. Materials like silver and zinc oxide (ZnO) nanoparticles have shown strong antibacterial effects. ZnO, in particular, has demonstrated safety and superior action against S. mutans [8].

Mechanically, elastomeric ligatures must maintain strength and elasticity throughout treatment. However, oral conditions like saliva, temperature changes, and fluoride exposure can weaken them over time [9]. Maintaining mechanical integrity while enhancing antibacterial function is critical for improved clinical outcomes.

This study aims to compare the antibacterial efficacy and tensile strength of silver and zinc oxide nanocoated orthodontic elastomeric modules. The null hypothesis states that nanocoating has no effect on antibacterial and has effect on mechanical properties.

Materials and Methods:

This in vitro study received approval from the Institutional Review Board and the Institutional Ethical Committee of K.S.R. Institute of Dental Science and Research, Tiruchengode (IEC Ref No: 379/KSRIDSR/IEC/2023). Sample size was determined using GPower software (version 3.1.9.7; Heinrich Heine University, Düsseldorf, Germany). Based on a correlation coefficient of 0.60 with $\alpha = 0.05$ and 95% study power, a minimum of 122 samples was required. To accommodate potential sample loss, the number was rounded up to 135, divided equally into three groups:

Group A: Uncoated elastomeric ligatures in artificial saliva

Group B: Silver nanoparticle (AgNP) coated ligatures in artificial saliva

Group C: Zinc oxide nanoparticle (ZnONP) coated ligatures in artificial saliva

Nanocoating of Silver and Zinc Oxide:

Silver and zinc oxide nanoparticles were synthesised via chemical reduction and coated onto orthodontic elastomeric ligatures. AgNPs were produced by reducing silver nitrate ($AgNO_3$) with sodium borohydride ($NaBH_4$), while ZnONPs were formed by reducing zinc sulphate ($ZnSO_4$) with sodium hydroxide ($NaOH$).

A total of 135 grey elastomeric ligatures (3M Modules A1-Grey) were randomly assigned to the three groups. Ligatures in the experimental groups were immersed in the respective precursor solutions, followed by the addition of reducing agents. The reactions were allowed to proceed for 12 hours. A dark brown colour indicated AgNP formation (Figure 1), and a milky white appearance confirmed ZnONP synthesis (Figure 2) [10]. Ligatures were then removed and air-dried. Nanoparticle presence and distribution were verified using scanning electron microscopy (SEM) [10]. Particle sizes were approximately 278.1 nm for AgNPs (Figure 3A,3B,3C) and 289.3 nm for ZnONPs (Figure 4A,4B,4C).

Antibacterial Activity:

The antibacterial efficacy of coated and uncoated ligatures was assessed using agar diffusion tests. Ligatures were stored in artificial saliva for 2, 4, and 8 weeks to simulate oral conditions.

Petri dishes containing nutrient agar were inoculated with a 24-hour culture of Streptococcus mutans, standardised to 0.5 McFarland. Wells were created in the agar, and test samples were placed into them. Gentamicin was used as a positive control. After 24 hours of incubation at 37°C, zones of inhibition were measured to assess antibacterial activity at different time intervals: 2,4 and 8 weeks (Figure 5) [11]. Results were analysed using GraphPad Prism version 6.0.

Mechanical Properties:

To evaluate any changes in mechanical properties, testing was conducted on 10 ligatures from each group using a universal testing machine with a 500 N load cell at a crosshead speed of 100 mm/min [6]. Modules were mounted on a custom jig using two 0.6 mm stainless steel loops to ensure uniform loading (Figure 6). Maximum tensile force and elongation at break were recorded to determine the impact of nanoparticle coatings on mechanical performance.

Results:

The data were organized using Microsoft Excel and analyzed using IBM SPSS Statistics (version 21.0). The Shapiro-Wilk test confirmed that all variables zone of inhibition, peak load, percentage elongation, and ultimate tensile strength were normally distributed, allowing for the use of parametric tests. A one-way ANOVA was performed to compare the means among three groups: uncoated ligatures, silver nanoparticle (AgNP) coated ligatures, and zinc oxide nanoparticle (ZnONP) coated ligatures across multiple time intervals. Tukey's post-hoc test was used for pairwise comparisons when the ANOVA indicated significant differences.

Antibacterial testing revealed a clear difference in efficacy between the groups. ZnONP-coated ligatures consistently demonstrated the greatest antibacterial activity against Streptococcus mutans, followed by AgNP coated ligatures, while uncoated ligatures showed no measurable antibacterial effect. Although AgNP coatings initially exhibited substantial antibacterial properties, a noticeable decline was observed over time. ZnONP coatings maintained a stronger and more consistent antibacterial effect throughout all time points. These findings are detailed in Table 1, with supporting statistical significance confirmed by ANOVA in Table 2.

In contrast, evaluation of mechanical properties showed no statistically significant differences among the three groups at any measured interval. Variables such as peak load, percentage elongation, and ultimate tensile strength remained stable regardless of the nanoparticle coating applied. While slight numerical variations were observed, none were statistically significant. This consistency across all time points suggests that the addition of AgNP or ZnONP coatings did not adversely affect the physical integrity or mechanical performance of the elastomeric ligatures.

Tukey's post-hoc comparisons (summarized in Table 3) further confirmed the absence of significant differences in mechanical outcomes between the coated and uncoated groups. This implies that both silver and zinc oxide nanoparticle coatings are compatible with orthodontic elastomers from a mechanical standpoint.

In summary, ZnONP coated ligatures showed superior and sustained antibacterial efficacy compared to both AgNP coated and uncoated ligatures. While AgNP coatings were initially effective, their antibacterial effect diminished over time. Importantly, both types of nanoparticle coatings preserved the mechanical integrity of the ligatures.

Discussion:

White spot lesions (WSLs) are a common complication associated with fixed orthodontic appliances. These lesions develop due to plaque accumulation, often within the first few weeks of treatment, as the appliances hinder effective oral hygiene [1]. Even after orthodontic therapy, enamel does not fully recover from early demineralisation. Patients frequently face challenges in maintaining oral hygiene immediately after appliance placement, leading to a heightened risk of WSLs [6]. Studies have estimated the prevalence of WSLs in orthodontic patients to range between 25% and 73% [5]. As a preventive strategy, incorporating antibacterial properties into orthodontic materials such as brackets, adhesives, or ligatures—has gained interest, offering protection independent of patient compliance.

Elastomeric ligatures are commonly used in orthodontics and come into close contact with the enamel surface, making them ideal for delivering antibacterial agents [6]. Nanoparticles, due to their high surface area and interaction potential, are effective antimicrobial agents, particularly against Streptococcus mutans and Lactobacillus, the primary bacteria responsible for dental caries. Silver and zinc oxide nanoparticles have shown promising antibacterial effects and are being incorporated into orthodontic products [8]. However, a few studies have evaluated and compared the long-term antibacterial activity and mechanical integrity of ligatures coated with silver (AgNP) and zinc oxide (ZnONP) nanoparticles.

In this study, ZnONP coated elastomeric ligatures demonstrated the strongest and most sustained antibacterial activity against S. mutans throughout an 8-week period. Their antibacterial effect was significantly greater at all time points compared to both AgNP coated and uncoated control ligatures. While AgNP coated ligatures showed effective antibacterial properties at the beginning, their efficacy declined progressively by Week 4 and further by Week 8. The uncoated control group showed no measurable antibacterial activity at any point. These findings suggest that ZnONP coatings offer more reliable, long-term protection against bacterial colonisation in orthodontic applications.

Regarding mechanical properties, there were no statistically significant differences among the three groups at any of the measured intervals. Variables such as peak load, percentage elongation, and ultimate tensile strength remained consistent, regardless of whether the ligatures were coated with nanoparticles or left uncoated. This indicates that neither AgNP nor ZnONP coatings negatively affected the structural integrity or performance of the elastomeric ligatures over time.

Hernández-Sierra et al. [12] previously reported that AgNP's were highly effective against S. mutans due to their low inhibitory concentrations, aligning with the strong antibacterial activity observed in our study at Week 2. However, our findings differed over time, as the AgNP's efficacy declined significantly after Week 4.

Kim et al. [13] observed no significant effect of silver releasing elastomers on S. mutans in plaque samples, which contrasts with our in vitro results showing measurable antibacterial action. Meanwhile, Caccianiga et al. [14] found reductions in plaque and gingival inflammation with silver releasing ligatures, supporting our Week 2 findings.

Kasraei et al. [15] found ZnONPs had greater antibacterial activity than AgNPs, which supports our observation of superior and sustained ZnONP efficacy. Ramazanzadeh et al. [16] also highlighted the effectiveness of ZnO in reducing *S. mutans* growth on brackets. Kamarudin et al. [6] stressed the value of sustained antibacterial protection in orthodontics, which was achieved with ZnONP in this study.

Although this study used a suitable sample size and reliable in vitro methods, clinical validation is necessary to account for the dynamic oral environment. Future research should evaluate these findings under in vivo conditions to confirm the effectiveness and stability of nanoparticle coatings in daily orthodontic use.

Clinical Implication:

Clinically, ZnONP coated elastomeric ligatures present a promising approach to reducing WSL development. Their sustained antibacterial activity across typical orthodontic appointment intervals, combined with unchanged mechanical performance, suggests they could provide practical, patient-independent protection during treatment.

Conclusion:

There was a statistically significant difference in the antibacterial efficacy between the silver and zinc oxide nanocoated elastomeric ligatures and the uncoated group.

Zinc oxide nanoparticles (ZnONPs) demonstrated superior and sustained antibacterial efficacy against Streptococcus mutans throughout the 8-week study period, suggesting their potential as a long-term antimicrobial coating for orthodontic elastomeric ligatures.

The use of ZnO or Ag nanoparticle coatings had **no significant effect** on mechanical properties such as peak load, ductility, or ultimate tensile strength.

Table 1: Comparison of zone of inhibition of S mutans (Antimicrobial assay):

Time of test	**Zone of inhibition**	**Groups**	**N**	**Mean**	**Standard Deviation**	**P value**
Week 2	S. mutans	AgNP	45	15.6	0.14	.000*
		ZnONP	45	18.6	0.14	
		Uncoated	45	0	0.14	
Week 4	S. mutans	AgNP	45	11.6	0.14	.000*
		ZnONP	45	17.45	0.35	
		Uncoated	45	0	0.14	
Week 8	S. mutans	AgNP	45	10.35	0.21	.024*
		ZnONP	45	15.85	0.21	
		Uncoated	45	0	0.21	

* p<0.05 - statistically significant

Table 2: Inter group Comparison of Zone of Inhibition (Tukey Post-Hoc):

Time of test	**Group**	**Mean Difference (I-J)**	**p Value**
			Zone of Inhibition
Week 2	AgNP	ZnONP	.001*
		Uncoated	.042*
	ZnONP	AgNP	.001*
		Uncoated	.001*
	Uncoated	AgNP	.042*
		ZnONP	.001*
Week 4	AgNP	ZnONP	.000*
		Uncoated	.002*
	ZnONP	AgNP	.000*
		Uncoated	.039*
	Uncoated	AgNP	.002*
		ZnONP	.039*
Week 8	AgNP	ZnONP	.037*
		Uncoated	.022*
	ZnONP	AgNP	.037*
		Uncoated	.030*
	Uncoated	AgNP	.022*
		ZnONP	.030*

* p<0.05 - statistically significant.

Table 3: Inter group comparison of peak load, percentage elongation, and ultimate tensile strength of nanoparticle-coated orthodontic modules at different time intervals as against uncoated orthodontic modules:

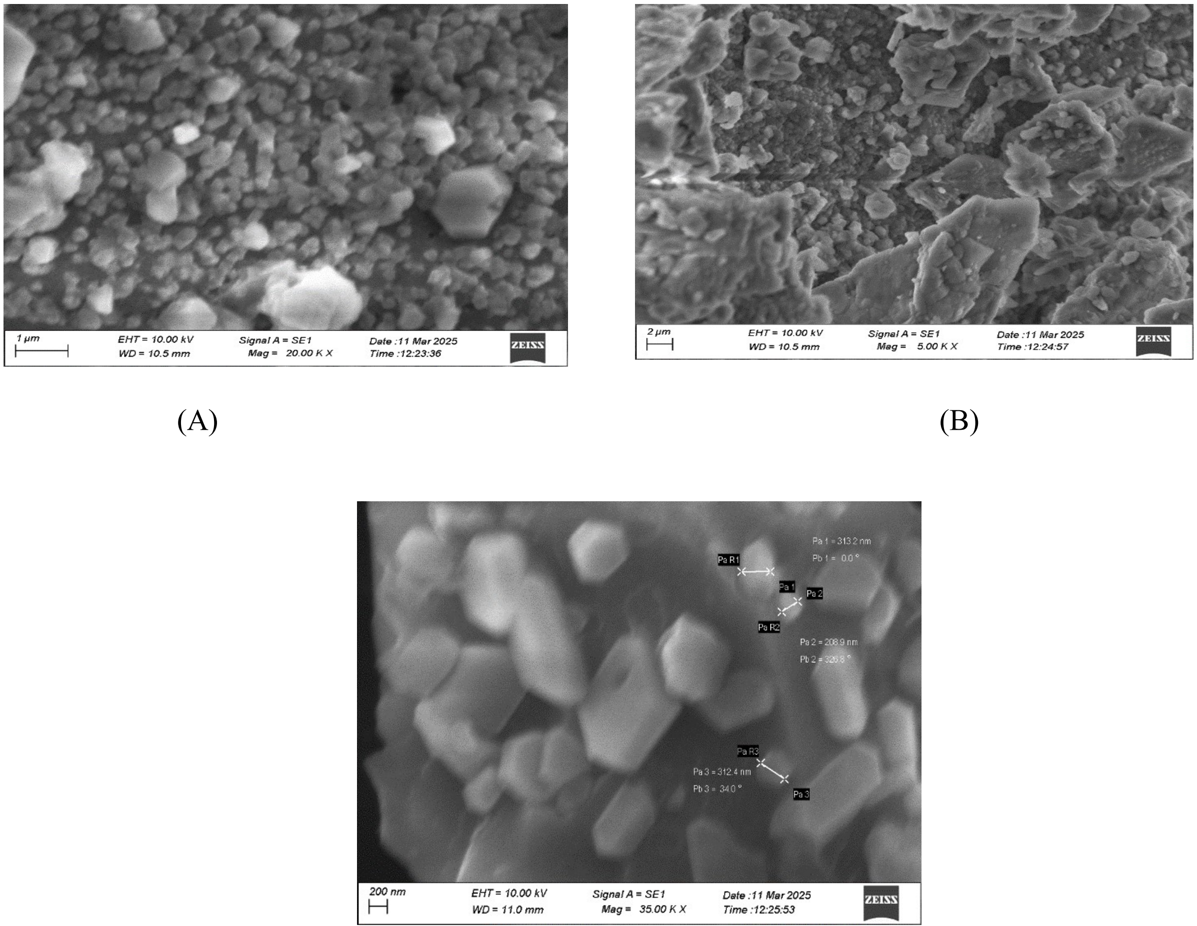
Time of test	**Group**	**Mean Difference (I-J)**	**p Value**		
			Peak Load	**% Elongation**	**UTS**
Week 2	AgNP	ZnONP	.195	.142	.899
		Uncoated	.061	.078	.076
	ZnONP	AgNP	.195	.142	.899
		Uncoated	.059	.918	.078
	Uncoated	AgNP	.061	.078	.076
		ZnONP	.059	.918	.078
Week 4	AgNP	ZnONP	.058	.260	.058
		Uncoated	.074	.675	.061
	ZnONP	AgNP	.058	.260	.058
		Uncoated	.549	.695	.557
	Uncoated	AgNP	.074	.675	.061
		ZnONP	.549	.695	.557
Week 8	AgNP	ZnONP	.075	.996	.070
		Uncoated	.568	.963	.541
	ZnONP	AgNP	.075	.996	.070
		Uncoated	.343	.983	.344
	Uncoated	AgNP	.568	.963	.541
		ZnONP	.343	.983	.344

*p<0.05 - statistically significant

Figure 1: Synthesised Silver nanoparticles

Figure 2: Synthesised Zinc oxide nanoparticles

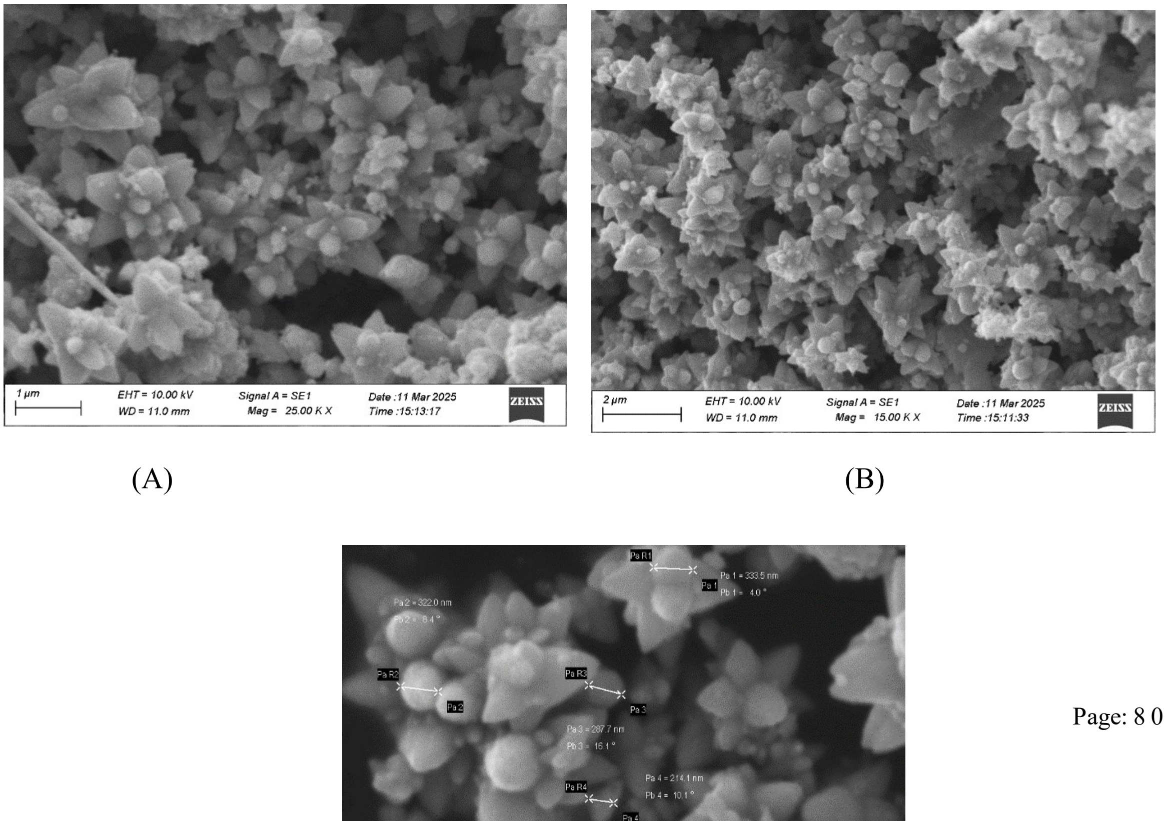
Figure 3: Scanning electron micrographs of Silver coated elastomeric ligature surfaces



(A) (B)

(C)

Figure 4: Scanning electron micrographs of Zinc oxide coated elastomeric ligature surfaces



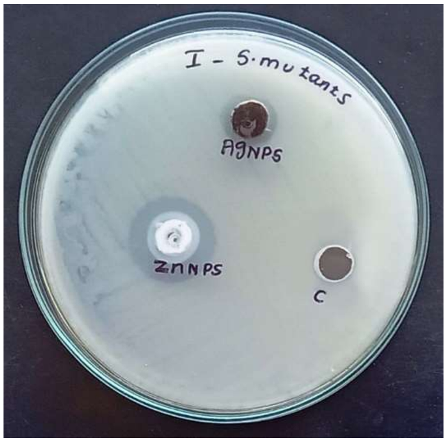
(A) (B)

(C)

Figure 5: Antibacterial activity of Silver and Zinc oxide nanocoated elastomeric ligatures (Zone of inhibition)

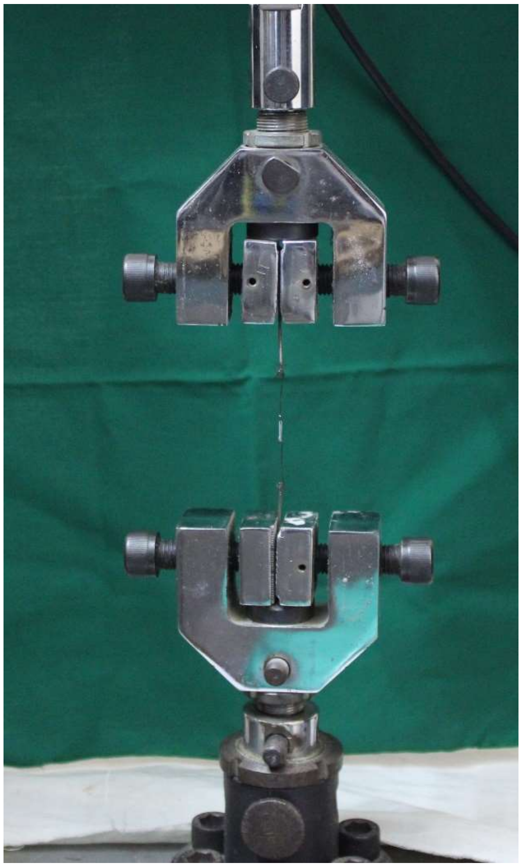
A. 2 Weeks

B. 4 Weeks



C. 8 Weeks

Figure 6: Testing the mechanical properties using Universal testing machine



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