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Antimicrobial Efficacy of Triclosan Coated Orthodontic Elastomeric Modules: An In-Vitro Study

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Abstract

Aim: During fixed orthodontic treatment, elastomeric modules contribute to increased plaque accumulation by providing a favorable surface for bacterial adhesion and biofilm formation. This study evaluated the antimicrobial efficacy of triclosan-coated orthodontic elastomeric modules against *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*.

Methods: Sixty elastomeric orthodontic modules were randomly divided into two groups: triclosan-coated and non-coated controls (n=30 each). A 1% triclosan coating solution with a cellulose acetate binder was prepared and applied using a standardized three-cycle dip-coating technique. Surface characterization was performed using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS). Antimicrobial activity was assessed quantitatively using colony-forming unit (CFU) analysis and qualitatively via zone of inhibition (ZOI) testing against *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Normality of data was assessed using the Shapiro-Wilk test, and intergroup comparisons were performed using independent samples t-tests or Mann-Whitney U tests ($P<0.05$).

Results: SEM and EDS analyses confirmed the successful and uniform deposition of the triclosan coating on the elastomeric module surfaces without compromising their visual appearance. Triclosan-coated modules demonstrated measurable zones of inhibition against all tested microorganisms, whereas non-coated controls showed no antimicrobial activity. Furthermore, CFU counts were significantly reduced in the coated group compared with the control group for *Staphylococcus aureus* (5.60±1.51 vs. 465.40±15.42), *Candida albicans* (2.90±0.88 vs. 339.60±24.99), and *Streptococcus mutans* (4.20±0.79 vs. 156.40±6.08) ($P<0.001$). The most pronounced microbial reduction was observed against *Candida albicans*.

Conclusions: Within the limits of this in vitro study, triclosan-coated modules yielded lower viable microbial counts than uncoated modules, with no apparent change in appearance. The durability of this effect, triclosan release, and biosafety remain undetermined and require in vivo and clinical evaluation.

Keywords: Antimicrobial Coating, Elastomeric Modules, Orthodontic Materials, Streptococcus Mutans, Triclosan.

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Background

During fixed orthodontic treatment, elastomeric modules contribute to increased plaque accumulation by providing a favorable surface for bacterial adhesion, thereby increasing the risk of biofilm formation. This biofilm is predominantly composed of cariogenic microorganisms, such as *Streptococcus mutans*, which are closely associated with enamel demineralization, white spot lesions, gingival inflammation, and periodontal complications during orthodontic treatment (1–3).

In addition to elastomeric modules, Abutayyem et al., in an in vitro study, demonstrated significant adhesion of *Staphylococcus aureus* and *Candida albicans* to orthodontic brackets and archwires, highlighting the susceptibility of fixed appliance materials to bacterial and fungal colonization (4).

Maintaining oral hygiene alone is often insufficient in orthodontic patients; therefore, adjunctive material-based strategies are needed for better microbial control (5). Growing interest in material-based antimicrobial approaches aims at reducing microbial adhesion directly at the appliance surface. Triclosan is a broad-spectrum antimicrobial agent that inhibits bacterial fatty acid synthesis; it also exhibits anti-inflammatory effects that reduce gingivitis (6).

A systematic review by Almeida et al. supported the safety and antibacterial efficacy of triclosan in various dental applications, including antimicrobial sutures and adhesive systems (7). Similarly, experimental orthodontic adhesives containing triclosan-loaded halloysite nanotubes demonstrated sustained inhibition of *Streptococcus mutans*, without affecting bond strength or polymerization characteristics (8). Yang et al. reported that polymerizable triclosan derivatives incorporated into universal dental adhesives maintained long-term antimicrobial efficacy without compromising the material's mechanical properties (9).

In orthodontics, triclosan has mainly been studied in a 0.3% triclosan-containing toothpaste, which has shown reduced plaque accumulation and gingival inflammation during fixed mechanotherapy (10,11). However, no research exists on its direct incorporation into orthodontic components. Therefore, this in vitro study aimed to address this gap by evaluating the antimicrobial efficacy of triclosan-coated elastomeric modules.

Methods

Ethical Approval

The present in vitro experimental study was conducted in the Department of Orthodontics and Dentofacial Orthopedics and the Department of Microbiology of the ITS Dental College. The study protocol was approved by the Institutional Ethics Committee (IEC/ORTHO/06/25).

Preparation of Samples

Sixty grey elastomeric orthodontic modules (PANAMA Orthodontics, Delhi, India) were selected. The samples were divided into two groups: group 1: triclosan-coated modules (n=30) and group 2: uncoated control modules (n=30). The coated and non-coated samples were subsequently allocated for Scanning Electron Microscope/Energy - Dispersive X-Ray Spectroscopy analysis, zone of inhibition testing, and colony-forming unit (CFU) analysis.

Sample Size Estimation

*Sample size estimation was performed using G*Power software for means (difference between two independent means, two-tailed). With an effect size of 1.6, an α error probability of 0.05, and a target power of 0.90, a minimum total sample size of 18 samples (n=9 per group) was required. To account for potential dropouts or improve robustness, a total of 20 samples (n=10 in each group) were included, yielding an actual statistical power of 0.922.

Triclosan Coating Procedure

0.1 g of triclosan powder (company BRM Chemicals, BRM Enterprises, Delhi, India) was used to prepare a 1% weight/volume (w/v) solution, dissolved in 10 mL of an ethanol–water solvent system (9:1) (obtained from Changshu Hongsheng Fine Chemical Co., Ltd., Changshu, Jiangsu Province, China). To improve solubility and ensure uniform dispersion, 2% acetic acid (RANKEM Chemicals, Avantor, Delhi, India) was added to the formulation.

A cellulose acetate binder solution (0.5–3% w/v) (Central Drug House Ltd., CDH Fine Chemical, Delhi, India) was added to improve adhesion of the coating to the elastomeric surface and promote formation of a uniform coating layer. The mixture was prepared using a BEXCO magnetic stirrer with controlled heating (40–60°C) for 40 minutes, until a homogeneous viscous coating solution was obtained.

The coating was applied by dip-coating. Each elastomeric module was immersed in the triclosan solution for 10 seconds and withdrawn at a constant slow speed to ensure a uniform film. The coated modules were then dried at 57°C for 3 minutes in an incubator (JINDAL, S.M. Scientific Instruments, Private Limited, Delhi, India).

To improve the triclosan deposition and coating uniformity, the dip-dry procedure was repeated for three consecutive cycles.

After completing the three coating cycles, all coated and non-coated orthodontic modules were stored separately in sterile Eppendorf tubes at 37°C in an incubator for 24 hours before further analysis.

Surface Characterization of Triclosan

Surface characterization was performed using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS).

Characterization Techniques

1. Elemental composition analysis was performed using EDS integrated with a scanning electron microscope (ZEISS EVO 18; Carl Zeiss Microscopy GmbH, Oberkochen, Germany).

2. Surface morphology was evaluated using a scanning electron microscope operating at an accelerating voltage of 10 kV and a working distance of 11 mm. Images were obtained at magnifications ranging from ×123 to ×1,473 using a secondary electron detector.

Antimicrobial Tests

Antimicrobial activity was assessed using zone of inhibition testing and colony-forming unit analysis. Three microbial strains were used in the study: *Streptococcus mutans* (MTCC 497) and *Staphylococcus aureus* (MTCC 96) were procured from MTCC-CSIR (Chandigarh, India), and *Candida albicans* (NCMR 1437, Hayati Chemical Corporation, Gujarat, India.) was procured from the National Center of Microbial Resources, Pune, India.

Zone of Inhibition Test

Each microbial strain was cultured in brain-heart infusion (BHI) broth for 24 hours at 37°C. Microbial suspensions were evenly inoculated onto Mueller–Hinton agar (CDH Fine Chemical, Delhi, India) plates using sterile swabs. Triclosan-coated and non-coated modules were placed on the inoculated plates and incubated at 37°C for 24 hours. The zones of inhibition (ZOI) surrounding the module were measured in millimeters using a digital measuring scale (R-Tek India Inc., Delhi, India; accuracy: 0.003 ± 0.02 mm).

Colony-Forming Unit Analysis

Following the zone of inhibition assay, antimicrobial activity was quantitatively evaluated using a colony-forming unit (CFU) assay. Five milliliters of brain heart infusion (BHI) broth was inoculated separately with *Streptococcus mutans*, *Staphylococcus aureus*, or *Candida albicans*, followed by incubation at 37 °C for 24 hours. Growth was confirmed by turbidity. Subsequently, 0.5 mL of microbial suspension was transferred to 1 mL of artificial saliva and incubated for an additional 24 hours.

The media samples were also incorporated with orthodontic modules. The sample without modules served as the negative control. After incubation, 50 µL from each sample was spread on nutrient agar plates using a sterile spreader and incubated at 37°C for the next 24 hours. Colony counting was performed using a digital colony counter (JINDAL, S.M. Scientific Instruments Private Limited, Delhi, India) (Figure 1).



Figure 1. Digital Colony Counter.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS (version 31.0) (IBM Corp., Armonk, NY, USA). Descriptive statistics, including means and standard deviations, were calculated. A significance level of $P<0.05$ was set.

The normality of the data was assessed using the Shapiro-Wilk test. Based on the data distribution, intergroup comparisons were performed using the independent-samples t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

Results

Surface Characterization

EDS analysis was performed to evaluate the elemental composition of the surface deposits following triclosan coating. The coated modules demonstrated increased carbon (C) and oxygen (O) content, while chlorine (Cl) was not detected (Table 1). The coated modules demonstrated differences in elemental composition compared with the non-coated controls. However, the absence of a detectable chlorine signal limits the ability to confirm triclosan deposition based solely on EDS analysis.

The non-coated orthodontic modules showed elemental peaks for carbon (C), nitrogen (N), oxygen (O), and trace amounts of chlorine (Cl) (Table 2). The presence of nitrogen may be attributed to additives used during module fabrication, while trace chlorine could be related to residual processing compounds.

Following EDS, SEM analysis revealed clear morphological differences between the non-coated and the triclosan-coated modules (Figure 2).

At ×123 magnification, non-coated modules exhibited a smooth homogeneous surface, whereas triclosan-coated modules showed a continuous uniform coating layer. At ×500 magnification, coated samples showed increased surface roughness with evenly distributed deposits, suggesting triclosan accumulation. At higher magnifications (×1,000 to ×1,473), fine granular microstructures and localized micro-agglomerates were observed without surface cracks or coating defects.

Overall, SEM analysis confirmed effective, uniform surface modification after repetitive dip-dry coating.

Table 1: Energy-dispersive X-ray spectroscopy (EDS) analysis of the coated samples.

Parameter	Carbon (C)	Oxygen (O)	Chlorine (Cl)
Weight %	10.12	3.99	0
Atom %	47.48	52.52	0.00
Atom % Error (± 1 Sigma)	54.63	45.37	0.00

Table 2: Energy-dispersive X-ray spectroscopy (EDS) analysis of the non-coated samples.

Parameter	Carbon(C)	Nitrogen(N)	Oxygen(O)	Chlorine(Cl)
Weight %	13.26	9.5	56.7	1.2
Atom %	38.27	12.60	48.15	0.98
Atom % Error (± 1 Sigma)	44.73	12.63	42.25	0.39

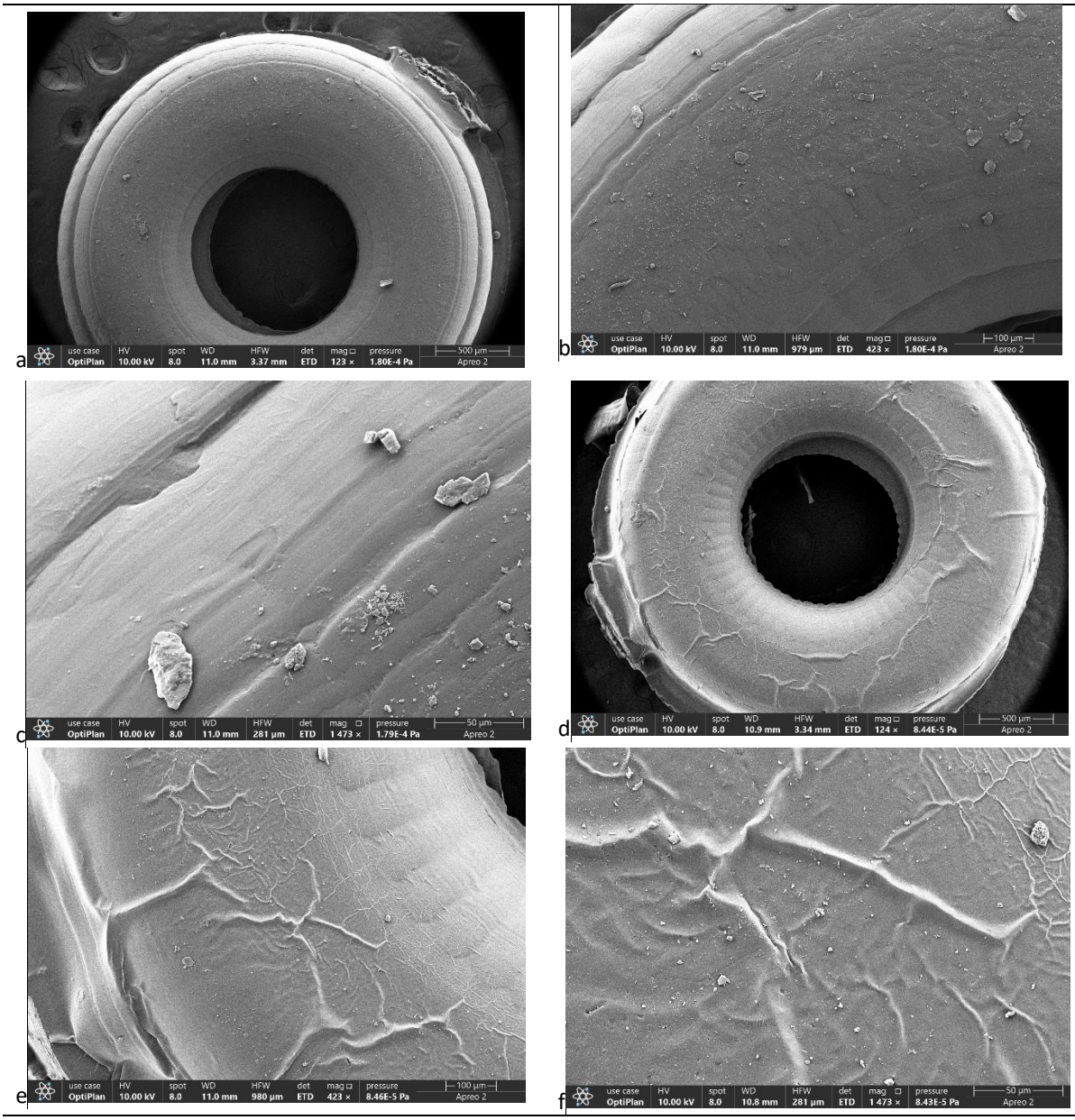


Figure 2. Representative scanning electron microscopy (SEM) images of non-coated and triclosan-coated orthodontic elastomeric modules. (a) Low-magnification view of a non-coated module showing a relatively smooth and uniform surface morphology. (b, c) Higher-magnification views of the non-coated module showing surface features with scattered particulate deposits. (d) Low-magnification view of a triclosan-coated module showing surface irregularities following coating application. (e, f) Higher-magnification views of the coated module demonstrating surface deposits and a network of fissure-like features, consistent with alterations in surface morphology following triclosan coating.

Antimicrobial Effect of Triclosan-coated Modules

The zone of inhibition (ZOI) demonstrated antimicrobial activity against all tested microorganisms. A zone of 5 mm was observed against Streptococcus mutans and 3 mm against *Staphylococcus aureus* and *Candida albicans* in the triclosan-coated group, whereas the non-coated group showed no inhibition zones. As shown in Figure 3, panel A displays a distinct zone of inhibition around the coated module, while panel B shows only the physical impression of the non-

coated module, confirming the antimicrobial effectiveness of triclosan coating (Figure 3).

Triclosan-coated elastomeric modules demonstrated a statistically significant reduction in CFU counts compared with non-coated modules for all tested microorganisms (P<0.001).

Among the tested microorganisms, the greatest reduction in CFU counts was observed for *Candida albicans*, followed by *Streptococcus mutans*, while the least reduction was noted for *Staphylococcus aureus* (Figure 4).

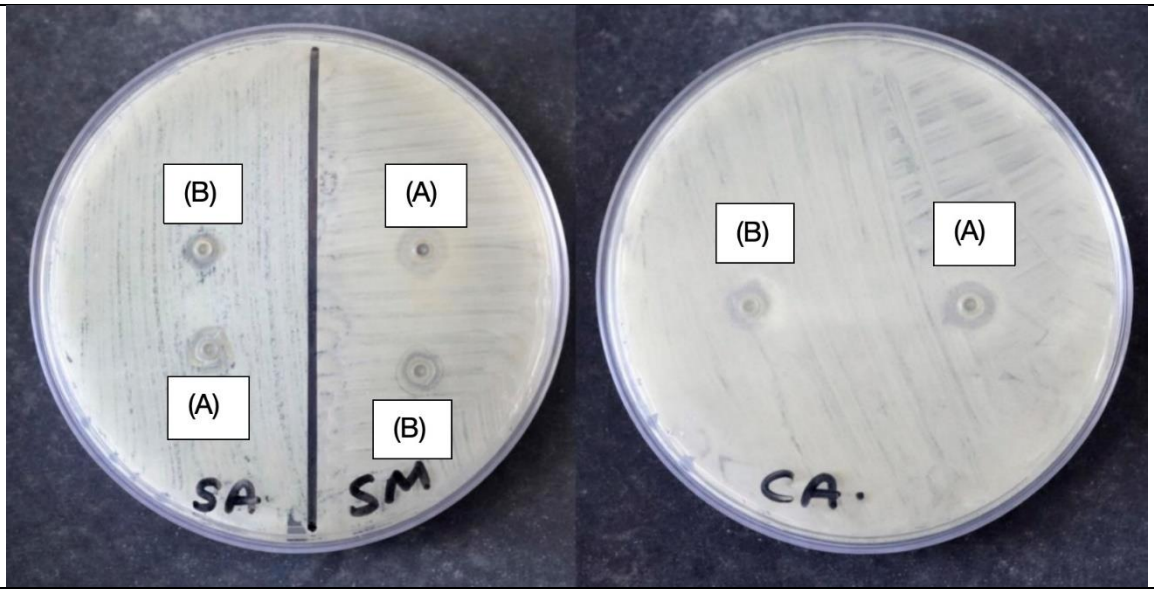


Figure 3. Diffusion assay of Staphylococcus aureus , Streptococcus mutans and Candida albicans on nutrient agar plate. Image A illustrates the triclosan-coated module with a clear zone of inhibition indicating antimicrobial efficacy. Image B represents the non-coated control module, which shows only the physical impression of the module without any zone of inhibition, reflecting absence of antimicrobial activity.

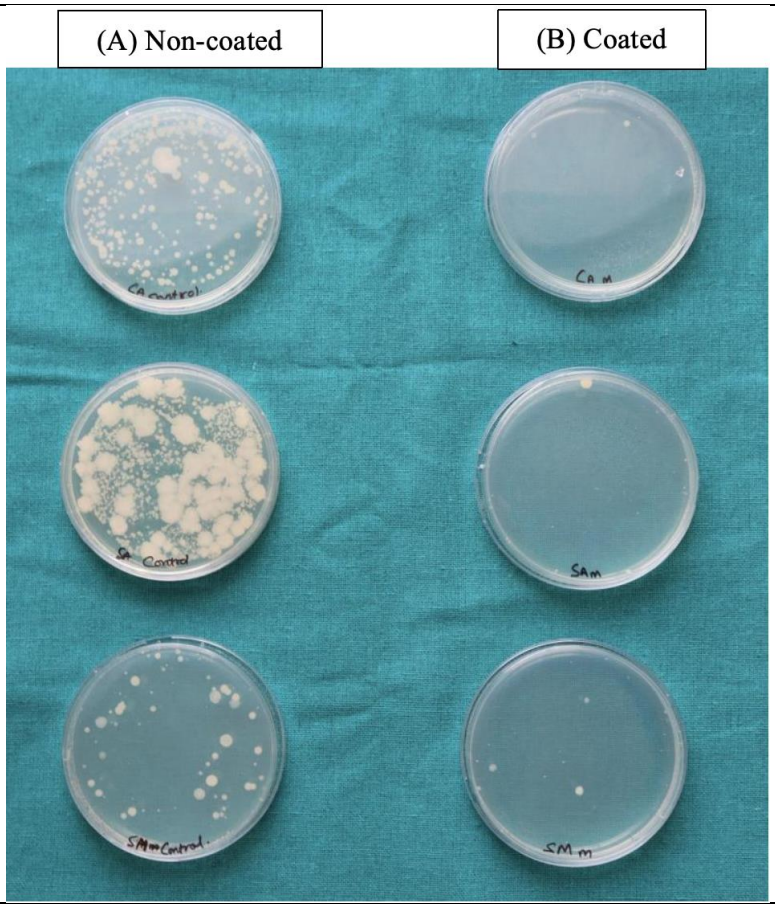


Figure 4. Colony-forming unit (CFU) assay plates showing microbial growth patterns on nutrient agar plate for (A) non-coated and (B) coated modules.

Normality Assessment

The Shapiro-Wilk test showed normal distribution of CFU count data for coated and non-coated *Staphylococcus aureus* groups (P>0.05). However, the coated groups of *Candida albicans* and *Streptococcus mutans* showed significant deviation from normality (P≤0.05). Therefore, parametric testing was used for *Staphylococcus aureus,* while non-parametric tests were used for *Candida albicans* and *Streptococcus mutans* (Table 3).

Comparison of CFU Counts between Coated and Non-coated Elastomeric Modules

As shown in Table 4, coated elastomeric modules had significantly lower CFU counts than non-coated modules for all tested microorganisms.

For *Staphylococcus aureus,* the mean CFU count in the non-coated group was 465.40±15.42, whereas the coated group showed a significantly lower mean CFU count of 5.60±1.51 (P<0.001).

For *Candida albicans*, the mean CFU count in the non-coated group was 339.60±24.99, while the coated group demonstrated a significantly lower mean CFU count of 2.90±0.88 (P<0.001).

Similarly, for *Streptococcus mutans*, the mean CFU count in the non-coated group was 156.40±6.08, whereas the coated group showed significantly lower mean CFU counts of 4.20±0.79 (P<0.001).

Discussion

Triclosan is a broad-spectrum antimicrobial agent that inhibits bacterial fatty acid synthesis and cell membrane formation (6). Because of its antibacterial and anti-inflammatory properties, it has been widely incorporated into dental materials. Jones et al. reported effective antimicrobial activity of triclosan with a favorable safety profile in healthcare applications (12). Researchers have also investigated triclosan for its potential effects on bacterial attachment to material surfaces (13). Therefore, the present study evaluated the antimicrobial efficacy of triclosan-coated elastomeric modules against *Staphylococcus*

aureus, *Candida albicans*, and *Streptococcus mutans*.

Table 3: Normality assessment

Micro-organism	Modules	Statistic	df	P- value
Staphylococcus aureus	Non-coated	0.90	10	0.24
	Coated	0.85	10	0.06
Candida albicans	Non-coated	0.89	10	0.20
	Coated	0.80	10	0.01*
Streptococcus mutans	Non-coated	0.97	10	0.93
	Coated	0.82	10	0.02*

Shapiro-Wilk test; *p < 0.05 indicates significant deviation from normal distribution.

Table 4: Comparison of CFU counts between non-coated and coated elastomeric modules

Microorganism	Modules	Mean ± SD	Test Statistic	p-value	Median (IQR)
Staphylococcus aureus	Non-coated	465.40 ± 15.42	93.839	<0.001*	470 (20.25)
	Coated	5.60 ± 1.51			5 (3)
Candida albicans	Non-coated	339.60 ± 24.99	-3.807	<0.001*	338 (19.75)
	Coated	2.90 ± 0.88			3 (2)
Streptococcus mutans	Non-coated	156.40 ± 6.08	-3.810	<0.001*	156 (10.25)
	Coated	4.20 ± 0.79			4 (1.25)

Independent t-test was used for comparison of *Staphylococcus aureus*, while Mann-Whitney U test was used for *Candida albicans* and *Streptococcus mutans*. Values are expressed as mean ± standard deviation and median (interquartile range). *p ≤ 0.05 indicates statistical significance.

Triclosan-coated modules showed a significant reduction in microbial counts for all tested microorganisms compared with non-coated controls, indicating notable antimicrobial effects in vitro. Similarly, Beyth et al. (14) demonstrated reduced *Streptococcus mutans* biofilm formation with antimicrobial-modified dental materials, while Cheng et al. reported effective inhibition of bacterial colonization using antibacterial dental nanocomposites without affecting material properties (15).

Previous studies have mainly focused on incorporating triclosan into orthodontic adhesive systems rather than auxiliary orthodontic materials. Degrazia et al. reported inhibition of *Streptococcus mutans* while maintaining bonding performance and promoting mineral deposition (8). Similarly, Sehgal et al. demonstrated antibacterial activity in orthodontic composites containing antimicrobial agents, including triclosan, without compromising their physical properties (16). Similarly, Yang et al. reported that polymerizable triclosan derivatives preserved antimicrobial efficacy, without compromising the mechanical properties of universal adhesives (9). Despite these findings, limited attention has been directed toward antimicrobial modification of elastomeric modules, which are known to favor plaque accumulation during fixed orthodontic treatment.

In a split-mouth clinical study, Forsberg et al. reported significantly greater microbial accumulation around teeth ligated with elastomeric rings than with stainless steel ligatures during orthodontic treatment. Increased colonization by *Streptococcus mutans* and Lactobacilli was observed around elastomeric ligatures, emphasizing their plaque-retentive nature and potential role in enamel decalcification and gingival inflammation (17,18).

Other antimicrobial approaches in orthodontics have included nanoparticle-based coatings. Hemashree and Padmanabhan demonstrated reduced *Streptococcus mutans* levels with zinc

oxide nanoparticle-coated elastomeric modules in a randomized split-mouth clinical trial (1).

In addition, it was shown that chitosan and glutaraldehyde-cross-linked chitosan coatings effectively inhibit bacterial colonization while maintaining high gingival fibroblast viability. These surface modifications preserve the essential mechanical properties of orthodontic elastomeric modules without causing adverse structural impairment. Therefore, chitosan-based coatings represent a promising strategy to mitigate plaque accumulation and enhance the clinical performance of auxiliary orthodontic materials (19).

However, Gwinn and Vallyathan highlighted concerns regarding cytotoxicity and biological risks associated with nanoparticle exposure (20). In contrast, triclosan has an established history of use in regulated dental concentrations for plaque and gingivitis control (5,10).

SEM and EDS analyses in the present study confirmed successful surface deposition of triclosan on elastomeric modules. The observed reduction in CFU counts may be attributed primarily to triclosan's antimicrobial activity. Although surface morphological changes were observed following coating, their potential influence on microbial adhesion was not directly evaluated in the present study. Repeating the dip-dry coating cycle three times may also have improved coating uniformity and surface coverage, as previously reported by researchers (21,22).

Another favorable finding was the absence of visible color alteration after triclosan coating, which is clinically relevant because elastomeric materials are prone to staining and discoloration in the oral environment. Ardeshna and Vaidyanathan reported significant color changes in elastomeric modules after exposure to dietary pigments and beverages, particularly in clear elastomers (23).

Although triclosan has been used in dental products, its safety with prolonged exposure remains a concern. In vitro studies have reported cytotoxic effects on human gingival cells, highlighting the need to determine the amount of triclosan released from coated modules and its potential exposure to oral tissues (24). Prolonged use has also raised concerns regarding reduced microbial susceptibility and antimicrobial resistance (25). In addition, triclosan can enter wastewater and aquatic environments, where it may adversely affect aquatic organisms (26). These concerns do not diminish the antimicrobial activity observed in the present study but emphasize the need to assess safety and long-term performance before clinical applications.

The present study evaluated antimicrobial activity only after 24 hours; therefore, the duration of the effect, triclosan release, and coating durability could not be determined. This is relevant because antimicrobial activity may depend on the amount of triclosan released and retained on the module surface. Previous studies, such as that by Kockisch et al. have reported varying release patterns for triclosan depending on the polymeric carrier, including sustained release in certain formulations. (27). In addition, the effect of the coating on the mechanical properties of elastomeric modules requires evaluation. Andhare et al. reported substantial force decay of orthodontic elastomeric chains and modules during the initial weeks of use (28). Future studies should therefore assess triclosan release, coating durability, force decay, elasticity, long-term antimicrobial activity, and biocompatibility under simulated intraoral and in vivo conditions. Clinical studies should also evaluate staining and discoloration during orthodontic treatment.

Limitations

Notwithstanding the promising findings of this study, several limitations must be acknowledged. The evaluation was restricted to a 24-hour endpoint under static in vitro conditions; consequently, the long-term substantivity, cumulative release kinetics, and sustained antimicrobial durability over a standard 4- to 6-week orthodontic appointment interval remain to be determined. Furthermore, the reliance on single-species planktonic models and agar assays fails to fully replicate the intricate polymicrobial biofilms, salivary flow, continuous pH fluctuations, and masticatory shear stresses that characterize the dynamic intraoral environment. In addition, the potential impact of the solvent system and thermal drying cycles on the intrinsic mechanical properties, tensile strength, and force-decay characteristics of the elastomeric modules was not evaluated. Quantitative cytotoxicity profiling against human oral cells and precise drug-release quantification were also beyond the scope of this preliminary investigation and warrant comprehensive assessment in future in vitro and in vivo studies.

Conclusion

Within the limitations of this in vitro study, triclosan-coated elastomeric orthodontic modules

exhibited a significant CFU reduction compared with conventional uncoated modules. The antimicrobial effect was most pronounced against *Candida albicans*, followed by *Streptococcus mutans* and *Staphylococcus aureus*.

No appreciable alteration in the aesthetic appearance of the coated modules was observed following coating. These findings indicate that triclosan coating can reduce the viable microbial load recovered from elastomeric orthodontic modules under the conditions tested. However, the clinical relevance of this finding cannot be established from the present in vitro data. Further in vivo and clinical studies are therefore required to determine the clinical safety, durability, mechanical properties, release characteristics, and long-term efficacy of triclosan-coated orthodontic elastomeric modules.

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None.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

Data Availability

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

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Authors' Contributions

AP: conceptualization, project administration, supervision, validation, review and editing. EK: data curation, formal analysis, visualization, writing - original draft and investigation. AN: writing - review and editing and supervision. KM: methodology, resources, software. AS: supervision.

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