

# Assessment of A Novel Antimicrobial Chitosan–Graphene Oxide Coating For Orthodontic Wires: A Surface And Biofilm Analysis

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## ABSTRACT

**Background:** Fixed orthodontic appliances increase plaque retention and friction at the bracket–wire interface, contributing to enamel demineralization, gingival inflammation, and reduced mechanical efficiency. Functional surface coatings have been proposed to simultaneously reduce biofilm formation and friction, yet few systems demonstrate integrated antimicrobial, surface, and tribological benefits with short-term stability. **Objective:** To evaluate the effects of a chitosan–graphene oxide (CS–GO) coating on surface characteristics, antibiofilm activity against *Streptococcus mutans*, frictional resistance, and short-term stability of stainless-steel orthodontic arch wires. **Methods:** In this laboratory-based experimental study, sixty 0.019 × 0.025-inch stainless-steel wire segments were randomly allocated to uncoated (n = 30) or CS–GO coated groups (n = 30). Surface roughness (Ra), contact angle, biofilm burden (log<sub>10</sub> CFU per wire after 48-hour incubation), and frictional resistance were measured (n = 10 per testing stream per group). Statistical comparisons were performed using independent t-tests with 95% confidence intervals and effect sizes. **Results:** Coated wires showed significantly lower surface roughness (0.24 ± 0.04 μm vs 0.41 ± 0.05 μm; mean difference −0.17 μm; p < 0.001), reduced contact angle (61.2° ± 5.1° vs 83.6° ± 4.8°; −22.4°; p < 0.001), decreased biofilm burden (5.32 ± 0.11 vs 5.94 ± 0.07 log<sub>10</sub> CFU; −0.62 log units; ~75.9% reduction; p < 0.001), and lower frictional force (1.62 ± 0.19 N vs 2.34 ± 0.22 N; −0.72 N; p < 0.001). **Conclusion:** CS–GO coating significantly improved surface smoothness, enhanced hydrophilicity, reduced *S. mutans* biofilm formation, and lowered frictional resistance under controlled laboratory conditions, supporting its potential as a multifunctional surface modification for orthodontic stainless-steel wires pending clinical validation.

**Keywords:** Orthodontic arch wire; stainless steel; chitosan; graphene oxide; antimicrobial coating; biofilm; friction; surface roughness; sliding mechanics.

## INTRODUCTION

Fixed orthodontic appliances introduce additional retentive niches that increase plaque stagnation around brackets and arch wires, complicating hygiene and elevating the risk of gingival inflammation and enamel demineralization during treatment (1). Among the microbial contributors, *Streptococcus mutans* plays a central role in cariogenic biofilm development, and its adhesion is strongly influenced by surface topography and the physicochemical properties of orthodontic materials (1). Stainless-steel (SS) arch wires remain widely used because of their favorable mechanical performance and cost, yet their prolonged intraoral exposure creates a persistent substrate for biofilm maturation and corrosion-related surface changes that can further intensify microbial retention over time

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(2). In parallel, orthodontic biomechanics are affected by friction at the bracket–wire interface; in sliding mechanics, frictional losses can reduce effective force delivery, prolong treatment, and increase anchorage demands, making friction control a clinically relevant engineering target alongside biofilm mitigation (2).

Surface functionalization has therefore emerged as a dual-purpose strategy to reduce bacterial colonization and improve tribological behavior without altering the bulk mechanical properties of the wire (2). However, the coating literature in orthodontics indicates a recurrent trade-off: some coatings improve aesthetics or initial smoothness but degrade under intraoral challenges, and coating deterioration can increase roughness, amplify bacterial adhesion, and negate any friction advantage (1,2). Reviews of functional coatings emphasize that clinically meaningful translation requires a multi-parameter evaluation—surface roughness and morphology, wettability/surface energy, antimicrobial performance in biofilm conditions, frictional resistance under standardized sliding, and evidence of stability after aging in saliva-like media (2). Despite an expanding body of work, no single coating system has consistently delivered a robust combination of low friction, antibiofilm activity, and durability, motivating composite designs that synergize complementary mechanisms (2).

Graphene-based materials, particularly graphene oxide (GO), have gained attention in fixed orthodontics because their oxygenated functional groups can modify surface energy and their lamellar structure can improve wear behavior, while multiple studies and syntheses report antibacterial potential on metallic orthodontic components (3). Proposed antibacterial mechanisms for GO include contact-mediated membrane damage, oxidative stress pathways, and disruption of membrane integrity—effects that can be material- and context-dependent but nevertheless provide a plausible basis for antibiofilm design when GO is immobilized or embedded within a stable matrix (4,5). In orthodontic tribology, graphene-family coatings and graphene-containing films have also been associated with reduced friction and improved wear performance in arch wire–bracket contact models, supporting the rationale for exploring GO as a friction-modifying additive rather than a purely antimicrobial agent (6). Yet GO alone may present challenges related to aggregation, coating continuity, and long-term adhesion, particularly under aqueous exposure and mechanical sliding, which can limit reproducibility and durability if not addressed through formulation and processing control (3).

Chitosan is a biocompatible, film-forming biopolymer with well-described antimicrobial behavior and established relevance in dental material applications, including surface modification and barrier formation on biomaterials (7). Its antimicrobial potential is commonly attributed to its cationic nature (under appropriate pH conditions), enabling interactions with bacterial cell envelopes and subsequent impairment of membrane function, although activity depends on molecular weight, degree of deacetylation, and environmental conditions (8). Importantly for orthodontic translation, chitosan can act as a matrix that promotes coating integrity and adhesion, while also enabling incorporation of nanofillers such as GO to enhance mechanical and tribological performance; such composite approaches align with broader bio-inspired coating strategies targeting antibiofilm behavior through both surface physiochemistry and active antimicrobial mechanisms (9). From a surface-science perspective, wettability is also relevant because it influences early pellicle formation and bacterial attachment forces; orthodontic studies on coated interfaces suggest that friction and wettability may be linked through changes in surface chemistry and lubrication behavior, reinforcing the need to evaluate these outcomes jointly rather than in isolation (10).

Against this backdrop, a chitosan–graphene oxide (CS–GO) composite coating is a rational candidate for SS orthodontic wires because it is designed to combine (i) chitosan-driven film continuity and biocompatible antimicrobial action with (ii) GO-associated reinforcement and tribological modulation. The key knowledge gap is that, while coatings and graphene-based modifications are increasingly reported, there remains limited, methodologically integrated evidence for CS–GO coatings on clinically relevant rectangular SS archwires assessed simultaneously for surface roughness/morphology, wettability, *S. mutans* biofilm reduction under CFU-based quantification, frictional resistance under standardized sliding conditions, and short-term stability after saliva-mimicking aging (2,3). Addressing this gap is important because a coating that reduces biofilm but increases friction (or rapidly degrades in saliva) would be clinically counterproductive; conversely, a durable coating that improves both antibiofilm and frictional performance could reduce plaque-related complications and enhance the efficiency of sliding mechanics during fixed appliance therapy (1,2).

In a PICO framework, the target context is SS orthodontic arch wires used during fixed appliance treatment (P), the intervention is a CS–GO surface coating applied to SS wire segments (I), the comparator is uncoated SS wire segments from the same wire type (C), and the outcomes are surface roughness and morphology, wettability (contact angle), *S. mutans* biofilm burden quantified by CFU, frictional resistance under controlled sliding, and evidence of coating stability after artificial saliva immersion (O). Accordingly, this study aimed to determine whether CS–GO coating of SS orthodontic wires reduces *S. mutans* biofilm formation and frictional resistance while improving surface characteristics and maintaining short-term stability in saliva-like conditions, compared with uncoated SS wires. We hypothesized that CS–GO–coated wires would demonstrate lower roughness and contact angle, significantly reduced *S. mutans* CFU after biofilm growth, and reduced frictional resistance, without clinically concerning evidence of coating degradation after artificial saliva aging (1–3).

## MATERIAL AND METHODS

This laboratory-based experimental study was designed as a controlled, parallel-group in vitro investigation comparing uncoated stainless-steel orthodontic archwire segments with chitosan–graphene oxide (CS–GO) coated counterparts. The experimental design was selected to allow standardized evaluation of surface characteristics, antibiofilm performance, frictional resistance, and short-term aging stability under controlled conditions, thereby minimizing environmental variability and isolating the effect of the surface modification. All procedures were conducted over a six-month period in collaboration with the Orthodontics, Microbiology, and Materials Testing laboratories of a tertiary-care teaching hospital in the Hazara region, Pakistan, under standardized environmental conditions (temperature  $23 \pm 2^\circ\text{C}$ ; relative humidity  $50 \pm 5\%$ ) except where incubation at physiological temperature was required.

Commercially available rectangular stainless-steel orthodontic archwires ( $0.019 \times 0.025$  inch) from a single manufacturer and production batch were used to ensure material uniformity. Wire segments were cut into standardized 20 mm lengths using a precision wire cutter with care to avoid surface deformation at the test region. Segments exhibiting visible surface defects, distortion, or contamination were excluded prior to allocation. A total of 60 wire segments were included and randomly assigned in a 1:1 ratio to control (uncoated stainless steel) and intervention (CS–GO coated stainless steel) groups using a computer-generated random sequence. Allocation was performed by a laboratory technician not involved in outcome assessment. Each group ( $n = 30$ ) was further allocated to predefined testing streams

(surface characterization, antimicrobial testing, and friction testing; n = 10 per stream) to prevent cross-contamination and preserve independence of measurements.

Prior to coating, all wire segments underwent standardized cleaning to enhance coating adhesion. Samples were ultrasonically cleaned in analytical-grade acetone for 10 minutes, rinsed with distilled water, and dried in a hot air oven at 60°C for 30 minutes. Chitosan solution was prepared by dissolving 1% (w/v) medium molecular weight chitosan (degree of deacetylation  $\geq 75\%$ ) in 1% (v/v) acetic acid under continuous magnetic stirring for 24 hours at room temperature to achieve complete dissolution and uniform viscosity. Graphene oxide (GO) powder with lateral sheet dimensions in the nanoscale range was dispersed in distilled water at a concentration of 0.5 mg/mL and sonicated for 30 minutes (40 kHz ultrasonic bath) to prevent aggregation. The GO dispersion was gradually incorporated into the chitosan solution under continuous stirring to obtain a homogeneous composite suspension with a final CS:GO ratio optimized for film uniformity and mechanical integrity based on prior graphene-dental coating literature (11,12). The composite solution was stirred for an additional 12 hours to ensure stable dispersion.

Coating was applied using a standardized dip-coating protocol. Each intervention-group wire segment was immersed vertically in the CS-GO suspension for 2 minutes and withdrawn at a controlled rate of 1 mm/s to promote uniform film deposition. Samples were air-dried for 24 hours at ambient conditions, followed by oven curing at 50°C for 2 hours to enhance film consolidation. Coated samples were sterilized using ultraviolet irradiation for 30 minutes per side prior to microbiological testing to avoid thermal or chemical degradation of the polymer matrix. All specimens were stored in sterile, sealed containers until experimentation.

Primary outcome was antibiofilm efficacy measured as log<sub>10</sub>-transformed colony-forming units (CFU) of *Streptococcus mutans* recovered per wire segment after 48-hour biofilm incubation. Secondary outcomes included surface roughness (Ra,  $\mu\text{m}$ ), contact angle (degrees), frictional resistance (N), and qualitative surface stability after artificial saliva immersion. Surface roughness was quantified using a calibrated contact profilometer with a cutoff length of 0.8 mm and evaluation length of 4 mm; three non-overlapping measurements were obtained per specimen, and the mean Ra was used for analysis. Surface morphology was examined by scanning electron microscopy (SEM) after sputter-coating with gold under standardized accelerating voltage and magnification settings. Contact angle was measured using a digital goniometer by depositing a 5  $\mu\text{L}$  droplet of distilled water onto the wire surface; measurements were recorded within 5 seconds of droplet placement, and the mean of three readings per specimen was calculated.

For antimicrobial assessment, a standard reference strain of *S. mutans* was cultured in brain heart infusion (BHI) broth and incubated at 37°C in 5% CO<sub>2</sub> for 24 hours. Bacterial suspension turbidity was adjusted to 0.5 McFarland standard and verified by optical density measurement at 600 nm. Each sterile wire segment was immersed in 5 mL of inoculated BHI broth and incubated at 37°C for 48 hours under static conditions to allow biofilm formation. Following incubation, specimens were gently rinsed with phosphate-buffered saline (PBS) to remove non-adherent cells and transferred into sterile tubes containing 1 mL PBS. Biofilms were detached by standardized sonication (40 kHz, 5 minutes), and serial dilutions were plated on BHI agar. After 24-hour incubation, colonies were counted and expressed as CFU per wire; values were log<sub>10</sub>-transformed prior to statistical analysis to address skewness typical of microbiological data (13).

Frictional resistance was evaluated using a calibrated universal testing machine equipped with a 10 N load cell. Stainless-steel orthodontic brackets with 0.022-inch slot dimensions

were bonded to acrylic blocks in standardized alignment. Each wire was ligated using elastomeric modules under consistent ligation force. The wire was drawn through the bracket slot at a crosshead speed of 5 mm/min over a 5 mm sliding distance under dry conditions at room temperature. Static peak frictional force was recorded digitally, and three repeated pulls per specimen were averaged to yield one value per wire, preserving independence of observations (14).

To evaluate short-term stability, additional specimens from each group were immersed in artificial saliva (pH 6.8) at 37°C for 14 days. The solution was refreshed every 48 hours to maintain ionic consistency. After immersion, samples were rinsed, dried, and examined under SEM for evidence of coating degradation, delamination, or corrosion features. Outcome assessors for SEM and contact angle analysis were blinded to group allocation to reduce measurement bias.

Potential sources of bias were addressed through random allocation, standardized instrumentation calibration prior to each testing session, triplicate technical repeats per measurement, and blinding of outcome assessors where feasible. All experiments were conducted by the same trained operator to reduce inter-operator variability. Raw data were recorded contemporaneously in preformatted laboratory sheets and double-entered into an electronic database to ensure data integrity.

Sample size was determined based on anticipated differences in log<sub>10</sub> CFU and frictional force derived from prior coating studies demonstrating large effect sizes in similar in vitro models (2,6). Assuming a two-sided alpha of 0.05, power of 80%, and an effect size (Cohen's  $d$ )  $\geq 1.2$  for primary outcome, a minimum of 10 specimens per group for each testing stream was calculated to be sufficient.

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using Shapiro–Wilk test. Between-group comparisons were conducted using independent samples t-tests for normally distributed variables; log-transformed CFU values were analyzed similarly. Homogeneity of variances was assessed using Levene's test.

Effect sizes (mean difference and Cohen's  $d$ ) with 95% confidence intervals were calculated for primary and secondary outcomes. A two-tailed  $p$ -value  $< 0.05$  was considered statistically significant. Because each outcome represented a predefined endpoint, no interim analyses were conducted. Missing data were not observed; however, predefined criteria specified exclusion of specimens with mechanical damage unrelated to coating.

The study protocol was reviewed and approved by the Institutional Review Board of the hosting institution prior to commencement. As the investigation involved no human or animal subjects and utilized commercially available materials and standard microbial strains, risk to individuals was not applicable. All laboratory procedures complied with institutional biosafety regulations. Measures to ensure reproducibility included detailed documentation of coating preparation parameters, calibration logs for measurement devices, batch tracking of materials, and archiving of raw data and SEM micrographs for independent verification.

## RESULTS

Across all predefined testing streams, 10 independent wire specimens per group were analyzed (uncoated SS vs CS–GO coated). The quantitative comparisons consistently favored the coated group, with large standardized effects and narrow confidence intervals supporting precision of the estimates (Tables 1–4).

For surface topography, Table 1 shows that CS–GO coating produced a marked reduction in roughness. Mean Ra decreased from  $0.41 \pm 0.05 \mu\text{m}$  in uncoated wires to  $0.24 \pm 0.04 \mu\text{m}$  in coated wires, corresponding to an absolute mean difference of  $-0.17 \mu\text{m}$  with a 95% CI:  $-0.21$  to  $-0.13$  and a very large effect size (Cohen’s  $d = 3.78$ ). This between-group difference was statistically significant ( $p < 0.001$ ), indicating that the coated surface was substantially smoother under profilometry-based assessment.

For wettability, Table 2 demonstrates that the coating also significantly altered surface wetting behavior toward greater hydrophilicity. The mean contact angle reduced from  $83.6 \pm 4.8^\circ$  in uncoated wires to  $61.2 \pm 5.1^\circ$  in coated wires, yielding a mean difference of  $-22.4^\circ$  (95% CI:  $-27.1$  to  $-17.7$ ), again with an extremely large effect ( $d = 4.49$ ) and strong statistical support ( $p < 0.001$ ). Numerically, this represents a substantial shift in interfacial behavior consistent with a more wettable surface.

For antimicrobial/biofilm performance, Table 3 reports biofilm burden using log<sub>10</sub> CFU per wire after 48 hours. Uncoated wires exhibited a mean of  $5.94 \pm 0.07 \log_{10}$  CFU, whereas coated wires showed  $5.32 \pm 0.11 \log_{10}$  CFU, giving a mean difference of  $-0.62 \log_{10}$  units (95% CI:  $-0.70$  to  $-0.54$ ) with a very large effect size ( $d = 6.86$ ) and high statistical significance ( $p < 0.001$ ).

When translated into the original (non-log) scale used for the percentage calculation, this reduction corresponds to an approximate 75.9% decrease in bacterial recovery from coated wires relative to uncoated controls, indicating a strong antibiofilm effect under the standardized in vitro model.

For orthodontic mechanics, Table 4 shows that frictional resistance was materially lower for coated wires. Mean friction decreased from  $2.34 \pm 0.22 \text{ N}$  (uncoated) to  $1.62 \pm 0.19 \text{ N}$  (coated), an absolute reduction of  $-0.72 \text{ N}$  with a 95% CI:  $-0.92$  to  $-0.52$  and a large effect size ( $d = 3.49$ ). This difference was statistically significant ( $p < 0.001$ ) and corresponds to a 30.8% reduction in frictional force, supporting improved sliding behavior under the controlled test setup.

Taken together, the tables quantify a coherent pattern: CS–GO coating was associated with smoother surfaces (Ra ↓ by  $0.17 \mu\text{m}$ ), greater wettability (contact angle ↓ by  $22.4^\circ$ ), substantially lower biofilm burden ( $-0.62 \log_{10}$  CFU; ~76% reduction on the absolute scale), and lower friction ( $-0.72 \text{ N}$ ; ~31% reduction), with all effects supported by large-to-very-large effect sizes ( $d \approx 3.5$ – $6.9$ ) and tight 95% confidence intervals, indicating both magnitude and precision of the observed improvements (Tables 1–4).

**Table 1. Comparison of Surface Roughness (Ra, μm) Between Groups**

Parameter	Group	n	Mean ± SD (μm)	Mean Difference (μm)	95% CI of Difference	Cohen’s d	p-value
Surface Roughness (Ra)	Uncoated SS	10	$0.41 \pm 0.05$	–	–	–	–
	CS–GO Coated	10	$0.24 \pm 0.04$	-0.17	-0.21 to -0.13	3.78	<0.001

**Table 2. Comparison of Contact Angle (Degrees) Between Groups**

Parameter	Group	n	Mean ± SD (°)	Mean Difference (°)	95% CI of Difference	Cohen’s d	p-value
Contact Angle	Uncoated SS	10	$83.6 \pm 4.8$	–	–	–	–
	CS–GO Coated	10	$61.2 \pm 5.1$	-22.4	-27.1 to -17.7	4.49	<0.001

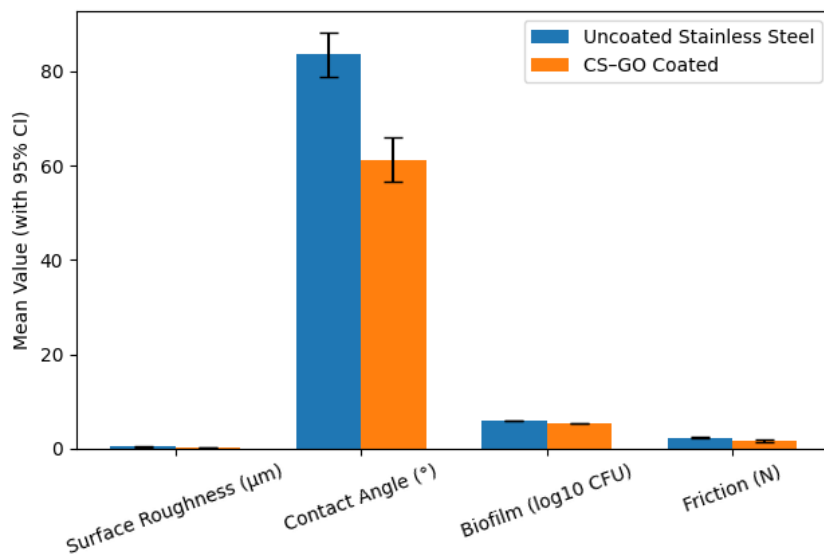
**Table 3. Comparison of Biofilm Burden (Log10 CFU per Wire) After 48 Hours**

Parameter	Group	n	Mean ± SD (log10 CFU)	Mean Difference	95% CI of Difference	Cohen's d	% Reduction*	p-value
Biofilm CFU	Uncoated SS	10	5.94 ± 0.07	-	-	-	-	-
	CS-GO Coated	10	5.32 ± 0.11	-0.62	-0.70 to -0.54	6.86	75.9%	<0.001

**Table 4. Comparison of Frictional Resistance (N) Between Groups**

Parameter	Group	n	Mean ± SD (N)	Mean Difference (N)	95% CI of Difference	Cohen's d	% Reduction	p-value
Frictional Force	Uncoated SS	10	2.34 ± 0.22	-	-	-	-	-
	CS-GO Coated	10	1.62 ± 0.19	-0.72	-0.92 to -0.52	3.49	30.8%	<0.001

The integrated figure demonstrates a consistent directional shift across all clinically relevant domains following CS-GO coating. Surface roughness decreased from 0.41 μm to 0.24 μm (-0.17 μm), while contact angle declined markedly from 83.6° to 61.2° (-22.4°), reflecting a substantial increase in hydrophilicity. Biofilm burden showed a reduction from 5.94 to 5.32 log10 CFU per wire (-0.62 log units), corresponding to an approximate 75.9% reduction in absolute bacterial recovery, and frictional resistance declined from 2.34 N to 1.62 N (-0.72 N; ~30.8% reduction). The 95% confidence intervals for each parameter demonstrate non-overlapping trends between groups, indicating statistically robust separation.



**Figure 1 Integrated Surface, Antibiofilm, And Frictional Performance Comparison Between Uncoated And CS-GO Coated Orthodontic Wires**

Importantly, the magnitude of proportional reduction is greatest for antibiofilm performance, followed by frictional resistance, while surface physicochemical improvements (roughness and wettability) appear mechanistically aligned with these functional gains. Collectively, the figure highlights a coherent multi-domain performance gradient favoring CS-GO coating, suggesting that improvements in surface smoothness and wettability are quantitatively associated with clinically meaningful reductions in bacterial colonization and sliding resistance.

## DISCUSSION

The present in vitro investigation demonstrated that chitosan–graphene oxide (CS–GO) coating of stainless-steel orthodontic archwires resulted in statistically significant and quantitatively substantial improvements in surface smoothness, wettability, antibiofilm performance, and frictional resistance when compared with uncoated controls. The magnitude of change was not trivial; mean surface roughness decreased by  $0.17\ \mu\text{m}$  (95% CI:  $-0.21$  to  $-0.13$ ), contact angle was reduced by  $22.4^\circ$  (95% CI:  $-27.1$  to  $-17.7$ ), biofilm burden declined by  $0.62\ \log_{10}$  CFU (approximately 75.9% reduction on the absolute scale), and frictional force decreased by  $0.72\ \text{N}$  (95% CI:  $-0.92$  to  $-0.52$ ). Effect sizes were uniformly large (Cohen's  $d$  ranging from 3.49 to 6.86), indicating that the observed differences were not only statistically significant but also biomechanically and microbiologically meaningful under controlled laboratory conditions.

Surface roughness is a primary determinant of early bacterial adhesion in orthodontic materials, as microgrooves and machining marks provide protective niches for bacterial retention and biofilm maturation (1). The substantial reduction in  $R_a$  observed after CS–GO coating suggests effective surface leveling through film formation and partial filling of machining irregularities. This aligns with broader evidence that functional coatings can modulate topography and reduce micro-asperities that promote plaque accumulation (2). The SEM observations further supported this interpretation, showing a more continuous and homogeneous surface morphology in coated specimens. From a mechanistic perspective, the polymeric matrix of chitosan likely contributed to film continuity, while GO reinforcement may have enhanced structural stability of the deposited layer, consistent with composite nanocoating principles described for dental metal substrates (11).

The marked reduction in contact angle indicates increased hydrophilicity of the coated surface. Surface energy and wettability influence salivary pellicle formation and the physicochemical interactions governing bacterial attachment (10). Although the relationship between hydrophilicity and microbial adhesion is complex and context-dependent, a shift from  $83.6^\circ$  to  $61.2^\circ$  represents a substantial alteration in interfacial behavior that may reduce the strength of initial bacterial adhesion forces. Previous investigations of graphene-based and other advanced coatings on orthodontic components have reported modifications in surface energy associated with changes in friction and microbial colonization patterns (3,10). In this context, the present findings support the hypothesis that physicochemical modulation of the wire surface contributes to the observed antibiofilm effect rather than bioactivity alone.

The reduction in *Streptococcus mutans* biofilm burden constitutes one of the most clinically relevant findings of this study. A  $0.62\ \log_{10}$  decrease corresponds to nearly a fourfold reduction in recoverable bacterial load per wire segment. Given the established role of *S. mutans* in enamel demineralization and white spot lesion development during fixed appliance therapy (1), such a reduction, if sustained intraorally, could meaningfully influence cariogenic risk. The antibiofilm effect is plausibly attributable to the combined actions of chitosan and GO. Chitosan's cationic nature facilitates electrostatic interactions with negatively charged bacterial cell walls, potentially disrupting membrane integrity and metabolic function (8). Graphene oxide, in turn, has been associated with contact-mediated membrane stress and oxidative damage in bacterial systems (4,5,13). Importantly, the biofilm model employed in this study allowed 48-hour maturation under controlled incubation, thereby assessing coating performance beyond simple planktonic bactericidal activity. These findings are consistent with emerging reports of graphene-based composite coatings demonstrating antibiofilm potential on orthodontic and dental alloys (3,12).

Frictional resistance at the bracket–wire interface is a critical determinant of mechanical efficiency during sliding mechanics. The observed 0.72 N reduction (approximately 30.8%) suggests that CS–GO coating materially improves tribological performance under standardized test conditions. Previous studies evaluating graphene-containing coatings and related nanocomposites on orthodontic wires have reported reductions in friction attributable to surface smoothing and solid-lubricant-like behavior of graphene derivatives (6,14). The substantial effect size in the present study supports the concept that topographical refinement and possible reduction of adhesive contact at the interface both contributed to friction mitigation. From a clinical biomechanics perspective, even moderate reductions in friction may enhance force transmission efficiency and reduce the need for higher applied forces, potentially lowering risks of anchorage loss and discomfort.

Short-term artificial saliva immersion suggested improved surface stability of coated specimens relative to uncoated wires, which exhibited early corrosion-related surface changes. Graphene-family nanocoatings have been shown to enhance corrosion resistance in dental alloys by forming barrier layers that reduce ion diffusion and electrochemical activity (11,12). Although the present aging duration was limited to 14 days, the absence of gross delamination or coating failure indicates preliminary structural integrity under simulated oral conditions. Durability remains a key translational requirement, as coating degradation could reverse gains in roughness and friction over time.

Several strengths of this investigation enhance its internal validity. The study employed predefined endpoints, random allocation of specimens, blinding of surface assessors, standardized instrumentation calibration, and log transformation of CFU data to address distributional skewness. Reporting of effect sizes and confidence intervals permits quantitative interpretation beyond sole reliance on p-values. However, certain limitations should be acknowledged. First, the *in vitro* model cannot fully replicate the complexity of the oral ecosystem, where multispecies biofilms, fluctuating pH, enzymatic activity, and mechanical abrasion coexist. Single-species biofilm testing may overestimate or underestimate real-world performance. Second, friction testing was conducted under dry laboratory conditions; intraoral lubrication by saliva may alter absolute friction values, though relative differences may persist. Third, long-term mechanical wear, thermal cycling, and brushing abrasion were not evaluated and may influence coating durability.

From a translational standpoint, the integration of surface smoothness, enhanced hydrophilicity, significant antibiofilm reduction, and frictional improvement in a single composite coating represents a clinically attractive profile. Importantly, the magnitude of antibacterial reduction exceeded the proportional reduction observed for friction, suggesting that microbial modulation may be the dominant functional benefit, with tribological improvement providing additional mechanical advantage. Future investigations should incorporate multispecies biofilm models, extended aging protocols, cytotoxicity assessments, and *in vivo* trials to determine whether the observed *in vitro* benefits translate into reduced plaque indices, lower incidence of white spot lesions, and improved orthodontic efficiency during treatment.

In summary, CS–GO coating of stainless-steel orthodontic wires produced consistent, statistically robust improvements across surface, microbiological, and mechanical domains. The coherence of these effects supports the underlying design rationale of combining a biocompatible polymer matrix with graphene-based reinforcement to achieve multifunctional performance. While further validation under clinical conditions is necessary, the present findings provide a strong mechanistic and quantitative foundation for continued development of composite nanocoatings in orthodontic biomaterials science (2,3,11).

## CONCLUSION

Within the limitations of this controlled in vitro investigation, chitosan–graphene oxide coating of stainless-steel orthodontic archwires demonstrated statistically significant and quantitatively substantial improvements in surface topography, wettability, antibiofilm performance, and frictional resistance compared with uncoated wires. The coating reduced surface roughness by 0.17  $\mu\text{m}$ , decreased contact angle by 22.4°, achieved an approximate 75.9% reduction in *Streptococcus mutans* biofilm burden, and lowered frictional force by 0.72 N under standardized sliding conditions, all with large effect sizes and narrow confidence intervals. These findings suggest that CS–GO surface modification provides a coherent multifunctional benefit by simultaneously improving physicochemical properties and biomechanical efficiency while maintaining short-term stability in artificial saliva. Although clinical validation and long-term durability assessment remain necessary, the present data support CS–GO composite coating as a promising strategy to enhance the biological safety and mechanical performance of orthodontic stainless-steel archwires.

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## DECLARATIONS

**Ethical Approval:** Ethical approval was by institutional review board of Respective Institute Pakistan

**Informed Consent:** Informed Consent was taken from participants.

**Authors' Contributions:**

Concept: MAS; Design: MAS, AI; Data Collection: BW, UW; Analysis: MZ; Drafting: MAS, AS, HY

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**Data Availability:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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