

Characterization of Antibacterial and Biofilm Inhibition in Tin and Zinc Oxide Nanoparticle-Doped Orthodontic Composites

Shifo Savio¹, Remmiya Mary Varghese^{2,*}, Prem Vishva Natarajan³, Pugal Mani⁴

Abstract

Background: This study investigated the antibacterial effectiveness of orthodontic adhesive enhanced with stannous oxide (SnO₂) nanoparticles against *Lactobacillus acidophilus* and *Streptococcus mutans*, in comparison to traditional adhesives without nanoparticle infusion. **Methods:** A modified orthodontic adhesive was prepared by incorporating 1% w/w of tin dioxide and zinc oxide nanoparticles (SnO₂ + ZnO₂ NPs) into Transbond XT. This nanoparticle-enhanced composite was used to fabricate discs for assessing antibacterial activity against *Lactobacillus acidophilus* and *Streptococcus mutans* using a biofilm inhibition test. To evaluate the antibacterial effect and biofilm inhibition properties of the modified adhesive, a total of 50 composite discs were fabricated. The antibacterial properties of composite discs infused with SnO₂ and ZnO₂ nanoparticles were evaluated against *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) using disc agar diffusion and biofilm inhibition assays. Inhibition zones were measured on Mueller-Hinton agar, while biofilm formation was assessed on light-cured and polished composite discs over multiple three-day cycles. Bacterial suspensions were prepared under controlled conditions, and CFU counts were used to quantify biofilm inhibition. Additionally, direct contact tests and microscopic analyses, including SEM and confocal laser scanning microscopy with DAPI staining, were conducted to examine bacterial attachment and early biofilm development on the composite surfaces. **Results:** The disc agar diffusion test showed significantly larger zones of inhibition around SnO₂+ZnO₂-infused composite discs compared to the control, indicating enhanced antibacterial activity against *Lactobacillus acidophilus* and *Streptococcus mutans*. SEM analysis revealed reduced *Streptococcus mutans* attachment and sparser biofilm formation on SnO₂+ZnO₂-infused composites compared to unmodified discs. CLSM imaging further confirmed lower biofilm density on nanofilled composites, demonstrating effective inhibition of early biofilm development. **Conclusion:** The antibacterial activity of orthodontic composites modified with SnO₂ NPs was significant compared with conventional composites. The control group showed less SBS when compared to the NP-infused composite, with a statistically significant difference in mean SBS values between both groups.

*Author for Correspondence

Remmiya Mary Varghese
E-mail: remmiyav.sdc@saveetha.com

¹Postgraduate Student, Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

²Associate Professor, Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

³Senior Lecturer, Department of orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

⁴Professor, Department of Biomaterials, Research Department Blue Lab, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

Received Date: September 06, 2025

Accepted Date: September 29, 2025

Published Date: November 08, 2025

Citation: Shifo Savio, Remmiya Mary Varghese, Prem Vishva Natarajan, Pugal Mani. Characterization of Antibacterial and Biofilm Inhibition in Tin and Zinc Oxide Nanoparticle-Doped Orthodontic Composites. Journal of Polymer & Composites. 2025; 13(6): 230–239p.

Keywords: antibacterial property, shear bond strength, composites, orthodontic adhesive, nanoparticles.

INTRODUCTION

In orthodontics, progress in material science has significantly transformed treatment approaches and

improved patient outcomes. Orthodontic composites are now fundamental in attaching brackets and various appliances, providing benefits such as enhanced aesthetics, stronger adhesion, and shorter treatment times. Despite these advancements, a persistent challenge during fixed appliance therapy is the demineralization of enamel surfaces [1]. Brackets and other fixed orthodontic appliances tend to create niches that encourage plaque accumulation due to their retentive design [2]. The resulting changes in the oral microbiota—particularly the proliferation of acidogenic bacteria like *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus*—lead to the production of acids that contribute to enamel demineralization and the formation of white spot lesions (WSLs) [2,3,4]. These lesions can begin forming as early as four weeks after appliance placement, highlighting the critical need for effective prevention and management strategies [5].

Recent advancements in orthodontic materials have aimed to address this issue, with increasing interest in the incorporation of nanotechnology in dental applications [6,7]. We are constantly bombarded with enthusiastic claims about how nanotechnology is benefiting every aspect of orthodontic society [8]. Nanoparticles (NPs), owing to their distinctive physicochemical properties, have demonstrated enhanced antibacterial effects by interacting with the negatively charged surfaces of bacterial cells. Researchers have explored embedding such nanomaterials into orthodontic adhesives to improve their antibacterial efficacy without compromising mechanical properties [9]. A study by Ahn et al. [10] found that integrating silver nanofillers into orthodontic bonding agents could effectively reduce enamel demineralization while preserving adhesive performance.

Moreover, nanocomposites, nano ionomers and fibres have been reported to meet clinically acceptable shear bond strength (SBS) standards, making them suitable candidates for bonding in orthodontics [11]. Similar study by Almeshaal et shown that fibers from *Grewia monticola* Sond (GMS) could potentially serve as a reinforcing material in biocomposites[12]. Kenaf/banana hybrid composites containing up to 40 wt.% fiber content have demonstrated suitability for wear-related applications, such as disc brakes, under real-world conditions. Even when fully saturated with water, these composites do not exhibit a significant reduction in mechanical strength[13]. The elemental analysis confirms the organic nature of cellulose due to its high carbon and oxygen content. Based on the physicochemical properties, these celluloses could serve as alternative sources for industrial applications, particularly as reinforcement fillers in polymer composites for value-added products[14]. Incorporating nanofillers can enhance the tensile strength retention of open-hole laminates and postpone the initiation of failure [15]. Among the various nanoparticles studied, zinc oxide, silver, gold, and titanium have shown significant antibacterial effects against *S. mutans* [11].

The selection of tin oxide (SnO₂) and zinc oxide (ZnO) nanoparticles is justified due to their strong and synergistic antimicrobial activity, cost-effectiveness, biocompatibility, and minimal discoloration compared to silver nanoparticles, which, despite their potency, can cause esthetic concerns. Recent studies have shown that combining SnO₂ with ZnO enhances the production of reactive oxygen species and improves contact-killing mechanisms against oral pathogens [16]. With their high surface area and strong interaction with microbial membranes, SnO₂-ZnO nanocomposites are emerging as promising additives in orthodontic composites.

This study, therefore, focuses on evaluating and comparing the antibacterial performance of a SnO₂-ZnO nanocomposite-infused orthodontic adhesive against a conventional, non-nano-modified composite. The primary aim is to assess the potential of the nano-enhanced material in minimizing bacterial activity associated with enamel demineralization

MATERIALS AND METHODS

The study was performed at Saveetha Dental College and Hospitals in Chennai, India. A tin dioxide (SnO₂) nanoparticle composite was created by mixing commercial SnO₂ nanoparticles with an orthodontic adhesive resin (3M™ Transbond™ XT Light Cure Adhesive - Figures 1 and 2).



Figure 1. Transbond XT adhesive.



Figure 2. The image shows a light-cured nanoparticle composite block and its powdered form

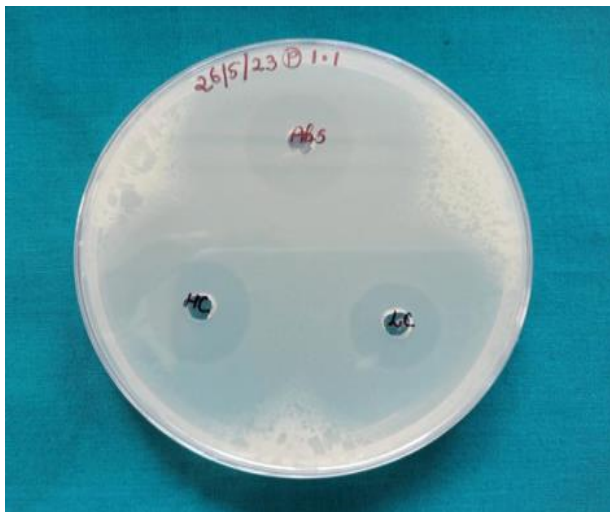


Figure 3. Abs, LC, and HC marked on the agar plate are standard antibiotics used for antimicrobial testing. NP, nanoparticle; SnO₂, tin dioxide and zinc oxide.

The nanoparticles, measuring 30–50 nm with >99.5% purity, were added at an optimized weight ratio. Mixing occurred in a dark room using a Retsch Emax high-energy ball mill.

Sample Preparation

A total of 50 composite discs were fabricated for this study. Following the methodology of Reddy et al., the sample size was calculated using G*Power software (version 3.1) with a power of 95%. Each disc was consistently prepared with a 3 mm thickness and 6 mm diameter, using a Bioplast transparent thermoplastic sheet (Figure 3).

Antibacterial Testing

To test for antibacterial properties, composite discs were used against *S. mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307). The disc agar diffusion test was employed to measure the zone of inhibition on Mueller-Hinton agar (Figure 3). Three discs were placed 2 cm apart: a conventional Transbond XT control and two infused with SnO₂+ZnO₂ nanomaterials. A 20 µL bacterial suspension (10⁸ CFU/mL) was added to each disc, and bacterial growth inhibition was measured after 48 hours.

Preparation of Bacterial Suspensions

Streptococcus mutans (MTCC 497) was grown in brain heart infusion (BHI) broth with 40 µg/mL Bacitracin at 37°C, shaking at 300 rpm for 48 hours. The bacterial suspension's turbidity was adjusted after centrifugation at 3180 g for 15 minutes.

Biofilm Inhibition Assay

To evaluate biofilm inhibition, twelve composite discs (n=12,100±3 mg) were light-cured (470 nm) for 20 seconds and polished with a Sof-Lex disc system. Biofilms were grown on these discs in 24-well plates using *S. mutans* and *L. acidophilus*. After three days, the biofilms were disrupted with PBS and glass beads, homogenized, and serial dilutions were performed to quantify CFU on BHI agar plates. This process was repeated six times.

Aging of Biofilm Composites

Biofilms were grown on the same discs for three separate, three-day cycles. Between each cycle, the discs were sonicated in double-distilled water for 15 minutes to eliminate residual proteins and biofilm matrix. Biofilm inhibition was then quantified using the same method as the initial experiment.

Direct Contact Inhibition and Microscopy

To observe bacterial attachment and early biofilm formation, composite discs were inoculated with bacterial solutions (10⁷ CFU) and incubated for 24 hours. The samples were fixed with 2.5% glutaraldehyde and sodium cacodylate buffer, then dehydrated with ethanol. Finally, the samples were visualized using a scanning electron microscope (S-3000N, Hitachi Sciences Systems) and a confocal laser scanning microscope (LSM 510 META, Carl Zeiss MicroImaging) with DAPI staining.

RESULTS

Disc Agar Diffusion Test

The disc agar diffusion test revealed significant differences in the zones of inhibition for both *Lactobacillus acidophilus* and *Streptococcus mutans*. The zones of inhibition were substantially larger around the nanofilled composite discs compared to the control group, indicating that the SnO₂+ZnO₂-infused composites demonstrated a stronger antibacterial effect against both bacterial strains. This can be visualized in Figures 4–6, where the inhibition zones for the nanocomposite discs are clearly more pronounced than those for the unmodified control discs.

The results clearly demonstrate enhanced antibacterial activity in nanoparticle-doped composites compared to controls, with statistically significant differences in inhibition zones ($p < 0.05$). The differences in biofilm inhibition were not only statistically significant ($p < 0.05$), but also clinically relevant, as the reduction in biofilm mass directly correlates with lower risk of enamel demineralization and white spot lesion formation in orthodontic patients.

Biofilm Inhibition by Microscopy

Cell adherence and biofilm growth patterns were qualitatively assessed on both the unmodified and nanofilled composite discs using Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM).

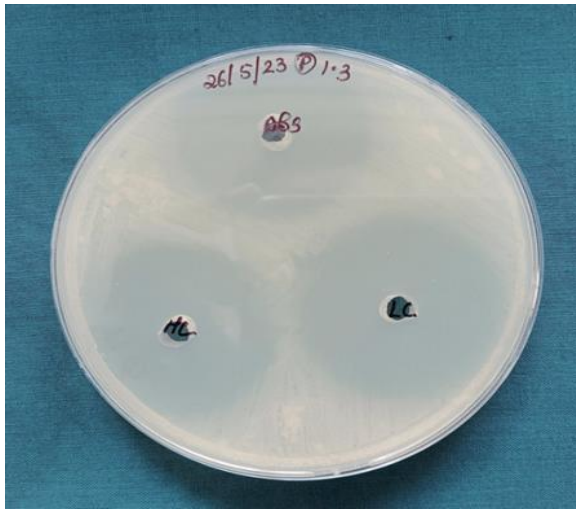


Figure 4. Zones of inhibition around conventional composite discs (control). The transparent circles around each composite disc indicate the zones with no bacterial growth, while the white areas represent regions with bacterial growth. Abs, LC, and HC are standard antibiotics used for comparison.

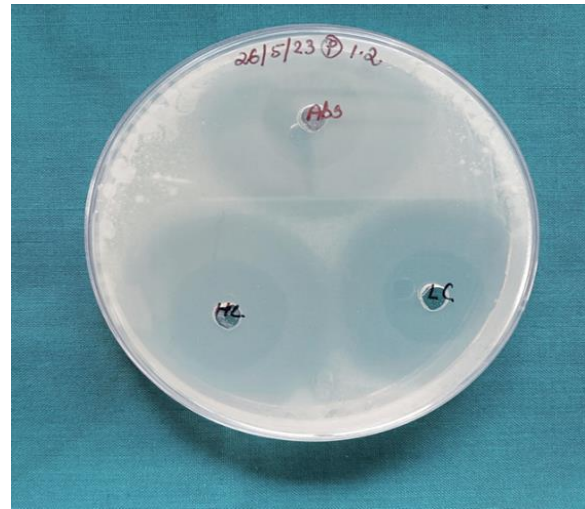


Figure 5. Zone of inhibition around SnO₂+ZnO₂-filled composite against *Lactobacillus acidophilus*, demonstrating a larger inhibition zone compared to the control. (NP, nanoparticle; tin dioxide and zinc oxide)

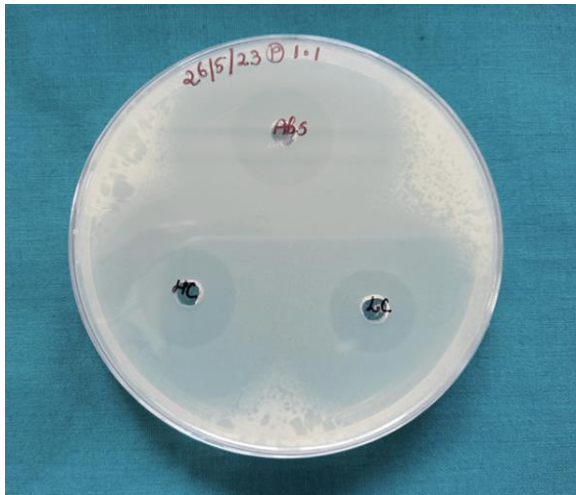


Figure 6. Zone of inhibition around the nanofilled composite discs against *Streptococcus mutans*, showing a larger inhibition zone compared to the control composite discs. (NP, nanoparticle; tin dioxide and zinc oxide).

SEM Observations (Figure 7)

Streptococcus mutans attachment after one day of growth was notably higher on the unmodified composite discs compared to the SnO₂+ZnO₂-infused composite discs. The nanofilled composites showed a sparse distribution of microbial cells and less dense biofilm formation.

CLSM Observations (Figure 8)

The CLSM images demonstrated a higher biofilm coverage on the unmodified composites after one day, with a continuous biofilm layer formed. In contrast, the SnO₂+ZnO₂-composites exhibited significantly lower biofilm density, indicating effective inhibition of biofilm formation.

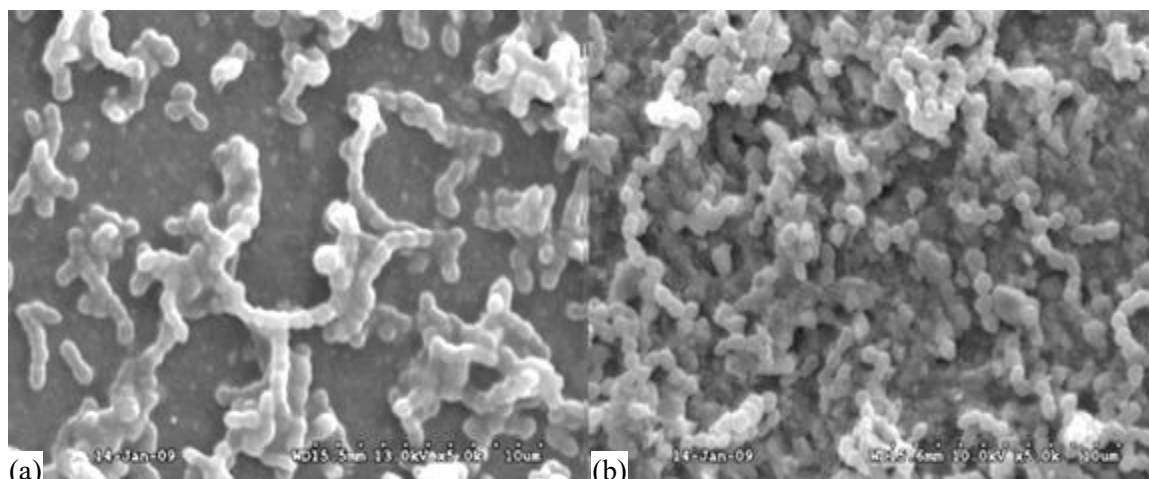


Figure 7. Streptococcus mutans biofilm attachment after one day of incubation, captured using scanning electron microscopy (5000× magnification). The images show: (a) SnO₂+ZnO₂-infused composite, where bacterial attachment is sparse, with fewer bacterial colonies and limited biofilm formation; (b) unmodified composite, displaying a denser and more widespread biofilm coverage, indicating significantly higher bacterial colonization. The differences in biofilm density and attachment illustrate the enhanced antibacterial effect of the SnO₂+ZnO₂ nanocomposite.

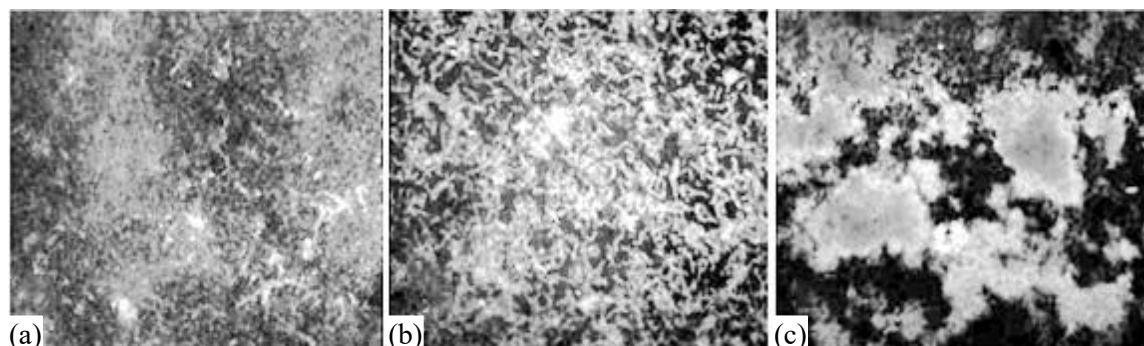


Figure 8. Visualization of Streptococcus mutans biofilm stained with DAPI using confocal laser scanning microscopy (CLSM). The images show biofilm attachment after one day of incubation on: (a) SnO₂+ZnO₂-infused composite, with sparse and less dense biofilm formation; (b) unmodified composite, exhibiting significantly higher biofilm coverage; (c) a low magnification view (120×) of the biofilm on the unmodified composite, revealing extensive biofilm formation and distribution across the composite surface. The contrast between the SnO₂+ZnO₂ nanocomposite and the unmodified composite highlights the superior biofilm inhibition of the nanofilled material.

Additionally, neither SEM nor CLSM images showed any bacterial cell attachment on the Ag control composites after one day of incubation (data not shown).

The differences in biofilm inhibition were not only statistically significant ($p < 0.05$), but also clinically relevant, as the reduction in biofilm mass directly correlates with lower risk of enamel demineralization and white spot lesion formation in orthodontic patients.

DISCUSSION

This study evaluated the antibacterial properties and biofilm inhibition potential of orthodontic composites infused with SnO₂ and ZnO₂ nanoparticles (NPs). The combination of these nanomaterials was incorporated into a commercially available orthodontic adhesive, 3M™ Transbond™ XT, and compared with unmodified composites for their efficacy against Streptococcus mutans and Lactobacillus acidophilus, two key bacterial species implicated in dental caries and biofilm formation

[17, 18]. The findings of this study provide important insights into the potential application of nanofilled composites in clinical orthodontics to reduce bacterial colonization and mitigate the risk of plaque accumulation around orthodontic appliances.

The disc agar diffusion test demonstrated a significant antibacterial effect of the SnO₂+ZnO₂-infused composite against both *S. mutans* and *L. acidophilus*. The nanofilled composite discs exhibited considerably larger zones of inhibition compared to the unmodified control discs. This confirms that nanoparticle doping significantly enhances the antibacterial properties of orthodontic composites [19]. These results suggest that the incorporation of SnO₂ and ZnO₂ nanoparticles into orthodontic adhesives enhances the antibacterial properties of the material [20-22].

Several studies have reported the antimicrobial properties of metal oxide nanoparticles, particularly SnO₂ and ZnO₂, which are known for their bactericidal activities due to their ability to generate reactive oxygen species (ROS) [23]. These ROS can induce oxidative stress in bacterial cells, leading to cell membrane disruption and DNA damage. This mechanism likely accounts for the superior antibacterial performance observed in the nanocomposite discs, as the nanoparticles release ROS upon contact with bacterial cells, creating an inhospitable environment that inhibits bacterial growth and proliferation [24-26].

Biofilm formation is a major challenge in orthodontic treatments, as it can lead to enamel demineralization, white spot lesions, and increased caries risk [27]. Therefore, evaluating the biofilm inhibition potential of the modified composite was a crucial aspect of this study. Both SEM and CLSM observations revealed that biofilm formation was significantly reduced on the SnO₂+ZnO₂ infused composites compared to the unmodified control composites [28-30].

After one day of incubation, *S. mutans* attachment and biofilm growth were markedly higher on the unmodified composite discs, while the SnO₂+ZnO₂ composites exhibited a sparse distribution of bacterial cells. This indicates that the nanocomposite effectively reduced the initial bacterial adhesion, which is a critical step in biofilm formation. The roughness and surface properties of the composite materials play a significant role in bacterial attachment. The presence of SnO₂ and ZnO₂ nanoparticles may have altered the surface characteristics of the composite, making it less conducive for bacterial colonization [31].

The CLSM images further corroborated the SEM observations, showing dense biofilm formation on the unmodified composites after one day, with a continuous biofilm layer covering the surface. In contrast, the biofilms on the nanocomposites were sparser and less structured, indicating that the SnO₂+ZnO₂ nanomaterials disrupted the biofilm formation process. This suggests that the nanoparticles not only inhibit bacterial growth but also interfere with biofilm maturation, reducing the overall biomass and structural integrity of the biofilm [32,33].

These findings are consistent with previous studies that have demonstrated the ability of metal oxide nanoparticles to inhibit biofilm formation. Specifically, recent reports indicate that SnO₂ and ZnO disrupt biofilm development by interfering with quorum sensing and increasing oxidative stress in pathogens [32].

SnO₂+ZnO₂, in particular, has been shown to disrupt bacterial quorum sensing, a cell-to-cell communication mechanism that regulates biofilm development. By interfering with this process, SnO₂+ZnO₂ may prevent bacteria from forming well-organized biofilm communities, thereby reducing the risk of plaque accumulation and subsequent enamel demineralization [34].

The absence of cell attachment on the Ag control composites, as observed in both SEM and CLSM images, further highlights the importance of selecting appropriate nanomaterials for dental applications. While silver nanoparticles are well known for their potent antibacterial activity, their

potential cytotoxicity and discoloration issues limit their widespread use in clinical settings, especially in esthetically sensitive areas such as orthodontic treatment [35, 36]. By contrast, the use of SnO₂ and ZnO provides a biocompatible and visually acceptable alternative, as supported by comparative in vitro cytotoxicity studies [32].

Limitations

This study was conducted under controlled laboratory conditions, and the clinical environment presents additional challenges that may affect the performance of the nanofilled composites. Factors such as salivary flow, diet, and oral hygiene practices could influence the antibacterial efficacy of the composite in a real-world setting. Furthermore, the sample size for biofilm inhibition studies was relatively small, and the results should be interpreted with caution. Larger-scale studies with more extensive replication are warranted to confirm the findings of this study.

CONCLUSION

In summary, adding SnO₂ and ZnO₂ nanoparticles to orthodontic composites significantly enhanced antibacterial and anti-biofilm effects against *S. mutans* and *L. acidophilus*, suggesting their potential to reduce bacterial colonization and prevent enamel demineralization during treatment. Further in vivo studies are needed to confirm long-term safety and efficacy.

REFERENCES

1. Altmann ASP, Collares FM, Leitune VCB, Samuel SMW. The effect of antimicrobial agents on bond strength of orthodontic adhesives: a meta-analysis of in vitro studies. *Orthod Craniofac Res* 2016;19:1–9.
2. Borzabadi-Farahani A, Borzabadi E, Lynch E. Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. *Acta Odontol Scand* 2014;72:413–7.
3. Manfred L, Covell DA, Crowe JJ, Tufekci E, Mitchell JC. A novel biomimetic orthodontic bonding agent helps prevent white spot lesions adjacent to brackets. *Angle Orthod* 2013;83:97–103.
4. Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M. Adhesion of *Streptococcus mutans* to different types of brackets. *Angle Orthod* 2007;77:1090–5.
5. Adel SM, El-Harouni N, Vaid NR. White Spot Lesions: Biomaterials, Workflows and Protocols. *Seminars in Orthodontics* 2023;29:68–78.
6. Vaid NR, Adel SM. Contemporary orthodontic workflows: A panacea for efficiency? *Semin Orthod* 2023;29:1–3.
7. Al-Eesa NA, Wong FSL, Johal A, Hill RG. Fluoride containing bioactive glass composite for orthodontic adhesives - ion release properties. *Dent Mater* 2017;33:1324–9.
8. Yves Gendron, Jane Andrew, Christine Cooper, Helen Tregidga, On the juggernaut of artificial intelligence in organizations, research and society, *Critical Perspectives on Accounting*, Volume 100, 2024, 102759, ISSN 1045-2354,
9. Padmanabhan S. Nanotechnology in Orthodontics. *Seminars in Orthodontics* 2023;29:79–84.
10. Ahn S-J, Lee S-J, Kook J-K, Lim B-S. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. *Dent Mater* 2009;25:206–13.
11. Uysal T, Yagci A, Uysal B, Akdogan G. Are nano-composites and nano-ionomers suitable for orthodontic bracket bonding? *Eur J Orthod* 2010;32:78–82.
12. Almeshaal, M., Palanisamy, S., Murugesan, T. M., Palaniappan, M., & Santulli, C. (2022). Physico-chemical characterization of *Grewia Monticola* Sond (GMS) fibers for prospective application in biocomposites. *Journal of Natural Fibers*, 19(17), 15276–15290.
13. Palanisamy, Sivasubramanian & Mayandi, K. & Azeez, Alavudeen & Palaniappan, Murugesan & Dharmalingam, Shanmugam & Rajini, N. & Santulli, Carlo. (2022). Wear Properties and Post-Moisture Absorption Mechanical Behavior of Kenaf/Banana-Fiber-Reinforced Epoxy Composites. *Fibers*. 10. 32. 10.3390/fib10040032.

14. Palaniappan, M., Palanisamy, S., Khan, R. *et al.* Synthesis and suitability characterization of microcrystalline cellulose from *Citrus x sinensis* sweet orange peel fruit waste-based biomass for polymer composite applications. *J Polym Res* **31**, 105 (2024). <https://doi.org/10.1007/s10965-024-03946-0>
15. Residual Tensile Properties of Natural-Fiber-Reinforced Composites: A Review. *Journal of Composites Science*. 2023; 7(4):136. <https://doi.org/10.3390/jcs7040136>
16. Hernández-Sierra JF, Ruiz F, Pena DCC, Martínez-Gutiérrez F, Martínez AE, Guillén A de JP, et al. The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. *Nanomedicine* 2008;4:237–40.
17. Rajamanickam, P., & Pugalmani, S. Evaluation of Cytotoxic Behavior of Orthodontic Composite Containing Zinc Oxide and Tin Oxide Nanoparticles. *Journal of Chemical Health Risks*, 13(4).2023
18. de Soet JJ, de Graaff J. Microbiology of carious lesions. *Dental Update* 1998;25.
19. Wilson RM, Donly KJ. Demineralization around orthodontic brackets bonded with resin-modified glass ionomer cement and fluoride-releasing resin composite. *Pediatric Dentistry* 2001;23.
20. Missier, M. S., Mahesh, R., Saravana Dinesh, S. P., & Rajeshkumar, S. (2024). Commercial ZnO NPs Coating on Orthodontic Bands and Its In-Vivo Evaluation. *Zenodo*. [<https://doi.org/10.1007/s10965-024-03946-0>]
21. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine* 2007;3:95–101.
22. Miura N, Shinohara Y. Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells. *Biochem Biophys Res Commun* 2009;390:733–7.
23. Salehi P, Babanouri N, Roiein-Peikar M, Zare F. Long-term antimicrobial assessment of orthodontic brackets coated with nitrogen-doped titanium dioxide against *Streptococcus mutans*. *Prog Orthod* 2018;19:35.
24. Guggenheim B, Giertsen E, Schüpbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. *J Dent Res* 2001;80:363–70.
25. Imazato S, Ebi N, Takahashi Y, Kaneko T, Ebisu S, Russell RRB. Antibacterial activity of bactericide-immobilized filler for resin-based restoratives. *Biomaterials* 2003;24:3605–9.
26. Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. *Biomaterials* 2006;27:3995–4002.
27. Zhou G, Li Y, Xiao W, Zhang L, Zuo Y, Xue J, et al. Synthesis, characterization, and antibacterial activities of a novel nanohydroxyapatite/zinc oxide complex. *J Biomed Mater Res A* 2008;85:929–37.
28. Adams LK, Lyon DY, McIntosh A, Alvarez PJJ. Comparative toxicity of nano-scale TiO₂, SiO₂ and ZnO water suspensions. *Water Sci Technol* 2006;54:327–34.
29. Peter K. Stoimenov †., Rosalyn L. Klinger †., George L. Marchin ‡. And, Kenneth J. Klabunde* †. Metal Oxide Nanoparticles as Bactericidal Agents 2002. <https://doi.org/10.1021/la0202374>.
30. Sunada K, Watanabe T, Hashimoto K. Bactericidal activity of copper-deposited TiO₂ thin film under weak UV light illumination. *Environ Sci Technol* 2003;37:4785–9.
31. Kritheka, C. K., & Bandari, S. (2024). Antimicrobial Activity of Piperine Based Zinc Oxide Nanoparticle against Dental Pathogens. *Journal of Research in Medical and Dental Science*, 12(2), 15-19.2024
32. Kumaraguru M, et al. In vitro evaluation of microhardness of hydrophilic pit and fissure sealants reinforced with green-synthesized silver nanoparticles. *World J Dent*. 2024;15(3):257-261.
33. Rajamanickam, P., & Pugalmani, S. Evaluation of Cytotoxic Behavior of Orthodontic Composite Containing Zinc Oxide and Tin Oxide Nanoparticles. *Journal of Chemical Health Risks*, 13(4).2023
34. Rajeshkumar, A. S., Pavithra, B. D., Tharani, C. M., Sulochana, D. G., & Jayasree, E. A. (2024). Green Nanomaterials: Zinc Oxide and Chitosan for Antimicrobial Activity Against Oral Pathogens. *In *Smart Nanomaterials for Infectious Diseases* (pp. 74–129).2024

-
35. Missier, M. S., Mahesh, R., Saravana Dinesh, S. P., & Rajeshkumar, S. (2024). Commercial ZnO NPs Coating on Orthodontic Bands and Its In-Vivo Evaluation.* Zenodo.
 36. Goutham, E. R. S., Hussain, S. S., Muthukumar, C., Krishnasamy, S., Kumar, T. S. M., Santulli, C., Palanisamy, S., Parameswaranpillai, J., & Jesuarockiam, N. (2023). Drilling Parameters and Post-Drilling Residual Tensile Properties of Natural-Fiber-Reinforced Composites: A Review. *Journal of Composites Science*, 7(4), 136. <https://doi.org/10.3390/jcs7040136>.