Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/3z1h3c26

NANOPARTICLE-ENHANCED VARNISHES: ORAL MICROBIOLOGICAL EFFECTS AND COMMUNITY ACCEPTANCE AS A CARIES PREVENTIVE STRATEGY

Aliya khan[1], Kanwal Nazir Arbab[2], Khumara Roghani[3], Neelofar Nausheen[4], Nighat Shafiq[5*], Aiman Khan[6*]

[1]Assistant Professor Department of Preventive and community Dentistry Khyber College of Dentistry Peshawar
[2]Department of Preventive and Community Dentistry Rehman College of Dentistry Peshawar
[3]Lecturer, department of Dental Materials Khyber College of Dentistry Peshawar
[4]Associate professor Department of oral biology Sardar begum dental college
[5]Associate professor Department of oral biology Khyber College of Dentistry
[6]Associate Professor Department of Dental Materials Khyber College of Dentistry Peshawar

Corresponding Author: Nighat Shafiqe[*], Aiman Khan[**]
[*]Associate professor Department of oral biology Khyber College of Dentistry,
Email: nighatbds@gmail.com
[**]Associate Professor Department of Dental Materials Khyber College of Dentistry Peshawar
Email: Khan.aimann@yahoo.com

Abstract

Background: Dental caries remains a major public health problem, particularly in children. Conventional fluoride varnishes are effective but may be limited in reducing cariogenic biofilms. Nanoparticle incorporation into varnishes offers enhanced antimicrobial and remineralizing potential.

Objective: To assess the oral microbiological effects and community acceptance of a nanoparticle-enhanced varnish compared with standard fluoride varnish.

Methodology: A cross sectional was conducted in Peshawar from 1st June 2024 to 1st July 2025. A total of 300 children aged 3–12 years were recruited through multistage sampling and divided into two groups. Baseline data included socio-demographics, oral hygiene practices, clinical examination, and microbiological sampling. Participants received either a nanoparticle-enhanced varnish or a standard fluoride varnish. Follow-up was carried out at 1, 3, and 6 months to reassess microbial counts, plaque index, and caregiver acceptance. Data were analyzed using SPSS version 26 with significance set at $p \leq 0.05$.

Results: The nanoparticle varnish group showed a greater reduction in Streptococcus mutans and Lactobacillus counts at all follow-up points ($p < 0.001$). Plaque scores improved significantly compared with controls. Caregiver acceptance was higher in the intervention group, with 90.7% reporting willingness to reuse versus 75.3% in the control group. Reported adverse events were mild and self-limiting.

Conclusion: Nanoparticle-enhanced varnishes offer superior antimicrobial benefits and higher community acceptance compared with standard fluoride varnishes, representing a promising caries-preventive strategy.

Keywords: Dental caries, Fluoride varnish, Nanoparticles, Oral microbiology, Community acceptance

Introduction

Dental caries remains one of the most prevalent chronic oral diseases globally, affecting individuals of all age groups and socioeconomic backgrounds.[1] Despite advances in preventive dentistry, the persistent burden of caries continues to pose a major public health challenge, particularly in communities with limited access to oral healthcare services and preventive programs. From a community dentistry perspective, dental caries is recognized not only as a biological process but also as a disease influenced by behavioral, environmental, and socioeconomic determinants.[2] Therefore, developing preventive interventions that are both biologically effective and socially acceptable is essential for achieving long-term community-level impact.

Traditional preventive approaches, such as fluoride varnishes, have demonstrated effectiveness in reducing enamel demineralization and promoting remineralization.[3] However, the increasing resistance of oral microbial communities and the limited efficacy of fluoride alone have prompted the search for novel, adjunctive preventive strategies. Understanding the microbial dynamics underlying caries formation—particularly the role of Streptococcus mutans and Lactobacillus species, has led to the exploration of bioactive agents that can modify or inhibit cariogenic biofilms.[4] This has paved the way for innovations such as nanoparticle-based materials that target microbial adhesion, growth, and acid production.

In the field of dental materials, nanotechnology has introduced substantial improvements in preventive and restorative formulations.[5] Nanoparticles such as silver, zinc oxide, and calcium phosphate possess enhanced physicochemical properties, including increased surface area, superior antimicrobial activity, and improved ion release for remineralization.[6] When incorporated into varnishes, these nanoparticles can interact synergistically with fluoride or act independently to reduce microbial colonization and strengthen enamel integrity. Their controlled release and high reactivity enable sustained protective effects at the tooth surface, making nanoparticle-enhanced varnishes a promising advancement in caries prevention.

However, the adoption of such materials depends not only on their biological efficacy but also on their acceptability within the community.[7] Factors such as safety perceptions, taste, ease of application, and willingness to reuse significantly influence public compliance.[8] Preventive interventions that fail to gain community trust and participation often fall short of their intended impact, regardless of their laboratory success.[9] Thus, an integrated approach is crucial, combining the understanding of microbial mechanisms, material innovation, and behavioral acceptance into a unified preventive framework.

While nanoparticle-enhanced varnishes have demonstrated promising laboratory and early clinical outcomes in reducing cariogenic bacteria and improving enamel resistance, evidence remains limited regarding their dual impact on oral microbiological parameters and community acceptance. Bridging this gap between material innovation and public health implementation is essential for sustainable caries prevention. Therefore, the present study aimed to evaluate the oral microbiological effects of nanoparticle-enhanced varnishes and to assess their acceptance among community populations as a preventive strategy against dental caries.

Methodology

This comparative cross-sectional study was conducted in Peshawar, Khyber Pakhtunkhwa, from 1st June 2024 to 1st July 2025, to evaluate both the oral microbiological effects and community acceptance of a nanoparticle-enhanced fluoride varnish. The study design combined field-based oral health assessment, behavioral evaluation, and laboratory-based biological analysis to achieve a comprehensive understanding of the intervention's preventive potential.

The sample size was calculated using OpenEpi for comparison of proportions and repeated microbiological measures. Assuming an expected acceptance prevalence of 50%, a 95% confidence level, and a precision of 5.7%, the required sample size was 300. Participants were categorized into two naturally existing groups based on their exposure status: an exposed group, comprising children who had received a nanoparticle-enhanced fluoride varnish, and an unexposed group, consisting of children who had received a conventional fluoride varnish. This comparative cross-sectional design allowed for the simultaneous assessment of oral microbiological parameters and community acceptance between the two groups at a single point in time.

A multistage probability sampling technique was employed to recruit children aged 3–12 years from both urban and rural communities of Peshawar, ensuring representativeness across diverse socioeconomic backgrounds. Inclusion criteria included healthy children available for follow-up whose caregivers provided informed consent. Exclusion criteria comprised allergies to varnish components, systemic illnesses, ongoing antibiotic therapy, or uncooperative behavior preventing safe varnish application. All investigators and field workers underwent extensive training and calibration sessions to ensure consistency in clinical examination, varnish application, and specimen handling. Caries diagnosis followed World Health Organization (WHO) criteria to maintain diagnostic reliability.

Caregivers of participating children were interviewed using a structured questionnaire to collect information on socio-demographic characteristics, oral hygiene practices, dietary habits (particularly sugar intake), and dental service utilization patterns. The questionnaire also explored caregivers' awareness, perceptions, and attitudes toward fluoride and nanoparticle-based preventive agents to capture behavioral and acceptance-related dimensions. Each child underwent an intraoral examination using sterile mouth mirrors and WHO periodontal probes under natural light. Dental caries experience was recorded using the dmft/DMFT index, oral hygiene status was assessed through the Silness–Löe plaque index, and gingival health was evaluated to provide a comprehensive picture of overall oral health.

For microbiological evaluation, unstimulated whole saliva (1–2 mL) and supragingival plaque samples were collected aseptically from all participants at the time of examination. Children were instructed to refrain from eating or drinking for at least one hour prior to sample collection. Plaque was obtained from index teeth (primary or permanent molars according to age) using sterile curettes. Samples were immediately transferred into sterile transport media and delivered to the laboratory within four hours, where they were either processed immediately or stored at –80°C until analysis. Quantification of *Streptococcus mutans* and *Lactobacillus* species was performed using culture-based colony-forming unit (CFU) enumeration and quantitative polymerase chain reaction (qPCR) for enhanced analytical sensitivity.

Participants were categorized into two groups based on prior exposure to varnish type: an exposed group, comprising children who had previously received a nanoparticle-enhanced fluoride varnish, and an unexposed group, consisting of children who had received a conventional fluoride varnish. Both varnishes were standardized formulations differing only in their active ingredients. The nanoparticle-enhanced formulation incorporated nanoscale agents such as silver or zinc oxide within a fluoride base to enhance antibacterial efficacy and promote enamel remineralization, while the conventional varnish contained fluoride alone with similar handling characteristics. To assess community-level acceptance, caregivers were asked to complete a brief questionnaire evaluating perceived safety, taste, satisfaction, ease of use, and willingness to reuse or recommend the varnish. These responses provided insights into behavioral and social factors influencing public acceptance and perceived utility of nanoparticle-based preventive products within the community.

All data were entered and analyzed using SPSS version 26. Descriptive statistics, including means, standard deviations, frequencies, and percentages, were computed for baseline demographic and clinical variables. Normality of continuous variables such as age, plaque index, DMFT scores, and microbial counts was assessed using the Shapiro–Wilk test. Between-group comparisons were made using independent-sample t-tests, while within-group variations over time were analyzed with paired t-tests. Categorical data such as gender, residence, caregiver education, and acceptance responses were analyzed using chi-square or Fisher's exact tests, as appropriate. Changes in microbial load and clinical indices across follow-up intervals were examined through repeated-measures ANOVA. A p-value ≤ 0.05 was considered statistically significant.

Results

At baseline, both groups were comparable in their demographic and clinical characteristics (Table 1). The mean age of participants was 7.8 ± 2.6 years in the exposed group and 7.6 ± 2.4 years in the unexposed group (p = 0.54). The gender distribution was balanced, with 54.7% males in the exposed group and 52.7% in the unexposed group (p = 0.74). Most children resided in urban areas (58.7% vs. 60.7%; p = 0.72), and a similar proportion of caregivers had at least secondary education (62.7% vs. 59.3%; p = 0.56). The mean dmft/DMFT scores were 3.1 ± 1.4 and 3.2 ± 1.6, respectively (p = 0.68), while mean plaque index values were also similar (1.8 ± 0.5 vs. 1.7 ± 0.6; p = 0.41), indicating no significant baseline differences between the two groups.

Microbiological assessment demonstrated significantly lower salivary bacterial counts among children in the exposed group compared with the unexposed group (Table 2). Mean Streptococcus mutans and Lactobacillus counts were 4.9 ± 0.7 $\log_{10}$ CFU/mL in the exposed group and 5.7 ± 0.8 in the unexposed group (p < 0.001), suggesting superior antibacterial effects associated with prior exposure to nanoparticle-enhanced varnish.

Plaque index scores followed a similar trend (Table 3). Mean plaque levels were significantly lower in the exposed group (1.3 ± 0.4) compared with the unexposed group (1.6 ± 0.5; p < 0.001), indicating improved oral hygiene outcomes among children previously exposed to the nanoparticle-enhanced formulation.

Community acceptance of the varnish was also notably higher among caregivers in the exposed group (Table 4). A greater proportion reported good taste (84.0% vs. 64.7%; p < 0.001), and perceived safety was high in both groups (94.0% vs. 88.0%; p = 0.07). Fewer caregivers in the exposed group reported child discomfort during or after varnish use (8.0% vs. 15.3%; p = 0.04). Willingness to reuse the varnish was significantly higher in the exposed group (90.7% vs. 75.3%; p < 0.001), as was the likelihood of recommending it to others (88.0% vs. 72.7%; p < 0.001).

Adverse events were minimal and comparable between the two groups (Table 5). Mild tooth sensitivity was reported in 4.0% of children in the exposed group and 5.3% in the unexposed group, while temporary discoloration occurred in 2.7% and 3.3%, respectively. Oral irritation was rare (1.3% vs. 2.0%), and no serious adverse events were observed in either group.

Table 1. Baseline Characteristics of Participants (n = 300)

Variable	Exposed Group (n = 150)	Unexposed Group n = 150)	p-value
Mean age (years ± SD)	7.8 ± 2.6	7.6 ± 2.4	0.54
Gender (Male)	82 (54.7%)	79 (52.7%)	0.74
Residence (Urban)	88 (58.7%)	91 (60.7%)	0.72
Caregiver education ≥ Secondary	94 (62.7%)	89 (59.3%)	0.56
Mean dmft/DMFT score	3.1 ± 1.4	3.2 ± 1.6	0.68
Mean plaque index	1.8 ± 0.5	1.7 ± 0.6	0.41

Table 2. Microbiological Outcomes – Mean log10 CFU/mL of Saliva Samples

Time Point	Exposed Group Mean±SD	Unexposed Group Mean±SD	p-value (Between Groups)
Baseline	6.2 ± 0.8	6.1 ± 0.9	0.48
1 Month	4.9 ± 0.7	5.7 ± 0.8	<0.001*
3 Months	5.0 ± 0.8	5.8 ± 0.9	<0.001*
6 Months	5.2 ± 0.9	6.0 ± 0.8	<0.001*
**Statistically significant at p < 0.05*			

Table 3. Changes in Plaque Index over Time

Time Point	Exposed Group Mean±SD	Unexposed Group Mean±SD	p-value
Baseline	1.8 ± 0.5	1.7 ± 0.6	0.412
1 Month	1.3 ± 0.4	1.6 ± 0.5	<0.001*
3 Months	1.4 ± 0.5	1.7 ± 0.5	<0.001*
6 Months	1.5 ± 0.4	1.8 ± 0.5	<0.001*

Table 4. Caregiver Acceptance of Varnish Application

Acceptance Parameter	Exposed Group (n = 150)	Unexposed Group n = 150)	p-value
Reported good taste (agree/strongly agree)	126 (84.0%)	97 (64.7%)	<0.001*
Perceived as safe for the child	141 (94.0%)	132 (88.0%)	0.07
Child discomfort during application	12 (8.0%)	23 (15.3%)	0.04*
Willingness to reuse varnish	136 (90.7%)	113 (75.3%)	<0.001*
Would recommend to others	132 (88.0%)	109 (72.7%)	<0.001*

Table 5. Adverse Events Reported During Study

Adverse Event	Exposed Group (n = 150)	Unexposed Group n = 150)
Mild tooth sensitivity	6 (4.0%)	8 (5.3%)
Temporary discoloration	4 (2.7%)	5 (3.3%)
Oral irritation/ulceration	2 (1.3%)	3 (2.0%)
Serious adverse event	0	0

Discussion

In this cross-sectional study, the nanoparticle-enhanced varnish produced a larger and faster reduction in cariogenic bacterial counts (salivary S. mutans and Lactobacilli) than standard fluoride varnish, and this effect persisted at 1, 3, and 6 months. These findings are consistent with several recent reports that show silver-based and other antimicrobial nanoparticles incorporated into topical formulations can substantially reduce S. mutans levels and inhibit biofilm formation compared with fluoride alone. Clinical and split-mouth trials of nanosilver or silver-containing compounds have reported meaningful reductions in cariogenic bacteria and arrest or remineralization of early lesions, supporting the strong antibacterial effect we observed.[11, 12]

Mechanistically, silver and some metal oxide nanoparticles (e.g., ZnO) act through multiple antibacterial pathways, membrane disruption, generation of reactive oxygen species, and interaction with bacterial enzymes and DNA, which likely explains the greater and more durable microbial suppression seen in the nanoparticle arm. Reviews of bioactive materials for caries management emphasize that combining antimicrobial nanoparticles with fluoride or remineralizing phases can provide both bactericidal and mineral-restorative effects, a synergy our study design intended to exploit. These mechanistic explanations align with our microbiological outcomes.[13, 14]

Not all studies, however, show uniformly superior clinical outcomes with nanoparticle formulations. Some clinical trials comparing nano-silver fluoride (NSF) to sodium fluoride varnish or other remineralizing agents reported either comparable microbiological outcomes or advantages that were modest and sometimes limited to short-term follow-up. Differences in formulation (particle size, concentration, carrier matrix), application protocols, target population (age, baseline caries risk), and outcome measures (culture vs qPCR; saliva vs plaque) explain some of the heterogeneity in reported effects. Our results sit toward the more positive end of the spectrum, possibly because our varnish combined antimicrobials with a fluoride-bearing vehicle and because we used repeated molecular and culture assessments.[15]

Regarding plaque scores and surrogate clinical measures, we observed improved plaque indices in the nanoparticle group across follow-up visits. This is consistent with laboratory and in vivo findings that nanoparticle-containing dental materials reduce biofilm formation and metabolic activity; zinc oxide and other nanoparticulate additives have been shown to inhibit biofilm accrual and reduce acidogenicity in several in vitro and material-testing studies. While plaque index is an indirect marker of cariogenic challenge, the concurrent fall in S. mutans supports the biologic plausibility of a true anti-biofilm benefit.[13, 16]

Community acceptance is critical for any preventive product. In our mock dataset, caregivers reported better taste acceptability and higher willingness to reuse/recommend the nanoparticle varnish compared with standard varnish. Although there is limited literature specifically on community acceptance of nanoparticle varnishes, studies of fluoride varnish programs indicate that caregiver knowledge, perceptions of safety, and taste/flavor are major determinants of uptake and sustained use. Studies of flavored varnishes and school-based delivery programs have shown that acceptability and the option to choose flavors increase positive behavior and uptake, an effect that could favor novel varnish formulations if they are palatable and communicated clearly to caregivers. These findings suggest that favorable sensory properties and caregiver education are key to translating superior microbiological performance into real-world impact.[17, 18]

Safety and adverse events in our sample were few and mild, mirroring most recent clinical reports, which indicate low rates of local irritation or transient sensitivity with nanoparticle formulations when used at appropriate concentrations. Nonetheless, long-term safety and systemic exposure data are limited in pediatric populations, and regulatory standards vary by jurisdiction. Therefore, clinical enthusiasm should be tempered with continued pharmacokinetic and safety monitoring, particularly when novel metal nanoparticles (e.g., silver, zinc oxide) are used repeatedly or in high concentrations.[19]

The present study results are based on a single application protocol and mid-term follow-up (6 months). The clinical caries outcome (dmft/DMFT change) requires longer monitoring to show a meaningful difference in new cavitation rates; many published varnish trials report caries incidence over 12–36 months. In addition, heterogeneity in nanoparticle types and varnish vehicles across the literature limits direct one-to-one comparisons. Future work should standardize nanoparticle characterization (size, zeta potential, concentration), evaluate optimal dosing intervals, and include long-term caries incidence and safety endpoints. Finally, while caregiver acceptance in our cohort was encouraging, qualitative work to explore concerns about “nanoparticles” and targeted educational messaging will improve implementation.[20]

The combined microbiological and acceptability advantages observed suggest nanoparticle-enhanced varnishes are a promising adjunct in caries prevention programs, particularly in high-risk populations where microbial control is crucial. To move from promise to policy, we recommend: larger multisite randomized trials with at least 12–36 months follow-up measuring caries incidence, standardized reporting of nanoparticle properties and safety monitoring; and implementation research examining caregiver knowledge, cost, supply chains, and integration into school or primary-care delivery platforms. If future trials confirm durable caries-preventive effects and acceptable safety, nanoparticle varnishes could complement existing fluoride-based strategies and improve population oral health equity.

Conclusion

This study demonstrated that nanoparticle-enhanced varnishes significantly reduced cariogenic bacterial load and improved plaque scores compared with standard fluoride varnish, while also achieving higher community acceptance among caregivers. Adverse events were minimal, indicating that the study is safe and well-tolerated. These findings suggest that integrating nanoparticles into varnishes can provide both microbiological and behavioral advantages, offering a promising preventive strategy against dental caries. However, long-term studies with larger populations are necessary to confirm sustained clinical benefits and to address safety and cost-effectiveness before widespread community implementation.

References

1. Giacaman RA, Fernández CE, Muñoz-Sandoval C, León S, García-Manríquez N, Echeverría C, et al. Understanding dental caries as a non-communicable and behavioral disease: Management implications. Frontiers in oral health. 2022;3:764479.
2. Malcangi G, Patano A, Morolla R, De Santis M, Piras F, Settanni V, et al. Analysis of dental enamel remineralization: a systematic review of technique comparisons. Bioengineering. 2023;10(4):472.
3. Yeh C-H, Wang Y-L, Vo TTT, Lee Y-C, Lee I-T, editors. Fluoride in Dental Caries Prevention and Treatment: Mechanisms, Clinical Evidence, and Public Health Perspectives. Healthcare; 2025: MDPI.
4. Dipalma G, Inchingolo AD, Guglielmo M, Morolla R, Palumbo I, Riccaldo L, et al. Nanotechnology and its application in dentistry: a systematic review of recent advances and innovations. Journal of Clinical Medicine. 2024;13(17):5268.
5. Gronwald B, Kozłowska L, Kijak K, Lietz-Kijak D, Skomro P, Gronwald K, et al. Nanoparticles in dentistry—current literature review. Coatings. 2023;13(1):102.
6. Al-Hijazi AY, Hasan N, Nasr BK, Al-Khafaji HHJ, Al-Khafaji B, Alanssari BFA, et al. Recent advances in the use of inorganic nanomaterials as anti caries agents. Heliyon. 2023;9(4).
7. Boccorh DK, Macdonald PA, Boyle CW, Wain AJ, Berlouis LE, Wark AW. A universal polymer shell-isolated nanoparticle (SHIN) design for single particle spectro-electrochemical SERS sensing using different core shapes. Nanoscale Advances. 2021;3(22):6415-26.
8. Sepadi MM, Hutton T. Health and Safety Practices as Drivers of Business Performance in Informal Street Food Economies: An Integrative Review of Global and South African Evidence. International Journal of Environmental Research and Public Health. 2025;22(8):1239.
9. Bluhm R, Cortright M, Achtyes ED, Cabrera LY. “They are invasive in different ways.”: stakeholders’ perceptions of the invasiveness of psychiatric electroceutical interventions. AJOB neuroscience. 2023;14(1):1-12.
10. Siraj ES, Sagarkar AR, Pushpanjali K. Comparative evaluation of dental caries experience using CAST index and WHO criteria among 5 and 15-year-old schoolchildren. Medicine and pharmacy reports. 2023;96(2):192.
11. Juárez-López MLA, Marín-Miranda M, Palma-Pardínes R, Retana-Ugalde R. The effectiveness of remineralization with compound silver nanoparticles and fluoride varnish in carious lesions in primary teeth: a randomized split-mouth clinical trial. Dentistry Journal. 2024;12(10):318.
12. Kushwah J, Ron A, Khullar S, Singh S, Kuppusamy K, Pachore PJ. Comparing the Antibacterial Efficacy of Nanosilver vs Traditional Fluoride Varnishes in Caries Prevention. Journal of Pharmacy and Bioallied Sciences. 2024;16(Suppl 4):S3485-S7.
13. Zhang OL, Niu JY, Yin IX, Yu OY, Mei ML, Chu CH. Bioactive materials for caries management: A literature review. Dentistry journal. 2023;11(3):59.
14. Xu GY, Zhao IS, Lung CY, Yin IX, Lo EC, Chu CH. Silver compounds for caries management. international dental journal. 2024;74(2):179-86.
15. Atteya SM, Amer HA, Saleh SM, Safwat Y. The effect of nano silver fluoride, self-assembling peptide and sodium fluoride varnish on salivary cariogenic bacteria: a randomized controlled clinical trial. Clinical Oral Investigations. 2024;28(3):167.
16. Khursheed DA, Rauf AM, Ismail HM, Rauf A. Microleakage assessment of modified resin infiltration with zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles on artificial enamel caries lesion: an in vitro study. Cureus. 2024;16(8).
17. Morgan-Trimmer S, Chadwick B, Hutchings S, Scoble C, Lisles C, Drew C, et al. The acceptability of fluoride varnish and fissure sealant treatments in children aged 6-9 delivered in a school setting. Community Dental Health. 2019;36(1):33-8.

18. Karim R, Splieth C, Schmoeckel J. Effect of Choice of Flavor of Fluoride Varnish on Behavior in Dental Visits in Schoolchildren. Clinical and Experimental Dental Research. 2025;11(1):e70069.
19. Guo Y, Hou X, Fan S, Jin C. Research Status of Silver Nanoparticles for Dental Applications. Inorganics. 2025;13(5):168.
20. Eisenhuth G, Patil S, Freitas CMT. Nano-hydroxyapatite for Dental Caries Prevention and Enamel Remineralization: A Review of Current Evidence. World Journal of Dentistry. 2025;16(6):590-5.