



ORIGINAL RESEARCH

SYNTHESIS AND IN VITRO CHARACTERIZATION OF A PROBIOTIC-ENRICHED PROPHYLACTIC POLISHING PASTE

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ABSTRACT

Background: Scaling roughens the tooth surface, increasing susceptibility to bacteria, and making polishing past essential post-procedure. A paste with probiotic effects and nanoparticles efficiently smooths enamel and repairs micro damage. Lactobacillus helps prevent harmful bacteria, supporting oral health. Nano-silica enhances polishing, while nano-hydroxyapatite remineralizes enamel.

Aim: Our study aimed to develop a prophylactic polishing paste incorporating Lactobacillus plantarum MTCC 5690 nano-silica, and nano-hydroxyapatite particles and to evaluate its in vitro properties, such as cytotoxicity activity antimicrobial activity, and surface roughness.

Materials and Methods:This is an in vitro study conducted in Green Lab, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India between August 2024 and September 2024. A novel prophylactic polishing paste was formulated with bioactive and probiotic components. The base comprised glycerin (25%) and sorbitol (25%) in a 1:1 ratio, with deionized water and Carbopol (1–2%) for viscosity. SiO₂ and hydroxyapatite nanoparticles were added for abrasive and remineralizing effects. Peppermint oil (0.2%), sodium fluoride (0.32%), and sodium benzoate (0.15%) were included for flavor, anti-caries action, and preservation, respectively. The pH was adjusted to ~7.0. After cooling below 40 °C, *Lactobacillus plantarum* MTCC 5690 (10⁸–10⁹ CFU/mL) and xylitol (2–5%) were added, followed by pH adjustment (5.5–7.0) to maintain probiotic viability. The final paste was homogenized and filtered for oral application and evaluated for its in vitro properties.

Results: The SEM image at 23,000× magnification revealed densely packed, heterogeneous angular structures with individual nanoparticles distinguishable at the 0.5 µm scale. In contrast, at 19,000× magnification, the particles appeared more compactly merged, suggesting the incorporation of *L. plantarum*, while irregular clusters and plate-like formations were identified as hydroxyapatite aggregates. The cytotoxicity analysis showed increased cell viability with higher concentrations of the prophylactic paste, peaking at 75 µL/mL. Fluorescence microscopy of MG-63 cells treated with prophylactic polishing paste showed dose-dependent viability. The control had sparse cells with low fluorescence, while 50 µL/mL treatment yielded the highest cell density and healthy morphology. Lower doses and control showed smaller, less spread cells, indicating mild cytotoxicity; higher doses promoted cell growth. The antimicrobial activity at low concentrations with inhibition zones slightly smaller than antibiotics. At high concentrations, the paste produced the largest inhibition zones, exceeding those of antibiotics, demonstrating strong antimicrobial efficacy. Surface roughness analysis revealed a significant reduction in roughness post-polishing, with area roughness (Sa) decreasing from 51.559 nm to 37.532 nm, highlighting the paste's ability to smooth the tooth surface and reduce plaque accumulation.

Conclusion:The formulated prophylactic polishing paste demonstrated strong antimicrobial activity and biocompatibility and enhanced cell viability in a dose-dependent manner and significantly reduced surface roughness post-polishing, and is beneficial for patients with gingivitis and periodontitis

Keywords -Oral prophylaxis, Lactobacillus plantarum, Nanoparticle, Hydroxyapatite

INTRODUCTION

Scaling is a procedure that involves the removal of plaque and calculus from the teeth ¹, and during this process, the tooth surface becomes more rough, making it more susceptible to bacterial accumulation. Thus, prophylactic polishing paste is an essential component in dental care, which is used primarily after scaling procedures to smooth the tooth surface and to prevent early plaque recolonization ². The polishing paste, typically containing fine abrasives, fluoride, and flavoring agents, is applied with a rubber cup or brush attached to a dental handpiece ³.

Lactobacillus bacteria are probiotics known for maintaining a healthy balance of bacteria in the oral cavity and preventing periodontal diseases ⁴. These bacteria also lower the counts of Streptococcus mutans, a primary bacterium responsible for tooth decay ⁵.

Nano-silica particles are highly effective abrasives due to their small size and high surface area, thus polishing tooth surfaces more efficiently. This results in a glossy, smooth enamel surface less likely to attract plaque and bacteria. They also enhance the paste's mechanical properties and have reduced abrasiveness compared to traditional polishing agents ⁶.

Nano-hydroxyapatite is a biomimetic material that closely resembles the natural mineral component of tooth enamel ⁷. When included in a polishing paste, they can help fill microscopic pores in the enamel surface, enhancing the tooth's natural defense against decay ⁸.

Commercially available chemical prophylactic polishing pastes present several potential drawbacks ⁹. They contain abrasive agents, thus causing increased tooth sensitivity. Some patients may find the paste's taste unpleasant, affecting their comfort during dental cleanings. These considerations emphasize the importance of exploring probiotic prophylactic polishing paste enriched with nanoparticles. Thus, our study aimed to develop a prophylactic polishing paste incorporating Lactobacillus plantarum MTCC 5690, nano-silica, and nano-hydroxyapatite particles, and to evaluate its in vitro properties.

MATERIALS AND METHODS

This is an in vitro study conducted between August 2024 and September 2024 in Green Lab, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. The Institutional Scientific Research Board (SRB/SDC/UG-2041/24/PERIO/467) approval was obtained before the start of the study.

Formulation of the Prophylactic Polishing Paste:

Base paste preparation

The base paste was prepared by mixing glycerin (25%) and sorbitol (25%) in a 1:1 ratio. Deionized water was gradually added to create a homogeneous mixture. Carbopol (1-2%) thickening agent was then incorporated, with continuous stirring until the desired viscosity was achieved.

Incorporation of nano-silica and nano-hydroxyapatite nanoparticles

After that, silicon dioxide (SiO₂) nanoparticles synthesized earlier by the modified Stöber process and hydroxyapatite (HA) nanoparticles synthesized by the coprecipitation method were added to the base. The silicon dioxide nanoparticles provide abrasive properties, while the hydroxyapatite nanoparticles help with remineralization. Once these nanoparticles were added, the formulation was blended well using a magnetic stirrer at a controlled speed of 500 rpm for 7 minutes to ensure a homogeneous and even distribution of the active nanoparticles throughout the base. The next step was the incorporation of additional ingredients to finalize the prophylactic polishing paste.

pH Adjustment

Peppermint oil (0.2%) was added to provide a pleasant flavor, and to enhance the paste's anti-caries properties, sodium fluoride (0.32%) was included. The pH of the formulation was carefully adjusted to around 7.0 using a phosphate buffer to ensure compatibility with the oral environment. Finally, the entire mixture was blended thoroughly to achieve a smooth and homogeneous paste, ready for application.

Incorporation of Lactobacillus plantarum MTCC 5690 bacteria strain

The obtained mixture was then cooled to below 40°C to preserve the viability of Lactobacillus plantarum MTCC 5690 (10⁸–10⁹ CFU/mL), and was added in liquid concentrate form. The pH of the mixture was measured and adjusted between 5.5 and 7.0 using the phosphate buffer solution. Xylitol (2–5%) was then mixed in, and

preservative sodium benzoate (0.15%) was added to protect against microbial contamination. The final product was then filtered to remove the impurities, as shown in **Figure 1**.



Figure1.The formulated Prophylactic polishing paste

Characterization of the prophylactic polishing paste

Scanning Electron Microscopy

The microstructure of Selenium-doped bioglass was studied using a Scanning Electron Microscope (SEM) (FEI Quanta FEG 650 SEM FEI company, Hillsboro, Oregon, United States) operated at 2,000 kV,

capturing images of the membrane's surface at 19000x and 23000x magnification.

Cytotoxic Activity

Cytotoxic analysis was done by Fluorescence microscopy. It is an imaging technique that uses fluorescent dyes or proteins to quantify cell structures, in which the green fluorescence indicates cell viability. The stronger the fluorescence, the higher the cell viability.

Antimicrobial Activity

Wells on Mueller-Hinton agar plates, each inoculated with *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Escherichia coli*, were loaded with 1-2mg/ml [Low concentration-LC] and 10-12mg/ml [High concentration-HC] of prophylactic polishing paste separately. The plates were then incubated at 37°C for 48 hours. Amoxicillin was used as the standard antibiotic control. Each pathogen's zone of inhibition was measured and replicated three times to establish the mean values.

Surface roughness analysis

In our study, scaling was first performed on an extracted natural human tooth to remove plaque and calculus, which typically increased the surface roughness. The surface roughness was then evaluated using Atomic Force Microscopy (AFM) to establish a baseline measurement. After scaling, using a low-speed dental handpiece with a soft rubber cup, the formulated prophylactic polishing paste was applied to the tooth uniformly, and polishing was carried out for a set duration of 30–60 seconds, ensuring consistent and even coverage over the entire surface. Water irrigation was applied after the polishing process to clear the excess paste.

After polishing, the surface roughness was re-evaluated using AFM. Surface roughness was evaluated using parameters such as area roughness (Sa), peak height (Sp), and valley depth (Sv), while line roughness was assessed through arithmetic mean roughness (Ra), peak height (Rp), and valley depth (Rv). The difference in surface roughness before and after polishing was calculated and compared to assess the effectiveness of the prophylactic paste in reducing surface roughness and improving the smoothness of the tooth surface.

RESULTS

Scanning Electron Microscopy

At 19,000x magnification, the SEM images revealed that the particles had merged into a more compact matrix, suggesting the presence of *Lactobacillus plantarum* potentially embedded within the structure **Figure 2A**.

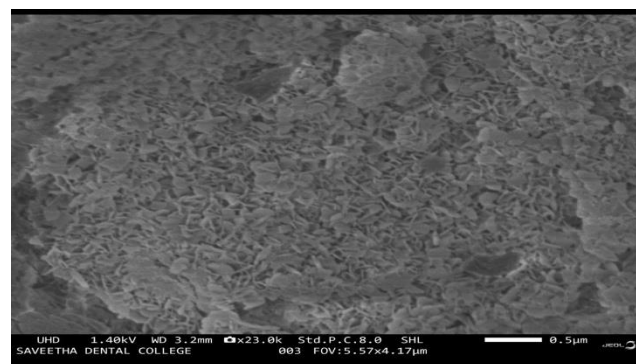


Figure.2A-SEM image of the formulated Prophylactic polishing paste at 19000x.

Irregular clusters and plate-like formations were identified as hydroxyapatite aggregates. The relatively continuous and roughened surface was considered favorable for controlled-release properties, which could help maintain probiotic viability and enable gradual release during use.

At 23,000x magnification, the SEM images showed densely packed, heterogeneous angular structures, including irregular, rod-like, and flake-like shapes **Figure 2 B**. This morphology indicated successful integration of nano-silica and nano-hydroxyapatite. Individual nanoparticles were distinguishable at the 0.5 μm scale, confirming that the nanoparticles preserved their nanoscale characteristics without significant agglomeration.

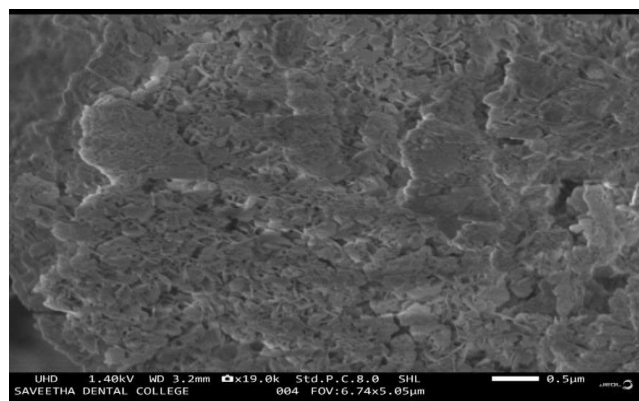


Figure 2 B. SEM image of the formulated Prophylactic polishing paste at 23000x.

Cytotoxic Activity

The bar graph (**Figure 3**) presents a cytotoxicity analysis of the prophylactic polishing paste tested on MG-63 cells, with concentrations ranging from 15 $\mu\text{L/mL}$ to 75 $\mu\text{L/mL}$ and a control group.

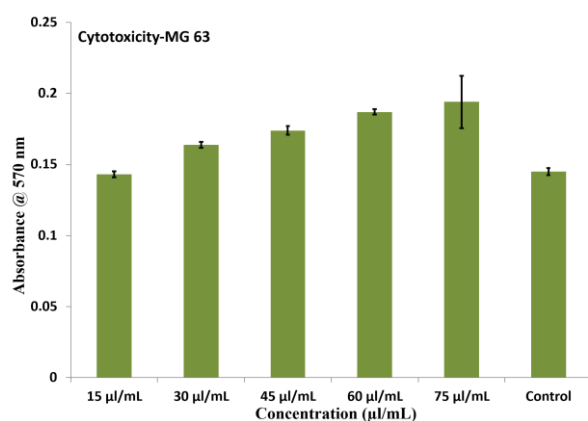


Figure 3. The bar graph depicts the cytotoxicity analysis of the prophylactic polishing paste tested on MG-63 cells, with concentrations ranging from 15 $\mu\text{L/mL}$ to 75 $\mu\text{L/mL}$ and a control group. The X-axis represents the different concentrations of standard used in $\mu\text{L/mL}$ and the Y-axis represents the absorbance at 570nm.

The absorbance at 570 nm reflects cell viability, and the control group exhibited lower absorbance compared to most experimental concentrations, indicating reduced cell viability. As the concentration of the paste increased from 15 $\mu\text{L/mL}$ to 75 $\mu\text{L/mL}$, there was a general rise in absorbance, peaking at 75 $\mu\text{L/mL}$. This suggests that higher concentrations of the paste may enhance cell viability or proliferation in this assay. The dose-response relationship revealed a positive correlation between paste concentration and cell viability, with the highest concentration (75 $\mu\text{L/mL}$) demonstrating maximum absorbance, implying minimal cytotoxicity and a potentially beneficial effect on cell viability at this concentration.

The fluorescence microscopy images (**Figure 4**) showed MG-63 cells treated with different concentrations of the prophylactic polishing paste, with live or viable cells visualized using a green fluorescent stain.

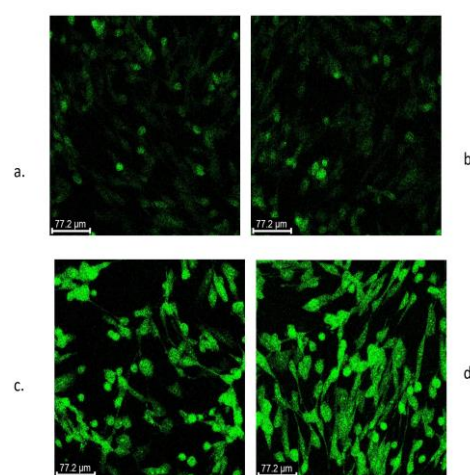


Figure.4 The fluorescence microscopy images show MG-63 cells treated with varying concentrations of the prophylactic polishing paste, including control, at 15 $\mu\text{L/mL}$, 30 $\mu\text{L/mL}$, and 50 $\mu\text{L/mL}$.

In the control, the cells appeared sparsely distributed with lower fluorescence intensity, indicating reduced cell viability or lower cell density at that concentration. At a concentration of 15 $\mu\text{L/mL}$, the cells appeared slightly denser, suggesting a minor increase in cell viability. At 30 $\mu\text{L/mL}$, the fluorescence intensity and cell density were significantly higher, indicating enhanced cell viability or proliferation compared to the control. At 50 $\mu\text{L/mL}$, the image showed the most intense fluorescence and highest cell density, indicating maximal cell viability or proliferation at this concentration of the prophylactic paste. At lower concentrations, the cells appeared smaller and less spread out, possibly indicating stress or cytotoxic effects. However, at increased concentrations, the cells seemed to be elongated and spread out, characteristic of healthy and proliferative cells, suggesting that the paste

was less cytotoxic and possibly supportive of cell growth at these higher concentrations.

Antimicrobial activity

The bar graph (**Figure 5**) displayed the anti-microbial activity of a prophylactic paste tested against four microorganisms: *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Anti-microbial activity assay

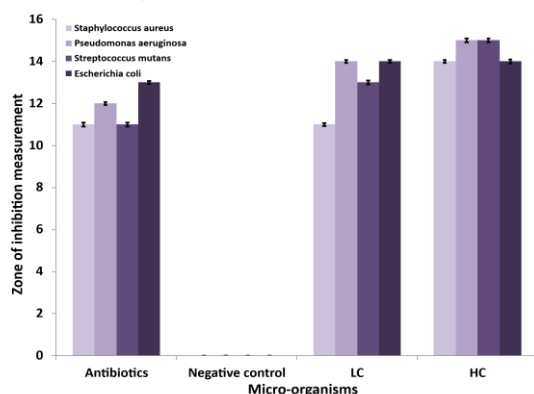


Figure 5. The bar graph displays the anti-microbial activity of a prophylactic paste tested against four microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Escherichia coli*.

The zone of inhibition, which measured the effectiveness of the paste in preventing microbial growth, was compared across different groups: Antibiotics [Amoxicillin], Negative Control, LC (Low Concentration), and HC (High Concentration). All four microorganisms showed significant zones of inhibition against the control, ranging from approximately 12 to 14 mm, confirming the effectiveness of the antibiotics used as a positive control. The negative control showed no inhibition zones, confirming that the medium without the prophylactic paste or antibiotics did not have antimicrobial properties. At low concentrations, the prophylactic paste produced zones of inhibition for all four microorganisms, with measurements similar to those seen with antibiotics, though slightly lower, indicating that even at a low concentration, the paste had considerable antimicrobial activity. At high concentrations, the prophylactic paste resulted in the largest zones of inhibition for all microorganisms, with measurements slightly higher than those of the antibiotics, suggesting that the paste at a higher concentration was highly effective in inhibiting the growth of these microorganisms.

Surface roughness analysis

Post-scaling, the tooth surface exhibited moderate roughness, with significant peaks and valleys. The surface roughness parameters indicated that the scaling process, while effective in removing surface

contaminants, left behind irregularities. The area roughness (Sa) was measured at 51.559 nm, with notable peak heights (Sp) of 171.72 nm and valley depths (Sv) of -208.23 nm, suggesting unevenness on the tooth enamel or dentin. Similarly, the line roughness parameters revealed an arithmetic mean roughness (Ra) of 58.349 nm, along with peak heights (Rp) of 158.29 nm and valley depths (Rv) of -142.25 nm, reflecting the rough, textured surface left after scaling (**Figure 6**).

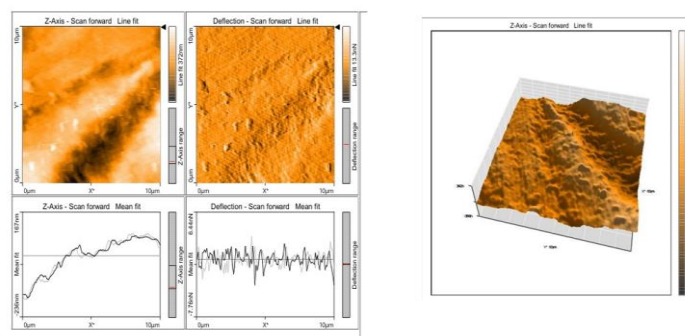


Figure 6. The Atomic Force Microscopy (AFM) image showing Surface roughness analysis of the tooth after scaling.

Following the application of the post-prophylaxis polishing paste, there was a marked reduction in both surface and line roughness, indicating the effectiveness of the treatment. The area roughness (Sa) decreased to 37.532 nm, with peak heights (Sp) reducing to 134.34 nm and valley depths (Sv) improving slightly to -201.04 nm. Similarly, the line roughness values dropped, with Ra reducing to 44.768 nm, Rp to 99.374 nm, and Rv to -110.72, highlighting the polishing effect of the paste (**Figure 7**).

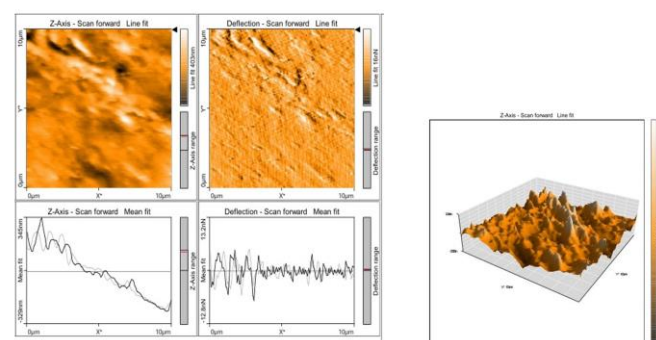


Figure 7. The Atomic Force Microscopy (AFM) image showing the surface roughness analysis of the tooth after applying prophylactic polishing paste

These improvements demonstrate that the polishing paste successfully flattened the peaks and valleys, creating a smoother and more uniform tooth surface, thereby aiding in reducing plaque accumulation (Table 1)

Table 1 shows the Surface Roughness Parameters Before and After Prophylactic Polishing Paste Application

Parameter	Post-Polishing -Baseline	Post-Polishing Paste Application
Area roughness (Sa)	51.559 nm	37.532 nm
Peak height (Sp)	171.72 nm	134.34 nm
Valley depth (Sv)	-208.23 nm	-201.04 nm
Arithmetic Mean Roughness (Ra)	58.349 nm	44.768 nm
Peak height (Rp)	158.29 nm	99.374 nm
Valley depth (Rv)	-142.25 nm	-110.72 nm

DISCUSSION

Scaling is a dental procedure that involves the removal of plaque and calculus from the surfaces of the teeth ¹⁰. This process is essential for preventing periodontal disease, as it helps to clean areas where regular brushing and flossing may not reach. Scaling helps to reduce inflammation and minimize the risk of periodontal disease ¹¹. Scaling leaves teeth with a rough texture due to the removal of plaque and calculus, making post-prophylaxis polishing necessary for a smooth finish.

Polishing is essential for smoothing the irregularities left after scaling. The polishing paste contains fine abrasives that gently buff the tooth surfaces, effectively removing any remaining rough patches ¹². This enhances the aesthetic appearance of the teeth and reduces plaque accumulation, also ¹³. Many polishing pastes contain fluoride, making them more resistant to cavities and sensitivity ¹⁴.

A prophylactic polishing paste incorporating *Lactobacillus plantarum* MTCC 5690, nano silica, and nano-hydroxyapatite particles offers a comprehensive approach to oral health. Both in vitro and in vivo studies have highlighted the individual benefits of these ingredients in oral health. By combining these three components into a single product, the aim was to maximize their synergistic effects.

Lactobacillus strains are renowned for their probiotic properties, which are crucial in restoring a healthy balance of oral microbiota ¹⁵. These beneficial bacteria help reduce the risk of dental caries and gingival diseases. Additionally, they play a role in maintaining oral pH balance, promoting fresher breath.

Nano-silica particles in polishing paste offer superior performance by leveraging their tiny size and large surface area while minimizing damage to the enamel ¹⁶. They can also be used to create a barrier on the tooth surface that helps reduce tooth sensitivity by

protecting exposed dentin.

Nano-hydroxyapatite particles are highly effective in the remineralization of tooth enamel as they mimic the natural mineral composition of teeth, facilitating the repair of early enamel lesions ¹⁷. They form a protective barrier over exposed dentin and fill in microscopic tubules, thereby reducing the sensitivity to any stimuli. This dual action underscores the versatility of nano-hydroxyapatite in both dental and medical applications ¹⁸. The objective of our study was to evaluate the in vitro characteristics of a prophylactic polishing paste formulated with probiotics and nanoparticles. Of course! Here's the same summary in the past tense:

The SEM revealed clear integration of nano-silica and hydroxyapatite without agglomeration, preserving their nanoscale benefits, and the roughened surface supported controlled-release, allowing gradual delivery of actives. Retained nanoparticle size increased surface area for better bioactivity, and diverse particle shapes contributed to improved mechanical and functional properties.

The cytotoxicity activity assay demonstrated a positive dose-response relationship, where higher concentrations resulted in minimal cytotoxicity, and in fluorescence microscopy, the highest viability was observed at 50 µL/mL, where the cells appeared healthy, indicating that the paste supported cell growth at higher concentrations. These findings align with previous research showing that *Lactobacillus* strains can selectively target and inhibit cancer cells while sparing normal cells from any damage ¹⁹.

The antimicrobial activity assay revealed strong antimicrobial activity, with higher concentrations of paste showing greater inhibition of microbial growth, even surpassing the effect of antibiotics. The negative control confirmed the paste's effectiveness, as it showed no inhibition. In addition, other studies have shown that both the nanoparticles and *Lactobacillus* strains possess strong antimicrobial activity against various Gram-

negative periodontal pathogens²⁰

In our study, a surface hardness test was conducted to assess the efficacy of the polishing paste on an extracted tooth. The results showed that the tooth surface had significant roughness post-scaling with noticeable peaks and valleys. However, after applying the prophylactic paste, both surface and line roughness were significantly reduced, resulting in a smoother, more uniform surface. This improvement highlights the paste's ability to minimize irregularities, potentially aiding in the reduction of plaque accumulation. An investigation evaluated the in vitro effects of one year of brushing with 10 wt. % nano-hydroxyapatite toothpaste. It concluded that while the toothpaste did not change enamel roughness, it positively contributed to remineralization and protection of the enamel surface^{21,22}.

Various studies have highlighted the significance of nanoparticle polishing pastes, especially those with nano-hydroxyapatite (n-HAp), as they enable targeted remineralization of micro-damage and their potential antimicrobial Efficacy against *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*²³. Probiotics, on the other hand, promote a balanced oral microbiome while also enhancing the immune response in the oral cavity²⁴.

While chemical post-prophylaxis polishing pastes effectively remove stains and smooth teeth, they have drawbacks, including enamel wear, increased sensitivity, and disruption of the oral microbiome²⁵. They also lack remineralization benefits and may cause allergic reactions. Additionally, environmental concerns arise from non-biodegradable components, such as microplastics. These issues emphasize the need for exploring probiotic polishing pastes with nanoparticles as safer alternatives.

Awareness of probiotic formulations and nanoparticles has been increasing recently²⁶. This is especially true as in vitro studies demonstrate the enhanced antibacterial efficacy of probiotic-based products against cariogenic pathogens^{27,28}. Thus, the combination of probiotics and nanoparticles in this polishing paste is intended for use in patients with gingivitis and periodontitis after oral prophylaxis, thereby providing a holistic approach to oral hygiene.

CONCLUSION

In conclusion, the prophylactic polishing paste demonstrated effective integration of *Lactobacillus plantarum* and nanoparticles, and exhibited strong antimicrobial activity, comparable to conventional antibiotics at higher concentrations, while maintaining effective biocompatibility and promoting cell viability in a dose-dependent manner. Additionally, the paste significantly improved the smoothness of tooth surfaces by reducing surface roughness, which can help minimize plaque accumulation and support oral health. Overall, these findings suggest that the paste is

a promising, multifunctional oral care product with potential benefits for preventing and managing periodontal conditions.

STRENGTHS AND CONSTRAINTS

The study's strengths include its innovative use of probiotics and nanoparticles, offering antimicrobial benefits with fewer side effects than chemical pastes. However, limitations include the absence of in vivo data and individual variations in oral microbiota, which may impact the paste's practical effectiveness.

DECLARATIONS

ETHICAL APPROVAL

Not applicable as this study is an in vitro study.

Institutional Scientific Review Board approval was o

Conflict of interest

The authors declare that they have no conflict of interest.

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