ORIGINAL RESEARCH

Formulation and Evaluation of Characteristics, Remineralization Potential, and Antimicrobial Properties of Toothpaste Containing Nanohydroxyapatite and Nanosilver Particles: An *In Vitro* Study

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Abstract

Aim and objectives: The current study is aimed to evaluate the antimicrobial and remineralizing properties of toothpaste containing nanosilver and nanohydroxyapatite (NH-NS TP).

Materials and methods: The toothpaste was prepared by incorporating NH-NS TP into a toothpaste base. Following this, a physicochemical analysis of the prepared toothpaste was carried out. The toxicity against human gingival fibroblast (HGF-1) cells was assessed. The antimicrobial activity of the toothpaste was evaluated against *Streptococcus mutans* and *Escherichia coli* using the disk diffusion method. Subsequently, 15 selected human premolar teeth extracted for orthodontic purposes were used to measure the remineralization potential. The surfaces of the selected teeth were demineralized using a prepared demineralizing solution [323.4 mg of calcium chloride ($CaCl_2$), M450 µL of calcium acetate $(CH_3COO)_2Ca$, 300 mg of potassium dihydrogen phosphate (KH_2PO_4) at pH 4.4–4.7] for 72 hours and were subjected to a toothbrush simulator applying the prepared toothpaste. The remineralization potential was evaluated by measuring the microhardness of the enamel surface before and after treatment with the toothpaste. Further, the remineralization potential was assessed based on scanning electron microscopy (SEM) imaging accompanied by energy-dispersive X-ray spectroscopy.

Results: The results showed that the NH-NS TP had significantly greater antimicrobial activity compared to the control toothpaste and no toxicity against HGF-1 up to 40 µg. Approximately, 7–9 mm inhibition zone against *S. mutans* and 4–6 mm inhibition against *E. coli* was achieved. Additionally, the toothpaste significantly increased (31.4%) the microhardness of the enamel surface, indicating its potential for remineralization. The SEM images revealed the presence of regular deposits, mostly pertaining to the demineralized spots, resulting in a regained smooth surface. Following this, physicochemical analysis of the prepared toothpaste was carried out, resulting in a pH of 7.38 and showing good extrusion, frothing, and organoleptic properties. Additionally, the toothpaste significantly increased the microhardness of the enamel surface, further indicating its potential for remineralization.

Conclusion: NH-NS TP may provide a promising approach for improving oral health by enhancing antimicrobial efficacy and promoting enamel remineralization, while exhibiting reduced toxicity.

Keywords: Antibacterial, *In vitro* study, Nanohydroxyapatite, Nanosilver, Remineralization, Toothpaste.

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Introduction

Dental caries remains a significant yet preventable oral health issue in India and globally. The Global Burden of Disease Study by the World Health Organization (WHO) in 2019 estimated that 520 million children suffer from caries in primary teeth, and 2 billion adults suffer from caries in permanent teeth.

Dental caries is now defined as "a dysbiosis in the oral microbiome."[1] It is a chronic disease with multifactorial etiology, primarily due to an imbalance between remineralization and demineralization processes. The known etiological factors for dental caries, such as sugar consumption, alterations in salivary flow, socioeconomic status, changes in oral pH, poor oral hygiene, and carbohydrate-rich diets, are now considered "etiological pressures" that contribute to dysbiosis in the oral environment.

Dental caries interferes with normal nutrition intake, speech, self-esteem, daily routine activities and impair cognitive development among children. Therefore, focusing on the prevention of dental caries is of utmost importance.[2]

Dental caries is considered a "complex" or "multifactorial" disease with no single causation pathway. Therefore, prevention does not depend solely on a single preventive method, such as the elimination of microorganisms, diet control, or behavioral modifications.

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Fluoride is a proven anticariogenic agent available in various forms that can be used both professionally and at home. The use of antibacterial agents like chlorhexidine and listerine as mouth rinses are effective in caries prevention. Silver diamine fluoride (SDF) is used globally to prevent and arrest caries, particularly in developing countries.[3]

Professionally applied agents require frequent dental visits as they are technique-sensitive and time-consuming. Therefore, researchers have developed self-applied products available in the form of gels, foams, and dentifrices.

Tooth brushing with dentifrice is evidenced to prevent dental caries as it facilitates plaque removal and provides necessary minerals for remineralization. As a routine oral hygiene measure, the recommended amount of toothpaste and toothbrush is used by the majority of the population for cleaning the oral cavity.

The use of effective toothbrushing techniques along with fluoride dentifrices is a globally accepted method of preventing dental caries. Fluoridated dentifrices containing 500–600 ppm of fluoride help in caries prevention in children. Calcium is delivered to the tooth with the help of calcium phosphate in dentifrices like Colgate and NovaMin, increasing enamel resistance to dental caries. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) prevents caries by neutralizing acids and remineralizing early carious lesions.[4]

Toothpaste with nanohydroxyapatite (NH) crystals has remineralization potential. Recently, ozone has been proposed for the prevention of dental caries. When delivered to the tooth surface for 10–40 seconds, remineralization occurs within 4–12 weeks.[5] Carbon dioxide laser irradiation increases the acid resistance of enamel and prevents demineralization.[6]

Independent *in vitro* and *in vivo* studies have proven the effectiveness of NH crystals in remineralizing incipient lesions and inhibiting plaque biofilm accumulation by nanosilver (NS) particles, which is crucial for preventing dental caries. To date, studies have not assessed the combined effect of NH and NS particles in caries prevention. Therefore, we have planned a study to assess the combined effect of NH crystals and NS particles in preventing dental caries.

As per protocol, a cell line study should be conducted for the newer formulation of a preventive agent to evaluate its characterization and biocompatibility.

Hence, an *in vitro* study was conducted with the objective of formulating and evaluating the physical and chemical properties of toothpaste containing nanohydroxyapatite crystals and nanosilver particles (NH-NS TP). The study also assessed its remineralization potential, antimicrobial properties, and biocompatibility.

Materials and Methods

The present study was conducted at the Cancer and Stem Cell Research Lab, Department of Pharmacology, Saveetha Dental College, Chennai, Tamil Nadu, India, over a period of 2 months. The Institutional Ethics Committee registration number for the current study is CSICDSR/IEC/0225/2022. The *in vitro* study was carried out after the preparation of NH silver toothpaste.

Preparation of Toothpaste

Nanohydroxyapatite NS toothpaste was formulated and prepared using the ingredients listed in Table 1. NH crystals and NS particles are the active ingredients. The inactive ingredients, including binders, surfactants, buffering agents, humectants, sweetening agents, abrasives, and preservatives, were also added. The composition of the toothpaste used for the present study is provided in Table 1.

Steps in the Preparation of Toothpaste

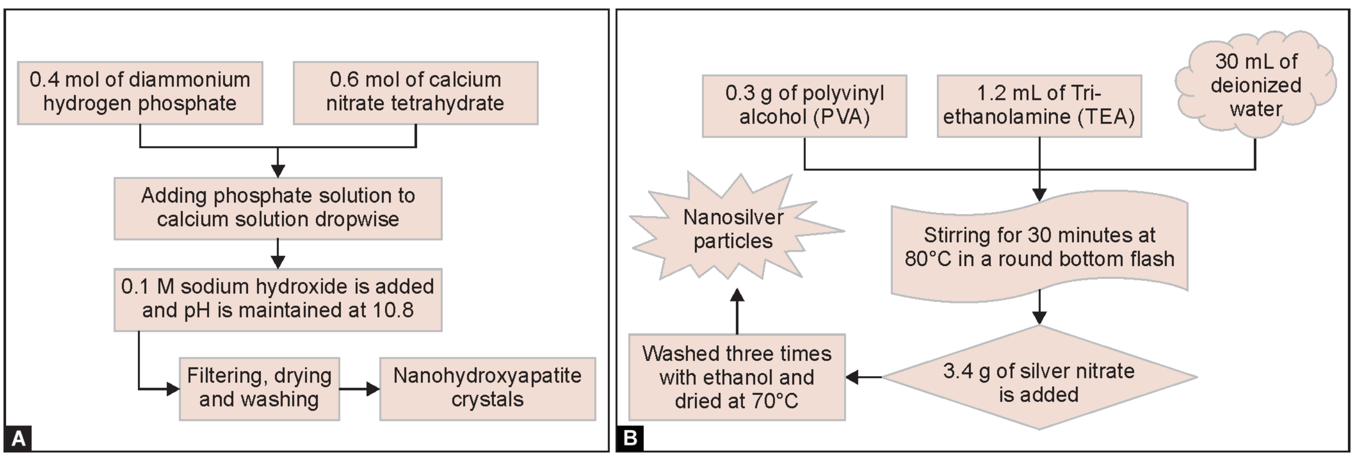
Preparation of Nanohydroxyapatite Crystals

Nanohydroxyapatite particles were prepared by the wet chemical method. 500 mL of 0.4 M diammonium hydrogen phosphate with a pH of 4.0 was vigorously stirred in a 2 L beaker at room temperature. Over a period of 4 hours, 500 mL of 0.6 M calcium nitrate tetrahydrate with a pH of 7.4 was added dropwise. Throughout the stirring process, the pH of the system was maintained at 10.8 using 0.1 M sodium hydroxide. The mixture was allowed to settle overnight, resulting in the formation of a white precipitate. The precipitate was vacuum-dried and subsequently cleaned with distilled water and ethanol, repeating the process three to four times. The steps are illustrated in Figure 1A.

Table 1: Composition of prepared toothpaste

Ingredients	*Quantity used*
Calcium carbonate	40%
Calcium hydroxide	2%
Silica	0.4%
Sodium lauryl sulfate	1.8%
Saccharin	0.8%
Peppermint oil	2.5%
Sorbitol	10%
Water	20%
CMC	0.5%
Sodium benzoate	0.8%
NS solution (20 nm)	500 µL
NH (8–10 nm)	10%

CMC, carboxy methylcellulose



Figs 1A and B: Steps in preparation of (A) NH crystals; (B) NS particles

Synthesis of Nanosilver Particles

Silver nanoparticles were synthesized using the following method—0.3 gm of polyvinyl alcohol (PVA; 0.03 mmol; MW 10,000), 12 mL (90 mmol) of triethanolamine (TEA), and 30 mL of deionized water were combined in a 250 mL round bottom flask. The solution was heated to 80°C and stirred at this temperature for 30 minutes. Then, 3.4 gm of solid silver nitrate ($AgNO_3$) (20 mmol) was added to the flask. The colorless solution turned brown, and heating and stirring continued for an additional 3 hours, resulting in a solution acquiring a shiny silver color. After cooling to room temperature, 200 mL of ethanol was added and stirred. The reaction mixture was then placed in a sonication bath for 1 hour. Unreacted polyvinyl alcohol, triethanolamine, and $AgNO_3$ remained soluble in ethanol, while the silver nanoparticles, being insoluble, precipitated out. The precipitate was subsequently dried in a hot air oven at 70°C for 5 hours. 22 gm of the obtained silver nanoparticles were mixed with 1 L of deionized water, and a silver solution of 22 ppm was obtained. The steps are described in Figure 1B.

Preparation of Nanohydroxyapatite Nanosilver Toothpaste

The composition of the ingredients required to formulate NH-NS TP is listed in Table 1. The toothpaste was prepared using the aforementioned ingredients, and its physicochemical characteristics were evaluated.

Organoleptic Properties

The color, odor, taste, and texture of the formulated toothpaste were evaluated through visual, physical, and sensory assessments. Color assessment was conducted through visual examination.

Tube Inertness

The inertness of the formulated NH-NS TP was evaluated using the tube inertness method. For this, the desired quantity of the prepared toothpaste was placed in a container under normal storage conditions up to 45°C for 10 days. Subsequently, the inner surface of the container and cross-sectional pieces were examined for any signs of corrosion resulting from chemical reactions.

pH of the Prepared Toothpaste

The required amount of the prepared NH-NS TP was placed on litmus paper and subsequently tested with a pH meter. The pH of the toothpaste falls within the range of 6–7.5, which is slightly acidic to neutral. A pH in this range helps in plaque removal and contributes to maintaining healthy teeth and gums.

Rheological Tests

Extrusion Test

The homogeneity of the formulated toothpaste was examined at normal room temperature (27 ± 2°C). A uniform force was applied to the tube containing the formulated NH-NS TP to evaluate if a consistent mass of toothpaste extrudes from the tube. Additionally, the bulk of the toothpaste must extrude from the nozzle of the tube when the crimp is rolled gradually.

Spreadability

Assessment of spreadability was carried out using the place and drag method. In this method, the slip and drag characteristics of the formulated NH-NS TP play a vital role. The prepared toothpaste (1–2 gm) was weighed and placed on a glass slide measuring 10 × 10 cm. Another glass slide of the same dimensions was placed over it, and the slides were pulled in opposite directions. The spreadability of the formulated NH-NS TP was then assessed by measuring the spread formed in centimeters. The experiment was repeated several times, and the average value was calculated.

Foamability

For this test, 2 gm of prepared NH-NS TP was mixed with 5 mL of water in a measuring cylinder. The initial level was marked and measured. The measuring cylinder was covered with a watch glass, and the mixture was allowed to sit for 30 minutes. After 30 minutes, the mixture was stirred initially to break down any lumps and then vigorously stirred for 10 minutes. The final volume of foam formed was then measured.

Moisture and Volatile Matter Determination

Thermogravimetric analysis (TGA) was adopted to measure the moisture and volatile content. Around 5 gm of prepared toothpaste was placed in a porcelain dish with dimensions of 6–8 cm in diameter and 2–4 cm in depth. The mixture was dried in an oven at 105°C. Moisture and volatile matter determination were calculated using the appropriate formula:

$$\% \text{ loss by mass} = (M - Ml/M) \times 100$$

Ml: Mass loss during drying.
M: Mass of the material used in the test.

Sharp and Edge Abrasive Particle Determination

Presence of sharp particles was determined by applying the prepared toothpaste on the fingertips and then scratching it on butter paper for 6 inches. This process was repeated several times. Additionally, the prepared toothpaste was placed in a dappen dish and rubbed vigorously for 30 seconds to check for the presence of abrasive particles with sharp edges.

Toothpaste Cleaning Ability

Eggshells were used to check the cleaning ability of toothpaste to effectively replicate tooth enamel, as they contain high amounts of calcium. Around 200 mL of water was brought to a boil, and then 15 mL of vinegar and 20 drops of red food coloring agent were added. To this mix, the hard-boiled eggs were added and allowed to sit for about 5 minutes until they attained desirable red staining. Then hard-boiled eggs were taken out and dried. A pea-sized amount of prepared NH-NS TP was placed in a wet toothbrush and was brushed 10 back-and-forth strokes. The eggshell was measured for any stain removal.

Antimicrobial Activity and Minimal Inhibitory Concentrations

The *in vitro* antibacterial activity against *E. coli* and *S. mutans* was performed using an agar well diffusion method using lysogeny broth (LB medium). Initially, the plates were streaked with pathogenic bacterial strains of *E. coli* and *S. mutans* and were cultured. Then, two wells/bores of size 5 mm were made in the cultured LB medium using a sterile cork borer. Then, the formulated NH-NS TP was placed in created bores, and the plates were wrapped with paraffin, labeled, and incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition (ZOI) was measured using a ruler in mm. The minimum inhibitory concentrations were also measured subsequently.

Toxicity against Human Gingival Fibroblast Cells

The toxicity against human gingival fibroblast (HGF-1) cells of prepared NH-NS TP was evaluated at concentrations of 5, 10, 20, 40, 60, and 100 µL. The prepared NH-NS TP was incubated with HGF-1 for 24 hours, and then the interaction of these was measured by evaluating the mitochondrial metabolic potential (MMP) assay. The permeability of the cell membrane of mitochondria was assessed using water-soluble fluorescent dyes such as 3,3′-dihexyloxacarbocyanine iodide. In mitochondrial cells with intact cell membranes, the dye aggregates in the mitochondrial matrix and produces red fluorescent under microscopy, while cells with depolarized and disintegrated cell membranes appear to be green. Thus, the red–green ratio present at the end indicates the mitochondrial cell membrane status and, in turn, the cell viability.

Remineralization Assessment

Maxillary premolars extracted for orthodontic purposes were assessed for caries and other anomalies. Fifteen caries and anomalies-free teeth were selected and included in this study. The selected teeth were then decoronated at the level of the cementoenamel junction, window preparation was done in the middle third of the remaining crown, and the samples were mounted in acrylic resin block. Microhardness was initially assessed using a Shimadzu HMV-G31DT microhardness tester, which applied a test load of 300 gm for 20 seconds. Then, the Vickers microhardness number was calculated. Then, all the collected samples were subjected to demineralization solution (323.4 mg of $CaCl_2$, 450 µL of calcium acetate $(CH_3COO)_2Ca$, and 300 mg of KH_2PO_4 at Ph of 4.4–4.7) for 72 hours. The postdemineralization microhardness was measured. Finally, the demineralized samples were subjected to a toothbrush simulator (SD Mechatronik) operating at a speed of 10,000 cycles consisting of a linear X-axis of 2,500 cycles, linear Y-axis of 2,500 cycles and circular motion of 5,000 cycles 9 (Fig. 2). Once the toothbrush simulation is complete, the microhardness after remineralization process was assessed finally.

For scanning electron microscopy (SEM) analysis, the demineralized and remineralized samples at their respective stages were visualized under SEM-energy dispersive X-ray (EDX) to analyze the chemical composition in the teeth surface.

Results

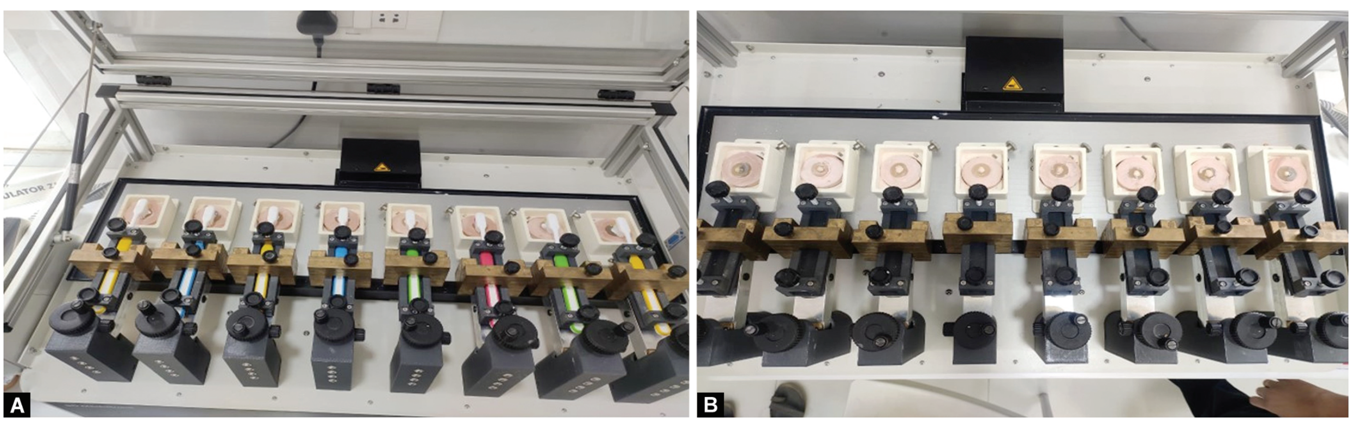
The NH-NS TP was prepared as proposed earlier, and desirable results were achieved. The results of the organoleptic physiochemical evaluation are tabulated (Table 2).

The NH-NS TP was evaluated visually for color, resulting in bright white, which has an aromatic and characteristic odor and the taste was checked manually and found to be sweet and palatable. The pH was found to be 7.38, which is within the acceptable range. From the abrasiveness test, it was concluded that the paste was found to be of smooth consistency and had no sharp and edge abrasive particles. The formulated NH-NS TP was found to be inert and didn't exhibit any degrading effects on the stored container, as the tube inertness test showed that no corrosion or degradation occurred as a result of chemical reactions in the cross-sectional pieces of the stored container. On the extrusion test, the formulated toothpaste was found to have a good extrusion property of quick, consistent, and uniform shape extrusion when applied with low extrusion force.

From the spreadability test, prepared NH-NS TP was shown to spread evenly without the formation of any lumps or irregularity, and it was of optimal consistency; in turn, this can be proved using the extrusion test. The toothpaste spread up to 7 cm in a glass slide and retained the shape further without undergoing any distortion or additional spreading. Thus, from the extrusion and spreadability

Table 2: Properties of the prepared NH-NS toothpaste

Properties	*Results*
Organoleptic properties	
Color	Bright white
Odor	Aromatic and characteristic
Taste	Palatable and sweet
Rheological properties	
Spreadability	7 cm, consistent
Tube extrusion	Good
pH	7.38
Tube inertness	Excellent
Foamability	Good
Sharp and edge abrasive particles	No sharp and edge abrasive particles
Cleaning ability	Good
Moisture and volatile content	17 and <1%, respectively
Antimicrobial activity	
E. coli	Highly active
S. mutans	Moderately active
Minimum inhibitory concentration	5–10 µg
Toxicity against HGF-1	No toxicity up to 40 µg



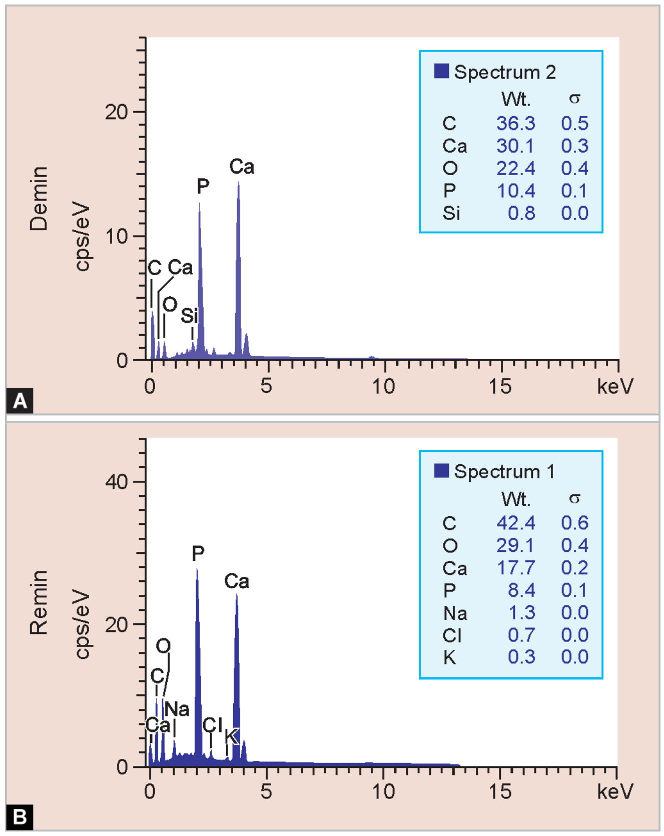
Figs 2A and B: Toothbrush simulator (SD Mechatroix) (A) During brushing simulation; (B) After brushing simulation test, it can be concluded that the prepared toothpaste showed to be homogeneous.

The foamability test resulted in foam formation for about 3 mL in the measuring cylinder. The cleaning ability of toothpaste was checked using stained hard-boiled eggs. A visible difference in the removal of stains was observed while using the prepared NH-NS TP. The prepared toothpaste resulted in a moisture content of 1.7% and a volatile substance of <1%. A minor change in mass of 0.2% for every 5 minutes occurred at 105°C.

On the antimicrobial test, a ZOI of 7–9 mm was obtained against *E. coli* strains, and 4–6 mm (Figs 3 and 4) was obtained against *S. aureus* stains. The prepared toothpaste completely inhibited the growth of gram-positive bacteria *S. aureus* and almost inhibited the growth of gram-negative bacteria such as *E. coli* at concentrations of 5–10 µg [minimum inhibitory concentration (MIC)]. The mechanism is the concentration-dependent bactericidal activity of NS and the NH particles. Prepared toothpaste showed to have no toxicity up to concentrations of 40 µg against HGF-1.

Discussion

Oral hygiene maintenance is one of the integral parts of body's general well-being. One effective and efficient technique to maintain oral hygiene is to brush teeth using toothpaste and a toothbrush.[7,8] Maintaining poor oral hygiene can result in a wide array of ill effects ranging from white spot lesions and dental caries to serious systemic complications. Dental caries is initially formed by the demineralization actions of the toxins produced by bacteria, resulting in the initial stage of white spot lesions.[9,10] Thus, proper and effective oral hygiene measures must be implemented to achieve optimal care. The primary aim of this *in vitro* study is to formulate a biocompatible toothpaste containing NH and NS, which has both remineralization and antibacterial potential. From the literature search, this is the first kind of study conducted using the NH-NS TP for caries prevention and remineralization. The results



Figs 4A and B: Energy dispersive X-ray analysis after demineralization and remineralization

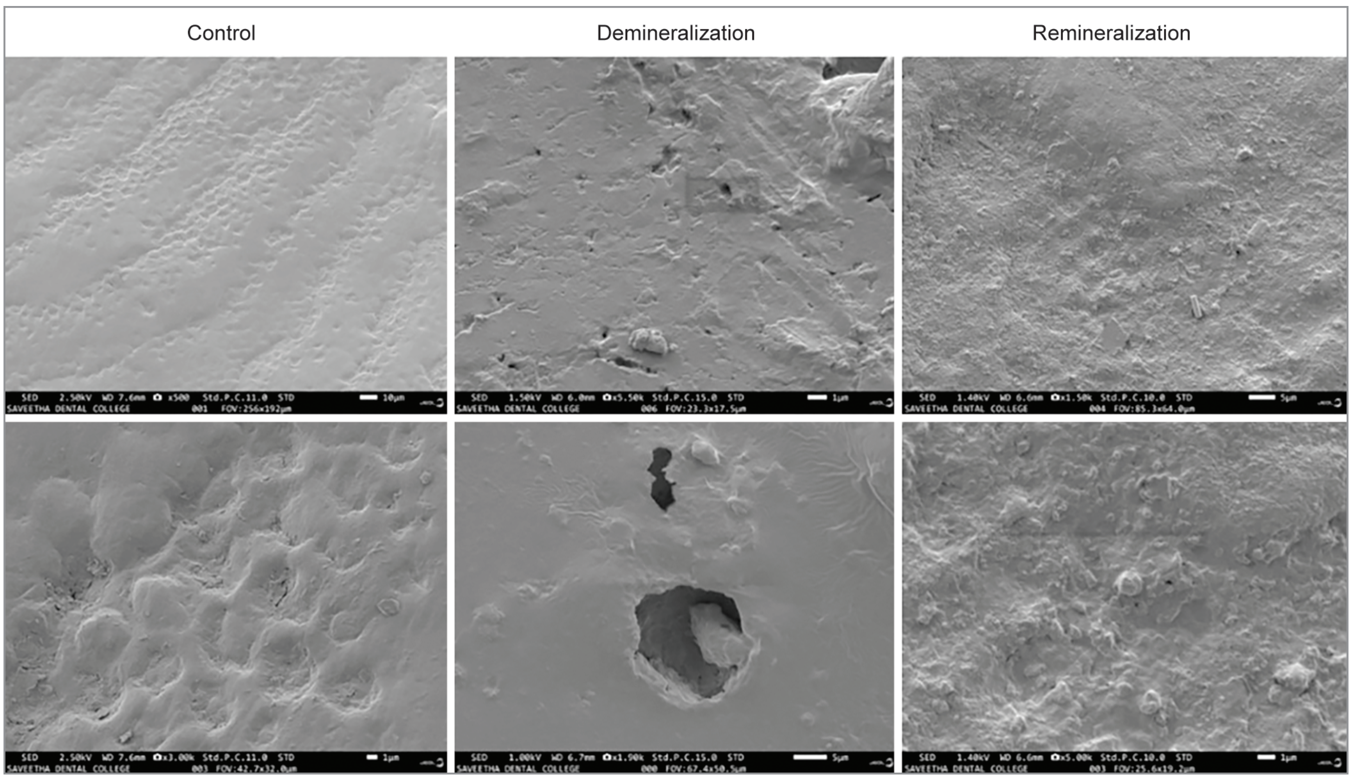


Fig. 3: Scanning electron microscopy images. Demin, demineralization; remin, remineralization

of the study highlight the finding that the formulated NH-NS TP showed to have high remineralization potential with antibacterial properties.

In recent times, the use of nanotechnology has been tremendously increased in the field of dentistry. They have potential effects of plaque control, caries prevention and antibacterial properties.[11] In this study, the NH-NS TP was formulated as per the composition given in Table 1. The main aim is to accentuate the synergistic effect of NH and NS particles combined together in toothpaste for caries prevention by antibacterial action and remineralization.

The prime idea behind using NH in toothpaste is dental biomimetics. The substance used should possess properties and nature similar to the enamel surface to achieve desired results. This can be supported by its remineralization ability. The remineralization ability of NH particles can be made clear by looking into the composition of enamel. Hydroxyapatite (HAP) is the main inorganic component of enamel; HAP is basically composed of calcium and phosphate ions. The well-organized enamel structure consisting of enamel rods is built from the compact and precise organization of HAP crystals.[12,13] This high organization of HAP crystals contributes to the strength and enamel's ability to withstand force, resist microbial attack to a limited extent and resist microfracture.[14] Thus, the dental caries process is a multifactorial one;[15] begins with the loss of mineral content from the components of enamel such as HAP.[16] Under normal circumstances, a net balance exists between the enamel surface minerals and saliva; when the pH of saliva drops below 5.5[16] and when a favorable environment exists, slow and steady demineralization of enamel begins, resulting in initial white spot lesions and dental caries. Calcium and phosphate ions predominantly couple with the H^+ ions present in saliva to favor demineralization and the oral conditions resulting in the dissolution of HAP.[17] This results in calcium and phosphorous-containing NH particles acting as potential remineralizing agents.[18] The NH particles have the ability to naturally assemble and bind to defective enamel surfaces and restore surface integrity,[19] the nano size of <20 nm NH particles potentiates their penetration into deeper layers reaching the base of carious lesions.[20] A randomized clinical trial by Paszynska et al.[21] concluded that the use of NH-containing toothpaste is in no way inferior to fluoridated toothpaste.

On the contrary, NS proves to be a potential antibacterial agent. Chiefly, three mechanisms of action have been put forward, the NS particles anchor to the bacterial cell membrane and disturb their essential functions of permeability and respiration;[22] they have the ability to inhibit the synthesis of bacterial cell wall synthesis, particularly in *S. mutans*[23] and finally through plasmolysis of bacterial cell wall.[24] The main reason behind this is the high affinity of silver toward phosphorous ions, found in abundance throughout the cell membrane.[25] The action of silver greatly depends on the size of the particles. The smaller the size, the greater the surface area available for bacterial interaction, resulting in augmented antibacterial action. Thus, NS particles (5–100 μm) have a better efficacy compared to conventional ones. Guzman et al., in their study, reported that the antibacterial effect of NS increases with a decrease in particle size.[26] Thus, a smaller dose of NS suspension is sufficient to achieve the desired results.

The prepared NH-NS TP was evaluated for antibacterial properties against *Staphylococcus aureus* and *E. coli*. *S. mutans* is the predominant pathogen associated with infections and dental caries mainly due to its immune-invasive mechanisms.[27] The growth of *S. mutans* was inhibited by prepared NH-NS TP at a concentration of 5–10 μg. One of the important mechanisms of action is the formation of pits in the bacterial surface by NS particles, thereby increasing the bacterial cell membrane penetration.[26] The antibacterial properties of NS are nonspecies specific, which helps to prevent the development of bacterial resistance.[27] The antibacterial activity was higher against *S. mutans* than against *E. coli*. The results indicate the synergistic effect of NH and NS. The action of NS particles is increased in the presence of additional substances, such as biodegradable polymeric substance coating or biomimetic substances. A study by Limban et al. demonstrated that NH-NS combined together exhibit good antibacterial properties, and the NH will diminish the adverse effects of NS particles.[28]

When assessing the remineralization potential, the prepared toothpaste was shown to have a good remineralization ability, which was revealed through SEM-EDX analysis. The images obtained through SEM show a paradigm shift from a demineralized irregular enamel surface to a nearly perfect one with optimal remineralization. The images also revealed the binding of NH particles to the enamel surface. EDX results supported the SEM findings. The concentrations of calcium and oxygen showed a considerable increase after remineralization. This proves that calcium and phosphorus deposition has occurred on the enamel surface, and the enamel integrity has been restored.

Additionally, the prepared toothpaste showed minimal levels of toxicity against the HGF-1 up to concentrations of 40 μm. The toxicity levels of nanoparticles are greatly dependent on their size, as the interactions between the nanoparticles and the surface take place at these levels.[29] NS-mediated cytotoxicity levels are highly dependent on properties like size, shape, surface charge, oxidation state, and method of preparation.[30] The optimized synthesis procedure with the use of stabilizing agents such as trisodium citrate has been done, minimizing the toxicity of NS particles. Additionally, the size, shape, and charge distribution of NS particles were thoroughly examined throughout the procedure. Fadeel et al., in their study, concluded that NS particles exhibited excellent biocompatibility against HGF-1 cells even after incubation for 7 days at a concentration of 1.0 μg.[30] The measured MMP levels were within the normal range, suggesting less toxicity. The MMP assay is a key indicator of cell health, and it is known that xenobiotic compounds have an impact on cell health. These compounds alter the MMP, leading to cell death and apoptosis at toxic doses. The prepared toothpaste showed minimal effects on MMP concentrations up to 40 μg, beyond which there were signs of membrane disintegration as the green fluorescence ratio increased.

The results of this study suggest that NH-NS TP is an effective alternative with benefits of both remineralization and demineralization. The toxic effects of NS particles, if present in high concentrations, can be neutralized by the presence of NH particles.[28] On the whole, the prepared NH-NS TP showed good antibacterial properties with remineralization capacity. The use of biocompatible doses of NS and NH resulted in a toothpaste with almost no effects on human cells. It is clear that NH-NS TP exhibits antibacterial properties at biocompatible doses with a nonspecies-specific mechanism, which synergistically increases in the presence of NH particles. Furthermore, it has a good remineralization potential. This study has a few limitations, such as being conducted in a lab setup using extracted teeth and a minimum number of tooth samples. Further, this study can be elaborated by proceeding with *in vitro* and *in vivo* broad-scale studies.[31]

Conclusion

Nanohydroxyapatite crystals have a variety of beneficial effects when used in dentifrices, including increased remineralization of initial enamel lesions and caries inhibition. NS particles in the toothpaste formulated show anti adherence to strains of *S. mutans*, causing a bacteriostatic effect. On the whole, the combination of NH crystals and NS particles in the formulated toothpaste (NH-NS TP) has proven to be caries preventive as well as effective in remineralizing initial carious lesions. SEM-EDX analysis confirms that nHAP at an optimal concentration used in the preparation showed remineralization of the eroded areas on the tooth specimen. NH-NS dentifrice can be employed as an effective caries prevention agent in the near future. Further, the toothpaste was found to have the least toxicity effects on HGF-1 cells. It showed comparatively good antimicrobial properties against *S. mutans* and *E. coli*. The physicochemical and organoleptic properties of the toothpaste were good with extrusion, foamability, tube inertness, and spreadability.

Clinical Significance

Dental caries is one of the prime oral hygiene-related burdens among pediatric patients worldwide. Proper measures at the right timing must be taken to prevent any complications related to dental caries. One effective method to prevent and arrest existing caries is by maintaining good oral hygiene with the aid of toothpaste, toothbrush, and mouthwash. In this article, the prepared NH-NS TP proves to be an effective agent in preventing dental caries owing to its antimicrobial properties and its role as a remineralizing agent due to the presence of NH crystals. The low level of toxicity of this toothpaste makes it beneficial and suitable for use in children.

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