EFFECTIVENESS OF NANOPARTICLE-BASED ACIDULATED PHOSPHATE FLUORIDE (APF) GEL ON SURFACE MICROHARDNESS OF HUMAN ENAMEL: AN *IN VITRO* STUDY

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ABSTRACT: *Aim:* To compare the effectiveness of nanoparticle-based acidulated phosphate fluoride (APF) gel and conventional APF gel on the surface microhardness of human enamel. *Materials and methods:* This study was performed on 30 sound human teeth extracted in a 2 month period for the purpose of orthodontic or periodontal reasons. The crown portion of each tooth was divided into 2 halves and treated for 4 min, using for the right half a nanoparticle-based APF gel and for the left half a conventional APF gel. The surface enamel microhardness was measured using the Vickers microhardness test at baseline, after 24 hr, and at 30 days. *Results:* On analysis, after 24 hr, in the nanoparticle-based APF gel group, the tooth samples showed a significant increase in surface microhardness compared to those treated with the conventional APF gel. After 30 days, the microhardness in both the groups was reduced compared to that found at 24 hr. *Conclusion:* After 24 hr of treatment, the incorporation of nanoparticles of sodium fluoride, the active component in the APF gel, resulted in a significant increase in the enamel surface microhardness of teeth samples compared to treatment with a conventional APF gel.

Keywords: APF gel; Hardness; *In-vitro*; Nanoparticles; Permanent teeth; Sodium fluoride.

INTRODUCTION

Fluoride ion (F) therapy, delivered both topically and systemically, has been the corner stone in the prevention of dental caries for over half a century.[1] The mode of delivery, concentration used, frequency, and dosage along with the age of exposure of the subject dictate the potential effect of fluoride on dental caries prevention and the occurrence of dental fluorosis.[2-6]

Among the professionally applied topical F agents, acidulated phosphate fluoride (APF) gel has long term proven efficacy, both in the deciduous and the permanent dentitions. APF gels yield around 1.23% fluoride (12,300 ppm F) and are usually applied using a tray, which is held in the patient's mouth for a period of 4 minutes. Longitudinal clinical trials assessing the efficacy of the bi-annual application of APF gel among different groups have shown an average reduction in the DMFS scores ranging from 21–60%, especially in high caries risk children.[7-9] A study among the Indian population has also shown a similar reduction of about 61%.[8]

The APF gel acts by improving the properties of the enamel crystals through the formation of fluorapatite crystals which are more resistant to acid dissolution.[10] Many researchers have reported an average increase of 50–60% in the surface microhardness of human enamel compared to treatment with neutral sodium fluoride (NaF). [11,12]

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Current technological advancements have pioneered the use of nanoparticles in all aspects of science including dentistry.[13] Nanodentistry refers to the application of this science and technology, using nano-structured material, for the purpose of diagnosing, treating, and preventing oral and dental diseases, relieving pain, and improving dental health.[14-16] The prevention of enamel dissolution after acid attack by microorganisms is mainly due to the fluoride ion, the active component in APF gel, which aids in the formation of fluorapatite crystals on the enamel surface. The NaF compound in APF has an octahedral lattice of 462 pm with each particle measuring approximately 99.47 μm in diameter. The novelty of this study was to reduce the particle size of the NaF and to incorporate this in the APF gel. It was hypothesized that, when applied to the tooth surface, the incorporation of the nanoparticles in APF gel would alter the F uptake and have a further effect on the tooth surface microhardness. Hence, the aim of the present study was to assess, in an *in vitro* setting, the changes in the enamel surface microhardness on extracted human teeth after the application of a nanoparticle-based acidulated phosphate fluoride (APF) gel as compared to a conventional APF gel.

MATERIALS AND METHODS

This was an *in-vitro* study carried out on 30 extracted human teeth. Ethical clearance was obtained from the Institutional Review Board of the Ragas Dental College and Hospital, Chennai, India (TN/RDC20170701). Only teeth with sound enamel, extracted for orthodontic reasons or due to mobility, were included in the investigation. The entire study was carried out in a laboratory setting at Evans Bio Pvt Ltd, East Ekambaram, Chennai, India.

Preparation and characterization of the NaF nanoparticles: Sodium fluoride (NaF) nanoparticles were synthesized by a simple co-precipitation method.[17,18] 0.02 M ammonium fluoride (NH_4F) was added into an aqueous solution of 0.01 M sodium chloride (NaCl) under vigorous stirring for 2 hr, until the transparent solution turned into a white opaque suspension indicating the formation of NaF nanoparticles. The obtained suspension was set aside for 24 hr without any disturbance. The settled precipitate was then filtered and washed with ethanol thrice to remove any residual chloride/ammonium ions. The NaF nanoparticles obtained were air dried and stored in a vacuum. The characteristics of the particles formed were confirmed with the help of a scanning electron microscope (SEM). The size of the NaF particles ranged between 20 nm to 80 nm and appeared as shown in Figure 1.

Preparation of the nanoparticle-based APF gel: The APF gel was synthesised by adding a hydroxyethyl cellulose to a NaF nanoparticle-based solution under constant stirring on a mechanical vibrator. This solution was prepared by dissolving 20 g of NaF nanoparticles in 1 litre of 0.1M phosphoric acid and to this 50% hydrofluoric acid was added such that the pH was adjusted to 3.0 and the F concentration was maintained at 1.23%. The gel was stored in an air tight container under refrigerated conditions until further use.

Specimen preparation and mounting: All the extracted teeth were cleaned thrice with distilled water and stored in normal saline before the commencement of the study. The samples were then individually sectioned with a carborundum disc on a slow speed hand piece, at the level of cementoenamel junction, followed by the palatal/ lingual portions for anterior teeth and the posterior teeth were hemi-sectioned mesiodistally to retain the buccal half (Figure 2)

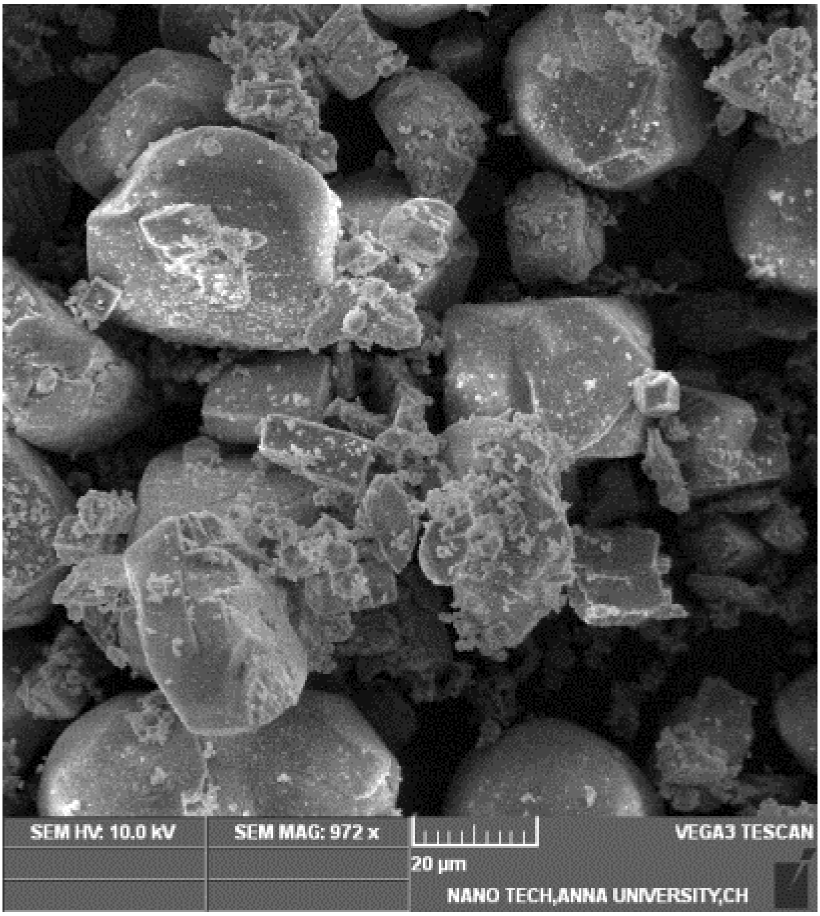


Figure 1. SEM image of NaF nanoparticles.

Figure 2. Specimen preparation using carborundum disc and mounting.

The specimens prepared were then individually mounted on an acrylic disc made of polymethyl methacrylate (PMMA) resin of diameter 2 cm. The mounting was done such that, the buccal surfaces of each tooth were flat, parallel to the base and 0.5 mm above the level of the disc. Each specimen was then divided into two equal halves vertically with the help of a paint marker such that each tooth acted as its own control.

Application of gel: The prepared nano-based APF gel was applied to the right half and the left commercially available APF gel (16 fluid ounces, Pascal International Incorporation, USA) was applied to the left half with the help of a micro brush for 4 min after which the specimens were rinsed with distilled water. The samples were then stored in artificial saliva for the rest of study period and pH was maintained between 6.5–7.0. The artificial saliva medium was freshly replenished each day to prevent biofilm formation.

The surface microhardness of the teeth samples were measured with a Micro Vickers Hardness tester as a Vickers microhardness number at a load of 0.025 N to obtain a rhomboidal indentation. The microhardness was recorded at baseline, 24 hr, and 30 days. Further, the enamel surface of a randomly selected tooth was taken for SEM analysis, to visualize the surface changes. The analysis was carried out at the Central Workshop Division, College of Engineering Guindy, Anna University, Chennai, India.

RESULTS

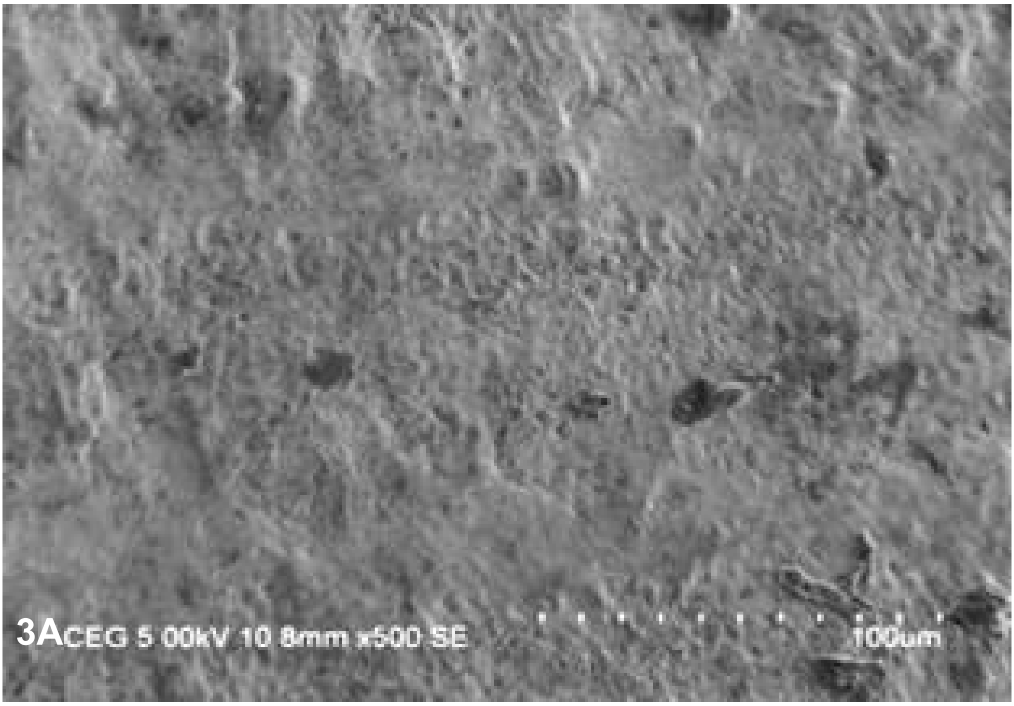
The results of the present study are depicted in Table 1. The intergroup comparison between the nano-based and the conventional APF gel groups at 3 time intervals revealed a significant increase in the mean enamel surface microhardness in the test group, at the 24 hr evaluation. The intragroup comparison for the same time interval showed a significant increase in the enamel surface microhardness in both the groups; between baseline and 24 hr. An insignificant decrease in the enamel surface microhardness was observed, in both the groups on the 30th day evaluation, in both the intragroup and intergroup analyses.

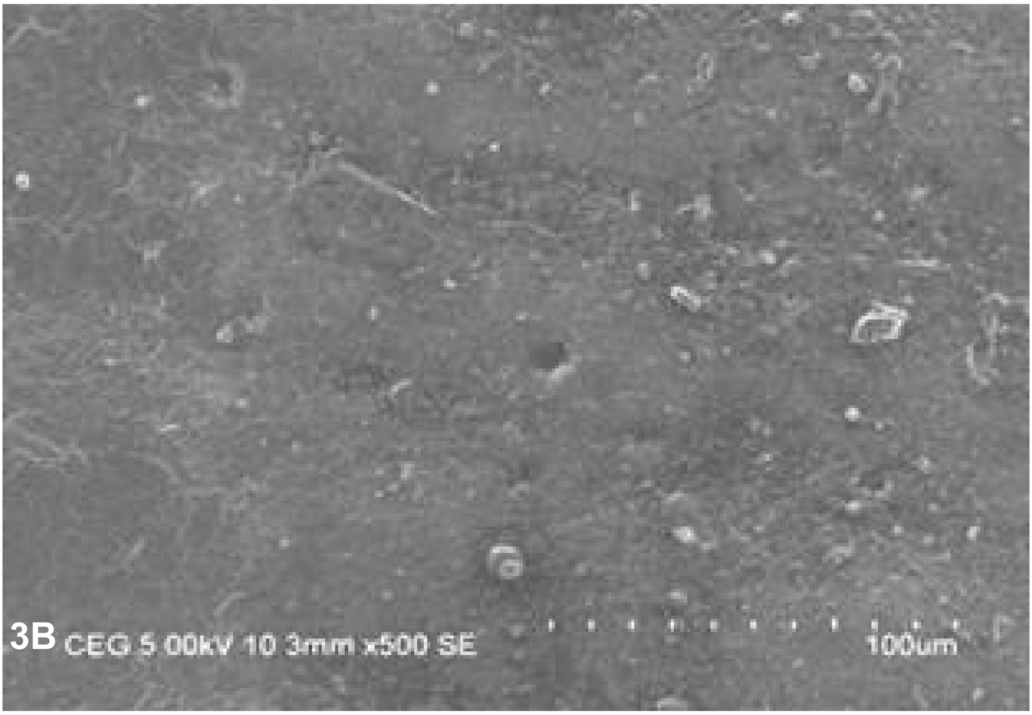
Table. The mean difference in surface hardness between the nanoparticle-based APF gel and the conventional APF gel (the comparisons are both between the groups and the within groups)

Time of testing	Nanoparticle-based APF gel (n=30)	Conventional APF gel (n=30)	p value
Baseline	363.17±71.80†	353.05±68.34†	0.578
After 24 hours	523.15±16.38†	512.12±17.32†	0.014*
After 30 days	351.92±67.68	341.71±70.87	0.570

*p value ≤0.05 indicates significance;
†p value =0.001 indicates significance for comparison within each group at baseline, after 24 hours, or after 30 days.

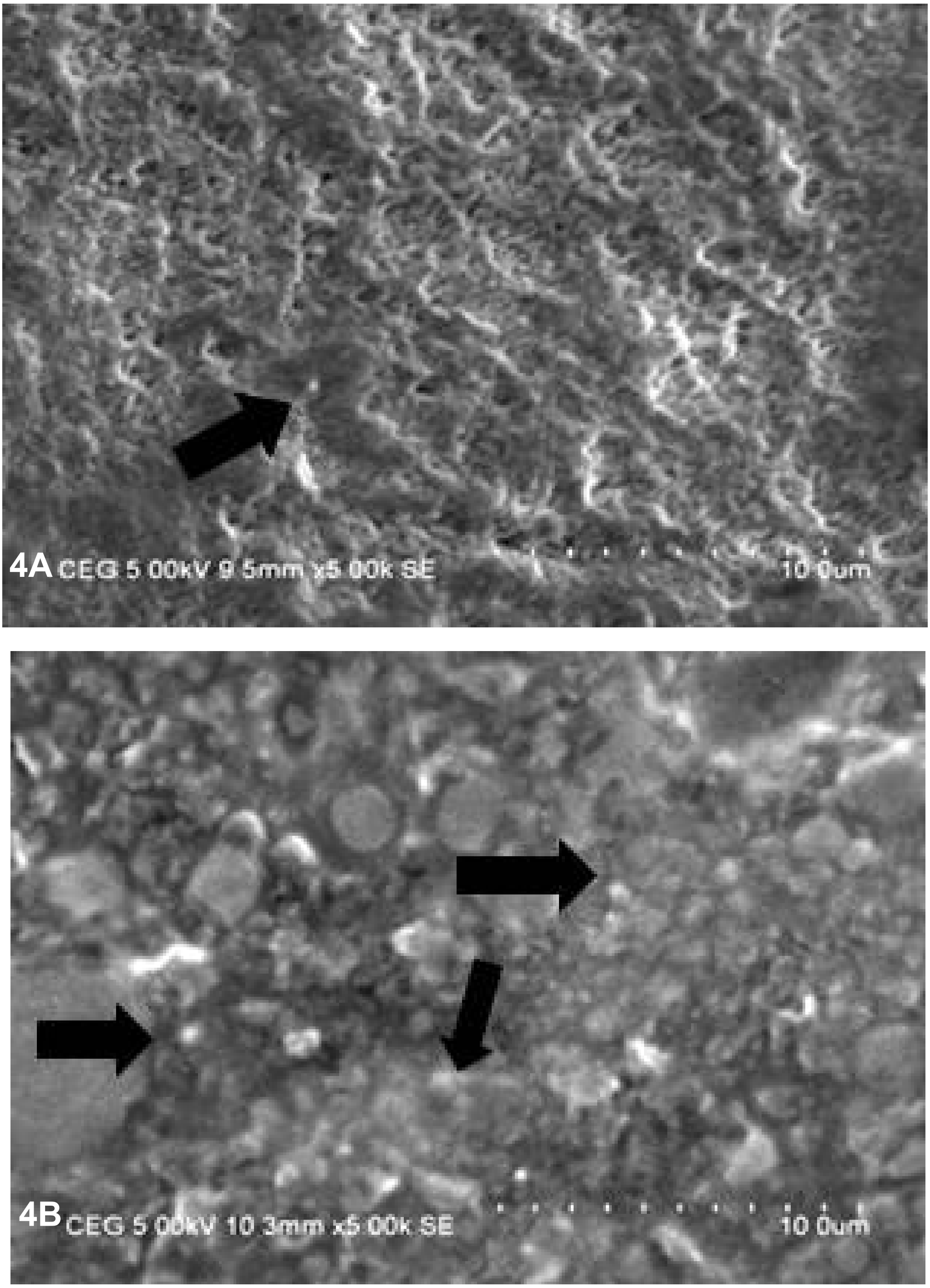
Scanning electron microscope images of both the teeth at 500× magnification showed a roughened enamel surface on the side which had conventional APF gel applied to it compared to the test side treated with nanoparticle-based APF (Figures 3A and 3B).





Figures 3A and 3B. Scanning electron microscope images of a premolar treated with: 3A: conventional APF get, and 3B: nanoparticle-based APF gel. (500×).

At 5,000× magnification, the conventional side showed etched enamel surface similar to the acid etch of enamel with phosphoric acid, although with the occasional presence of calcium fluoride crystals (Figure 4A). However, the test side showed globular structures with more calcium fluoride crystals (Figure 4B).



Figures 4A and 4B. Scanning electron microscope images of a premolar treated with: 4A: conventual APF get, and 4B: nanoparticle-based APF gel. The black arrows point to calcium fluoride (CaF_2) crystals. (5,000×).

Further observation of the test side only at 15,000× magnification showed a similar etched pattern with discrete calcium fluoride crystals (Figure 5).

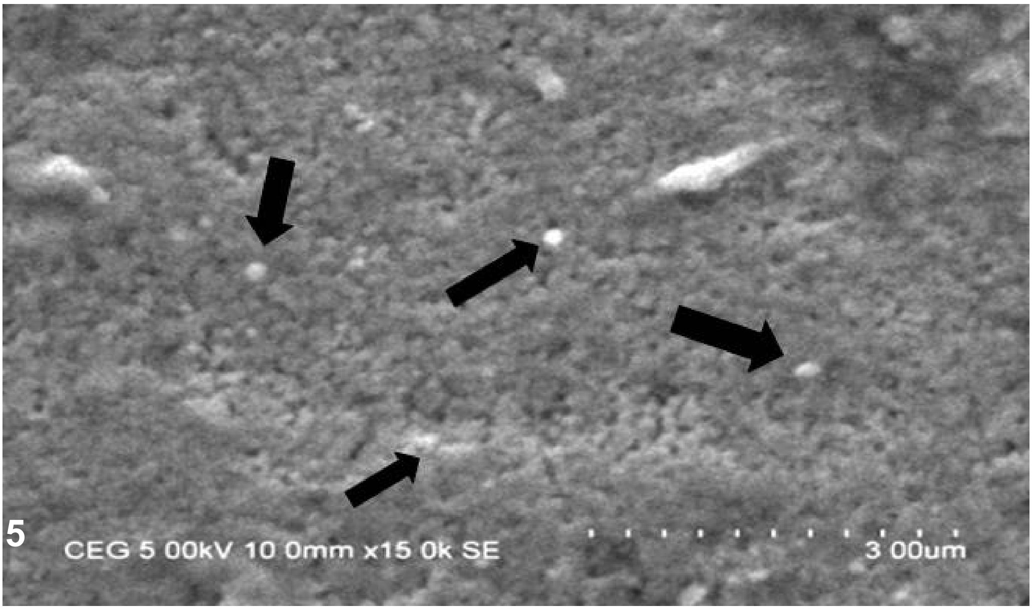


Figure 5. Scanning electron microscope images of a premolar treated with nanoparticle-based APF gel. The black arrows point to calcium fluoride (CaF_2) crystals. (15,000×).

DISCUSSION

Overall, treatment with both the nano-based APF and the conventional APF group resulted in an increase in the mean surface microhardness at the 24 hour evaluation. This can be attributed to the incorporation of the fluoride in the enamel after the topical application which improves the crystallinity through the formation of fluorapatite crystals and a calcium fluoride-like precipitate. Although similar results have been reported earlier,[19-21] one study reported the effect of APF gel on root dentin demineralization and the other reported the effect of APF gel on the mean hardness of endogenously eroded teeth.[20,21]

An *in vitro* model showed comparable mean surface microhardness as the effect of of the application of APF gel for different time periods but it was reported on bovine teeth with 9,000 ppm concentration.[19]

Another study on 56 enamel blocks[22] assessed the percentage changes in the surface microhardness of deciduous teeth among 4 different dentrifice groups, of which 3 of them contained NaF at concentrations ranging from 500 to 1,100 ppm. However, deciduous teeth have structurally different enamel, compared to the enamel of the permanent teeth used in our study.[23]

A comparison of the hardness among intact and demineralised enamel treated with the application of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and APF gel[24] also claimed APF gel to be more effective in remineralizing the smooth surface than CPP-ACP at 24 hr as the concentration of F provided by the APF gel was higher than that given by CPP-ACP.

A comparative evaluation of the surface microhardness of dentin treated with 4% titanium fluoride and 1.23% APF gel[25] on extracted premolars reported that the protective effect of F in APF gel is mainly due to the presence of calcium fluoride (CaF_2)-like precipitate on the tooth fluid interface. However, the major disadvantage with APF gel is the ready dissolution of this precipitate. This could be one of the reasons for the reduction in the mean surface microhardness values at the 30th day evaluation.

The scanning electron microscope images at the end of 30 days showed the presence of a CaF_2-like precipitate in both the nanoparticle-based and the conventional APF gel groups, though at different magnifications. This is similar to the results obtained on examination of the crystallographic changes in the enamel surface after topical fluoride application.[26,27] It was proposed that APF gel produced a surface coating, which appeared to consist of, a uniformly thick layer of densely packed 20 to 50 nm diameter particles which had fewer tendencies to aggregate. Even though the size of the CaF_2 particles were not determined in our present study, the images over all revealed more crystals in the nanoparticle-based APF group. Also, the enamel surface was more intact compared to the etched surface of the conventional group indicating a greater penetration and depth of action of the NaF nanoparticles.

In the present study, the topical application was carried out only once at the start of the study and the storage medium (artificial saliva) was replaced afresh every 24 hr to restrain biofilm formation. The outcome surface microhardness levels, were measured as Vickers hardness, which reflects the effect of APF gel application on the tooth. A limitation of this study was the variation in the extracted human teeth samples, as they were extracted from different people belonging to varied age groups. Further, with it being an *in vitro* experiment, the artificial saliva used can only mimic the oral environment in terms of mineral content, temperature, and pH and not completely simulate the dynamic changes of the oral cavity.

CONCLUSIONS

After 24 hr of treatment, the incorporation of nanoparticles of sodium fluoride, the active component in the APF gel, resulted in a significant increase in the enamel surface microhardness of teeth samples compared to treatment with a conventional APF gel. To the best of the knowledge of the authors, this is one of the very few studies to evaluate the effect of a one time, 4 minute application of APF gel on permanent teeth, and, the first of its kind to use an APF preparation containing sodium fluoride nanoparticles.[1,2,28,29] Further evaluations regarding the amount of fluoride uptake by the tooth surface and long term *in vivo* trials to assess the caries inhibitory effect are recommended to determine the advantage of APF containing sodium fluoride nanoparticles compared to the conventional preparations.

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