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Comparison of Remineralisation potential of Nano-Hydroxyapatite with Sodium fluoride, Sodium mono fluorophosphate in Initial Enamel Lesion – An In Vitro study

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Abstract

Background: Conventional inorganic fluorides sodium fluoride & sodium mono fluoro phosphate containing dentifrices have shown re-mineralization potential in many studies. Nano-hydroxyapatite in dentifrices is a novel re-mineralizing agent and provides an alternative from fluorides.

Aim: To evaluate & compare the re-mineralising potential of Nano-Hydroxyapatite with sodium fluoride and Sodium mono fluoro phosphate in initial enamel lesion**.**

Methods: Thirty sound non-carious human teeth were coated with acid resistant nail varnish leaving rectangular window of 2mm x 4mm on sound intact labial surface & then immersed in 500ml of demineralising solution (Glacial acetic acid) for 96 hr to produce artificial carious lesions. Sectioning was done longitudinally in Bucco-lingual direction and sections were then divided into three groups (Group I- Colgate total NaF, Group II- Colgate active salt Na MFP, Group III- Apagard M plus-Nano-hydroxyapatite). Remineralisation was assessed with SEM and EDS.

Results: Highly significant rise in Ca and Phosphate content on re-mineralization after standardized demineralization was seen in each group during intragroup comparisons depicting re-mineralization potential of all three re-mineralizing agents. In intergroup comparison NaF brought maximum increase in mineral content followed closely by Nano-HAP which was statistically non-significant and further followed by Na MFP which was statistically significantly lower than both NaF and NHA**.,** Surface morphology of enamel slabs treated with sodium mono-fluoro phosphate showed a homogeneous pattern of re-mineralization with formation of globular structures. Treatment with Sodium fluoride restored the normal smooth morphology of enamel comparable to sound enamel slab. In Nano-hydroxyapatite group, deposition of thick homo geneous apatite layer could be seen.

Conclusions: NaF exhibited highest remineralization potential in terms of mineral gain followed closely by Nano-HAP and then by sodium mono fluoro phosphate dentifrice.

Keyword: Homogeneous, Fluorophosphate, Sodium Fluoride

Introduction

Dental caries is the destruction of tooth tissue in the presence of organic acids produced by cariogenic bacteria.[1,2] Non cavitated white spot lesions (WSLs) are the first indication that the complex dynamic physico chemical processes that maintain healthy enamel have shifted in favour of demineralisation.

It is possible to reverse early stages of enamel caries, during which the surface remains intact and the net dissolution of calcium and phosphate ions occurs from the body of the lesion.[3]

Re-mineralising agent fluorides over the past 25 years, have been instrumental in causing the decline of dental caries experience in most industrialized countries. Topical fluorides promote the formation of fluorapatite in the presence of calcium and phosphate ions produced during enamel demineralization.[4]

Further, it has been found that high frequency use and low concentrations of fluoride agents are more beneficial. One cannot indefinitely increase the concentration of fluoride dentifrices at the risk of adverse effects such as dental fluorosis even without losing their cariostatic effect.[5]

Nano-HAP paste is a bioactive agent that contains calcium Nano- phosphate organized in a crystalline form of HA.[6]

Nano-Hy droxyapatite containing dentifrices has given an alternative to fluoride containing tooth pastes as it claims superior anti-caries activity, protection against hyper sensitivity and maintains gloss and natural translucent whiteness of the tooth. Its one of the most bio compatible and bioactive material.

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Considering the significance and superiority of preventive measures over invasive procedures for treatment of initial caries and the existing concerns regarding the use of fluoride due to its potential side effects, attempts have been made to use synthetic re-mineralizing agents as an alternative to fluoride.

Thus, owing to the re-mineralizing properties of NHA, NaF and Na MFP and the significance of preventive and conservative treatments, this study aimed to assess and compare the re-mineralizing effects of NHA, NaF and Na MFP on artificially created carious lesions on human extracted teeth.

Material and methodology

The present in-vivo study was conducted in the Department of Pedodontics and Preventive dentistry of Himachal Dental College, Sunder Nagar H.P. Ethical clearance was taken from the institutional ethical committee review board. Study involves 30 sound non-carious human extracted teeth without any restorations (orthodontic extractions).

Exclusion criteria

Teeth with any visible caries., hypoplastic or white spot lesions, teeth with fracture lines.

Inclusion criteria

Caries-free teeth & teeth extracted for therapeutic purpose.

Procedure

Selected teeth were stored in 10% formalin following extraction and calculus was mechanically removed using hand scalers.

Artificial saliva was prepared by mixing 4200 mg/l $NaHCO_3$, 500mg/l NaCl, 200mg/l KCl in distilled water to have a standard PH of 7.3 which was measured using digital ph. meter.

Artificial carious lesion formation

Teeth were dried and coated with acid resistant nail varnish leaving rectangular window of 2mm x 4mm on sound intact labial surface. Immersed in 500ml of demineralising solution for a period of 96 hr to produce artificial carious lesions. Demineralization is done with Glacial Acetic acid at 4.5 ph. to produce initial carious lesions.

Teeth were longitudinally sectioned using diamond disc mounted on a low-speed hand piece in Bucco-lingual direction such that a part of demineralized and normal enamel is present in each section. Total 30 sections were obtained and divided into 3 groups: -

Group I – Colgate total NaF (1450 ppm $F^{-)}$

Group II – Clolgate active salt Na MFP (1000 PPM F^{-})

Group III – Apagard M plus – Nano-Hydroxyapatite (10%)

Remineralization

Assessment is done with Scanning Electron Microscopy (SEM) showing morphological changes and Energy Dispersive X- Ray Spectroscopy (EDS) to measure mineral content i.e Calcium and Phosphorous in various groups of teeth sections.

Remineralisation is done then, by treating sections with respective dentifrice solution which were prepared by 3:1 ratio of deionized water to dentifrice in a Ph cycling for a period of 10 days. Fresh slurry was prepared for all three dentifrices before each use.

Results & observations

The present study evaluated and compared the re-mineralization potential of Sodium fluoride, Sodium Mono-fluorophosphate and Nano-hydroxyapatite on enamel subsurface lesions using SEM-EDX.

In Intragroup comparisons, there was highly significant decrease in mean value of calcium on demineralization in Group I from mean value 29.87 to 18.9, in Group II from 34.8 to 25.5 & Group III from 38.1 to 26.1. But after remineralization with NaF, Na MFP and Nano-HAP respectively in three groups there was statistically significant rise in calcium content in group I to 26.5, in Group II to 30.9 and in Group III to 34.4 of enamel sections which was non significantly lower than base level.

Similarly for phosphorous content there was statistically significant decrease in phosphorous content of enamel slabs after demineralization in Group I from 16.3 to 10.1, Group II from 18.2 to 11.1 & Group III from 19.8 to 12.14.But remineralization with NaF, Na MFP and Nano-HAP respectively in three groups showed statistically significant rise in phosphorous content in Group I to 14.32, in Group II to 15.9 and in Group III to 17.8 of enamel sections which was non significantly lower than the base level.

To compare the effects of different treatments in the increase of calcium level from demineralized to re-mineralized state, the percent increments of minerals following different treatments were calculated by one-way ANOVA. The results are summarized in the following tables (table 1to 3).

Table 1: Descriptive statistics for percentage of calcium level increment following re- mineralization by three treatment methods

Treatment	Mean	SD	Minimum	Maximum
NaF	41	6.39	33	51.3
Na MFP	23	12.61	5.5	50.8
NHA	33	16.73	7.2	61.5

Table 2: Descriptive statistics for percentage of calcium level increment following re-mineralization by three treatment methods.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1566.728	2	783.4	4.898	0.015
Within Groups	4318.055	27	159.9		
Total	5884.783	29			

Table 3: Multiple post hoc Tukey test for comparison among three re-mineralization treatment methods.

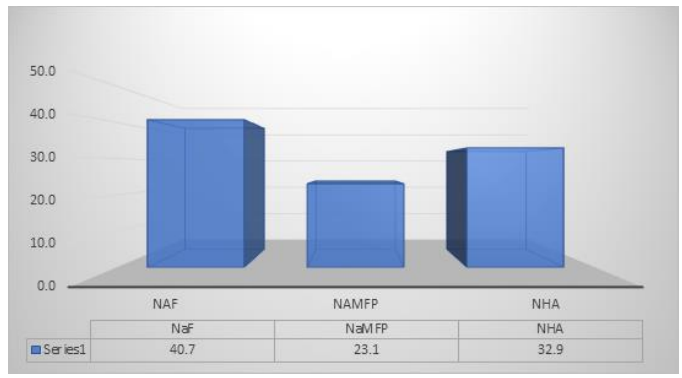
Treatment group (I)	Treatment group (J)	Mean Difference (I-J)	Std. Error	Sig.
NaF	Na MFP	17.7*	5.66	0.01
	NHA	7.78	5.66	0.37
Na MFP	NaF	-17.7	5.66	0.01
	NHA	-9.88	5.66	0.21
NHA	NaF	-7.78	5.66	0.37
	Na MFP	9.88	5.66	0.21

*The mean difference is significant at the 0.05 level.

From table 1 and graph 7, it appeared that the NaF re-mineralization treatment had maximally increased the calcium level by 41 %, followed by NHA (33%) and least increment was achieved by the Na MFP treatment (23%). A one-way ANOVA showed that a statistically significant difference (P≤.05) in calcium level was observed between NaF group (40.7%) and Na MFP group (23.1%). However, when NaF was compared with NHA group it was found that although Calcium level were high with NaF group it was not statistically significantly higher than NHA group.

When Na MFP group was compared with NHA group it was found that NHA did better re-mineralization where Calcium level was higher (32.9%) after re-mineralization than Na MFP group (23.1%) after re-mineralization.

Graph 1: Per-cent increment in calcium level following re-mineralization by three different treatments.



To compare the effects of different treatments in the increase of phosphate level from demineralized to re-mineralized state, the per-cent increments of minerals following different treatments were calculated by one-way ANOVA. The results are summarized in the following tables.

From table 4, it appeared that the NHA re-mineralization treatment had maximally increased the phosphate level by 50%, followed by Na MFP (49%) and least increment was achieved by the NaF treatment (44%).

A one-way ANOVA showed that no statistically significant difference in phosphate level was observed between the different treatments; (F (2, 27)) = 0.125, p = 0.883. The results of ANOVA are presented in table 5.

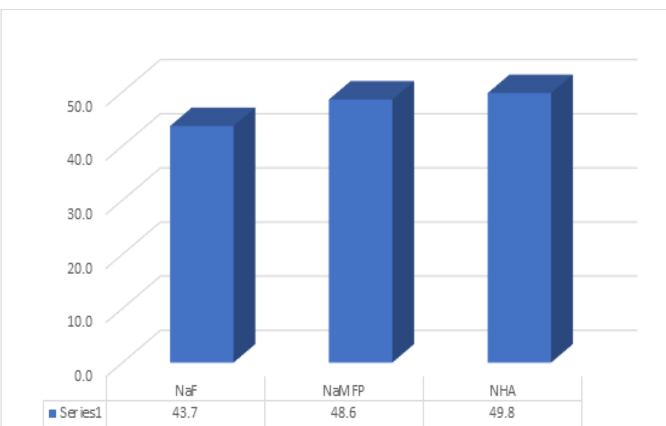
Table 4: Descriptive statistics for percentage of phosphate level increment following Re-mineralization by three treatment methods

Treatment	Mean	SD	Minimum	Maximum
NaF	44	27.7	12.5	97.8
Na MFP	49	34.6	3.2	112.8
NHA	50	22.9	8.7	82.2

Table 5: ANOVA results for the effects of three re-mineralization treatment methods in increasing phosphate level.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	207.6	2	103.8	0.125	0.883
Within Groups	22374.9	27	828.7		
Total	22582.6	29			

Graph 2: Per-cent increment in phosphate level following re-mineralization by three different treatments.



SEM images evaluated at 2000 magnification revealed that sound enamel had honeycomb appearance. [Fig.1] Loss of mineral content gives a porous appearance and depressions in defined honeycomb pattern [Fig.2] and [Fig.3].

Enamel slabs treated with sodium mono-fluorophosphate showed a homogeneous pattern of re-mineralization with formation of globular structures [Fig.4].

Treatment with Sodium fluoride restored the normal smooth morphology of enamel comparable to sound enamel slab [Fig.5]. In Nano-hydroxyapatite group [Fig.6], deposition of thick homogeneous apatite layer could be observed.

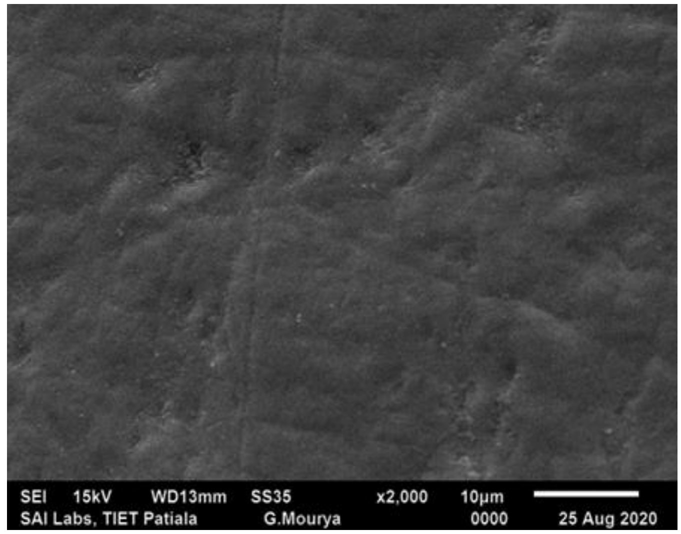


Fig 1: Sound Enamel

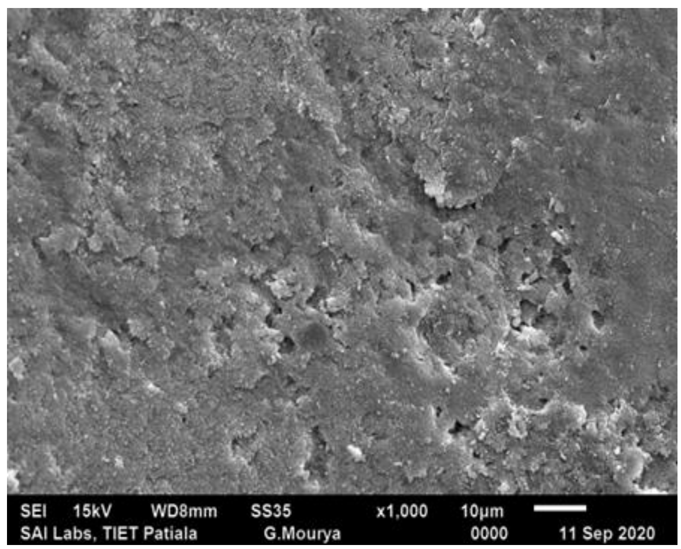


Fig 2: Demineralized Enamel (x 1000)

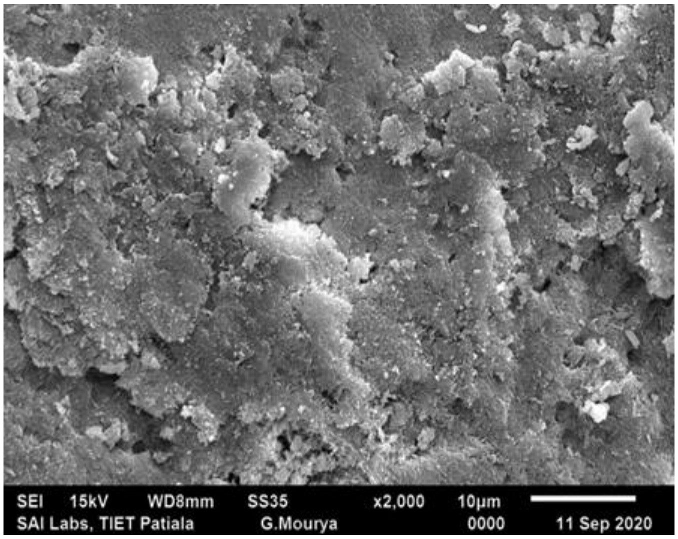


Fig 3: Demineralized Enamel (x 2000)

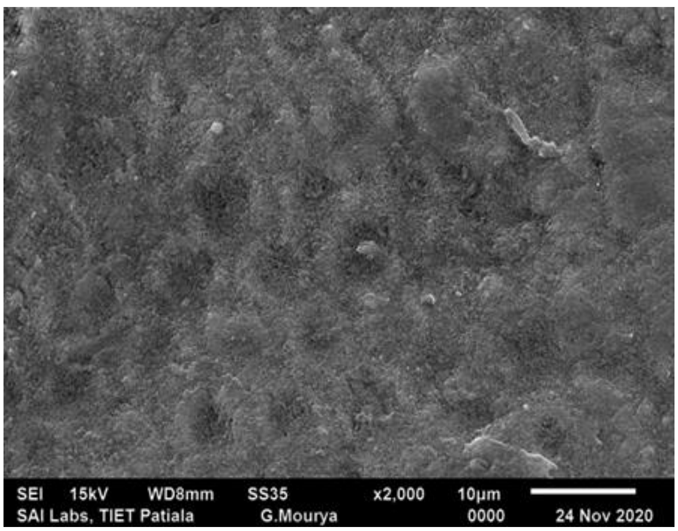


Fig 4: Sodium Mono fluoro phosphate remineralization.

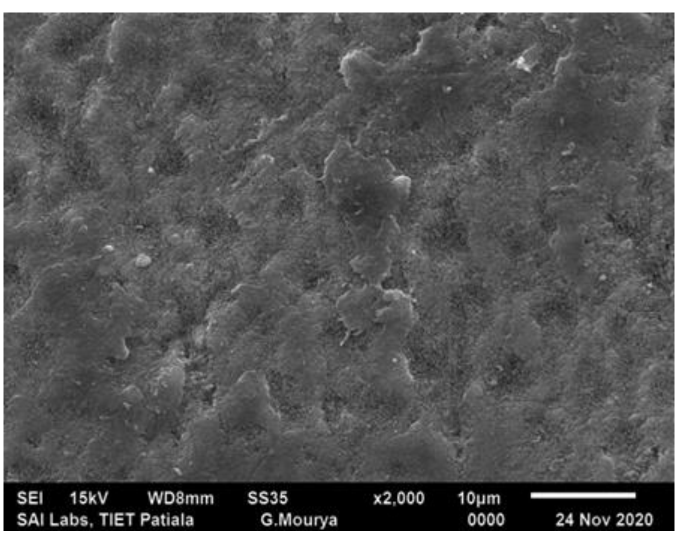


Fig 5: Sodium Fluoride remineralization

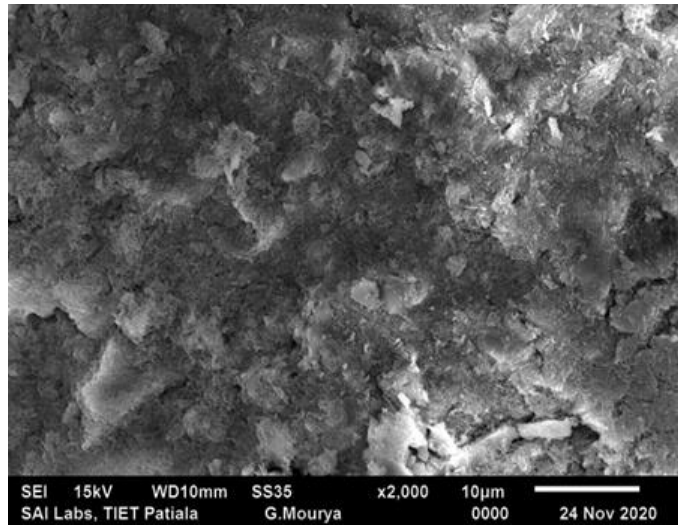


Fig 6: Nano- Hy droxyapatite remineralization.

Discussion

Caries is a pH dependent process, as the salivary pH drops below 5.5 (critical pH) dissolution of enamel begins which is the first step in demineralisation process. This marks the beginning of early enamel caries Near neutral pH of saliva has natural buffering capacity hence, demineralisation of the tooth enamel is reversed by saliva in early stages.[9]

Fluorides have been used for re-mineralization of dental caries since many years. Wolfgang H Arnold et al. (2006) in his study showed that fluoride dentifrices slow down the process of de-mineralization by producing an acid resistant component, fluorapatite.

Nano-hydroxyapatite as a biomimetic material for the reconstruction of tooth enamel suffering from mineral loss have been discussed in Santosh KP et al. (2009)[11] study.[12]

During carious lesion formation in oral cavity various acids produced by bacteria are Lactic acid, acetic acid, propionic acid and Iso-butyric acid. Hence, we have chosen acetic acid as the de-mineralizing agent at PH 4.5 in the present study.

In group I (NaF), when demineralization was done calcium and phosphorous level decreased significantly from the baseline values similar to Tschoppe P et al.

(2011)[14] & Edlund K et al. (1977)[15] studies. Acetic acid like demineralizing agents leaches out the mineral content of hydroxyapatite. After re-mineralization with NaF the calcium and P content again rose significantly to a level where they were non-significantly lower than the baseline values. Similar results were found in Shivashankar et al. (2018)[8] studies.

In Group II (Na MFP), calcium and phosphorous level decreased significantly below the baseline values due to the demineralization and remineralization raised Ca and P content significantly to a level where they were non significantly lower than the baseline values as in Manikandan et al. (2011)[16] and Grewal N et al. (2018)[17] studies.

In Group III (NHA) Calcium and phosphorous level decrease after demineralization was statistically significant and rose significantly after remineralization to a level where they were non significantly lower than the baseline values. Similar results were seen in Manchery N et al. (2018)[18] and Grewal N et al. (2018)[17]studies.

While comparing group I NaF and group II Na MFP, percentage level reduction in Ca and P levels was determined to make a stan dardization after remineralization. Ca and phosphorous level although it increased both in NaF and Na MFP group but, rise was more in NaF group and this rise was statistically significant when compared to Na MFP. Similar results were obtained by Toda S et al. (2008)[19] and Siva Sankar K et al. (2018)[8]. NaF is a highly ionisable compound and provides free fluoride easily causing its higher remineralization potential than Na MFP. Na MFP requires enzymatic hydrolysis to cleave the covalent bond between the phosphate molecule and fluoride. Enzyme system required for MFP hydrolysis is absent in in-vitro study (Beiswanger et al.1989[20]).

Between group II Na MFP and group III NHA, Ca and phosphorous level although increased in both but, rise was statistically significantly more in NHA group similar to Grewal N et al. (2018)[17] and Manchery N et al.[18] studies. Better efficacy of Nano-HAP can be attributed to its high affinity and attachment onto enamel surface as suggested by Lil et al. (2008).[12]

Between group I NaF and group III NHA, Ca level increased in both NaF and NHA groups but NaF showed slightly better increase in Ca content than NHA which was statistically non-significant. For phosphate level although both NaF and NHA increased the P level but rise was more in NHA than NaF and it was statistically not significant. But, in Manikandan E et al. (2011)[16] study NaF dentifrice demonstrated remineralization of carious lesion by virtue of an increase in mineral content and significant decrease in lesion depth. Roveri N et al. (2012) showed that nano-hydroxyapatite of the particle size 20 nm has the potential to re-mineralize initial enamel caries under in vitro conditions when compared with 2% sodium fluoride. In present study NaF brought maximum increase in mineral content followed closely by nano-HAP which was statistically non-significant and further followed by Na MFP which was statistically significantly lower than both NaF and NHA.

NaF being highly ionisable compound provides free fluoride easily causing its higher re-mineralization potential. Fluoride bioavailability is important for caries prevention and it is particularly dependent on the solubility of the fluoride containing compound and the adhesion of the fluoride compounds to the surface.[22] Bioavailability of Fluoride is better in NaF because of its high ionizability.

Limitation of the present study can be the fact that it was an in vitro study and although we have used all the recommended solutions including artificial saliva however, it was not possible to include many biological enzymes and proteins which are naturally occurring in human saliva. However, many clinical trials like study by Edlund & Koch (1977)[15] & Beiswanger et al. (1987)[20] have also proved NaF to be most effective in clinical trials and even nano hydroxyapatites clinical efficacy has been proved by Jeong S H et al. (2006)[22] & Roveri et al. (2009)[21] Hence, in accordance with our study.

Conclusion

All the three re-mineralizing agents i.e NaF, Na MFP and Nano-HAP demonstrated re-mineralization by virtue of increase in mineral content and showed morphological changes .NaF showed superior remineralizing efficacy followed by NHA and then by Na MFP. Nano-HAP paste is an alternative re-mineralizing agent that could be beneficial for children, pregnant females and those who are at risk of dental fluorosis. Considering cost effectiveness of both NaF and NHA, NaF would provide a cost-effective alternative.

References

1. Douglass JM, Douglass AB, Silk HJ. A practical guide to infant oral health. Am Fam Physician. 2004; 70:2113–20.
2. New burn E, Albrektsson TO, Bratthall D, Glantz PJ, Lindhe JT. Tissue Preservation in Caries Treatment. 3rd ed. Great Britain: Quintessence publication CO. ltd; 2001. Fluoride in caries Prevention and remineralization of partially demineralized enamel and dentin; p. 19.
3. Dowker SEP, Anderson P, Elliott JC, Gao XJ. "Crystal chemistry and dissolution of calcium phosphate in dental enamel," Mineralogical Magazine. 1999; 63 (6)791-800.
4. Pepla E, Besharat LK, Plia G, Ten ore G, Migliau G. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry; A review of literature. An Stomatol (Roma) 2014 July – Sep ;(3): 108-114
5. Marinelli C B, Donly K j, Wefel J S, Jacobson J R, Denehy G E. An in vitro comparison of three fluorides regimes on enamel remineralization. Caries Res 1997; 31: 418 – 422
6. Daas Issa, Badr Sherine, Osman Essam. Comparison between fluoride and nano- hy droxyapatite in remineralising initial enamel lesion: an in vitro study. J Contemp Dent Pract 2018;19(3):306-312.
7. Kani T, Kani M, Is Ozaki A, SHintani H, Ohashi T, Toku moto T. Effect of apatite-containing toothpastes on dental caries in school children. J Dent Health. 1989; 39:104–9.
8. Siva Shankar K, et al., Remineralizing potential of commercially available pediatric dentifrices: An In vitro study, Pediatric Dental Journal (2018).
9. Walsh Laurence J. Evidence that demands a verdict: latest developments I in remineralisation therapies. Australasian Practice 2009:48-59.
10. Wolfgang H Arnold; Andreas Do row; Stephanie Langen horst; Zeno Gintner; Jolan Bánóczy; Peter GA Engler (2006). Effect of fluoride toothpastes on enamel demineralization., 6 (1), 8–0. doi:10. 1186/ 1472- 6831-6-8.
11. Santosh KP, Chu MC, Balakrishnan A, Lee YJ, Kim TN, Cho SJ. Synthesis of nano hydroxyapatite powder that simulate teeth particle morphology and composition. Curr Appl Phys. 2009; 9:1459–62.
12. Li Li, a Hai Hua Pan, a Jinhui T. Repair of enamel by using hydroxyapatite nanoparticles as the building blocks ao, a Xurong Xu, a Caiyun Mao, b Xinhua Gub and Ruikang Tang*a
13. Geddes DA. The production of L (+) and D (-) lactic acid and volatile acids by human dental plaque and

the effects of plaque buffering and acidic strength on ph. Arch Oral Biol 1972; 17:537-45.

14. Tschoppe P, Zan dim DL, Martu's P, Kielbasa AM: Enamel and dentine remineralization by nano-hydro xyapatite toothpastes. J Dent 2011; 39: 430–437.

15. Edlund K, Koch G. (1977) Effect on caries of daily supervised toothbrushing with sodium mono fluoro phosphate and sodium fluoride dentifrices after three years. Scand. J. Dent. Res.,85,41-45.

16. Ekambaram, M., Itthagarun, A., & King, N. M. (2010). Comparison of the remineralizing potential of child formula dentifrices. International Journal of Paediatric Dentistry, 21 (2), 132–140. doi: 10. 1111/ j. 1365-263x.2010. 01101.x

17. Navneet Grewal, Neha Sharma, Nirapjeet kaur. Surface remineralization potential of nano-hydro xyapatite, sodium mono fluoro phosphate and amine fluoride coating dentifrices on primary and permanent enamel surfaces: An in vitro study. J ISPPD 2018 Aug; 36:158-156.

18. Manchery N, John J, Nagappan N, Subbiah GK, Premnath P. Remineralization potential of dentifrice containing nano hydro xyapatite on arti ﬁ cial carious lesions of enamel: A comparative in vitro study. Dent Res J 2019; 16:310-7

19. Toda, S., & Featherstone, J. D. (2008). Effects of Fluoride Dentifrices on Enamel Lesion Formation. Journal of Dental Research, 87(3), 224–227. doi:10.1177/ 154 4059 108087 00303

20. Beiswanger. The comparative cariostatic efficacy of sodium fluoride and sodium mono fluoro phosphate dentifrices: a review of trials. ASDC J Dent Child 1989; 56:337e47.

21. Roveri N, Battistella E, Bianchi CL, Foltran I, Foresti E, Iafisco M, et al. Surface enamel remineralization: bio mimetic apatite nano crystals and fluoride ions different effects. Journal of Nano materials 2009. 10.1155/ 2009/ 746383.

22. Jeong S.H, Jang S.O, Kim K.N, Kwon H.K, Park Y.D, Kim B.I Remineralization Potential of New Tooth paste Containing Nano- Hydro xyapatite. Key Engineering Materials Vols. 309-311 (2006) pp. 537-540.