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REMINERALIZATION EFFICIENCY OF NANOHYDROXYAPATITE, NANO-BIOACTIVE GLASS, AND SODIUM FLUORIDE ON INITIAL ENAMEL CARIES OF PRIMARY TEETH

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ABSTRACT

Objectives: This in vitro study was carried out to evaluate the remineralization efficiency of nanohydroxyapatite (Nano-HAp), nano-bioactive glass (Nano-BAG), and sodium fluoride (NaF) on initial enamel caries of primary molar teeth. **Material and Methods:** The extracted primary molar teeth's enamel was used to create a total of 108 enamel samples. A particularly prepared demineralized solution was used to perform artificial caries. The demineralized enamel samples from primary molar teeth were remineralized using three solutions, each with a different concentration. According to the remineralization regime, the enamel samples were separated into three groups (n=36): the first group included enamel samples that had been treated with 10% Nano-HAp; the second group: enamel samples treated with 10% Nano-BAG; the third group: enamel samples treated with 2% NaF. The surface morphology, remineralization potential, and microhardness of enamel samples were assessed using the scanning electron microscope (SEM), energy dispersive X-ray (EDX), and Vickers microhardness tester, respectively, at the baseline, after demineralization, and after remineralization. **Results:** SEM analysis after remineralization intervention, all three study groups displayed favorable constructive surface changes. EDX analysis revealed a statistically significant increase in the Ca/P ratio (remineralization potential) for the enamel samples treated with Nanohydroxyapatite and Nano-bioactive glass in comparison with the sodium fluoride-treated group, however, no significant difference between Nanohydroxyapatite and Nano-bioactive glass treated groups. Also, statistically significant differences between the baseline (before demineralization), and after remineralization values in comparison with post-demineralization, were seen in the intragroup comparison of Ca/P ratios, however, no significant difference between baseline, and post-remineralization. The microhardness test showed a significant improvement in the surface hardness after remineralization of the demineralized enamel samples for all treated groups. However, the NaF treated sample showed a significantly lower hardness than the enamel samples at the baseline. **Conclusion:** The use of a 10% Nano-HAp and 10% Nano-BAG displayed positive constructive surface modifications and had the potential to remineralize the initial enamel caries and improve the microhardness of the demineralized enamel under in vitro conditions.

KEYWORDS: Nanohydroxyapatite, Nano-bioactive glass, Primary teeth, Remineralization, Sodium fluoride.

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INTRODUCTION

Dental caries in enamel is unique among diseases as enamel is both acellular and avascular.[1] Therefore, dental enamel does not have a regenerative capacity after tooth eruption and carious lesions may occur at any time in the course of a lifetime. [2] The signs of the initial caries process cover a continuum from the first molecular changes in apatite crystals of the tooth, to a visible white spot lesion, to dentin involvement, and eventual cavitation. [3]

Thus, the focus of modern preventive dentistry should be the protection and preservation of dental hard tissues through the treatment of early carious lesions with non-invasive approaches.[4] However, the basic enamel building blocks are typically 20–40 nm hydroxyapatite nanoparticles, and the enamel structure is too complicated to be altered.[4,5] Because of this, recent research has focused on creating the optimum remineralizing agent, which diffuses into the subsurface and can deliver calcium and phosphate. [4,6]

Topical fluorides cause the decline of dental caries experienced in most cases as well as promote the formation of fluorapatite. [7,8] However, it was stated that the topical fluorides do not intend to encourage the mineralization of apatite crystals or the replacement of the lost minerals, but rather to reduce apatite dissolution. [5,9] This is because, for every 2 fluoride ions, 10 calcium ions, and 6 phosphate ions are required to form a one-unit cell of fluorapatite. [10] Hence, the availability of calcium and phosphate ions can be the limiting factor for net remineralization to occur. [11]

Modern biomaterials such as Nano-hydroxyapatite and Nano-bioactive glass have recently been developed as remineralizing agents. [12,13] BAG is a composite of synthetic minerals that can effectively improve remineralization, containing calcium, sodium, phosphorous, and silica. [2,12] A substantial layer of hydroxyapatite (HA) is formed on the surfaces of the enamel and dentin when microscopic particles of BAG exposed to humidity release millions of mineral ions. [4]

Due to recent advancements in nanotechnology, calcium phosphate compounds have a stronger penetration capability for the demineralized porosity region as remineralizing agents. These compounds are extremely bioactive and even have their particle size and shape changed. [14] Therefore, the present study was conducted to explore the effects of synthetically processed solutions containing nanosized-HAp and nanosized-BAG particles in causing remineralization of the early enamel lesions in comparison with a solution of sodium fluoride. The null hypothesis is that there was no significant difference in the remineralization potential between the Nan-HAp and Nano-BAG solutions in comparison with sodium fluoride solution.

MATERIAL AND METHODS

After ethical approval was obtained from Ethical Committee, Faculty of Dentistry, Al-Azhar University (No. ECR-665/3844); a suitable number of human non-carious primary molars teeth, extracted for normal exfoliation without any restorations were included in this study were collected from the out-patient clinic of the Pediatric Dentistry and Public Health Department, Faculty of Dentistry, Al-Azhar University.

The eligibility requirements for choosing teeth included the absence of any prior restorations or carious lesions. The study did not include any teeth with hypoplastic or white spot lesions, teeth with any stains from early caries, or teeth with any macroscopic cracks or fractures. [1,5] The collected teeth were thoroughly cleaned and disinfected by soaking in 2.5% NaOCl for 24 hours to remove any remaining residual loose tissue and debris and stored in a normal saline (0.9% sodium chloride solution) at room temperature until used. [5]

The sample size of 18 samples per replicate was obtained based on the results of previous research by Swarup et al, 2012 [5]. A total of 108 enamel samples of primary molar teeth were used in this study and were divided into three equal main groups (n=36) according to the remineralization protocol as follows: group I: enamel samples that had been treated with 10% Nao-HAp group II: enamel samples treated with 10% Nano-BAG; and group III: enamel samples treated with 2% NaF (control group). The samples of each main group were further subdivided into two equal subgroups (n=18). A total of 18 samples in each subgroup were subjected to surface morphology and remineralization evaluation, and the other 18 samples were tested for surface microhardness.

Sample preparation:

The teeth crowns were separated and sectioned into two halves using a high-speed diamond disc. The abraded surfaces were polished using pumice polishing paste. Enamel samples of thickness 100–150 μm were prepared.[1,4,5] During the storage time intervals, the enamel samples were stored in the previously prepared artificial saliva. The artificial saliva was prepared by the mixing of 0.4 –KCl (gm/l), 0.4 –NaCl (gm/l), 0.906 –CaCl2·2H2O (gm/l), 0.690 –NaH2PO4·2H2O (gm/l), 0.005 –Na2S·9H2O (gm/l), and 1–urea (gm/l) and adjusted at pH nearly equal 6.5.[5]

Carious lesion formation (demineralizing regimen):

The demineralizing solution was prepared at the Faculty of Science, Al-Azhar University. The solution containing 2.2 mM calcium chloride ($CaCl._2H_2O$), 2.2 mM sodium phosphate, and 0.05 M acetic acid was prepared, and the pH was adjusted with 1 M potassium hydroxide to 4.4. The previously prepared enamel samples of each group were placed in 150 ml of a demineralization solution for 48 hours for the formation of incipient chalky white caries-like lesions.[1,4,15]

Remineralization regimen:

For the control group; a 2% neutral sodium fluoride solution was prepared by dissolving 20 grams of sodium fluoride powder in 1litter of distilled water. However, for Nano-HA and Nano-BAG groups; a 10% Nano-HA and Nano-BAG solution was prepared by dissolving 100 grams of Nano-HA and Nano-BAG powders in 1-litter of distilled water. The demineralized samples of each group were immersed in their assigned solution for 3 hours and then immersed in artificial saliva at 37°C in an incubator. This process was repeated for 21 days. All the solutions were changed every 24 hours.[1,16]

Testing procedures:

A. Evaluation of surface morphology:

A scanning electron microscope with a built-up EDX unit (ZEISS, EVO 15, UK) at the Egyptian Atomic Energy Authority (EAEA) was used for analyzing the surface morphology of the samples at the baseline, after demineralization (enamel lesion), and after remineralization at different magnifications. Before the scanning of the samples with SEM, the samples were gold sputtered to be conductive.[1] The gold coated was removed after each scanning session with a solution of potassium iodide of a concentration of (0.2% iodine in 1% potassium iodide) prepared at the Faculty of Science, Al-Azhar University. This is because the X-ray emission spectra revealed that exposing a coated surface to a stirred solution of 0.2% molecular iodine in 1% potassium iodide totally removed the gold in 30 seconds.[17]

B. Evaluation of mineral density (Ca/P ratio):

Energy dispersive X-ray was used to measure the calcium and phosphate content in the enamel samples. The readings were then converted to the Ca/P ratio (wt.%). Ca/P ratio was assessed at baseline, post-demineralization, and after remineralization.[1,5]

C. Evaluation of surface microhardness:

The surface microhardness of the enamel samples at the baseline, post-demineralization, and after the remineralization regimen of the teeth was measured using a Vickers microhardness tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) by applying a 25 grams load for 5 seconds using its square-based pyramid "diamond" shape indenter. Micro-hardness was obtained using the following equation: [18,19]

$$HV=1.854\ P/d^2$$

Where; HV is Vickers hardness in Kgf/mm^2, P is the load in Kgf and d is the length of the diagonals in mm.

Statistical analysis

The normality of the distribution was examined using the Kolmogorov-Smirnov test. A mean and standard deviation were used to describe the numerical data. To compare more than two groups using normally distributed quantitative variables, the F-test (One-way ANOVA) was performed, followed by Post-hoc comparisons using Bonferroni corrections. A P-value less than 0.05 was chosen as the significant level.

RESULTS

A. SEM Analysis of surface morphology results:

The SEM photomicrographs of the untreated enamel surface of the primary molars demonstrated the generalized smooth surface architecture. The SEM photomicrographs of the demineralized enamel surface of the primary molars demonstrated generalized surface irregularities with a honeycomb appearance were noted after the demineralization process. The SEM photomicrographs of the enamel surface of the primary molars that were remineralized with 10% Nan-HAp demonstrated that the enamel surface becomes relatively smoother at low magnification due to the precipitation of the HAp minerals on the surface. While at the higher magnification the precipitated minerals appeared as a dense apatite layer that bonded to the demineralized enamel surface. Furthermore, the generalized surface irregularities that were noted after the demineralization process completely disappeared. **(Figure 1)**

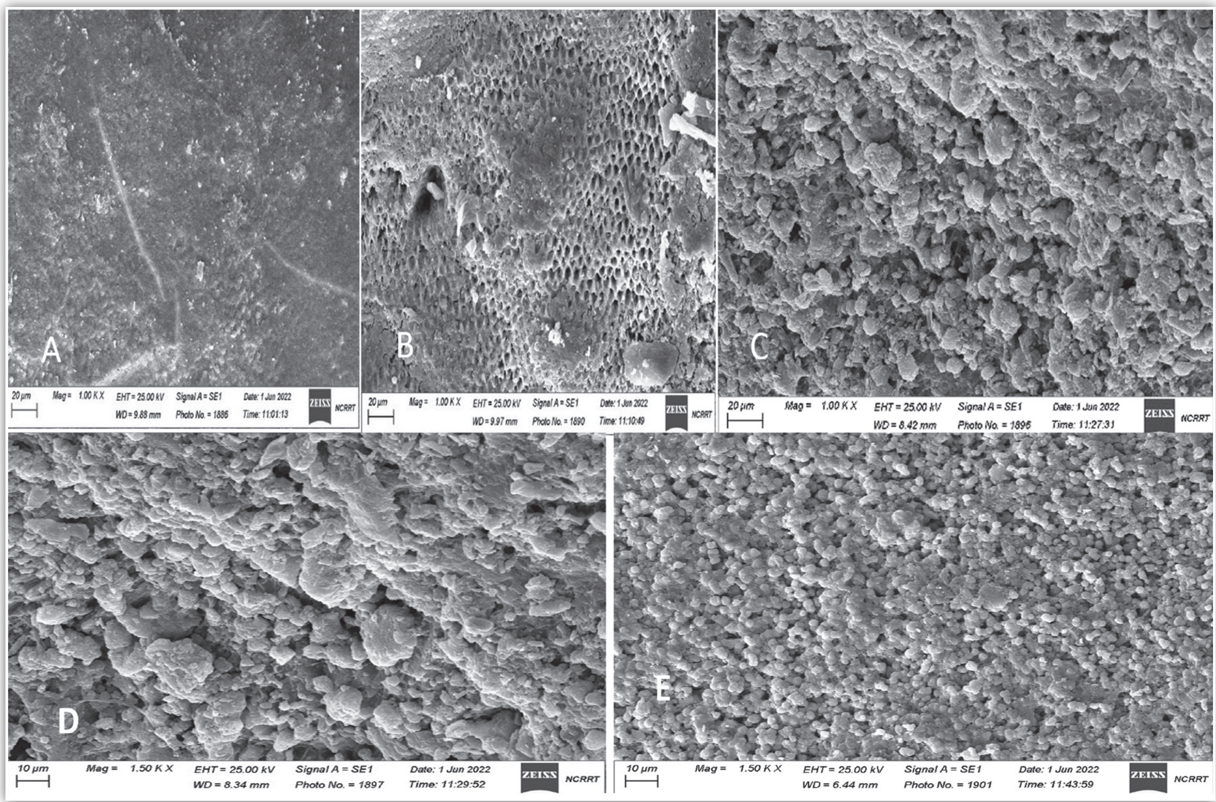

FIG (1) SEM photomicrographs of the primary enamel surface (A); at the baseline; (B); demineralized; (C); remineralized with 10% Nano-HAp; (D); remineralized with 10% Nano-BAG; and (E); remineralized with 2% NaF.

B. Remineralization efficiency (Ca/P ratio) results:

The EDX analysis for Ca/P ratio results revealed that the ratio of Ca/P of all tested groups at baseline and after demineralization was statistically non-significant (P>0.05). However, the Nano-HAp treated group showed a higher Ca/P ratio than the other two groups but the statistics revealed a significant increase in the Ca/P ratio (remineralization potential) for the enamel samples treated with Nano-HAp and Nano-BAG in comparison with the NaF-treated group, however, no significant difference between Nano-HAp and Nano-BAG treated groups. Also, statistically significant differences between the baseline, and after remineralization values in comparison with post-demineralization, were seen in the intragroup comparison of Ca/P ratios. However, no significant difference between baseline, and post-remineralization. (Table 1).

C. Micro-hardness test results:

The statistical analysis of the microhardness results among all tested groups showed a statistically significant difference (P<0.05) with a significant improvement in the surface hardness of the mineralized enamel samples in comparison with demineralized samples for all treated groups. The results showed that there was a statistically significant difference (P<0.05) between the enamel specimens at the baseline and after demineralization and NaF treated groups. While there was no statistically significant difference (P>0.05) between the enamel specimens at the baseline and those treated with 10% Nano-HAp and 10% Nano-BAG. (Table 2).

TABLE (1) Ca/P ratio among all tested groups:

	10% Nano-HAp	10% Nano-BAG	2% NaF	P-value
Baseline	1.398 ± 0.025^{Aa}	1.393 ± 0.018^{Aa}	1.392 ± 0.021^{Aa}	0.8072
After demineralization	1.276 ± 0.016^{Ab}	1.266 ± 0.009^{Ab}	1.272 ± 0.011^{Ab}	0.2325
After remineralization	1.385 ± 0.017^{Aa}	1.381 ± 0.012^{Aa}	1.350 ± 0.019^{Ba}	0.0001*
P-value	<0.0001*	<0.0001*	<0.0001*	

**; significant (p < 0.05) ns; non-significant (p>0.05)*
The different uppercase letters in the same raw indicate statistically significant differences (P< 0.05).
The different lowercase letters in the same column indicate statistically significant differences (P< 0.05).

TABLE (2) Comparison of the microhardness test results among all tested groups:

Variables	Mean	SD	f-ratio	P-value
Baseline	176.4^{A}	8.34		
Demineralized	64.6^{B}	10.18		
10% Nano-HAp	170.8^{AC}	4.70	1483.94	<0.00001*
10% Nano-BAG	172.7^{AC}	4.94		
2% NaF	165^{C}	8.27		

**; significant (P< 0.05) ns; non-significant (P>0.05); The different uppercase letters indicate statistically significant differences (P< 0.05).*

DISCUSSION

In this present study, the null hypothesis was partially rejected as the Nano-BAG and Nano-HAp treated enamel samples showed a similar microhardness result to the NaF, however, the NaF showed a significantly lower remineralization potential than the Nano-BAG and Nano-HAp.

The SEM was used in this present study to examine the surface morphology of the samples to confirm the ability of the nano-particles to bind to demineralization-induced pores in a manner similar to the biological enamel. As it was found that the HAp-NPs can cluster into micro clusters on the enamel surface and multiply after attaching to the surface to generate a homogeneous apatite layer that completely encases interprismatic and prismatic enamel. [5] This was proved during the SEM examinations in this present study.

The concentration of 10% Nano-HAp and Nano-BAG was chosen for the current study because they had been shown to have the potential to remineralize early enamel caries in earlier research under in vitro circumstances. [4,5] Moreover, NaF solution with a 2% concentration was chosen for the current study in order to simulate a higher concentration that might be present in the product that is currently on the market, as it was found that an increase in fluoride concentration increased its remineralization effect. [5,20]

Sodium fluoride is a typical fluoridated product that is commonly used in pediatric dentistry to remineralize the initial caries lesions.[21] Therefore, sodium fluoride was selected as a positive control group for testing the efficacy of the other remineralizing materials in the present in vitro study. One of the shortcomings of fluoride in remineralization, especially the high fluoride concentration products, is surface-zone remineralization at the expense of the lesion body, resulting in lesion arrest and not full remineralization of the lesion.[22] Therefore, the present study aimed to evaluate and compare the remineralizing potential of Nano-HAp and Nano-BAG on early carious lesions by considering sodium fluoride as a positive control group.

The results of the current study revealed that; following the demineralization process there is a fall in the surface Ca/P ratio of the demineralized enamel in all tested groups in comparison with the baseline when examined with EDX, in addition to the creation of porous surfaces when scanned with the SEM. This agrees with the results of the previous studies due to the loss of minerals from the surface resulting from the action of acid. [23] Moreover, after the application of the remineralizing agent, the Nano-HAp and Nano-BAG groups had a significantly higher mineral content of Ca and P than baseline. This affirms that the biomimetic agents could remineralize initial enamel lesion and enhance its mineral content leading to harder dental tissue. [23]

The results of this study revealed that after the remineralization there was a statistically significant difference between the remineralization efficiency of Nano-HA and Nano-BAG when compared with the NaF on artificially induced initial carious lesions. The higher remineralization efficiency was recorded in this study with Nano-BAG followed by Nano-HAp. This finding suggests that in the NaF treated group, the only structural modification of apatite occurs and is restricted to a partial hydroxyl group replacement by fluoride ions without influencing the calcium and phosphate content. [5] This is because the reaction of calcium with fluoride renders both Ca and F to be inactive and prevents the Ca-P reaction with F and the formation of calcium fluoride. [23] Furthermore, the use of high F concentration in the present study helps to increase the supersaturation state of Ca-F and prevent the normal remineralization process of enamel, especially in the presence of saliva. [23]

However, in the Nano-HAp and Nano-BAG groups, the treated samples exhibited a surface Ca/P ratio close to that of the biological enamel, and the synthetic nanohydroxyapatite used indicated an appetite coating deposition on the demineralized enamel surface. This agreed with the results of the previous studies which concluded that the use of Nano-HAp and Nano-BAG as remineralization systems is more effective than NaF.[5,24] This is because the Nano-particles have a greater solubility, greater surface energy, and higher biocompatibility.[24]

Hua et al, 2013[25] proved that dehydration increases the surface microhardness of swine enamel. Therefore, in the current in vitro study to mimic the intraoral environment and to prevent dehydration of the samples which can affect the microhardness of the enamel, the tooth samples were stored in artificial saliva.

In this study, the Vickers surface microhardness test had been selected to evaluate the remineralization potential of the test materials owing to the importance of the surface layer in caries progression also microhardness measurement is appropriate for a material having a fine microstructure, inhomogeneous structure, or prone to cracking such as enamel. [15] The surface microhardness test indentations provide a relatively simple, nondestructive, and rapid method in demineralization and remineralization studies. [26] In the present study, the Vickers surface microhardness test was chosen over the Knoop microhardness test because the shape of indent obtained in Vickers surface microhardness was easy and accurate to measure. [27] Thus, Vickers hardness number values are indirect measurements of remineralization. [15]

The results of the surface microhardness test in this study revealed a significant decrease in surface microhardness values of the demineralized enamel samples "after demineralization" in comparison with the baseline. This is because the effect of the demineralizing agent resulted in the softening of the enamel surface due to the removal of the mineral content. [20]

The significant increase in surface microhardness in the Nano-HAp and Nano-BAG groups establishes that this new material rehardens the softened enamel by gradual deposition of the mineral that precipitates and nucleates in the dark zone of demineralization thereby offering complete biomimetic regeneration of the lost enamel crystallites. [20] The efficacy of the Nano-HAp and Nano-BAG can be attributed to their particle size, which is fairly small, can enter into the enamel surface continuously and fill the vacant position of the enamel crystal. [15]

The present study also pictures that the increase in surface microhardness of the enamel samples treated with Nano-HAp and Nano BAG reaches to be close enough to the biological enamel surface hardness which might be because of the increased contact time with the test material and also the blockade of the surface layer. [5,28]

With regard to the baseline measurement of the microhardness, the results of the NaF surface microhardness treated group revealed significantly lower surface microhardness values, however, the Nano-HAp and Nano-BAG showed insignificant lower values. This could be attributed to the higher remineralization by hydroxyapatite crystals in the Nano-HAp and Nano-BAG groups with an increase in Ca/P ratio, however, the Ca/P ratio of the samples treated with fluoride did not resemble the Ca/P ratio of biologic enamel. [5]

However, the remineralization results of the three tested groups (Nano-HAp, NaF, and Nano-BAG) in this present study showed a non-statistically significant difference this could be attributed to the ability of the precipitated minerals (calcium fluoride, and hydroxyapatite) to occlude the pores and fill the vacant position of the enamel crystal. [5,15]

CONCLUSION

The use of a 10% Nanohydroxyapatite and a 10% Nano-bioactive glass has the potential to remineralize initial enamel caries and display positive constructive surface modifications as well as it able to improve the microhardness of the demineralized enamel under in vitro conditions.

REFERENCES

1. Geeta RD, Vallabhaneni S, Fatima K. Comparative evaluation of remineralization potential of nanohydroxyapatite crystals, bioactive glass, casein phosphopeptide-amorphous calcium phosphate, and fluoride on initial enamel lesion (scanning electron microscope analysis) - An in vitro study. J Conserv Dent. 2020; 23:275-79.
2. Körner P, Schleich JA, Wiedemeier DB, Attin T, Wegehaupt FJ. Effects of additional use of bioactive glasses or a hydroxyapatite toothpaste on remineralization of artificial lesions in vitro. Caries Res. 2020; 54:336–42.
3. 3. Rajan R, Krishnan R, Bhaskaran B, Kumar SV. A Polarized light microscopic study to comparatively evaluate four remineralizing agents on enamel viz CPP- ACPF, Reminpro, Shy-nm and Colgate strong teeth. Int J Clin Pediatr Dent. 2015; 8:42-47.
4. Khandelwal JR, Bargale S, Dave BH, Poonacha KS, Kariya PB, Vaidya S. Comparative evaluation of remineralizing efficacy of bioactive glass agent and nano-hydroxyapatite dentifrices on artificial carious lesion in primary teeth: An in vitro study. Adv Hum Biol. 2020; 10:129-33.
5. Swarup JS, Rao A. Enamel surface remineralization: Using synthetic nanohydroxyapatite. Contemp Clin Dent. 2012; 3:433-36.
6. Nithin M, Joseph J, Nagappan N, Kumar SG, Parvathy P. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative in vitro study. Dent Res J. 2019;16: 310-17.
7. Goldberg M. Fluorides in dental tissues: caries prevention and fluorosis. JSM Dent. 2020; 8: 1123-31.
8. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: A review of literature. Int J Clin Pediatr Dent. 2019; 12:139-44.
9. Juntavee A, Juntavee N, Hirunmoon P. Remineralization potential of nanohydroxyapatite toothpaste compared with tricalcium phosphate and fluoride toothpaste on artificial carious lesions. Int J Dent, 2021:5588832.
10. Kim H-J, Bae HE, Lee J-E, Park I-S, Kim H-G, Kwon J, et al. Effects of bioactive glass incorporation into glass ionomer cement on demineralized dentin. Scient Rep. 2021; 11:7016-22.
11. Philip N. State of the art enamel remineralization systems: the next frontier in caries management. Caries Res. 2019; 53:284-95.
12. Hafsa S, Mull GP, Krishnaswamy MM. Evaluation of the remineralization potential of bioactive glass, nanohydroxyapatite and casein phosphopeptide- amorphous calcium phosphate fluoride-based toothpastes on enamel erosion lesion –An Ex Vivo study. Indian J Dent Res. 2020; 31:670-77.
13. Karumuri S, Mandava J, Pamidimukkala S, Uppalapati LV, Konagala RK, Dasari L. Efficacy of hydroxyapatite and silica nanoparticles on erosive lesions remineralization. J Conserv Dent. 2020; 23:265-69.
14. Carrouel F, Viennot S, Ottolenghi L, Gaillard C, Bourgeois D. Nanoparticles as anti-microbial, anti-inflammatory, and remineralizing agents in oral care cosmetics: a review of the current situation. Nanomater. 2020; 10:140-72.
15. Joshi C, Gohil U, Parekh V, Joshi S. Comparative evaluation of the remineralizing potential of commercially available agents on artificially demineralized human enamel: An in vitro study. Contemp Clin Dent. 2019; 10:605-13.
16. Kind L, Stevanovic S, Wuttig S, Wimberger S, Hofer J, Müller B, Pieles U. Biomimetic remineralization of carious lesions by self-assembling peptide. J Dent Res. 2017; 96:790-97.
17. Charig A, Winston A, Magee S, McNeil D. Removal of sputtered gold from specimens after scanning electron microscope examination. Anal Biochem. 2004; 329:154-56.
18. Prabhakar AR, Paul MJ, Basappa N. Comparative evaluation of the remineralizing effects and surface microhardness of glass ionomer cements containing bioactive glass (S53P4): An in vitro Study. Int J Clin Pediat Dent. 2010; 3:69-77.
19. Ghanbarzadeh M, Ahrari F, Akbari M, Hamzei H. Microhardness of demineralized enamel following home bleaching and laser-assisted in-office bleaching. J Clin Exp Dent. 2015;7: 405-9.
20. Haghgoo R, Rezvani MB, Zeinabadi MS. Comparison of nano-hydroxyapatite and sodium fluoride mouth rinse for remineralization of incipient carious lesions. J Dent Tehran Univ Medic Sci. 2014; 11:406-11.
21. Amaechi B T. Remineralization Therapies for Initial Caries Lesions. Curr Oral Health Rep 2015;2: 95–101.
22. Tang S, Dong Z, Ke X. Advances in biomineralization-inspired materials for hard tissue repair. Int J Oral Sci 2021;13: 42-9.
23. Saudi R, Ibrahim MA. Comparison between nano-hydroxyapatite and CPP-ACPF in remineralizing early carious lesions (in vitro study). J Creative Sust Develop. 2020; 2: 1-5.

24. Elasser DM, Niazy MA, Elsharkawy DA, Mansour MS. The remineralizing potential of nano bioactive glass versus nanohydroxyapatite on dentine as affected by PH cycling. ADJ-for Girls. 2018; 5: 327: 34.

25. Hua LC, Zheng J, Zhou ZR. Effect of water content on the hardness and friction coefficient of swine teeth enamel. Tribology 2013; 33:262-6.

26. Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: An in vitro comparative evaluation. J Conserv Dent 2010; 13:42-6.

27. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in-vitro pH-cycling study. J Conserv Dent 2014; 17:3-7.

28. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed Mater. 2009; 4:1–6.