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IN-VITRO ASSESSMENT OF THE EFFECT OF DIFFERENT REMINERALIZING AGENTS ON CARIES-LIKE LESIONS IN PRIMARY TEETH

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ABSTRACT

Objective: The objective of this study is to evaluate and compare the effect of different remineralizing agents on early enamel lesions in primary teeth using Vickers microhardness testing.

Methodology: This in-vitro experimental study included fifty primary anterior teeth. Early enamel lesions were created by placing the samples in demineralizing solution for 4 days, then the specimens were randomly divided into 5 groups according to variable testing agent (control, sodium fluoride, CCP-ACP, CCP-ACP/F, nHAp) which were applied throughout a pH-cycling model. Surface microhardness was measured at three time points, at baseline, following demineralization and finally after pH-cycling (SMH-B, SMH-D and SMH-R), then percent microhardness recovery was calculated (%HR).

Results: Data was analyzed using Kruskal-Wallis test for multiple-groups followed by Mann Whitney U test for two-group comparisons. There was no statistically significant difference between the groups at baseline and demineralization measurements. However, a statistically significant difference was detected between the groups after pH-cycling, such that the control group recorded the least mean hardness number, followed by the CCP-ACP group. At the same time, there was no statistically significant difference between the groups of NaF, CPP-ACP/F and nHAp groups. Similarly, %HR statistical relation followed the same pattern as the SMH-R.

Conclusions: Based on the results of this study, it can be concluded that nHAp toothpate, CCP-ACP/F and (1000 ppm) fluoridated toothpaste are more effective than CCP-ACP in early enamel lesions remineralization.

KEYWORDS: toothpastes, enamel remineralization, nano-hydroxyapatite, CCP-ACP, primary enamel lesions

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INTRODUCTION

For decades, fluoride had been considered as a key component for caries prevention and remineralization of initial non-cavitated lesions as it inhibits mineral loss from the enamel surface by promoting surface adsorption of calcium and phosphate ions from oral environment and the deposition of the large fluorhydroxyapatite crystals into the demineralized areas to form a firm surface layer that is more acids resistant. This surface layer, however, is impervious to calcium and phosphate ions penetration thus the subsurface zone cannot be efficiently remineralized, as a consequence the opaque whitish appearance of the incipient lesioms will not be completely eliminated [1,2].

In addition, safety concerns had risen regarding the unsupervised use of fluoride containing toothpastes for long durations in children below 6 years, due to posing a risk of dental fluorosis especially when children are exposed to fluoride from multiple sources [3,4].

Nowadays, a variety of fluoride free toothpastes have recently been made available in the market with variable active ingredients that are promoted as safer alternatives. This occurred in confluence to the increased interest in the biomimetic strategies which help remineralize early carious lesions through increasing the bioavailability of free calcium and phosphate ions in the oral environment thus permitting noninvasive healing of these lesions [5] and amid advocacies that these products are more capable of further mineralization over products containing only fluoride since it was proposed that fluoride only minimizes further dissolution of apatite rather than enhancing mineralization to compensate for the mineral loss in the apatite crystal [6,7].

One of these products is casein phosphopeptide amorphous calcium phosphate (CPP-ACP) which is bioactive material derived from the milk protein (Casein) which can act as a reservoir of bio-available calcium and phosphate ions and can maintain a supersaturated state in the ambient fluid. It has extensively been investigated and recognized for its remineralizing potential and efficiency in reverting early enamel lesions according to numerous in-vitro and in-vivo studies [8-11].

Furthermore, the introduction of hydroxyapatite (HAp) particles for enamel remineralization, was based on the capacity of the material at micro and nano scales (nHAp) to penetrate deeper and repair enamel lesions while conforming to the biomimetic principal of tissue healing [12, 13]. Since it was cleared as an anti-caries agent in Japan back in 1993, it has been subjected to testing, which showed evidence of caries prevention and remineralization as it acts as a depot for calcium and phosphate ions thus maintaining a state of supersaturation which results in crystallite deposition and growth [14].

Owing to the availability of various topical formulations that are claimed to repair the demineralized enamel, the current study was conducted to compare the remineralization potential of various remineralizing agents on demineralized primary enamel specimens.

MATERIALS AND METHODS

Sample size estimation

The sample size was calculated using SPSS software version 20 based on the study by Lata et al. [1] to include N=10 specimens in each study group, assuming a confidence interval of 95%, Using an alpha (α) level of (5%) and Beta (β) level of 0.20 i.e., power = 80%.

Specimen preparation

A total of 50 freshly extracted primary anterior teeth were included in the study sample, such that the coronal portion is sound and free from any caries, discoloration, developmental defects, restorations, or cracking. Once extracted, the teeth were rinsed under running water and thoroughly cleaned with non-fluoridated pumice to remove any debris from their surfaces [15].

Specimens were embedded in acrylic resin with their labial surface facing upwards after the teeth were sectioned at the cemento-enamel junction with a water-cooled diamond disk to help discard the root portion [16]. The specimens were coated with an acid-resistant varnish excluding a 3mm × 3mm window in the middle 1/3 of the crown that was covered with an adhesive tape. A flat and smooth surface was obtained by polishing the area not covered with the varnish (after removing the tape) using sandpapers of grits 1000, 1500, 2000, and 2500 consecutively. After that they were stored in deionized water at room temperature until used within one month [17].

Demineralization stage

The demineralizing solution was prepared with the following constituents (2.2 mM CaCl2, 2.2 mM NaH_2PO_4, and 50 mM acetic acid with a pH adjusted to 4.4 with KOH pellets). The specimens were immersed in the demineralizing solution for 4 days in an incubator adjusted to 37 °C to produce 60–100 µm depth of enamel demineralization lesions following the regimen adopted by Rirattanapong et al. [18]. Later, the specimens were rinsed with deionized water to stop further demineralization and then blotted dry with tissue paper.

Remineralizing solution (Artificial Saliva)

Remineralizing solution was prepared by combining (500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to adjust the pH to 6.8 in order to mimic artificial saliva), and it was renewed daily throughout the period of the study [1]. Both the demineralizing solution and the artificial saliva were prepared at the laboratories of the Faculty of Pharmacy, Ain-Shams university.

Later, the specimens were randomly allocated into five groups (I-V) according to the surface treatment they received:

Group I: (Control group) No treatment {specimens were left in artificial saliva}

Group II: Fluoride toothpaste Colgate® Kids (1000 ppm NaF), (Colgate Kids; Palmolive Co., New York, USA)

Group III: Casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) (GC Tooth Mousse®, GC International Tokyo, Japan)

Group IV: Casein phosphopeptide-amorphous calcium phosphate fluoride paste (CPP-ACP/F) (GC MI Paste Plus, GC America Inc., Alsip, Ill., USA)

Group V: Nanohydroxyapatite (nHAP) containing toothpaste (Apagard®; Sangi Co., Ltd., Tokyo, Japan)

Specimens of groups (II, III, IV and V) were brushed with a cotton applicator smeared with the corresponding toothpaste for 1 minute two times a day, then rinsed with deionized water and immersed back into the artificial saliva. While group (I) specimens were followed by pH cycling model [19].

pH-cycling model

The pH-cycling model was intended to simulate the pH fluctuations that take place in the oral environment, interfered with toothpaste aided tooth brushing twice daily for 7 days. This pH cycling model corresponds to the acid challenge that take place 3 times a day at mealtimes. Each specimen was immersed in 10 mL of the demineralizing solution for 3 hours and in 10 mL artificial saliva for the rest 21 hours every day while being incubated at 37°C, the solutions were renewed every day and deionized water was applied before each step [1,20].

Micro-hardness (SMH) Testing

Surface micro-hardness testing for the enamel specimens was calculated using a digital microhardness tester (NEXUS 4000™)[1] with a Vickers elongated diamond pyramid indenter using a load of 200 g that was applied to the surface for 15 Sec. Each specimen received three indentations, spaced by 100 µm and an average hardness value was obtained.

The test was performed at three stages: baseline (SMH-B), after demineralization (SMH-D), and after remineralization (at the end of pH cycling) (SMH-R) and percentage of SMH recovery (%HR) was calculated according to the following equation: (%HR) = (SMH-R– SMH-D) / (SMH-B – SMH-D) X 100 [21].

Statistical analysis:

The obtained data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation and ranges.

The following tests were done:

Kruskal-Wallis test: for multiple-group comparisons in non-parametric data & Mann Whitney U test: for two-group comparisons in non-parametric data.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the Probability (P-value) was considered as follows:

P-value <0.05 was considered significant.

P-value <0.001 was considered as highly significant.

P-value >0.05 was considered insignificant.

Vickers Microhardness Test results

The descriptive results of surface microhardness testing in terms of minimum, maximum, mean, standard deviation, median and the IQR of microhardness of baseline (intact) enamel, demineralized enamel, and remineralized enamel specimens of the study groups are represented in (table 1) .

Kruskal–Wallis test was performed and Multiple comparisons between groups through Mann-Whitney test showed that, there was no statistically significant difference between the groups at baseline and demineralization measurements. However, a statistically significant difference was detected between the groups at the remineralization measurement, such that the control group recorded the least mean hardness number, followed by the CCP-ACP group. On the other hand, there was no statistically significant difference between the groups of NaF, CPP-ACP/F and nHAp groups, yet the three groups scored statistically significant higher means compared to the control and CPP-ACP group as described in (Table 2, Figure 1).

Comparing the percentage of hardness recovery after pH cycling between the study groups demonstrated varying hardness recovery in all the test groups except for the control group. The highest recovery was recorded in the CPP-ACP/F group followed by the NaF and then the nHAp group with no statistically significant difference between the three groups. The least recovery was recorded in the CPP-ACP group which showed a statistically significant lower mean value compared to the previously mentioned groups, as shown in (Table 3 and Figure 2).

TABLE (1): Descriptive results of Vickers microhardness in all the study groups at baseline, demineralization and remineralization measurements

Vicker's Microhardness Test		Min.	Max.	Mean	±SD	95% C.I. for Mean		Median	Percentile (IQR)	
						Lower	Upper		25th	75th
Baseline Measurement	Control Group	294.69	346.82	320.74	20.03	306.41	335.07	318.97	302.69	344.72
	NaF Group	289.60	344.15	317.26	17.21	304.95	329.57	317.52	303.07	329.10
	CPP-ACP Group	289.86	350.74	326.27	21.61	310.81	341.73	328.92	304.66	347.19
	CPP-ACP/F Group	291.90	353.78	313.99	18.56	300.71	327.26	314.40	298.81	322.29
	nHAp Group	269.86	370.64	314.36	36.64	288.15	340.57	298.45	285.62	352.09
Demineralization Measurement	Control Group	129.68	213.54	158.48	27.69	138.67	178.28	147.96	136.30	178.93
	NaF Group	121.96	185.52	155.27	21.86	139.64	170.91	151.28	135.67	180.30
	CPP-ACP Group	102.31	202.01	166.02	37.23	139.39	192.65	183.47	134.83	193.25
	CPP-ACP/F Group	139.31	182.56	161.81	15.44	150.76	172.85	162.39	147.65	179.57
	nHAp Group	145.21	195.54	165.38	17.73	152.69	178.06	161.94	148.87	181.22
Remineralization Measurement	Control Group	109.64	141.06	122.18	11.57	113.90	130.46	118.04	112.71	131.61
	NaF Group	196.54	261.60	226.65	21.52	211.26	242.04	226.60	207.58	243.64
	CPP-ACP Group	152.89	241.01	190.89	25.04	172.98	208.80	192.46	171.65	202.86
	CPP-ACP/F Group	213.57	281.56	238.39	20.50	223.73	253.05	233.96	221.41	252.85
	nHAp Group	185.64	245.50	219.50	21.98	203.78	235.22	223.73	198.40	240.56

TABLE (2): Comparison between groups according to Vickers hardness number at Baseline, Demineralization and Remineralization Measurements.

Vickers Micro-hardness Number	Control Group	NaF Group	CPP-ACP	CPP-ACP/F	nHAp Group	H-test	p-value
SMH-B	320.74±20.03	317.26±17.21	326.27±21.61	313.99±18.56	314.36±36.64	2.598	0.627
SMH-D	158.48±27.69	155.27±21.86	166.02±37.23	161.81±15.44	165.38±17.73	3.202	0.525
SMH-R	122.18±11.57C	226.65±21.52A	190.89±25.04B	238.39±20.50A	219.50±21.98A	33.080	<0.001**

Means that do not share same letter are significantly different at p-value (p<0.05).

p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS

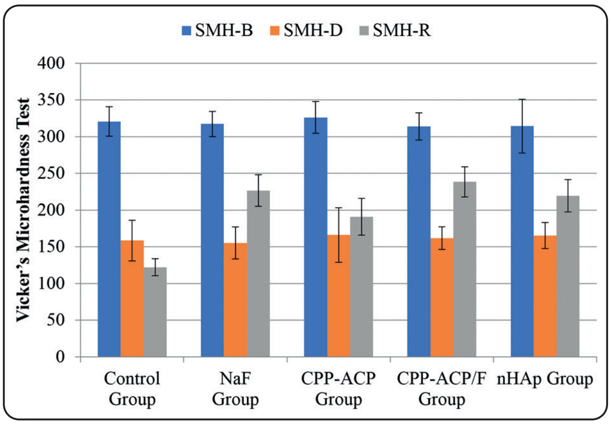

Fig. (1) Comparing the SMH of the study groups at the different testing intervals.

TABLE (3): Comparison of hardness recovery (% HR) among the study groups.

Percentage of hardness recovery (%HR)	Mean±SEM	Range
Control	-26.20±7.83 **C**	-88.64 - -0.33
NaF	42.61±5.26 **A**	10.19 - 68.37
CPP-ACP	11.99±8.66 **B**	-26.76 - 45.73
CPP-ACP/F	50.39±6.06 **A**	30.34 - 90.45
nHAp	39.24±7.75 **A**	-5.65 - 73.48
ANOVA-test	**18.865**	
p-value	**<0.001****	

Means that do not share same letter are significantly different at p-value (p<0.05).

*****p-value <0.001 HS***

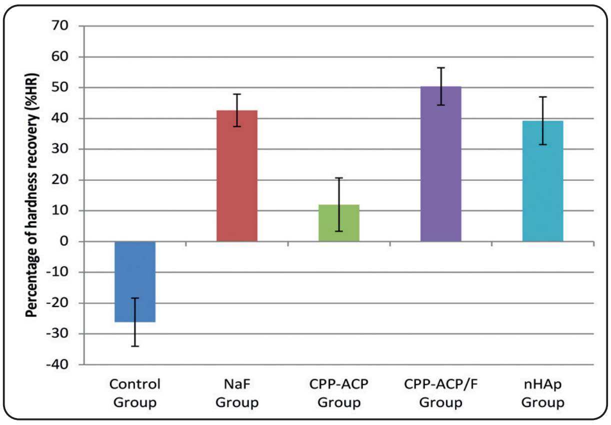

Fig. (2): Comparison between groups respect percentage of hardness recovery (% HR)

DISCUSSION

Remineralization of incipient lesions is one of the most vigorously advancing research arenas over the past decades. To date, the most convenient and acceptable approach for reducing the risk of demineralization and cavitation in primary and permanent teeth, is regular toothbrushing with toothpastes incorporated with active ingredients that stimulate the remineralization process and interfere with caries progression [22].

Since caries development and progression is a dynamic process in which periods of demineralization are alternated with others of repair [1], blocks with incipient enamel lesions utilized in this study were also subjected to pH cycling to mimic the oral environment respect the recurring acidic challenges in an attempt to assess the remineralization capacity of the toothpastes under study.

Fluctuations in the mineral content of enamel is reciprocated with changes in surface microhardness, thus microhardness testing is considered an effective method for appraising the possible effects of topical agents on the enamel surface. In addition, Vickers microhardness testing was used in the current study to assess the remineralization capacity of the study toothpastes, owing to its reliability, economic and non-destructive properties [23].

It has been reported that the average of Vickers microhardness number of sound enamel will fall between 250 and 360 [24, 25]. The baseline hardness number of all the enamel blocks in this study yielded measurements in the same range with no significant difference between any of the tested groups.

Similarly, there was no significant difference, between the groups following the demineralization step at (SMH-D), these findings would rather eliminate any confounding variables that might affect the mineralization outcome of variable toothpaste groups, since all the specimens were handled the same till the pH cycling commenced. Therefore, any changes to the SMH at the last reading would only reflect the variation between the groups that occurred in the final step where remineralization occurred throughout the pH-cycling model.

The results of this study showed that on comparing the test groups following remineralization, all the groups except the control group displayed an increase in the SMH compared to the readings at the demineralization stage. At the same time, it was displayed that with the exception of CPP-ACP group the which scored the least SMH-R mean value, there was no statistically significant difference between any of the NaF, CPP-ACP/F, and nHAp groups regarding their mean SMH numbers. This result also accorded the % hardness recovery values in all the study groups demonstrating that the remineralization potential was comparable in the NaF, CPP-ACP/F, and nHAp groups, and despite evidence of recovery was displayed in CPP-ACP group, yet it was still statistically inferior to the other groups.

Regarding the inferior remineralization potential of CPP-ACP, our results go in accordance with previous studies [2,26,27] which stated that CPP-ACP alone was not considered as *the best clinical practice* in terms of caries prevention and enamel lesion repair, and that CPP-ACP when combined with fluorides could yield the better clinically detectable effects, which also augment the result obtained in this study that refers to better performance of CPP-ACP/F formulation displayed as significantly higher remineralization power compared to the topical agent without fluoride.

Although there is a controversy concerning the remineralization advantage of the combined use of CPP-ACP and fluoride over fluoride therapy alone, a systematic review and meta-analysis published in 2018 [28], specified that fluoride could perform efficiently whether used alone (monotherapy) or in combination with other agents, especially for early enamel lesions affecting smooth surfaces, yet combined therapy would be rather advantageous when early occlusal carious lesions are concerned. This could be a reasonable explanation to the outcome of the current research which refers to the equivalent remineralization capacity of fluoride toothpastes alone and CPP-ACP/F demonstrated as lack of significant difference in the SMH-R or the %HR.

In the current investigation, it has been demonstrated that the remineralizing effect of nHAp toothpaste employed in the current study resulted in comparable remineralization to the 1000 ppm fluoridated toothpaste. Similar results were obtained in a recent study published in 2021 by Juntavee et al. [29], as the authors found no significant difference between the utilized fluoridated and nHAp toothpastes regarding the lesion depth or surface microhardness recovery of artificial enamel lesions created on premolar specimens. Also, when primary enamel specimens were tested by Kasemkhun and Rirattanapong [17], despite that the SMH following remineralization by 1000 ppm fluoride toothpaste (through pH cycling model) produced a higher remineralization mean value compared to that of nHAp, yet no statistically significant difference was detected among the groups regarding the hardness recovery.

Similarly, Chandru et al.[30], showcased the effectiveness of hydroxyapatite containing toothpaste in remineralization of early enamel lesions by achieving considerable SMH recovery following pH cycling, also the results of scanning electron microscope evaluation used in the same study revealed improved surface morphology compared to the demineralized structures. The authors proposed that zinc carbonated nano-hydroxyapatite included in the toothpaste formula they tested, penetrated the enamel pores and acted as foci for further mineral precipitation that sealed off the vacant crystal positions.

Conversely, another group of researchers [31], stated that the nano-hydroxyapatite toothpaste granted superior results compared to 2% fluoride gel used in their study, in terms of increased microhardness following applying the treatments twice daily for 10 days on primary enamel samples. Their results however could be argued by the fact that nHAp toothpaste they chose for their study had also xylitol and fluoride (1450 ppm concentration) in addition to nHAp, thus multiple remineralizing agents come to interplay in the same product which could have imparted a possible synergistic effect and thus the distinct role of nHAp cannot be assured in their investigation.

Although, we consider the results of the current research as crucial in evaluating the remineralizing products available in the market in order to help caregivers and clinicians make informed decisions about deciding on preventive protocol components for young patients, more clinical trials should ensue this attempt to counteract the limitations encountered in this study. These limitations however are mostly related to the absence of factors present in the oral cavity as bacterial plaque and salivary components, which could modify the tooth response to the acidic challenges and remineralization susceptibility.

CONCLUSION

Based on the results of this study, it can be concluded that nHAp toothpaste, CCP-ACP/F and (1000 ppm) fluoridated toothpaste, are more effective than CCP-ACP in early enamel lesions remineralization.

REFERENCES

1. 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.
2. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate.J Dent Res 2008; 87:344-348.
3. Wong MC, Clarkson J, Glenny AM, Lo EC, Marinho VC, Tsang BW, et al. Cochrane reviews on the benefits/risks of fluoride toothpastes. J Dent Res 2011;90:573-9.
4. Zohoori FV, Maguire A. Are there good reasons for fluoride labelling of food and drink?.Br Dent J 2018;224(4):215–217 doi:10.1038/sj.bdj.2018.123
5. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel.J Dent Res 2010; 89:1187-1197.
6. Manoharan V, Kumar RK, Sivanraj AK, Arumugam SB. Compara- tive Evaluation of Remineralization Potential of Casein Phosphopeptide- Amorphous Calcium Fluoride Phosphate and Novamin on Artificially Demineralized Human Enamel: An In vitro Study. Contemp Clin Dent. 2018;9:58-63.
7. Roveri N, Battisella E, Bianchi CL, Foltran I, Foresti E, Iafisco M, et al. Surface enamel remineralization: Biomimetic apatite nanocrystals and fluoride ions different effects. J Nanomater. 2009;2009:1–9.
8. Walker G, Cai F, Shen P, Reynolds C, Ward B, Fone C, Honda S, Koganei M, Oda M, Reynolds E. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. J Dairy Res. 2006;73(1):74–8.
9. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - An in vitro study using scanning electron microscope and DIAGNOdent®. Indian J Dent Res. 2011;22:77-82.
10. Robertson MA, Kau CH, English JD, Lee RP, Powers J, Nguyen JT. MI paste plus to prevent demineralization in orthodontic patients: a prospective randomized controlled trial. Am J Orthod Dentofac Orthop. 2011;140(5): 660–8.
11. Zhou C, Zhang D, Bai Y, Li S. Casein phosphopeptide-amorphous calcium phosphate remineralization of primary teeth early enamel lesions. J Dent. 2014;42(1):21–9.
12. Bordea IR, Candrea S, Alexescu GT, Bran S, Băciuț M, Băciuț G, Lucaciu O, Mihail Dinu C, Todea DA. Nano-hydroxyapatite use in dentistry: a systematic review. Drug Metab Rev. 2020;52:319–32.
13. O'Hagan-Wong K, Enax J, Meyer F, Ganss B. The use of hydroxyapatite toothpaste to prevent dental caries. Odontology. 2021 Nov. DOI: 10.1007/s10266-021-00675-4. PMID: 34807345.
14. Amaechi BT, AbdulAzees PA, Alshareif DO, et al. Comparative efficacy of a hydroxyapatite and a fluoride

toothpaste for prevention and remineralization of dental caries in children. BDJ Open. 2019;5:18. Published 2019 Dec 9. doi:10.1038/s41405-019-0026-8

15. Bossù M, Saccucci M, Salucci A, Di Giorgio G, Bruni E, Uccelletti D, Sarto MS, Familiari G, Relucenti M, Polimeni A. Enamel remineralization and repair results of Biomimetic Hydroxyapatite toothpaste on deciduous teeth: an effective option to fluoride toothpaste. J Nanobiotechnology. 2019 25;17(1):17-22.

16. Singhal RK, Rai B. Remineralization Potential of Three Tooth Pastes on Enamel Caries. Open Access Maced J Med Sci. 2017 Jul 29;5(5):664-666. doi: 10.3889/oamjms.2017.090. PMID: 28932310; PMCID: PMC5591599.

17. Kasemkhun P, Rirattanapong P. The Efficacy of Non-fluoridated Toothpastes on Artificial Enamel Caries in Primary Teeth: An In Vitro Study. J Int Soc Prev Community Dent. 2021;11(4):397-401. Published 2021 Jul 3. doi:10.4103/jispcd.JISPCD_64_21

18. Rirattanapong P, Vongsavan K, Tepvichaisillapakul M. Effect of five different dental products on surface hardness of enamel exposed to chlorinated water in vitro. Southeast Asian J Trop Med Public Health 2011;42:1293-8.

19. Dai Z, Liu M, Ma Y, Cao L, Xu H, Zhang K, et al. Effects of fluoride and calcium phosphate materials on remineralization of mild and severe white spot lesions. BioMed Res Int. 2019;16:1271523. https://doi.org/10.1155/2019/1271523

20. Delbem AC, Bergamaschi M, Sassaki KT, Cunha RF. Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study. J Appl Oral Sci. 2006;14(2):88-92. doi:10.1590/s1678-77572006000200005

21. Gabasso SP, Pinto CF, Cavalli V, Paes-Leme AF, Giannini M. Effect of fluoride-containing bleaching agents on bovine enamel microhardness. Braz J Oral Sci 2011;10:22-6.

22. Horst JA, Tanzer JM, Milgrom PM. Fluorides and Other Preventive Strategies for Tooth Decay. Dent Clin North Am. 2018;62(2):207-234. doi:10.1016/j.cden.2017.11.003

23. Şakar-Deliormanli A, Güden M. Microhardness and fracture toughness of dental materials by indentation method. J Biomed Mater Res B Appl Biomater 2006;76(2):257–264

24. Kamath U, Sheth H, Mullur D, Soubhagya M. The effect of Remin Pro® on bleached enamel hardness: An in-vitro study. Indian J Dent Res 2013;24:690-3.

25. Gangwar A, Jha KK, Thakur J, Nath M. In Vitro evaluation of remineralization potential of novamin on artificially induced carious lesions in primary teeth using scanning electron microscope and vickers hardness. Indian J Dent Res. 2019 Jul-Aug;30(4):590-594. doi: 10.4103/ijdr.IJDR_326_16. PMID: 31745058.

26. Twetman S, Axelsson S, Dahlgren H, Holm AK, Kallestal C, Lagerlof F, et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontol Scand 2003; 61(6):347–355. [PubMed] [Google Scholar]

27. Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. Cochrane SysRev 2013; 12(12):CD003809

28. Tao S, Zhu Y, Yuan H, Tao S, Cheng Y, Li J, He L. Efficacy of fluorides and CPP-ACP vs fluorides monotherapy on early caries lesions: A systematic review and meta-analysis. PLoS One. 2018 Apr 30;13(4):e0196660. doi: 10.1371/journal.pone.0196660. PMID: 29709015; PMCID: PMC5927405.

29. 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 Mar 20; 2021: 5588832. doi: 10.1155/2021/5588832. PMID: 33824661; PMCID: PMC8007336.

30. Chandru TP, Yahiya MB, Peedikayil FC, Dhanesh N, Srikant N, Kottayi S. Comparative evaluation of three different toothpastes on remineralization potential of initial enamel lesions: A scanning electron microscopic study. Indian J Dent Res. 2020 Mar-Apr;31(2):217-223. doi: 10.4103/ijdr.IJDR_745_18. PMID: 32436900.

31. Meshki R, Basir L, Rahbar N, Kazempour M. Comparison of the effect of fluoride gel and two toothpastes with different materials on remineralization of initial carious lesions in primary teeth. J Family Med Prim Care. 2021 Sep; 10(9): 3309-3313. doi: 10.4103/jfmpc.jfmpc_1046_21. Epub 2021 Sep 30. PMID: 34760749; PMCID: PMC8565127.