ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 6, November - 2020, Page No. : 294 - 304

Comparative evaluation of ability of three different dentifrices to remineralise artificial carious lesions in enamel

[1]Dr Anita J Kale,[2]Dr Ruchi S Rathi, [3]Dr Sunanda L Gaddalay, [4]Dr Ramchandra S Kabir, [5]Dr Amol A Badgire, [6]Dr Praveen F Dhore

[1-6]Dept of Conservative dentistry and Endodontics, MIDSR Dental College and Hospital, Latur-413512

Corresponding Author: Dr Ruchi S Rathi, Dept of Conservative dentistry and Endodontics, MIDSR Dental College and Hospital, Latur-413512

Citation of this Article: Dr Anita J Kale,Dr Ruchi S Rathi, Dr Sunanda L Gaddalay, Dr Ramchandra S Kabir, Dr Amol A Badgire, Dr Praveen F Dhore, "Comparative evaluation of ability of three different dentifrices to remineralise artificial carious lesions in enamel" IJDSIR- November - 2020, Vol. – 3, Issue - 6, P. No. 294 – 304.

Type of Publication: Original research Article

Conflicts of Interest: Nil

Abstract

Aim: the aim of this in vitro study was to compare the ability of three different dentifrices to remineralise artificial carious lesions in enamel; using a 14 day pH cycling model through surface microhardness (SMH) analysis.

Material and Method: 40 enamel specimens were prepared by flattening the buccal surfaces of freshly extracted human premolars. The samples were randomly assigned to experimental and control groups and baseline microhardness values were recorded using Vicker's microhardness testing machine. The samples were then immersed in demineralising solution for lesion formation followed by surface microhardness evaluation. This was followed by pH cycling for 14 days and surface microhardness evaluation. The solutions were replenished periodically.

Results: The difference between SHY-NM and ClinPro tooth creme was statistically non-significant. GC tooth mousse plus showed the least remineralising ability and the difference was statistically significant.

Conclusion: The samples treated with SHY-NM showed the highest remineralisation followed by ClinPro tooth crème and GC Tooth mousse Plus.

Keywords: Demineralisation, Dentifrice, Minimally Invasive Dentistry, Ph Cycling, Remineralisation, White Spot Lesions.

Introduction

Shafer identified Dental caries as an infectious disease of microbiologic origin but, recent research has led towards identification of this process as a dynamic one, where a delicate balance exists between remineralisation and demineralisation. [1, 2] Factors like good oral hygiene, balanced diet, saliva buffer, promote remineralization; while increased consumption of dietary sugars, colonization by S.mutans or poor oral hygiene result in the predominance of demineralisation. [3]

The overall incidence of dental caries in primary dentition is 46.6% and its prevalence in adults is 49-84 %. [4, 5] The traditional treatment involves excavation of carious tooth structure and restoration using a suitable material. [3, 6] The initial manifestation of dental caries occurs in the form of an opaque, subsurface demineralisation called a white spot lesion. These lesions are often undetected owing to an intact enamel surface; and if detected do not warrant traditional treatment. With the advent of minimally invasive dentistry, the focus has shifted on to the prevention of demineralisation and reduction of the incidence of such lesions. [3, 7, 8]

Fluoride is known for its anticariogenic potential. Several mechanisms have been proposed like formation of fluorapatite crystals, enhancement of subsurface remineralisation resulting in reduced caries progression, inhibition of bacterial glucose metabolism, etc. [8, 9] Despite its beneficial effects in remineralization; detrimental effects to the tooth surface have been noted if exposed to increased concentrations. [3] However, fluoride is known to have a synergistic effect when used in combination with certain compounds. [10]

Bioactive glass (BAG) was developed by Dr. Len Litkowski and Dr. Gary Hack at the Department of compositions Restorative Dentistry at the University of Maryland and by Dr. David Greenspan at NovaMin® Technologies Inc. It is composed of SiO2, Na2O, CaO, P2O5 and the incorporation of fluorine further increases its bioactivity. The active ingredient is amorphous calcium sodium phosphosilicate. In an aqueous environment, this compound releases bioavailable calcium, sodium and phosphate ions; which raise the pH of the solution, thus contributing to the process of remineralization. SHY-NM (Group Pharmaceuticals, Bengaluru, India) consists of glycerine, PEG 400, silica, calcium, sodium phosphosilicate, sodium lauryal sulphate, titanium dioxide, flavouring agents, carbomer, potassium acesulfame. It is well known for its desensitizing property and its effect in enamel remineralization are promising. When the glass comes in contact with saliva or any physiological fluid; it can induce apatite formation in the form of hydroxyapatite or fluorapatite. If fluoride is incorporated in the composition, BAG can demonstrate 'smart properties' by inducing remineralisation in low pH environments. Consequently, it has been made a part of various dentifrices, prophylactic gels or other materials for the treatment of enamel demineralisation. [11, 12, 13]

CPP-ACP is derived from a milk protein known as casein. In the oral environment, the adhesive property of ACPF component promotes the binding with enamel surface or biofilm. It results in delivery of Calcium and Phosphate ions at the local site while creating a reservoir of bio-available calcium and phosphate ions. The anticariogenic property of CPP-ACPF can be attributed to its potential to enter enamel rods and amend the crystalline apatite structure. [3, 10, 14, 15, 16] CPP-ACPF is manufactured as GC Tooth Mousse Plus (GC, India) and it consists of Casein phosphopeptide-amorphous calcium phosphate along with 900ppm of fluoride. CPP-ACP acts synergistically with fluorine and its remineralising potential has been found to be superior in comparison with CPP-ACP alone. [15]

Tricalcium phosphate helps create a protective barrier around calcium; which gets dissolved on coming in contact with saliva resulting in the release of free Ca, phosphate and fluoride ions. [16] ClinPro tooth crème (3M ESPE, USA) consists of functionalised tri-calcium phosphate or *f*-TCP along with 950 ppm of fluoride. It acts as a smart system in synergy with fluoride ions to form acid resistant crystals similar to hydroxyapatite; preventing initiation and progression of lesions thereby decreasing hypersensitivity. [16, 17, 18]

The current 'minimally invasive' approach is the science of detecting, diagnosing, intercepting and treating dental caries in such a manner that operative intervention may be deferred for as long as possible. Keeping in mind that dental caries is an infectious disease; the 'minimally invasive' approach includes many nonsurgical modalities, the focus being on maximum conservation of demineralized, noncavitated enamel and dentin. [19] Till date, no study has compared the enamel remineralising ability of BAG, CPP-ACPF against *f*-TCP. Therefore, the aim of this in vitro study was to compare the ability of three different dentifrices to remineralise artificial carious lesions in enamel; using a 14 day pH cycling model through surface microhardness analysis.

Material and Methods

This in-vitro prospective study was conducted in the Department of Conservative Dentistry and Endodontics. Human premolars extracted for orthodontic reasons were selected for the study. The teeth were cleaned and stored in 10% formalin solution until use.

Inclusion criteria: Teeth with intact enamel surfaces were used for the study.

Exclusion criteria: Teeth with any visible or detectable caries, stains, restorations, cracks, hypoplastic lesions or white spot lesions were excluded from the study. Teeth having undergone endodontic therapy were also excluded from the study.

Samples preparation: The buccal surfaces of teeth were flattened and polished using abrasive paper. Forty blocks were cut out from these teeth of dimensions 4x4mm using a diamond disc.

Baseline SMH: (B-SMH): B-SMH was tested using Vicker's microhardness testing machine for all specimen. Three indentations were made with VMT at the rate of 100g load for 10 seconds. An average value was obtained to avoid discrepancy.

Preparation of solutions: Analytical grade chemicals and deionized water were used to prepare the solutions.

Demineralisation: Lactic acid containing 0.2 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate adjusted at pH=4.3 was used to mimic the conditions during caries process. The samples were immersed in 40 mL of demineralising solution for 6hrs and were stored at 37ºC temperature in an incubator.

Remineralisation: Artificial saliva contained 1.5 mM of Calcium Chloride, 0.9 mM of Sodium Phosphate and 0.15 M of Potassium Chloride adjusted at a pH=7 to mimic saliva.

Lesion formation: All samples were individually immersed in 40 mL of demineralising solution for 6 hours to produce lesions approximately 75 µm deep with a mineral loss of 10-15%.

Demineralized SMH: (D-SMH): All samples were subjected to SMH evaluation using Vicker's microhardness testing machine. Three indentations were made with VMT at the rate of 100g load for 10 seconds. An average value was obtained to avoid discrepancy.

Groups: Forty samples were randomly divided into four groups of 10 samples each.
Group A: Control – No treatment;
Group B: CPP-ACPF (Tooth Mousse Plus)
Group C: Bioactive glass (SHY-NM)
Group D: *f*-TCP (ClinPro crème)

The pH cycling model: To simulate changes occurring in the oral cavity, the following pH cycling model was adopted. In each 24 hour period, the samples were subjected to:

- 6hrs of demineralisation: All samples were immersed in 40mL of demineralising solution and were then rinsed by deionized water.
- minutes of remineralisation: The samples of Group B, Group C and Group D were treated with the respective

remineralising agents for a period of 3 minutes, twice a day to mimic the daily recommended brushing. They were rinsed with deionized water.

- 17 hours of remineralisation: All the samples were then immersed in artificial saliva to mimic the action of saliva in remineralising carious lesions. The pH cycling was carried out for 14 days. The remineralising and demineralising solutions were replenished every 48 hours.
- **Remineralized SMH: (R-SMH)** All samples were subjected to SMH evaluation using Vicker's microhardness testing machine. Three indentations were made with VMT at the rate of 100g load for 10 seconds. An average value was obtained to avoid discrepancy.

Statistical analysis: Data obtained was subjected to analysis using SPSS software by applying ANOVA, paired t-test and post hoc tukey's test.

Results

Table/fig 1	N	Mean	Std. Deviation	Std. Error	Range	Minimum	Maximum
Baseline	10	275.70	55.08	17.42	140	230	370
Demineralised	10	236.00	6.51	2.06	23	230	253
Tooth mousse Plus	10	257.75	31.52	9.97	82.0	230	312
ClinPro	10	261.00	35.02	11.08	80.5	230	310.5
SHY NM	10	268.10	36.13	11.43	90	230	320

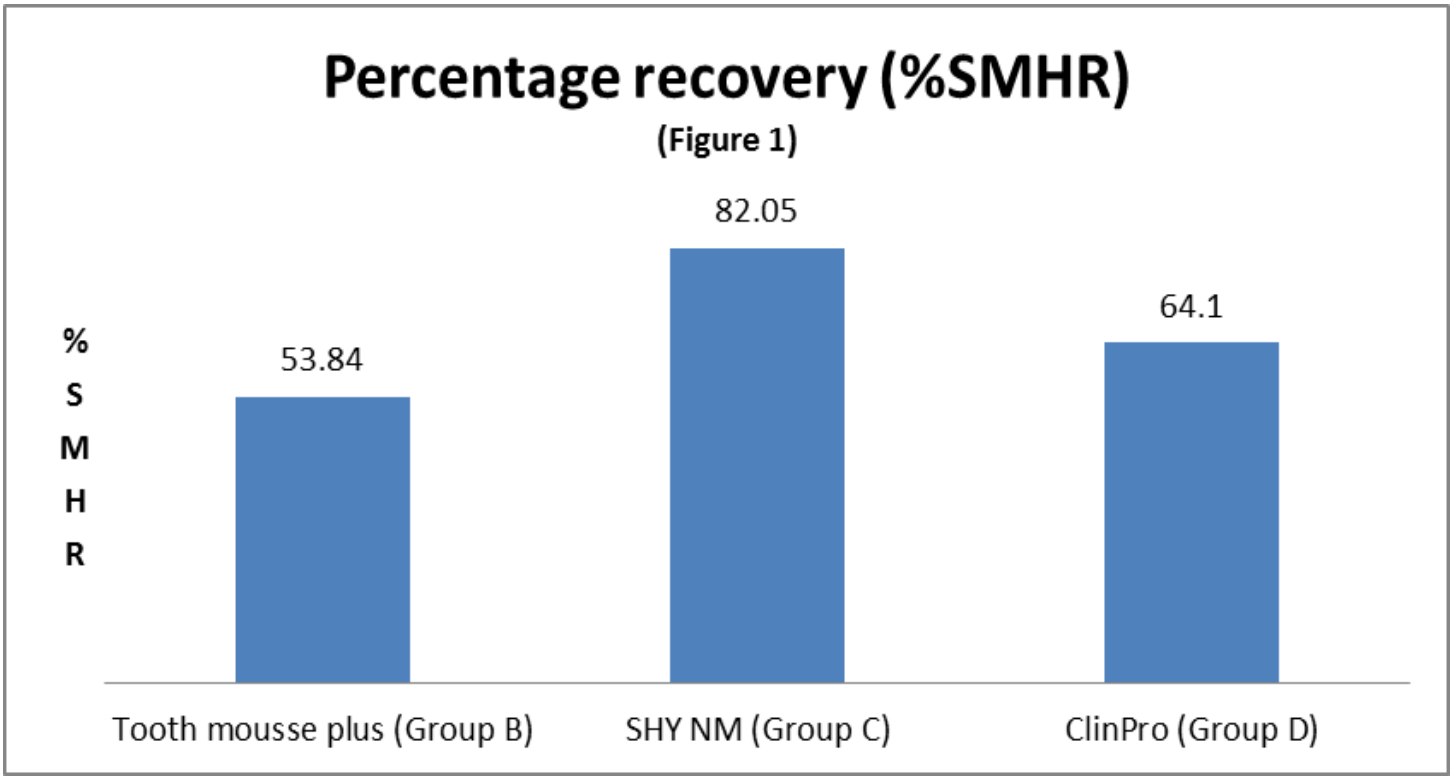
Table 1: Descriptive statistics of variables with their respective mean values and standard deviations

ANOVA Table 2	N	Mean	SD	F	P	Inference
Group B	10	249.30	28.91	9.88	0.045 (>0.05)	Significant
Group C	10	268.10	36.13			
Group D	10	261.00	35.02			

Table 2: On applying one way ANOVA, statistically significant differences were obtained between groups.

Table 3	Group D (Clin Pro)	Group C (SHY NM)
Group B (Toothmoose Plus)	-11.7*	-18.8*
Group D (Clin Pro)		-7.1

Table 3 - Post hoc tukey's HSD test was applied for intragroup comparisons where Statistically significant difference was obtained between Group B and Group C; Group B and Group D; whereas statistically nonsignificant difference was obtained between Group C and Group D.



The percentage recovery observed for all experimental groups is calculated using the following formula {[(R-SMH)-(D-SMH)] X 100}/ [(B-SMH)-(D-SMH)] (Figure 1). The highest amount of percentage SMH recovery was observed for SHY NM (group C= 53.84%)followed by ClinPro tooth crème (Group D = 64.1%) and Tooth mousse plus (Group B = 53.84%) respectively.

Discussion

Miles Markley stated regarding the dentist's role in the treatment of dental caries; that the loss of even a part of a human tooth should be considered 'a serious injury'. In relevance to his words, it may be implied that the goal of dentistry is to preserve healthy and natural tooth structure. [19] In order to achieve clinical success using the 'minimally invasive operative caries management strategy' (MI OCMS); a thorough understanding of the following critical factors, which constitute 'the golden triangle', is indispensible:

1. The histology of the dental substrate being treated
2. The chemistry/handling of the adhesive materials used to restore the cavity
3. Consideration of the practical operative techniques available to excavate caries minimally. [20]

Consequently, MID helps prevent or reverse caries in its early stages by interrupting the pathogenic process prior to cavitation; thereby fulfilling the treatment objective using the least invasive technique. [21]

An in vitro study model simulates a phenomenon of interest thus allowing investigators to derive information about the said phenomenon. Therefore, an in vitro study design was chosen which can mimic the dynamic variations in mineral saturation involved in the caries process. To obtain sufficient quantitative data, a pH cycling model was incorporated in the study design. The pH cycling model was developed by ten Cate and Duijster (1982) which was modified by Featherstone JDB et al in 1986. It is rapid, inexpensive and allows the study to be conducted with a smaller sample size. The pH cycling models allow a high scientific control, thereby reducing

the variability of outcomes in an in vitro study. The pH cycling protocol used in our study is adopted from the one given by Maia (2003)[22], which simulates an in vivo high caries risk condition; while simultaneously measuring the net result of the inhibition of demineralisation and enhancement of remineralisation in the in vivo set up.

In this model, the samples are immersed in an acidic (demineralising) buffer solution followed by supersaturated (remineralising) buffer solution to simulate the dynamic cycles of demineralisation and remineralisation. Dentifrice use as recommended twice a day is simulated by topical application of agents during the pH cycling. The demineralising solution was an acid buffer solution of lactic acid adjusted at pH 4.4 and the remineralising solution consisted of calcium and phosphate ions at a known degree of saturation, adjusted at pH 7.0 to mimic the effects of saliva. The compositions of the solutions were in accordance with the compositions employed by Buzalaf et al.[23]

The primary limitation with pH cycling models is the variability associated with the substrate and test conditions; following which single-section substrates are recommended in order to obtain accurate results. Also, certain mechanistic factors important in predicting prevention of caries are better elucidated in a single-section study. Enamel substrates demonstrate mineral loss and uptake at similar depths owing to low penetrability by acids in the interprismatic space. As a result, thin enamel sections of specified dimensions were obtained from freshly extracted human premolar teeth and utilised in our study as substrate. [9, 23, 24, 25]

The physical changes occurring on the surface layer of the enamel can be attributed to the interactions between enamel and the oral environment. [26] These changes can be measured quantitatively using hardness profiles and mineral content or qualitatively using SEM or Polarised light microscopy (PLM). [23] Of these, SMH testing has been the most popular method for measuring hardness of enamel and dentin. [27] SMH evaluation is simple, fast and a non-invasive method to measure mineral changes. It also reduces the experimental variations by allowing repeated measurements of the specimen over a given period of time. [26] A good correlation (r^2 = 0.94) has been established between measurement of net remineralisation using SMH. [28] In studies with a pre-post experimental design; measuring the SMH aids comparison between the baseline surface and the modified surface for the same indentation load.[29] A slight dissolution of the enamel surface marks the initiation of the caries process; while appearing unaltered owing to continuous regeneration as a result of mineral precipitation. Therefore, the evaluation of changes in this region is relevant and SMH measurement is a suitable technique for the same. [30]

In our study, the samples were tested prior to lesion formation to obtain baseline SMH values. The mean B-SMH value obtained was 339.35. After lesion formation, the samples were subjected to SMH and the mean D-SMH value obtained was 236.00 after 96 hours of demineralisation. (Table 1) The difference between the two values was statistically significant ($p<0.05$). These values are in accordance with the studies conducted by Gutiérrez-Salazar et al, Vieira AE et al, Soares R et al, Rao R et al and Neto FCR et al. [3, 26, 28, 31, 32] According to Koulourides and Reed, enamel surfaces exposed to weak acid (pH 5.5) result in calcium loss accompanied by a decrease in hardness due to changes of mineral density. [33, 34]

Various studies have carried out the pH cycling for varying number of days. [23] According to Featherstone and Glena (1990), their 14 day pH-cycling model was designed to simulate highly susceptible sites in vivo. They concluded that, an exponential quantitative relationship

exists between fluoride concentration and enhancement of remineralization. Therefore, in the present study, the pH cycling was carried out for a period of 14 days. During this time, the experimental agents were applied topically; twice daily for two minutes, to simulate the recommended daily oral prophylaxis. [9, 23] At the end of the pH cycling samples were subjected to SMH analysis (R-SMH) to evaluate the effect of the agents in net remineralisation. Statistically significant results were obtained between the experimental groups. (Table 2, 3)

SHY-NM consists of Bioactive Glass. Bioactive glasses are introduced into dentifrices as very fine particles to provide calcium and phosphorus at the tooth surface. According to Wang et al, toothpastes containing bioactive glass show significant reduction in dentine permeability and resistance to acid challenge which; owing to its remineralising ability, is advantageous for long term treatment of hypersensitivity. [35] In a study, Mehta et al, found that while both bioactive glass and CPP-ACP successfully remineralised early enamel caries; bioactive glass remineralised the carious lesion more effectively. BAG also demonstrated higher values of hardness owing to its ability to attach to the surface more compactly. [36]

Surface reaction of BAG begins immediately in three phases, i.e. leaching and exchange of cations, network dissolution of SiO_2 and precipitation of calcium and phosphate to form an apatite layer. In an aqueous environment i.e. presence of saliva in the oral cavity; sodium ions from the BAG particles rapidly exchange with hydrogen cations, resulting in release of calcium and phosphate ions at the surface. A localized, transient increase in pH occurs during the release of sodium ions which help to precipitate additional calcium and phosphate ions to form a calcium phosphate layer. As these reactions progress, this layer crystallizes into hydroxycarbonate apatite (HCA) which chemically similar to the natural teeth. [11, 35, 36]

In the present study, the highest levels of hardness were demonstrated by SHY-NM containing bioactive glass. This can be attributed to the compact attachment of BAG and precipitation of HCA layer on the surface. The difference between SHY-NM and ClinPro tooth crème was found to be statistically non significant; whereas statistically significant difference was found between SHY-NM and CPP-ACPF. Similar results were obtained in studies conducted by Kamath P et al, Mehta AB et al and Rajan R et al where they observed non-significant difference between SHY-NM and ClinPro tooth crème; whereas statistically significant difference was observed between SHY-NM and CPP-ACPF. [7, 36, 37]

Research has established that the use of ClinPro tooth crème results in increased surface microhardness. It is a hybrid material created by high impact grinding of beta-tricalcium phosphate and sodium lauryl sulfate or fumaric acid causing fusion of the constituents. This blending creates “functionalized” calcium and “free” phosphate ions which increase the efficacy of fluoride remineralisation. The Beta-TCP has a similar structure to apatite and possesses calcium environs that are capable of reacting with fluoride. The free floating phosphate protects the exposed calcium environs, preventing premature interaction between the calcium with fluoride. Therefore, TCP provides high levels of calcium to boost fluoride efficacy. On coming in contact with saliva, the protective barrier breaks down making the ions available. Subsequently, the fluoride and calcium react with the weakened enamel to provide a seed for enhanced mineral growth. Fluoride in combination with β-TCP provides greater recovery of microhardness and increased fluorine uptake; while decreasing the dose of fluoride required for the same degree of remineralisation. [37, 38] The statistically

non-significant difference obtained in our study; between SHY-NM and ClinPro tooth crème, can therefore be attributed to the above mentioned mechanism.

CPP-ACPF is advantageous as it delivers ACP and fluoride compounds to the tooth structure. On coming in contact with saliva, they readily solubilize to calcium, phosphate and fluoride ions; creating a supersaturated state of these ions around the tooth. In CPP-ACPF technology, ACP is stabilized by casein-derived peptides. The amino acid cluster sequence in CPP is –Ser(P)-Ser(P)-Ser(P)-Glu-Glu– which is reported to bind amorphous calcium phosphate; resulting in formation of small clusters of CPP-ACP. This prevents these clusters from reaching the critical size for precipitation, thereby, stabilizing calcium phosphates in close proximity of the tooth surface. These nanocomplexes act as calcium and phosphate reservoirs making the ions available when required. CPP-ACP acts synergistically with fluoride and CPP-ACPF has better remineralising ability. It localizes ACP in dental plaque, thereby helping to maintain a stage of super saturation around the tooth surface decreasing demineralization and enhancing remineralization. [10, 40] In the present study, the remineralising ability of ClinPro tooth crème was higher than CPP-ACPF and the difference was statistically significant. These results were in accordance with the findings of Elkassas D and Arafa A, Patil N et al and Hegde MN et al. [40, 41, 42]

Conclusion

Within the limitations of this study, we may conclude that while all the agents exhibited a statistically significant remineralising ability; SHY-NM containing BAG had the highest remineralising potential and it was closely followed by ClinPro tooth crème containing *f*TCP. Although, the difference between the two was not statistically significant; it was concluded that both were significantly better than GC Tooth Mousse containing CPP-ACPF. It must be noted that the conclusions drawn from an in vitro study are exclusive of factors which vary subjectively. Therefore, clinical extrapolations must be made accordingly.

References

1. John Hicks, Franklin Garcia-Godoy, and Catherine Flaitz. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent: April 2004, Vol. 28, No. 3:203-214.
2. Featherstone JD. Dental caries: a dynamic disease process. Aust dent J. 2008 Sep;53(3):286-91.
3. Soares R, De Ataide ID, Fernandes M, Lambor R. Assessment of enamel remineralisation after treatment with four different remineralising agents: A Scanning Electron Microscopy (SEM) Study. **J Clin Diagn Res**. 2017 Apr;11(4):ZC136.
4. Correa-Faria P, Paixao-Goncalves S, Paiva SM, Pordeus IA. Incidence of dental caries in primary dentition and risk factors: a longitudinal study. Braz oral res. 2016;30(1).
5. Janakiram C, Antony B, Joseph J, Ramanarayanan V. Prevalence of Dental Caries in India among the WHO Index Age Groups: A Meta-Analysis. Journal of Clinical & Diagnostic Research. 2018 Aug 1;12(8).
6. Pitts NB. Are we ready to move from operative to non-operative/preventive treatment of dental caries in clinical practice? Caries res. 2004;38(3):294-304.
7. 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(1):42-47.
8. Arnold WH, Dorow A, Langenhorst S, Gintner Z, Bánóczy J, Gaengler P. Effect of fluoride toothpastes

on enamel demineralization. BMC Oral Health. 2006 Dec;6(1):8.

9. Featherstone JD, Glena R, Shariati M, Shields C. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. J dent res. 1990 Feb;69(spec iss):620-5.
10. Jayarajan J, Janardhanam P, Jayakumar P. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization-An in vitro study using scanning electron microscope and DIAGNOdent®. Indian journal of dental research. 2011 Jan 1;22(1):77.
11. Narayana SS, Deepa VK, Ahamed S, Sathish ES, Meyappan R, Kumar KS. Remineralization efficiency of bioactive glass on artificially induced carious lesion an in-vitro study. J indian soc pedod prev dent. 2014 Jan 1;32(1):19.
12. Bassett DC, Meszaros R, Orzol D, Woy M, Ling Zhang Y, Tiedemann K, Wondraczek L, Komarova S, Barralet JE. 2014. A new class of bioactive glasses: Calcium–magnesium sulfophosphates. J Biomed Mater Res Part A 2014:102A:2842–2848.
13. Taha AA, Patel MP, Hill RG, Fleming PS. The effect of bioactive glasses on enamel remineralization: A systematic review. J dent. 2017 Dec 1;67:9-17.
14. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. J oral sci. 2007;49(2):115-20.
15. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP–ACP and CPP–ACP with 900 ppm fluoride on eroded human enamel: an in situ study. Arch of oral biol. 2010 Jul 1;55(7):541-4.
16. Karlinsey RL, Mackey AC, Schwandt CS, Walker TJ. SEM evaluation of demineralized dentin treated with professional-strength NaF topical pastes. American journal of dentistry. 2011 Dec;24(6):357-62.
17. Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a casein/calcium phosphate-containing tooth cream and fluoride on enamel erosion in vitro. Caries res. 2006;40(2):154-7.
18. Vanichvatana S, Auychai P. Efficacy of two calcium phosphate pastes on the remineralization of artificial caries: a randomized controlled double-blind in situ study. International journal of oral science. 2013 Dec;5(4):224-8.
19. Murdoch-Kinch CA, McLEAN ME. Minimally invasive dentistry. The J Am Dent Assoc. 2003 Jan 1;134(1):87-95
20. Banerjee A. Minimal intervention dentistry: part 7. Minimally invasive operative caries management: rationale and techniques. Br Dent J. 2013 Feb;214(3):107-11.
21. White JM, Eakle WS. Rationale and treatment approach in minimally invasive dentistry. J Am Dent Assoc. 2000 Jun 1;131:13S-9S.
22. Maia LC, de Souza IPR, Cury JA: Effect of a combination of fluoride dentifrice and varnishon enamel surface rehardening and fluoride uptake in vitro. Eur J Oral Sci 2003;111: 68–72.
23. Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem AC. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci. 2010 Aug;18(4):316-34.
24. Ten Cate JM, Timmer K, Shariati M, Featherstone JD. Effect of timing of fluoride treatment on enamel de- and remineralization in vitro: a pH-cycling study. Caries research. 1988;22(1):20-6.
25. White DJ. The application of in vitro models to research on demineralization and remineralization of

the teeth. Advances in Dental Research 1995;9:175–93.

26. Neto FR, Maeda FA, Turssi CP, Serra MC. Potential agents to control enamel caries-like lesions. J dent. 2009 Oct 1;37(10):786-90.
27. Meredith N, Sherriff M, Setchell DJ, Swanson SA. Measurement of the microhardness and Young's modulus of human enamel and dentine using an indentation technique. Arch Oral Biol. 1996 Jun 1;41(6):539-45.
28. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA,Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res. 2005;39:514–20.
29. Chuenarrom C, Benjakul P, Daosodsai P. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. Mater Res. 2009;12(4):473-6.
30. Argenta RMO, Tabchoury CPM, Cury JA. A modified pH cycling model to evaluate fluoride effect on enamel demineralization. Braz Oral Res 2003;17:241–6.
31. Gutiérrez-Salazar MD, Reyes-Gasga J. Microhardness and chemical composition of human tooth. Mater Res. 2003 Jun;6(3):367-73.
32. Rao R, Jain A, Verma M, Langade D, Patil A. Comparative evaluation of remineralizing potential of Fluoride using three different remineralizing protocols: An in vitro study. J Cons Dent. 2017 Nov;20(6):463.
33. Koulourides T, Feagin F, Pigman W. Remineralization of dental enamel by saliva in vitro. Ann NY Acad Sci. 1965 Sep;131(2):751-7.
34. Arends J, Ten Cate JM. Tooth enamel remineralization. J Cryst Growth. 1981 May 1;53(1):135-47.
35. Wang Z, Jiang T, Sauro S, Pashley DH, Toledano M, Osorio R, Liang S, Xing W, Sa Y, Wang Y. The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an in vitro study. Aust dent J. 2011 Dec;56(4):372-81.
36. 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 cons dent. 2014 Jan;17(1):3.
37. Kamath P, Nayak R, Kamath SU, Pai D. A comparative evaluation of the remineralization potential of three commercially available remineralizing agents on white spot lesions in primary teeth: An in vitro study. J Indian Soc Pedod Prev Dent. 2017 Jul 1;35(3):229.
38. Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Surfactant-modified β-TCP: structure, properties, and in vitro remineralization of subsurface enamel lesions. Journal of Materials Science: Mater Med. 2010 Jul 1;21(7):2009-20.
39. Karlinsey RL, Mackey AC, Stookey GK, Pfarrer AM. In vitro assessments of experimental NaF dentifrices containing a prospective calcium phosphate technology. Am J Dent. 2009 Jun;22(3):180-4.
40. Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. J dent. 2014 Apr 1;42(4):466-74.
41. Patil N, Choudhari S, Kulkarni S, Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. J Cons Dent. 2013 Mar;16(2):116.
42. Hegde MN, Devadiga D, Jemsily PA. Comparative evaluation of effect of acidic beverage on enamel

surface pre-treated with various remineralizing agents: An In vitro study. J Cons Dent. 2012 Oct;15(4):351