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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF REMINERALISATION POTENTIAL OF THREE DIFFERENT DENTIFRICES IN ARTIFICIALLY INDUCED CARIOUS LESIONS: AN INVITRO STUDY

***Dr. S. Sivaranjani, Dr. Shafie Ahamed, Dr. Bhavani and Dr. Rajaraman**

Department of Conservative Dentistry and Endodontics, Rajah Muthiah Dental College And Hospitals, Annamalai University, Annamalai naga, Tamilnadu, India

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ABSTRACT

Aim and Objectives: The present study was undertaken to evaluate and compare the remineralization potential of three different dentifrices with different composition (fluoro calcium phosphosilicate, nano-hydroxy apatite, calcium sucrose phosphate) on artificially induced carious lesions in vitro through scanning electron microscopy and quantitative assessment by energy dispersive X-ray spectroscopy. **Materials and method:** The present in vitro study conducted on 30 caries free impacted third molars with intact surfaces divided into three groups (10 each). Artificial demineralization was carried out, followed by remineralization using dentifrice slurry as per the group allocation. After 10 days period the entire test groups were evaluated with HRSEM and quantitative assessment by energy dispersive X-ray spectroscopy .the obtained data was analyzed statistically using one way ANOVA, student's t test and tukey's multiple comparison tests.$p \leq 0.05$ was considered to be significant. **Results:** All three dentifrices tested showed remineralization although insignificantly different from each other but highlights the concept of biomimetic bioactive glass as an effective remineralizing agent. **Conclusion:** To focus on the importance of minimal invasive treatment on incipient carious lesion by remineralization.

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INTRODUCTION

In the past, dental caries was considered as an infectious bacterial disease that was irreversible. Recent concept in caries states that caries is a complex multifactorial disease caused by an imbalance in physiologic equilibrium between tooth mineral and biofilm. The caries process is said to be active when demineralization periods are more than periods of remineralization. The new insight into caries provided the scope for remineralizing incipient carious lesion (Kawasaki, 2000). The demineralization process can be stopped by creating an environment conducive for remineralization by various remineralizing agents (Pradeep, 2011). The process of restoring lost mineral ions to the tooth structure and strengthening the lattice work is known as remineralization. The remineralized enamel crystallites are generally more resistant to decalcification and also have the same orientation as the original enamel crystallites (Tanaka, 2014). The early enamel lesions have a potential for remineralization with an increased resistance to further acid challenge, particularly with the use of enhanced remineralization treatments (Hicks, 2004). Thus invasive treatments of precavitated lesions are not required. Various remineralizing agents like fluoride (Arnold, 2006). Casein phosphopeptide stabilized amorphous calciumphosphate (CPP-ACP; Recaldent) (Oshiro, 2007 and Azarpazhooh, 2008), unstabilized ACP (ACP, Enamelon), CPP stabilized amorphous calcium phosphate with fluoride (CPP-ACPF; Recaldent) (Reynolds, 1997 and Reynolds, 2008), bioactive glass materials compared has been studied in both *in-vitro*(Kumar, 2008 and Jayarajan, 2011) and *in-vivo* studies (Reynolds, 2008 and Walker, 2009). Recently introduced fluoride cantaining bioactive glass(ELSENZ) to slowly release calcium, phosphate and fluoride ions over an 8-12 hr timeframe to form fluorapatite mineral to rebuild, strengthen and product tooth structure. The slow release of fluoride has been identified to be particularly beneficial in prevention of tooth decay. Calcium sucrose phosphate (ENAFIX) remineralizing agent is available only as dentifrice. Its quickly breaks down and release calcium, phosphate and sucrose phosphate ions into the saliva, calcium and phosphate ions, rapidly absorb onto the enamel, decrease the rate of enamel solubility under acidic conditions. Hydroxyapatite (HA) is one of the most biocompatible and bioactive materials and is widely applied to coat artificial joints and tooth roots. Medicinal Nanohydroxyapatite containing calcium hydrogen phosphate (APAGARD-PREMIO) remineralizing agent is available as dentifrice.

Corresponding author:* **Dr. S. Sivaranjani,
Department of Conservative Dentistry and Endodontics, Rajah Muthiah Dental College And Hospitals, Annamalai University, Annamalai naga, Tamilnadu, India

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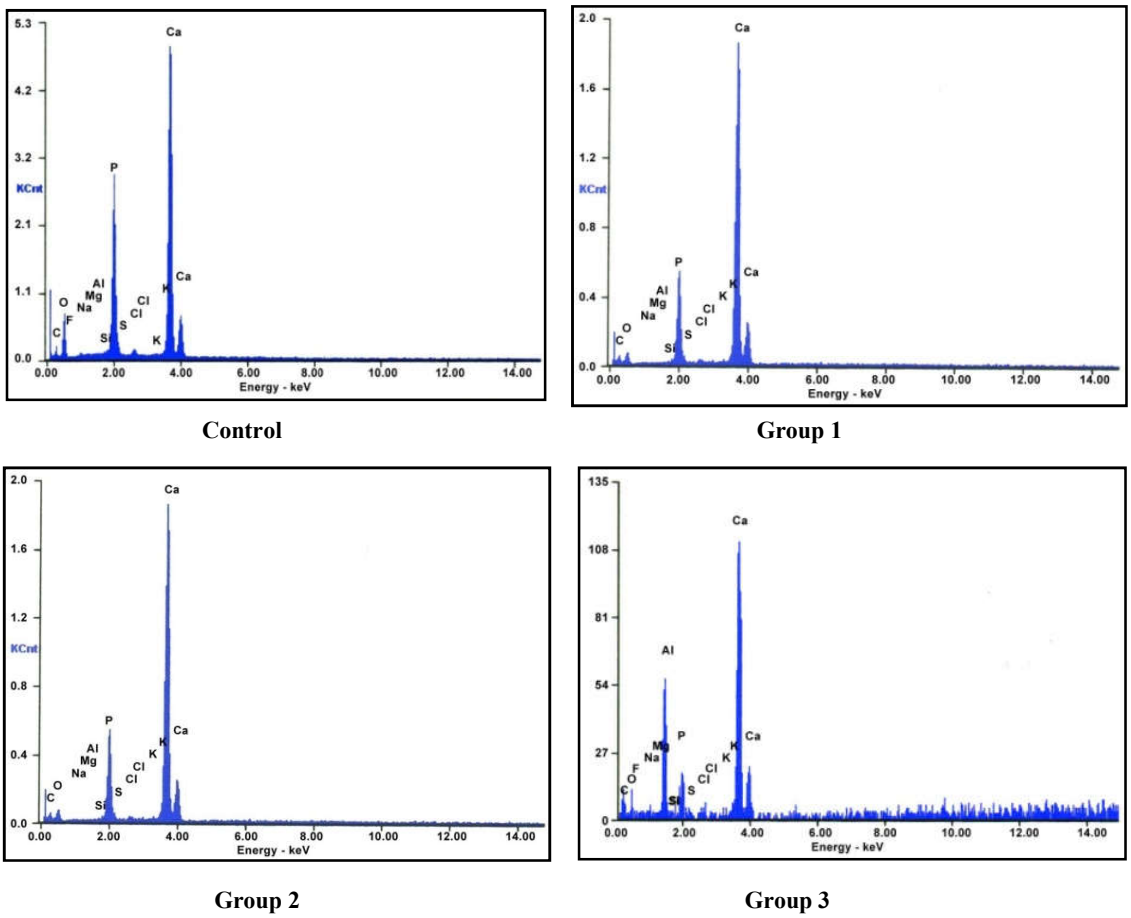


Graph 1. Micro analysis report of various elements by energy dispersive spectroscopy (edax)for different study groups

It aims to promote and support the naturally occurring minerals and functions that already take place in the mouth. However the remineralization potential of the above listed materials have not so for been evaluated and compared in invitro or invivo studies. Hence the literature is scarce on the remineralisation potential of these dentifrices. The topography of remineralized area was studied by high resolution scanning electron microscopy (HRSEM) & quantitative chemical change in mineral profile was assessed by energy dispersive X-ray spectroscopy (EDS).

MATERIALS AND METHODS

30 impacted third molars with intact crown structure without any visible caries, hypoplastic lesions and white spots on any surface of the tooth were collected for the present study. All the teeth were stored in 0.1% thymol solution until processing. The crown and root portions were separated using a low speed safesided diamond disc. Then the crown was sectioned sagittally to obtain sound buccal surface. Each specimen was divided vertically into three equal thirds using nail varnish [one-third positive control (sound surface), one-third negative control (demineralized) and the other third served as the study group (paediatric dentifrice)]. This method was in accordance with Kapoor *et al.* 2016 (Salonen, 2009).

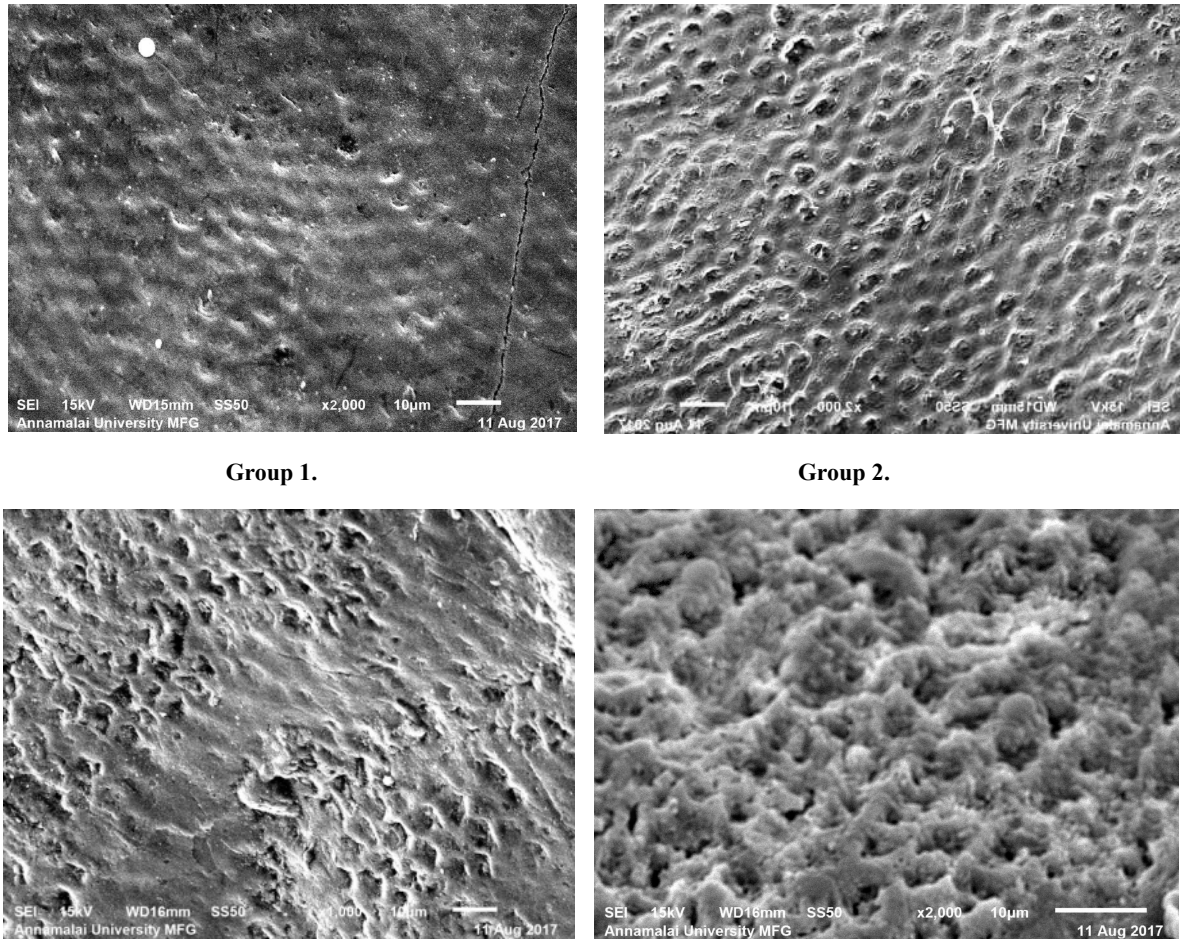
Demineralizing Procedure: The demineralizing solution was prepared with the help of the following components in equal proportions. 0.07 pH 4.7, Calcium chloride ($CaCl_2$) -2.2Mm, Sodium dihydrogen phosphate ($NaH2PO_4$)-2.2mM, Lactic acid-0.05M, Fluoride – 0.2%, pH Indicator paper.

All the 30 specimens were suspended with the floss immersing their two-thirds of the surface in the demineralizing solution, one-third of the surface was covered with nail varnish and served as positive control (sound surface). The specimens were suspended for 96 hrs in the demineralizing solution before washing them with double –distilled water for 15 seconds. After demineralization middle part of the buccal surface was coated with nail varnish. The test groups were divided as

Control: samples for negative control were selected randomly
Group 1: Tooth paste containing fluoro calcium phosphosilicate (ELSENZ-F533ppm)
Group 2: Medicinal Nanohydroxy apatite containing calcium hydrogen phosphate (APAGARD –PREMIO)
Group 3: Anticay 5% which is complex mixture of Calcium sucrose phosphate with inorganic amorphous calcium phosphate (ENAFIX)

Remineralizing Procedure

Dentifrice slurry was freshly prepared every time during the study period by thoroughly mixing 1 gm of test dentifrice in 2.5 ml of double distilled water and thoroughly centrifuging it at 4000 rpm for 20 minutes. During remineralization cycle, lower one-third of each specimen was immersed in 5 ml of freshly prepared dentifrice slurry for 5 minutes, leaving the middle third demineralized surface as negative control. The specimens were then removed, washed with double-distilled water for 15 seconds and placed in artificial saliva for 8 hours to simulate oral environment.



Group 1. **Group 2.**

Group – 3 **Control**

Figure 1. Scanning electron microscope pictures

The artificial saliva was prepared by mixing Na3PO4 - 3.90 mM, NaCl2 - 4.29 mM, KCl - 17.98 mM, CaCl2 - 1.10 mM, MgCl2 - 0.08 mM, H2SO4 - 0.50 mM, NaHCO3 - 3.27 mM in distilled water and the pH was set at a level of 7. 2. After 8 hours, the specimens were removed from the artificial saliva, washed with double-distilled water and again treated with freshly prepared dentifrice solution for 5 minutes in the same manner as described earlier, followed by overnight placement of the specimen in artificial saliva. The procedure was repeated daily for 7 days, followed by examination of all the specimens using (HRSEM WITH EDAX).

Scanning Electron Microscopy

Samples were rigidly mounted on a circular metallic sample holder with the help of sticky carbon tape. The samples were electrocoated with 20- to 50 nm thick gold using JEOL JFC - 1600 Auto Fine Coater. After gold sputtering, the samples were subjected to SEM analysis in the instrument SEM JSM-6610LV. The scans were automatically generated on a computer attached to the SEM. A magnification of 2000× was used to view sound, demineralized and remineralized surface of all the 30 specimens. The remineralization effect in the two test groups, positive and negative controls was noted by an independent examiner according to the evaluation parameter by, which was then compared and evaluated statistically (Gupta, 1998).

Evaluation criteria and statistical analysis

Qualitative topographical assessment was performed by HRSEM.

The specimens were viewed under 2000X magnification. The quantitative assessment of the changes in mineral profile was studied by EDS. The obtained data's were subjected to mean and standard deviation. The values were tabulated and statistically analyzed using one-way ANOVA.Comparison of each group with the control group was analyzed using "Student's *t*-test". The comparison of means between groups was conducted using Tukey's multiple comparison tests. $P \leq 0.05$ was considered to be significant.

RESULTS

The three test groups were not statistically different from one another in terms of mean remineralisation scores although Group 1 showed maximum remineralisation (score= 3.60+/- 0.52) followed by group 2 (score= 3.40+/- 0.52) and group 3 (score= 2.60+/- 0.52) respectively. All the three test groups showed significant remineralisation compared with demineralised enamel surface [Figure 1]. The following were the results obtained by mineral profile analysis for elements calcium, phosphorus and fluorine. One way ANOVA analysis showed a statistically significant difference in the mean value between the groups. Group 1 showed statistically significant results when compared to control for elements Ca and P. Group 2 showed statistically significant results when compared to control for elements Ca and P. Group 3showed statistically significant results when compared to control for elements Ca and P. The HRSEM pictures and energy dispersive X –ray analysis (EDAX) graph revealed mineral deposits on the surface when each test group was compared with the control group [Graph 1, Table 1].

Table 1. Depicts the comparison of individual group with the control for calcium, phosphorus, fluoride elements in weight and atomic percentage

Calcium (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	29.3270	1.3794	16.908	0.001 (S)
Group 1	10	46.6780	2.9373		

Calcium (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	14.6520	.8310	33.140	0.001 (S)
Group 1	10	37.4600	2.0115		

P (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.4930	1.1693	9.867	0.001 (S)
Group 1	10	14.3500	2.9346		

P (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.2220	.8950	13.429	0.001 (S)
Group 1	10	10.9820	1.3164		

F (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	1.6250	.8533	24.351	0.001 (S)
Group 1	10	11.4420	.9472		

F (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	2.4000	.4362	41.373	0.001 (S)
Group 1	10	14.3560	.8030		

Calcium (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	29.3270	1.3794	19.438	0.001 (S)
Group 2	10	47.2710	2.5728		

Calcium (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	14.6520	.8310	38.139	0.001 (S)
Group 2	10	32.1490	1.1892		

P (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.4930	1.1693	20.575	0.001 (S)
Group 2	10	13.6770	.7907		

P (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.2220	.8950	16.294	0.001 (S)
Group 2	10	10.2600	.7564		

F (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	1.6250	.8533	1.659	0.119 (NS)
Group 2	10	1.1080	.4926		

F (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	2.4000	.4362	14.246	0.001 (S)
Group 2	10	.4090	.7094		

Calcium (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	29.3270	1.3794	4.203	0.001 (S)
Group 3	10	33.4800	2.8034		

Calcium (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	14.6520	.8310	20.658	0.001 (S)
Group 3	10	22.3680	.8394		

P (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.4930	1.1693	4.158	0.001 (S)
Group 3	10	7.7670	2.1984		

P (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	4.2220	.8950	1.433	0.169 (NS)
Group3	10	5.4300	2.5116		

F (wt)	N	Mean	Std. Deviation	t- value	P- value
Control	10	1.6250	.8533	0.990	0.119 (NS)
Group 3	10	1.2930	.6298		

F (at)	N	Mean	Std. Deviation	t- value	P- value
Control	10	2.4000	.4362	2.898	0.001 (S)
Group 3	10	1.7200	.6002		

DISCUSSION

Morden prospective caries studies require the measurement of small changes in tooth mineral content. Quantitative measurements of changes in mineral content in a single caries lesion are desirable. One of the most recent techniques is scanning electron microscope with energy dispersive X-ray analysis (EDAX) attachment. It is a microanalytical technique that is employed to estimate quantitatively the amounts of mineral in a given tooth sample (Madan, 2009). HRSEM gives the topographical pictures and is used to assess the surface changes seen on enamel. EDAX gives quantification of various elements like Ca, P, F, O, Me, Na etc in both atomic and weight percentage (Jayarajan, 2011; Magalhães, 2009). The atomic and weight percentage of Ca, P and F are evaluated in the study groups as these ions take part in remineralization process. Bioactive glass is considered to be a breakthrough in remineralization technology. The probable mechanism of action of Bioactive glass could be that Unlike other calcium phosphate technologies, the ions that bioactive glass release from hydroxycarbonate apatite (HCA) directly, without the intermediate amorphous calcium phosphate phase. These particles also attach to the tooth surface and continue to release ions and re-mineralize the tooth surface after the initial application. Ultimately these particles will completely transform into hydroxycarbonate apatite which is the mineral our teeth and bones are made of hydroxycarbonate apatite (HCA) layer is beleved to contribute to the re-mineralization process of tooth surface (Arnold, 2006). Bioactive glass in aqueous environment immediately begins surface reaction in three phase leaching exchange of cations, network dissolution of SIO2 and precipitation of calcium and phosphate to form an apatite layer (Lynch, 2007). Hassanein et al investigated remineralization of bioactive glass using Raman spectroscopy and scanning electron microscope concluded that bioactive glass has the potential for remineralizing artificially carious enamel and dentin (Hassanein, 2006). Recently introduced remineralizing products based on calcium phosphate remimeralization systems are, flouride containing bioactive glass –fluoro calcium phosphosilicate remineralizing agent is available only as dentifrice. which is able to slowly release calcium, phosphate and fluoride ions over an 8-12 hr timeframe to form fluorapatite mineral to rebuild, strengthen and product tooth structure. The slow release of fluoride has been identified to be particularly beneficial in prevention of tooth decay.

In recent years, an increasing number of reports have shown that nano-hydroxyapatite (APAGARD) has the potential to remineralize artificial carious lesions following addition to toothpastes, mouthwashes.etc (Hicks, 2004). Nano-sized particles have similarity to the apatite crystal of tooth enamel in morphology, crystal structure and crystallinity. The results of our study was similar with the results of S. H. Jeong et al, who studied the remineralization effect of nano–hydroxyapatite tooth paste, contains nano-sized hydroxyapatite and fluoride, and other contains nano-sized HA excluding fluoride on artificial incipient caries lesion, that tooth paste containing nano-sized hydroxyapatite has the potential to remineralization. The addition of fluoride no synergistic effect on remineralization (Jeong, 2006). Calcium sucrose phosphate (ENAFIX) remineralizing agent Along with acting as an anticay, this paste has also been shown to act as a desensitizing agent, by occluding the dentinal tubules. The results of our study was similar with the results of S. Thabitha Rani et al investigated remineralization of calcium sucrose phosphate and casein phosphate The remineralization was studied by high–resolution scanning electron microscope (HRSEM) and energy dispersive x-ray spectroscopy. In oral cavity, the pH alteration are more frequent depending on individual's dietary and oral hygiene habits. Therefore itis difficult to exactly simulate the oral condition that prevail in the mouth.So nevertheless there is greater control over these variables in an in vitro model which may be difficult to obtain in mouth. The present study tried to stimulate the oral condition as far as possible, viz., 5 minutes suspension in dentrifrice slurry every 8 th hour (simulating 5 minutes of brushing twice daily) and suspension in artificial saliva (simulating the effect of saliva in oral cavity). Various methods have been used by different authors to demineralize enamel. However the present method was modified from tencate and Duijstars because of the convenience in the reduced time period of immersion and easy availability of chemicals. Time period of 96 hours was used for Rirattanapong et al to produce 60 to 100 micro meter deep artificial carious lesion. Various methods have been used for evaluating the remineralization of white spot lesion. So the present study employed the HRSEM and EDAX because of reported high sensitivity toward early reaction occurring at crystal level.

Conclusion

From the study, it can be concluded that all the three dentrifrices showed remineralising potential, which was sifnificantly high compared to control demineralised surface. Within the limitations of the study, group 1 showed higher percentage of remineralisation of calcium and phosphate salts followed by group 2 and group 3. Further studies on enamel crystal formation and chemical structure using advanced quantification technique and the resistance of acid solubility of these remineralized crystallites have to be investigated in order to achieve more conclusive results.

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