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Comparative assessment of enamel surface remineralisation with three different dentifrices: An energy dispersion x-ray spectroscopy analysis

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ABSTRACT

Aim and Objective: Comparative assessment of remineralisation of enamel surface when treated with three different dentifrices.

Materials and Methods: Sixty-six extracted upper right first premolars were demineralized to form artificial white spot lesions. The samples were divided into 3 groups: Group 1-Biomin F, Group 2- Clinpro ToothCreme, Group 3- Amflor. They were treated with three different dentifrices (22/ group) for 14 days in simulated oral conditions. Energy dispersion X-Ray Spectroscopy was done pre and post remineralistaion.

Results: The primary evaluation parameter for this study was the Ca/P ratio. As per the results, the maximum increase in Ca/P ratio was seen in Group1, followed by Group 3 and 2 respectively. The results were found to be statistically significant. ($p=0.045$ i.e. $p<0.05$) The secondary evaluation parameter for this study was the change in Fluoride content. As per the results, the maximum increase in Fluoride content was seen in Group 2 which was followed by Group 3 which is equivalent to Group 1. However, the results were found to be statistically non-significant. ($p=0.219$ i.e. $p>0.05$)

Conclusion: Biomin F has the highest remineralisation efficiency when compared to the other 2 dentifrices. Biomin F contains an optimum combination of fluoride (600 ppm), phosphate and calcium ions, incorporated within the structure of the glass itself. The glass gradually dissolves over up to 12 hours neutralizing acids in the mouth and deposit fluorapatite. Thus, it can be used in patients undergoing orthodontic treatment to prevent or reduce the formation of white spot lesions.

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1. Introduction

Orthodontic treatment is designed to improve the quality of life by enhancing facial and dental esthetics, along with providing functional efficiency and a structural balance between the tissue systems, which influence the tooth position. An orthodontist achieves these goals by employing complex appliances in the patient's mouth for a prolonged period. However, this complex appliance design necessitates the need to maintain diet restrictions and diligent care of the appliance and teeth. It becomes imperative that the patient follows this maintenance protocol as a part of the treatment. However, due to negligence or unawareness, the inability to do so leads to the formation of undisturbed plaque retentive areas around these appliances. Oral environmental factors like pH, temperature, salivary flow, protective factors in the saliva like immunoglobulins, fluoride, etc., and patient factors like carbohydrate consumption; create a dynamic environment that further influences this process.

During the initial period of the treatment, the patient is unaware of the consequences of negligence in terms of oral hygiene maintenance, unless explained so. Owing to the continued plaque retention around the appliance, the

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microbial flora of plaque shifts towards acidogenic bacteria. This results in acid diffusion in the intact and subsurface enamel, initiating the process of demineralization. This demineralization enhances the porosity and reduces the microhardness of enamel and in about 1 month, it starts presenting as opaque, white, chalky spots around the appliance. These are referred to as "White Spot Lesions". Thus, it becomes extremely vital to understand the etiology of these lesions and the associated risk factors. Early detection of WSL is important and challenging. This would allow the clinicians to apply preventive measures to control the demineralization process before lesions progress.

There are various modalities available to combat this adverse effect of white spot lesions by preventing their formation or limiting their development. Amongst these, fluoride-containing delivery systems are the most important agent to prevent decalcification and lesions from developing and progressing. We will be using three different fluoride-containing dentifrices, Biomin F (Bioactive glass based dentifrice), Clinpro 3M Tooth Crème 0.21% NaF Anticavity Toothpaste (Tri calcium phosphate-based dentifrice), and Amflor (Amine fluoride-containing dentifrice) which claim to be having remineralising potential. No study has been carried out to compare the efficiency of all three.

At present, a wide array of both macroscopic and microscopic methods are available which can be used in in-vitro and in-vivo studies. The most desirable method is the one that is accurate, reproducible, easy to use, and should validate mineral loss from the enamel. Various methods like caries models, polarized light microscopy, and Scanning Electron Microscope have been used for microscopic in-vitro evaluation An improvement on the Electron microscope has been the EDS analysis which is a micro-analytical technique employed to estimate quantitatively the amounts of minerals in a given tooth sample.

Thus, the aim of this study is to carry out a comparative assessment of the remineralisation of enamel surface when treated with three different dentifrices by an Energy Dispersion X-Ray Spectroscopy Analysis. This would help determine the remineralisation potential in terms of reducing/preventing white spot lesions, and increasing the microhardness of enamel in patients undergoing fixed orthodontic treatment.

2. Materials and Methods

This in-vitro study was carried out in the Department of Orthodontics and Dentofacial Orthopedics in our college. It was done in association with the Indian Institute of Technology (IIT), Bombay for laboratory testing needed as an essential part of this research. The inclusion and exclusion criteria has been mentioned in Table 1.

As per the sample size determined, 66 extracted upper right first premolars were collected After collection, teeth were thoroughly cleaned with saline solution (0.9% Sodium Chloride) to clear them of any debris or physical impurities. An ultrasonic scaler was used to remove hard deposits from the tooth surface. The teeth were then stored in 10% formalin in an incubator at 37^0C till in use. The use of formalin ensures that there is no effect on the inorganic content of enamel while being stored.[1,2] The stored teeth were washed thoroughly with distilled water 24 hours before the start of sample preparation.

A bracket was bonded on each of the sample at the FACC point. A 4X4 mm window was created around the bracket with the help of a cardboard jig. The remainng tooth area was coated with a nail polish. The teeth were immersed in the demineralisation solution containing 2.2mM ($CaCl_2$),2.2mM (NaH_2PO_4), and 0.05 M acetic acid adjusted to pH 4.4 with 1 M KOH[3] for 48 hours. The demineralization process was started 2 days prior to when they had to be sent for testing. On completion of 48 hours, the teeth were dried overnight as per the requirement of the laboratory to carry out the EDS-SEM study.

FEI Quanta 200 Scanning electron machine with EDAX APEX software was used to carry out the Scanning Electron Microscopy(SEM) and Energy Dispersion X-ray Spectroscopy (EDS), used to determine the elemental composition of the desired area of concern. Calcium, Phosphorus, Fluoride, Silicon, Carbon, and Oxygen were the chosen elements for analysis. Along with the graph, a table was also displayed which contained the atomic and weight percentage of every chosen element. The analysis was done at 500X magnification, 3.5 nm resolution, 100uA emission current, 200 V voltage, a vacuum pressure of at least 3 Pa, and CPS (Count per second) of above 900. The SEM images were taken at 1000X, 2500X, and 5000X magnification. A higher voltage of 15 kV was used. (Figures 1 and 2)

The embedding of the tooth was done after carrying out the pre-remineralisation EDS-SEM analysis for convenience purposes, as requested by the laboratory. The mounting was removed before carrying out EDS-SEM analysis again, post remineralisation

Molds were used to embed the tooth samples to standardize the size of all the samples. For ease, Orthokal(Type III Dental stone) was used for embedding the tooth samples. A layer of nail polish varnish was applied on the orthokal holding the teeth, to prevent any release of Calcium which could alter the results.

The teeth were divided into 3 groups, each containing 22 teeth, using the method of random sampling. All the tooth samples were then numbered as per the group to which they were allocated. Three different plastic containers labeled with each group's sticker were used to carry the teeth to the laboratory to avoid any errors.

1. Group 1: Biomin F Toothpaste, samples numbered 1-22

2. Group 2: Clinpro 3M Tooth Crème, samples numbered 23-44
3. Group 3: Amflor Toothpaste, samples numbered 45-66

A customized tooth-brushing machine was constructed to simulate manual toothbrushing, standardizing it for all three groups. The simulated tooth-brushing machine was made such that toothbrushing was done at 90 degrees to the labial surface under a constant loading of 150 strokes/minute for 3 minutes.[4] The tooth brushing with the three dentifrices was done twice a day at an interval of 12 hours, for 3 minutes per tooth sample.[3] The wooden stick containing the brush head was changed for every group and the bristles were cleaned with only distilled water after use. The amount of toothpaste being used for standardized by using a GIC scoop. (Figures 3 and 4)

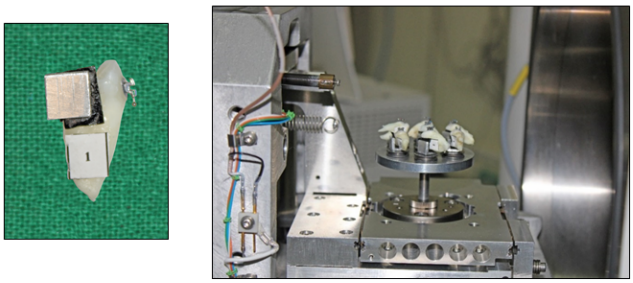

Figure 1: Tooth samples mounted forloading it in the FEI Quanta 200 Scanning Electron Microscope Machine for EDSanalysis

The samples were stored in artificial saliva containing 400mg/L NaCl, 400 mg/L KCl, 795 mg/L $CaCl_2$, 690 mg/L $Na_2H_2PO_4.H_2O$, 300 mg/L KSCN, 5 mg/L $Na_2S.9H_2O$, 1000 Mg/L Urea[5–7](Prepared at PharmaChem Research and Development Laboratories) in an incubator at 37^0C to simulate oral conditions. The same procedure was repeated to carry out EDS-SEM analysis post remineralisation. All the settings were kept the same to avoid any bias. A flow chart demonstrating the steps carried out in the study has been shown in Figure 5.

Data was compiled on MS Excel and subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Descriptive statistics like Mean & SD for numerical data have been depicted. The normality of numerical data was checked using the Shapiro-Wilk test & was found that the data did not follow a normal curve; hence non-parametric tests have been used for comparisons. (refer to annexure 1 table) Inter-group comparison (>2 groups) was done using Kruskall Wallis ANOVA followed by pair-wise comparison using the Mann-Whitney U test. Intra-group comparison was done using the Wilcoxon Signed rank test (upto 2 observations). For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving power to the study as 80%.

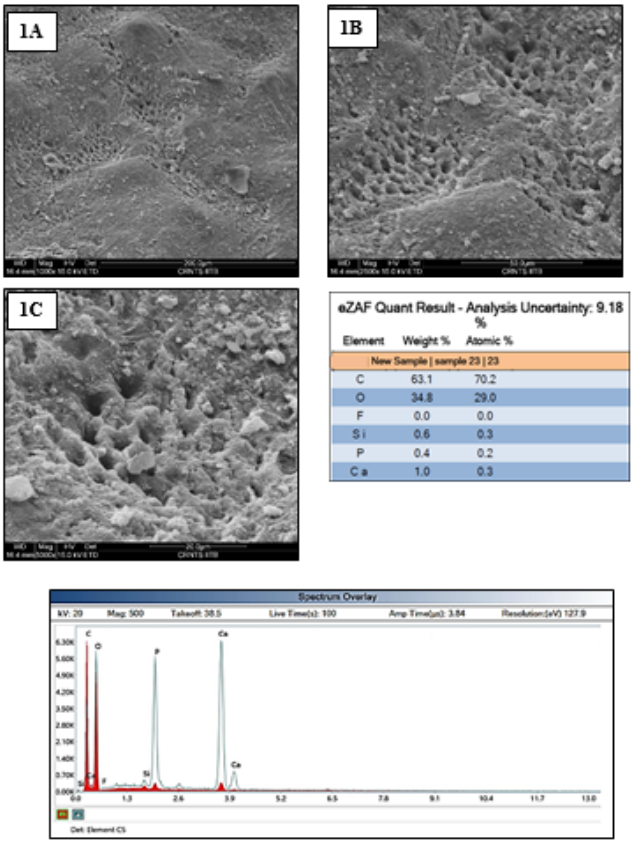

Figure 2: 1. SEM Images at A. 1000XB. 2500X C. 5000X & 2. EDX Images of Pre-Remineralisation of Group 2 sample

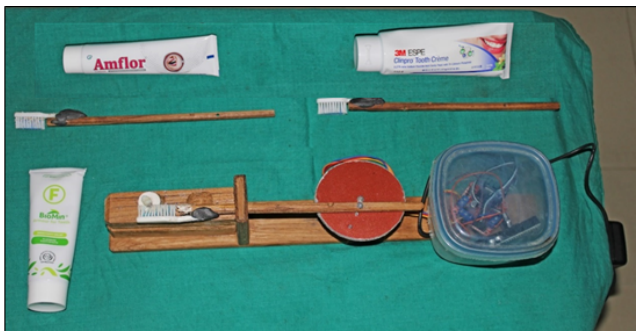

Figure 3: Simulated tooth brushingmachine

3. Results

Comparison of pre and post weight percentages of Ca, P, F, Si, C and O along with the Ca/P ratio between the three groups have been carried out. Also, the comparison of the difference between the pre and post values have been done for each parameter. The difference values highlight the change that has occurred and helps determine the final outcome of the test more evidently. Thus, more emphasis has been put on the difference values.

Table 1: Inclusion and exclusion criteria

Inclusion Criteria:	
1.	Extracted maxillary first premolar teeth with intact buccal surface, no developmental defects, visible cracks, physical damage due to extraction, and no buccal surface caries or white spot lesions.
2.	Biomin F Toothpaste Clinpro 3M Tooth Crème 0.21% NaF Anticavity Toothpaste
3.	Amflor Toothpaste
4.	Enlight bonding kit (ORMCO Corporation)
5.	Premolar bracket with an MBT prescription; slot size of 0.022" X 0.028".
Exclusion Criteria	
1.	Extracted maxillary first Premolar with developmental defect, cracks, or caries.
2.	Any toothpaste other than Biomin F toothpaste, Clinpro 3M Tooth Crème 0.21% NaF
3.	Anticavity Toothpaste and Amflor toothpaste.
4.	Bracket with a prescription other than MBT; slot size of 0.022" * 0.028"
5.	Any bonding kit other than Enlight (ORMCO Corporation).

Table 2: Comparison of outcomes

			Mean	Std. Deviation	Std. Error	Median	Chi square value	p value of Kruskal-Wallis Test
	1	22	34.136364	10.0175463	2.1357480	34.3000	12.588	0.002**
Diff C	2	22	30.365455	13.2464449	2.8241515	26.0250		
	3	22	20.100000	14.2944545	3.0475879	19.1500		
Diff O	1	22	12.772727	6.0217014	1.2838310	12.6500	18.437	0.000**
	2	22	12.459091	4.8973909	1.0441272	12.5000		
	3	22	6.095455	4.7787702	1.0188372	5.2000		
Diff F	1	22	.322727	.1540928	.0328527	0.3000	3.038	0.219
	2	22	.886364	.7528014	.1604978	0.6500		
	3	22	.813636	1.0096182	.2152513	0.3000		
Diff Si	1	22	5.045455	2.7310782	.5822678	4.7000	15.155	0.001**
	2	22	2.872727	1.9823463	.4226377	2.5500		
	3	22	3.145455	4.8583485	1.0358034	1.6000		
Diff P	1	22	3.831818	1.7395638	.3708763	3.7000	2.722	0.256
	2	22	3.736364	2.9572422	.6304862	3.0500		
	3	22	3.072727	2.7290643	.5818385	1.8500		
Diff C a	1	22	12.940909	5.3626050	1.1433112	13.1750	2.322	0.313
	2	22	12.506364	9.7322774	2.0749285	9.7500		
	3	22	10.368182	8.3923654	1.7892583	7.0500		
Diff Ca/P	1	22	1.823205	.7917117	.1687935	1.9182	6.197	0.045*
	2	22	1.236271	.8071969	.1720950	1.3636		
	3	22	1.601611	.6381002	.1360434	1.8902		

The first evaluation parameter for this study was the Ca/P ratio. As per the results, the maximum increase in Ca/P ratio was seen in Group1, followed by Group 3 and Group 2 respectively. The results were found to be statistically significant. ($p=0.045$ i.e. $p<0.05$) The second evaluation parameter for this study was the change in Fluoride content. As per the results, the maximum increase in Fluoride content was seen in Group 2 which was followed by Group 3 which is equivalent to Group 1. However, the results were found to be statistically non-significant. ($p=0.219$ i.e. $p>0.05$). Maximum increase in Silicon and decrease in carbon was seen with Group 1 followed by Group 2 and 3 respectively.

Results have been shown in Table 2.

4. Discussion

Esthetics form an essential part of the treatment objectives and improving that, is often the chief motive of the patient to undergo Orthodontic treatment. Treating the malocclusion improves the esthetics of the patient, providing the patient with a better smile and improving the self-image of many. Along with this, Orthodontic treatment also helps in delivering a long term health advantage by setting the teeth in positions which are easier to clean and maintain. Like every rose has its own thorn, in spite of its post treatment advantage, the appliance itself makes it difficult to maintain oral hygiene.[8] Oral hygiene maintenance is a vital adjunct to every Orthodontic treatment. Fixed

Figure 4: Group 1: Biomin F Toothpaste Group 2:Clinpro 3M Tooth Crème 0.21% NaF Anticavity Toothpaste Group 3:Amflor Toothpaste

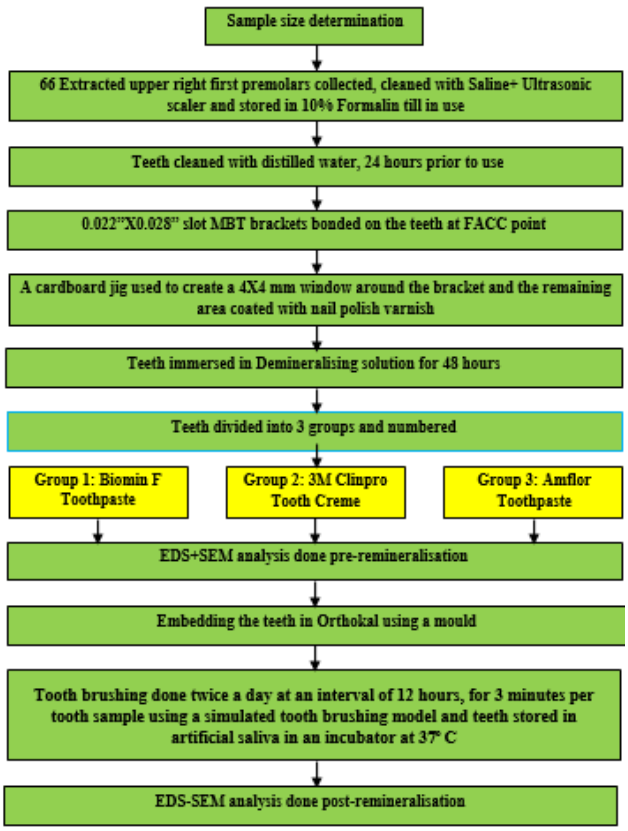

Figure 5: Flowchart demonstrating steps carried out in the study

appliances act as retentive areas for plaque accumulation. Orthodontic patients have about 2 to 3 times higher levels of plaque coverage when compared to normal adults with high cariogenic biofilm formation.[9]

Various studies have been carried out to compare different types of remineralising agents as well as between the subtypes. This study aims at assessing the remineralisation potential of three such dentifrices which have proven to be better than their counterparts according to the previous studies, but have been never been compared to each other. The fluoride concentration in any dentifrice plays a critical role in determining the mechanism of action of a dentifrice. A sustained slow release of fluoride with restoring the tooth enamel by formation of apatite crystals is required. The aim is to prevent or reduce white spot lesions without arresting the lesion completely, since it can affect aesthetics. Thus, the three dentifrices chosen in this study have fluoride concentrations between 600 ppm and 1000 ppm. We have chosen a Bioglass based Dentifrice, a functionalized Tri-calcium Phosphate based Dentifrice and an Amine Fluoride based Dentifrice. All the three dentifrices possess unique properties and mechanism of actions through which they affect the mineralization process.

Novamin^{R} is a patented ingredient introduced by Dr. LenLitkowsky and Dr. Gary Hack, composed of the traditional bioglass ingredients. It is a non-fluoride containing Bioglass dentifrice. Fluoride is an essential ingredient in any dentifrice, the presence of which promotes the formation of Fluor apatite, which is more resistant to acid attack, in comparison to Hydroxyapatite. Thus, recently a new bioglass containing fluoride dentifrice, Biomin F has been developed by the Laboratories of Queen Mary University of London, UK, by Prof. Robert Hill and his team. According to studies by Palananiswamy et al[10], Mohanty et al[3], Wang et al[11], Narayana et al.[12] and Jagga et al.[13], Novamin containing dentifrices showed better remineralisation potential. However, after the introduction of Biomin F, which is a fluoride containing Bio glass based dentifrice, few studies have been carried out which compare Novamin and Biomin F. According to Farooq et al.[14] and Omran et al.[15] Biomin F has better remineralisation potential than Novamin. According to Alhussain et al.[16] and Bakry et al,[9] Biomin F has a better remineralisation potential when compared with Fluoride dentifrices. Thus, Biomin F was chosen over Novamin^{R} in the Bioglass category for the study.

Jagga et al.[13] compared the remineralisation effect of Novamin and 3M Clinpro Tooth crème. They reported TCP to have a higher value of remineralisation, compared to Novamin. Rao et al carried out a study to evaluate the remineralising potential of three different topical Fluoride agents, Duraphat Fluoride varnish (22,600 ppm F), ReminPro paste (1450 ppm F) and Clinpro Tooth crème

(950 ppm F). They reported Clinpro Tooth crème to show the best remineralisation potential.[17] Tulumbaci and Oba carried out a study to compare the efficacy of different remineralizing agents on primary and permanent teeth. They concluded that Clinpro 5000 is the most efficient amongst all the agents. However, due to risk of high Fluoride concentration in children, Clinpro Tooth Crème (950 ppm Fluoride) or GC MI Paste Plus are better alternatives.[18] Although, high concentrations of fluoride high demineralization inhibiting efficiency, they tend to arrest the lesion. This will cause the lesion to remain of the same size, which often becomes stained due to the organic debris.[19] It is believed that dentifrices consisting of high Fluoride concentrations causes plugging of hydroxyapatite crystals diffusion pathways and cause hyper remineralisation.[20] Thus, 3M Clinpro Tooth Crème consisting of 950 ppm Fluoride was chosen for this study.

There are two basic types of fluoride: organic (e.g. Amine Fluoride) and inorganic fluorides (e.g. Sodium Fluoride). It was found that organic fluorides like amine fluoride have superior anti-cariogenic properties to inorganic ones.[21] A study done by Shetty et al in 2016 showed similar results where organic fluorides restored the enamel micro hardness more efficiently than inorganic fluorides.[22] A study done by Arnold et al shows similar results. They concluded that amine fluoride containing toothpaste has superior remineralisation effect on caries like enamel lesions followed by Sodium Fluoride and Sodium Monofluorophosphate respectively.[23]

Various studies have reported the caries inhibitory potential of Amine Fluoride. Amine fluoride (1000 ppm F) is a surface active agent manifesting anti-glycolytic and tensioactive properties. Owing to its surface active property, it causes the hydrophobic part of the agent to be self-aligned towards the oral cavity and the hydrophilic part close to the tooth surface. This results in fluoride accumulation close to the tooth surface.[24] The accumulated Fluoride acts as a labile fluoride reservoir which directly provides for the formation of calcium fluoride.[25,26] There is fast distribution and a uniform coating on the tooth surface for a long period of time. The end result of using amine fluorides is increased availability of fluoride with protection against acid attack. Thus, amine fluoride containing dentifrice (Amflor) was chosen for this study[3,27–30]

The primary elements that were analyzed for the remineralisation potential of the dentifrices were Calcium/Phosphorus ratio and Fluoride. Any significant changes seen with other element, that is, Carbon, Oxygen and Silicon with reference to the mechanism of action of any of the dentifrices were also reported. The greatest increase in Calcium, Phosphorus and Ca/P ratio was shown by Group I (Biomin F) followed by Group 3 and 2 respectively. This shows that the positive difference in Ca/P ratio is the greatest for Biomin F, indicating that the remineralising efficiency of Biomin F is better than the other two. There was increase in fluoride content seen in all three groups but the maximum was with Group 2. However, the results were not statistically significant. Amongst the other findings, interesting changes were seen in Carbon, Oxygen, Silicon and Phosphate content with reference to Biomin F. The maximum decrease in Carbon content and an increase in Silicon and Phosphate content was seen with Biomin F. Study by Omran et al showed similar results with statistically significant increase in Phosphorus content but there was no significant increase in Ca/P ratio.[15] Study by Bakry et al reported similar results in which the SEM-EDS analysis of the surface showed a layer rich in calcium and phosphorus and traces of silica. SEM-EDS analysis of the interface showed a rich layer of Calcium phosphate and a smooth subsurface layer.[9] Narayana et al reported similar results wherein they concluded bioactive glass to have better remineralising potential when compared to Amflor.[12]

Bioactive glasses are silicate-based materials that have the ability to form a strong chemical bond with both soft and hard tissue. Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica (sodium calcium phosphosilicate), which are all elements naturally found in the body. When in contact with saliva or water, bioactive glass first releases sodium ions. This elevates the pH into the range essential for Hydroxyapatite formation (7.5-8.5). The calcium and phosphate are released to supplement the normal levels found in saliva. This increase in ionic concentration, combined with an increase in pH causes the ions to precipitate onto the tooth surface and form calcium hydroxycarbonate apatite (HCA) to remineralize the defect and to occlude open tubules. Biomin F contains Fluoride along with the traditional Bioglass composition. This gives an additional advantage of formation of Fluor apatite, which is more acid resistant. The higher phosphate content and silicon helps in maintaining the network, which ensures Fluor apatite formation.[31] All the ions are embedded in the glass network which dissolves over 12 hours. To exaggerate the effect, Biomin F contains a polymer which adheres the glass network onto the tooth surface for longer periods of times,ensuring a slow, sustained release of mineral ions and maintaining a constant Fluoride level.[14]

Featherstone has described newly mineralized bone and teeth to actually be an impure Hydroxyapatite, that is, Carbonated Hydroxyapatite. This carbonate ion makes the tooth less acid resistant as it forms a defect by replacing the phosphate ion in the crystal lattice. This defect leads to lattice distortion which ultimately causes micro-stresses in the network. All this has a significant effect on the solubility of apatite crystals.[15] Thus, an increase in phosphate and decrease in carbon shows more replacement of carbonate by phosphate, ultimately leading to the formation of more amount of Fluorapatite and Hydroxyapatite. This makes the

enamel more resistant to acid attack.

Amflor reported a higher diff Ca/P ratio than Clinpro Tooth crème but the increase in Calcium, Phosphate and Fluoride and a decrease in carbon content was more for Clinpro Tooth crème. Thus, as we have seen in the mechanism of action of Biomin F, a higher amount of phosphate and decreased amount of carbon can be attributed to a more stable crystal lattice formation (decreased amount of Carbonated Hydroxyapatite formation) which is more resistant to acid attack. Thus, Clinpro Tooth Crème is being considered to have a better remineralising potential than Amflor.

4.1. Limitations of this study

No pH cycling was used in this study. The use of pH cycling would have simulated the oral conditions and helped in determination of the remineralisation efficiency of the dentifrices.

4.2. Future scope of the study

Biomin F is a relatively a recent technology and few studies have tested it. Thus, more comparative in-vitro studies which are prospective, comparative and controlled and clinical trials need to be carried out.

Comparative studies need to be carried out to determine the acid resistance capability of the crystal structure formed on remineralisation by these dentifrices.

5. Conclusion

The present in-vitro study aimed at assessing the remineralisation potential of three different dentifrices using Energy Dispersion X-Ray Spectroscopy. The three different dentifrices used in this study are Biomin F Toothpaste, 3M Clinpro Tooth Crème and Amflor Toothpaste.

1. Considering Ca/P ratio and change in Fluoride composition post dentifrice application, Biomin F showed the best remineralisation efficiency when compared to the other two.
2. The change in fluoride concentration was not statistically significant when the three groups were compared.
3. Carbon, Oxygen and Silicon were assessed. Carbon significantly decreased in Biomin F group followed by Clinpro Tooth crème and least decrease was seen with Amflor toothpaste. This was attributed to more conversion of Carbonated Hydroxyapatite into Hydroxyapatite and Fluor apatite.
4. It was concluded that Biomin F possesses the best remineralisation efficiency with the most biomimetic deposition of enamel.

6. Source of Funding

None.

7. Conflict of Interest

None.

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