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COMPARATIVE EVALUATION OF REMINERALIZING POTENTIAL OF DIFFERENT REMINERALIZING TOOTHPASTES ON INTIAL CARIES AND EROSIVE LESION; A SEM STUDY

Dentistry

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ABSTRACT

The oral cavity and teeth are frequently exposed to different pH variations due to the diet. These changes can cause enamel demineralization and erosion. These days due to high carbohydrate diet and frequent consumption of carbonated beverages, there is increased chances of dental caries and erosion of tooth. The aim of this study was to evaluate the remineralizing potential of different remineralizing toothpastes on initial caries and erosive lesion using SEM-EDX analysis and surface profilometer. **Method:** Sound 24 molar teeth with no defects or caries were taken for the study. They were divided into 3 groups-Group A was exposed to demineralizing solution and artificial caries was created on it; Group B was exposed to 1% citric acid and Group C was exposed to sports drinks and erosive lesion was created on it. SHY-NM (subgroup A1,B1,C1) ACLAIM (subgroup A2,B2,C2) COLGATE SENSTIVE PLUS (subgroup A3,B3,C3) and REMIN(A4,B4,C4) were applied on the teeth to remineralize. Ca/P% was measured by SEM-EDX analysis and surface roughness by SURFACE PROFILOMETRY. These scans were taken in three intervals- before demineralization, after demineralization and erosion, after remineralization. **Result:** In Group A, the mean Ca/P% for SHY-NM was the highest (2.243±0.228), but it was not statistically significant (p=0.680). REMIN exhibited significant improvement in Group B (1.848±0.065, p<0.001), while SHY-NM excelled in Group C (5.067±1.018, p<0.001). Surface roughness analysis revealed significant Ra/Rq values for SHY-NM and REMIN across groups (p<0.001) **Conclusion:** all remineralizing toothpastes demonstrated their efficacy but SHY-NM and REMIN comparatively had higher efficacy against initial caries and erosive lesion.

KEYWORDS

carbonated beverages, erosion, initial caries lesion, remineralization

INTRODUCTION

Enamel is the hardest tissue of the body made up of hydroxyapatite crystals arranged in prisms. [1]This microporous structure of the enamel keeps the demineralization and remineralization balance dynamic by providing ion exchange between the oral environment and i.e., saliva, plaque and tooth surface. [2] When oral pH surpasses 5.5, saliva is supersaturated and minerals precipitate otherwise the minerals dissolves in unsaturated saliva. Dental caries results of a microbial metabolism on tooth surface and leads to significant loss of tooth minerals. Studies suggest that the prevalence of dental caries is a pressing health concern affecting at least half of the Indian population.[3] The first clinical sign of dental caries is the appearance of white spot lesion on tooth surface which is due to demineralization of enamel subsurface.[4] Dental erosion is considered a partial chemical dissolution which is caused mainly by repeated exposure to acids of both microbiological and nonmicrobiological origin.[5] Lately there is increased consumption of carbonated beverages which have high titres of pH and energy drinks show high loss of tooth structure and minerals.[6] Substances with low pH and strong buffering capacity demineralize more, while those rich in calcium and phosphate are less damaging to teeth.[7] It is known that sodium fluoride prevents demineralization by forming fluorapatite crystals.[8] Multiple in-vitro studies have examined the efficacy of remineralization agents against initial caries with different methods as an alternative to sodium fluoride.[9,10] Many studies have used SEM as a qualitative method to assess the surface morphology of initial caries and erosive lesion. In addition, non-destructive techniques allow long term evaluation of effects of remineralizing agents on enamel. [10,11] This study aimed to compare and evaluate the remineralizing potential of different remineralizing toothpastes on initial caries lesion and erosive lesion by using SEM-EDX and surface profilometry. The null hypothesis was, there would be no difference in remineralizing potential of various remineralizing toothpastes used for initial caries lesion and erosive lesion.

MATERIAL AND METHODS:

The sample size has been estimated by using G power software, using ANOVA: fixed effects, omnibus, one –way. The minimum sample size was 24.The type α err prob was 0.05, the power (1-β err prob) was 0.95 and the effect size was 1.0377259.The samples were divided into 3 groups: GROUP A (demineralizing sol), GROUP B (1%citric acid) and GROUP C (sports drink).Each group had four subgroups: SHY-NM (sub group A1,B1,C1), ACLAIM (A2,B2,C2), COLGATE SENSITIVE PLUS (A3,B3,C3) REMIN (A4,B4,C4). Each subgroup had 8 samples. Samples were randomly assigned to each group.

Sample Preparation

24 caries free extracted molars were used for the study. Decayed, fractured, cracked, hypomineralized, hypocalcified and restored teeth were excluded from the study. Tissue residues and stains on the surface of teeth were cleaned using an ultrasonic scaler. The teeth were stored in a 0.1% thymol solution at room temperature until the day of study. The teeth were sectioned horizontally using a diamond disc at the level of CEJ, separating the crown and root. The crowns were then sectioned at the level of central fossa. The teeth were placed on acrylic blocks with their buccal and lingual surface and coated with acid-resistant nail varnish.

Methodology Of The Experiment

Flow chart of the experiment is demonstrated in figure 1. Following the initial scan the teeth were exposed to GROUP A: DEMINERALIZING SOL (pH-4.4 ;2.2mM $CaCl_2$, 2.2mM $NaHPO_4$, 0.5M CH_3COOH, 1M KOH) for 6hrs and remineralizing sol (pH-7; 1.5mM $CaCl_2$, 0.9mM $NaHPO_4$, 0.15M KCl, 1M KOH) for 18hrs.To create erosion teeth were exposed to GROUP B: 1%CITRIC ACID SOL (pH-4.2) and GROUP C: SPORTS DRINK- REDBULL (pH: 3.3) for 2 min for 4 times/day and remineralizing solution for the remainder of day. The experiment was done in room temperature for 7 days. The second SEM-EDX and SURFACE ROUGHNESS analysis was done after demineralization and erosion.

The four remineralizing toothpastes SHY-NM (batch number-SN23062; Group Pharmaceuticals ltd), ACLAIM (batch-A1202; Group Pharmaceuticals ltd), COLGATE SENSITIVE PLUS (batch-B0951; Colgate-Palmolive India ltd) & REMIN (batch-C232321223; ICPA Health Products Ltd) were applied over the tooth surface twice for 2 min/day using a soft bristled toothbrush and were placed in demineralizing solution for 1hr every day to simulate the oral pH and remineralizing sol for 23hr to simulate saliva buffer. This treatment was done for 15 days and final SEM-EDX and SURFACE

ROUGHNESS analysis was performed.

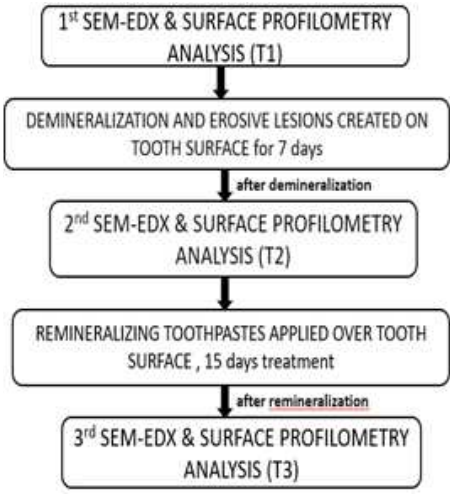


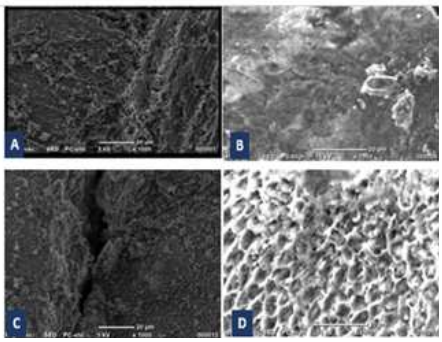
Fig 1: flowchart of the study

RESULTS:

IBM SSP software was used for statistical analysis. One-way analysis of variance (ANOVA) with post-hoc turkey test was used to determine the remineralization efficacy of the agents in terms of the factors such as Ca/P% and Ra/Rq. P<0.05 values were considered significant.

SEM-EDX analysis

FIGURE 2:



In this study, SEM-EDX done initially showed a healthy enamel structure having a "keyhole" appearance (Fig 2A). After demineralization & erosion (Fig 2B,2C,2D) it was observed that the keyhole like appearance had disappeared and there was increased micro porosities seen in all the groups. Group A showed more porous structure. The prism structure of enamel was destroyed in Group B. Group C showed more micro porosities and irregular prismatic structure. Group A (2.243±0.228) showed a higher mean Ca/P% compared to Group B (2.061±0.074) and Group C (2.013±0.267) had highest demineralization after treatment with sports drink. But this was not statistically significant (p value=0.1414).After remineralization against initial caries and erosive lesion, SHY-NM (Fig 3) showed a granular appearance and the porosities were worn out having a smoother surface; ACLAIM (Fig 4) showed half-filled pits and globular structures; COLGATE SENSITIVE PLUS (Fig 5) had noticeable porosities and voids; REMIN (Fig 6) appeared more homogenous and porosities had reduced giving a flatter surface.

As mentioned in table 1, on comparing between all four toothpastes in Group A, SHY-NM (2.243±0.228) had the higher mean Ca/P %, but it was not statistically significant (p value=0.680). In Group B, REMIN (1.848±0.065) had the higher mean Ca/P %, which was statistically significant (p value<0.001). In Group C, SHY-NM (5.067±1.018) had the higher mean Ca/P %, which was statistically significant (p<0.001). Pairwise comparison of Group A has no statistically significant values; Group B showed REMIN with high Ca/P% followed by COLGATE SENSITIVE PLUS, ACLAIM & SHY-NM each subgroup having a statistical significance (p<0.05). SHY-NM (p<0.05) was statistically significant in Group C.

Surface Roughness Analysis

Surface profilometry was done at three intervals: pre-treatment, post demineralization and erosion & post remineralization therapy. Roughness had increased in Group C(mean=0.771), followed by Group B (mean=0.960) and Group A(mean=1.007), none of which were statistically significant. As mentioned in table 2, on comparing between all four toothpastes in Group A, SHY-NM (1.417±0.081) had the higher mean Ra/Rq, which was statistically significant (p value<0.001). In Group B, REMIN (4.073±0.791) had the higher mean Ra/Rq, which was statistically significant (p<0.001). In Group C, SHY-NM (2.013±0.232) had the higher mean Ra/Rq, which was statistically significant (p=<0.001)

DISCUSSION:

Enamel a calcified structure, does not exhibit additional biological activity once formed. Enamel rods illustrate the embryology of enamel. Saliva, diet, drinks and other substances come into direct contact with human enamel daily. Following a high carbohydrate or highly acidic diet, the pH of the oral cavity decreases, increasing the risk of enamel demineralization, as described in Stephen's curve. [11,12] The primary components are phosphate and calcium ions with a molecular ratio of Ca/P of 1.6 far from the surface and 1.4–1.45 within it[18] An initial carious lesion results from a predominance of demineralization processes driven by bacterial biofilms and their acid production. Conversely, a net loss of mineral leading to dissolution of dental hard tissues can result in a clinically visible caries lesion. [13] With more people consuming carbonated beverages such as soft drinks, sports drinks and citrus fruit juices, the extrinsic element is becoming increasingly significant in modern society. [14,15,16]

SEM analysis provides a distinctive three-dimensional image that is helpful in analyzing the surface characteristics. The impact of precipitates that are superficially deposited and result from the dissolution of minerals by different agents have been demonstrated using SEM analysis. Surface profilometry quantifies the loss of tooth structure and can analyze deep erosion pits. [17,18,19]

One evolving area of study is the early, non-invasive treatment of developing lesions through remineralization of the enamel surface. Innovative products with more effective bio-reactive and biomimetic qualities have been developed due to the rapid rise of biomaterials and nanotechnologies. Additionally, nanostructured materials perform better than counterparts containing macro-dimensioned particles due to their increased surface/volume ratio. [20,21,22] Bioactive glass, novamin/ calcium sodium phosphosilicate is the remineralizing agent used in SHY-NM and REMIN, which has antiplaque and hypersensitivity action. Calcium sodium phosphosilicate/ novamin in contact with saliva allows sodium ions to exchange with hydrogen ions, which forms a silica-rich layer of calcium phosphate raising the pH and forms a layer of hydroxycarbonate apatite layer promoting remineralization. This layer seals the dentinal tubules. [23] Previous studies done on novamine, have shown that calcium and phosphate ions replaced the mineral loss in initial caries and erosive lesion forming a protective layer on tooth surface enhancing remineralization efficacy. [24,25,26] In the presented study, SHY-NM demonstrated higher remineralization capacity against demineralization solution & Redbull sports drink. This was supported by other studies as well. [27,28,29]REMIN (newly launched) containing 5% calcium sodium phosphosilicate/ novamin showed higher remineralization against the 1% citric acid group. In our study, ACLAIM containing hydroxyapatite showed lower statistical significance compared to the other two toothpastes containing novamin. Aclaim showed improved remineralizing effect but failed to fill pores with hydroxyapatite layer in accordance with other studies. [30,31,32] Colgate Sensitive Plus containing sodium monofluorophosphate and arginine bicarbonate did not show any statistical significance and had the least remineralization capacity, similar to results seen in other studies [33,34]

On SEM scanning after demineralization and erosion, multiple voids and increased porosities were seen increased in REDBULL sports drink then, demineralizing solution and 1% citric acid having the least microporous structure. On SEM imaging done after remineralization, SHY-NM and REMIN both showed a homogenous surface with increased depicting a flatter surface. ACLAIM showed half-filled porosities. COLGATE appeared rough with multiple microporosities and voids.

In surface profilometry done after demineralizing solution and erosion, highest surface roughness was seen in Redbull followed by

demineralizing solution and 1% citric acid. In Surface profilometry done after remineralization, surface roughness was highest in COLGATE followed by ACLAIM and REMIN and SHY-NM having the least surface roughness.

Limitations Of The Study Were As Follow: (i) short duration of experiment, (ii)inclusion of primary teeth, (iii)inability to depict in vivo conditions and (iv) standardization of the SEM-EDX and SURFACE ROUGHNESS analysis.

Future Scope: Micro-CT analysis can be used to determine mineral density. In vivo simulation can be used to determine the erosive and remineralizing properties of toothpastes.

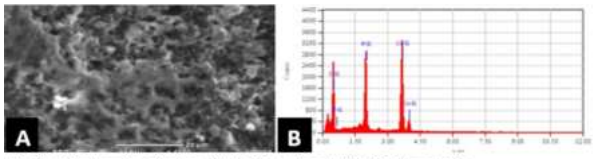


FIG 3: A) Post remineralization of SHY NM toothpaste B) EDX interpretation

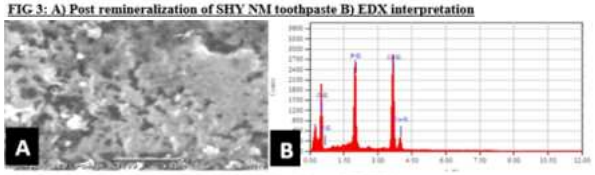


FIG 4: A) Post remineralization of ACLAIM toothpaste B) EDX interpretation

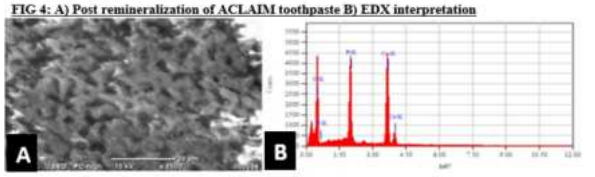


FIG 5: A) Post remineralization of COLGATE SENSITIVE PLUS toothpaste B) EDX interpretation

CONCLUSION:

In conclusion, this research emphasizes enamel's vulnerability to demineralization from diet and bacteria. Innovative toothpaste like SHY-NM and REMIN effectively reverse initial tooth demineralization. Using these alongside mechanical plaque removal helps protect enamel from demineralization, maintaining dental health.

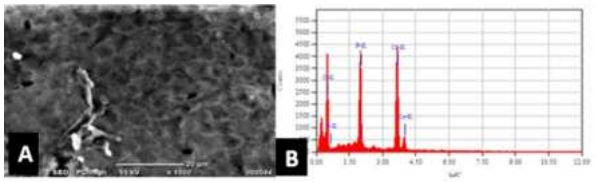


FIG 6: A) Post remineralization of REMIN toothpaste B) EDX interpretation

Table 1: Comparison of Ca/P between pastes under different solutions

Ca/P %		Mean	Std. Deviation	F	P value
DEMINERALIZING SOLUTION	SHY-NM	2.243	.228	.520	.680
	ACLAIM	2.199	.330		
	COLGATE	2.044	.024		
	REMIN	2.162	.077		
1% CITRIC ACID	SHY-NM	.448	.046	192.609	**<0.001**
	ACLAIM	.922	.129		
	COLGATE	1.467	.017		
	REMIN	1.848	.065		
REDBULL	SHY-NM	5.067	1.018	23.699	**<0.001**
	ACLAIM	2.455	.754		
	COLGATE	1.393	.033		
	REMIN	1.173	.084		

P value by ANOVA

P<0.05 statistically significant, statistically significant p value in BOLD

Table 2: Comparison of Ra/ Rq between pastes under different solution

Ra/ Rq		Mean	Std. Deviation	F	P value
DEMINERALIZING SOLUTION	SHY-NM	1.417	.081	22.595	**<0.001**
	ACLAIM	.783	.067		
	COLGATE	.797	.021		
	REMIN	1.006	.186		
1% CITRIC ACID	SHY-NM	.707	.040	40.420	**<0.001**
	ACLAIM	2.566	.229		
	COLGATE	3.763	.099		
	REMIN	4.073	.791		
REDBULL	SHY-NM	2.013	.232	141.688	**<0.001**
	ACLAIM	.308	.052		
	COLGATE	.212	.011		
	REMIN	.258	.092		

P value by ANOVA

P<0.05 statistically significant, statistically significant p value in BOLD

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