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Comparative Evaluation of Remineralization Potential of Commercially Available Remineralization Agents - An *In-Vitro* Micro-Hardness Study.

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Abstract

Introduction: Dental erosion has become a common etiology for dentinal hypersensitivity with increase in the amount of cola drinks in the recent years. This in-vitro study was aimed to compare and evaluate remineralization potential of commercially available remineralization agents GC Tooth Mousse, Toothmin, Aclaim and Conybio plus after immersion in cola solution using Vickers micro hardness test.

Materials and Methods : Twenty-five extracted human premolars tooth were taken for the study. Each tooth was split into four enamel blocks of dimensions (3mm long, 3mm width and 2mm thick) and embedded in acrylic blocks of size 5 x 5 x 5 mm. Each specimen was then immersed in carbonated drink with pH 2.7 for 8 min and washed with deionized water after every 2 min simulating in vivo soft drink consumption for one week. After the erosion process was completed, all the specimens were rinsed in deionized water and blotted dry . Fresh remineralizing paste was applied to the treatment halves for 3 minutes at 0, 8, 24, 36 and 48 hours. Vickers microhardness measurements was performed at 24- and 48-hour post treatment.

Conclusion: Toothmin claims to produce better remineralization compared to other commercially available toothpaste.

Key words: GC Tooth Mousse, Toothmin, Aclaim and Conybio plus

Introduction

Dentin hypersensitivity is a common complaint, affecting the teeth of many individuals. The etiology is multifactorial; however, over recent years with increase in junk food and aerated drink consumption, there is increase in occurrence of teeth erosion [1]. Dental erosion is a localized loss of the tooth surface by a chemical process of acidic dissolution of nonbacterial origin [2]. Demineralization is the process of removing minerals ions from hydroxyapatite crystals of hard tissues. A typical demineralization regime occurs during an acidic attack, which causes chemical dissolution of both the organic and inorganic matrix components. Excessive consumption of soft drinks decreases the pH in the oral cavity that cause dental erosion by facilitating acid diffusion in and mineral content out of tooth thus causing demineralization [3].

Many remineralizing formulations are available and the most commonly used remineralizing agents are GC Tooth Mousse (casein phosphopeptide - amorphous calcium phosphate paste) and Toothmin (Bioactive glass). Oshiro et al in their invitro study on evaluating the effect of CPP-ACP paste on demineralization by observing the treated tooth surface using an FE-SEM showed that the CPP-ACP paste was effective in preventing demineralization of enamel and dentin more effectively than the placebo paste (CPP-ACP free) [4]. Casein phosphopeptide can deliver amorphous calcium phosphate and can also help the ACP to bind with the dental enamel [5]. The sticky CPP part of the CPP-ACP complex binds readily to the enamel, biofilm and soft tissues, delivering the calcium and phosphate ions to reform the apatite crystals [6]. Toothmin, a Bioactive glass – calcium sodium phosphosilicate which delivers silica and ionic calcium, phosphorus and sodium, which are necessary for bone and tooth mineralization. Anurag Agarwal et al in their study evaluated the protective potential of toothmin and novamin containing toothpastes on enamel surface under confocal microscope and showed that enamel surfaces treated with calcium sucrose phosphate (Toothmin) paste exhibited the least lesion depths followed by enamel surfaces treated with the novamin tooth paste and control group respectively [7]. There are new remineralizing formulas in the market that gives promising results among those are Aclaim and Conybio. Aclaim consists of nano hydroxyapatite crystals, which are similar to apatite crystals of the tooth enamel in morphology and crystal structure [8]. Conybio contains chitosan, a natural biopolymer which has excellent properties like biocompatibility, anti-inflammatory, bio-adhesive which makes it suitable for treating dentine hypersensitivity [9].

On thorough review of literature, there are no studies comparing these four remineralizing agents and thus the aim of our study is to evaluate the remineralization potential of commercially available remineralization agents after application on tooth surface affected by cola solution using knoop microhardness. The null hypothesis states that there is no difference in remineralization potential among all the four commercially available remineralizing toothpaste.

MATERIALS AND METHODS:

The study was approved by the research committee (01042105). Twenty-five intact human premolars extracted for orthodontic reasons were selected for the study. Teeth with caries, fracture, discoloration or any developmental anomaly like enamel hypoplasia were excluded to avoid the discrepancies in mineralization . All selected teeth were used within three months of extraction as per recommended Occupational Safety & Health Administration (OSHA). The samples were sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc, so that four enamel blocks of dimensions (3mm long, 3mm width and 2mm thick) were made from each sample. These samples were embedded in acrylic blocks of size 5 x 5 x 5 mm. The basleine vickers indenter test was performed using Vickers Microhardness Testing Machine (SRM Dental College, Ramapuram, Chennai).Each specimen was then immersed in carbonated drink with pH 2.7 for 8 min and washed with deionized water after every 2 min simulating in vivo soft drink consumption for one week. After the erosion process was completed, all the specimens were rinsed in deionized water and blotted dry. Vickers test was performed again to observe the changes in the enamel due to demineralization.

The specimens were randomly divided into 5 groups of 20 samples each for remineralization protocols as follows: Group 1 was treated with remineralizing paste containing CPP-ACP (GC Tooth Mousse, Group Pharmaceuticals LTD,) group 2 was treated with remineralizing paste containing Bioactive glass (Toothmin), group 3 was treated with remineralizing paste containing nano-sized hydroxyapatite (Aclaim), group 4 was treated with remineralizing paste containing chitosan (Conybio) and group 5 (control group) received no treatment. The corresponding remineralizing agents were applied on the samples for 4 minutes and left in artificial saliva (AS) with 1ppm fluoride at 37°C. The Group 5 samples were left in artificial saliva without any treatment. Fresh remineralizing paste was applied at 8, 24 and 48 hours. Before applying the paste, the samples were washed twice with distilled water. After remineralization process vickers hardness was measured for all the specimens at 24 and 48 hours. [10] Statistical analysis of Vickers microhardness at various time intervals within each group were compared using one-way ANOVA at a significance level 0.05 using SPSS Software (Version 22.0)

RESULTS:

It is observed that Toothmin showed better remineralizing potential followed by Conybio plus, Aclaim and GC tooth moose at 24 hours. At 48th hour Toothmin and Conybio plus showed statistically significant remineralization potential compared to the other groups. (Table-1)

Table1: Remineralisation and demineralization potential at 24 and 48 hours.

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Baseline	GC TOOTH MOUSSE	NOVAMIN	9.310*	1.842	.000	4.35	14.27
		ACLAIM	16.150*	1.842	.000	11.19	21.11
		CHITOSAN	4.810	1.842	.060	-.15	9.77
	NOVAMIN	CPP-ACP	-9.310*	1.842	.000	-14.27	-4.35
		ACLAIM	6.840*	1.842	.004	1.88	11.80
		CHITOSAN	-4.500	1.842	.087	-9.46	.46
	ACLAIM	CPP-ACP	-16.150*	1.842	.000	-21.11	-11.19
		NOVAMIN	-6.840*	1.842	.004	-11.80	-1.88
		CHITOSAN	-11.340*	1.842	.000	-16.30	-6.38
	CHITOSAN	CPP-ACP	-4.810	1.842	.060	-9.77	.15
		NOVAMIN	4.500	1.842	.087	-.46	9.46
		ACLAIM	11.340*	1.842	.000	6.38	16.30
Demineralization	GC TOOTH MOUSSE	NOVAMIN	-5.130	4.877	.720	-18.26	8.00
		ACLAIM	-1.280	4.877	.994	-14.41	11.85
		CHITOSAN	-4.900	4.877	.748	-18.03	8.23
	NOVAMIN	GC TOOTH MOUSSE	5.130	4.877	.720	-8.00	18.26

		ACLAIM	3.850	4.877	.859	-9.28	16.98
		CHITOSAN	.230	4.877	1.000	-12.90	13.36
	ACLAIM	GC TOOTH MOUSSE	1.280	4.877	.994	-11.85	14.41
		NOVAMIN	-3.850	4.877	.859	-16.98	9.28
		CHITOSAN	-3.620	4.877	.879	-16.75	9.51
	CHITOSAN	GC TOOTH MOUSSE	4.900	4.877	.748	-8.23	18.03
		NOVAMIN	-.230	4.877	1.000	-13.36	12.90
		ACLAIM	3.620	4.877	.879	-9.51	16.75
Remineralization - 24hrs	GC TOOTH MOUSSE	NOVAMIN	-6.890	4.281	.386	-18.42	4.64
		ACLAIM	-64.830*	4.281	.000	-76.36	-53.30
		CHITOSAN	-30.850*	4.281	.000	-42.38	-19.32
	NOVAMIN	GC TOOTH MOUSSE	6.890	4.281	.386	-4.64	18.42
		ACLAIM	-57.940*	4.281	.000	-69.47	-46.41
		CHITOSAN	-23.960*	4.281	.000	-35.49	-12.43
	ACLAIM	GC TOOTH MOUSSE	64.830*	4.281	.000	53.30	76.36
		NOVAMIN	57.940*	4.281	.000	46.41	69.47
		CHITOSAN	33.980*	4.281	.000	22.45	45.51
	CHITOSAN	GC TOOTH MOUSSE	30.850*	4.281	.000	19.32	42.38
		NOVAMIN	23.960*	4.281	.000	12.43	35.49
		ACLAIM	-33.980*	4.281	.000	-45.51	-22.45
Remineraization - 48hrs	GC TOOTH MOUSSE	NOVAMIN	8.720	5.294	.366	-5.54	22.98
		ACLAIM	-49.220*	5.294	.000	-63.48	-34.96
		CHITOSAN	-16.230*	5.294	.020	-30.49	-1.97
	NOVAMIN	GC TOOTH MOUSSE	-8.720	5.294	.366	-22.98	5.54
		ACLAIM	-57.940*	5.294	.000	-72.20	-43.68
		CHITOSAN	-24.950*	5.294	.000	-39.21	-10.69
	ACLAIM	GC TOOTH MOUSSE	49.220*	5.294	.000	34.96	63.48
		NOVAMIN	57.940*	5.294	.000	43.68	72.20
		CHITOSAN	32.990*	5.294	.000	18.73	47.25
	CHITOSAN	GC TOOTH MOUSSE	16.230*	5.294	.020	1.97	30.49
		NOVAMIN	24.950*	5.294	.000	10.69	39.21
		ACLAIM	-32.990*	5.294	.000	-47.25	-18.73

*. The mean difference is significant at the 0.05 level.

DISCUSSION:

Dental erosion is defined as loss of dental hard tissue by a chemical process that does not involve bacteria (Pindborg, 1970) [11].Dental erosion can be due to extrinsic or intrinsic causes. Extrinsic causes include demineralizing acidic foods-such as citrus fruits and acidic beverages (Eccles and Jenkins, 1974; Smith and Knight, 1984; Asher and Read, 1987) [12,13,14] and some medicines such as effervescent vitamin C preparations, chewable vitamin C tablets (Eriksson and Angmar-Mansson, 1986; Meurman and Murtomaa, 1986) [15,16], and iron tonics (James and Parfitt, 1953) [17]. Nowadays, exposure to extrinsic acid can also be associated with leisure activities such as frequent swimming in chlorinated pool water (Centerwall et al., 1986) [18]. Intrinsic causes of erosion include recurrent vomiting because of psychological disorders, e.g., in anorexia (Hellstrom, 1977; Knewitz and Drisko, 1988) [19], or regurgitation of gastric contents because of some abnormality in the gastrointestinal tract (Ismail-Beigi et al., 1970; Eccles, 1978; Pope, 1982; Myllirniemi and Saario, 1985) [20,21,22]. One important additional factor in dental erosion is low salivary flow, which, naturally, results in inadequate rinsing and buffering of demineralizing acids on tooth surfaces (Dawes, 1970).[23]

Enamel is the most mineralized tissue of the body, forming a very hard, thin, translucent layer of calcified tissue that covers the entire anatomic crown of the tooth. It varies in color (typically from yellowish to grayish white) depending on variations in the thickness, quality of its mineral structure and surface stains. Enamel is composed primarily of inorganic materials roughly 95% to 98% of it is calcium and phosphate ions that make up strong hydroxyapatite crystals. Yet, these are not pure crystals, because they are carbonated and contain trace minerals such as strontium, magnesium, lead, and fluoride. These factors make "biological hydroxyapatite" more soluble than pure hydroxyapatite. Approximately 1– 2% of enamel is made up of organic materials, particularly enamel-specific proteins called enamelins, which have a high affinity for binding hydroxyapatite crystals. Water makes up the remainder of enamel, accounting for about 4% of its composition.The inorganic, organic, and water components of enamel are highly organized: millions of carbonated hydroxyapatite crystals are arranged in long, thin structures called rods that are 4 µm to 8 µm in diameter. In general, rods extend at right angles from the dento–enamel junction (the junction between enamel and the layer below it called dentin) to the tooth surface. Surrounding each rod is a rod sheath made up of a protein matrix of enamelins. The area in between rods is called interrod enamel, or interrod cement. While it has the same crystal composition, crystal orientation is different, distinguishing rods from interrod enamel. Minute spaces exist where crystals do not form between rods. Typically called pores, they contribute to enamel's permeability, which allows fluid movement and diffusion to occur, but they also cause variations in density and hardness in the tooth, which can create spots that are more prone to demineralization – the loss of calcium and phosphate ions – when oral pH becomes too acidic and drops below 5.5. In demineralization, the crystalline structure shrinks in size, while pores enlarge. When a tooth erupts, it is also not fully mineralized. To completely mineralize the tooth, calcium, phosphorous, and fluoride ions are taken up from saliva to add a layer of 10 µm to 100 µm of enamel over time. The mineral composition and structure of the enamel surface are partially products of the dynamic demineralization and remineralization process. The enamel demineralization process begins when these acids lower the pH of the biofilm to below 5.5. The acids result in the loss of calcium and phosphates from the surface and subsurface enamel. The demineralization process is reversible provided that the acidogenic properties of the biofilm are neutralized. The buffering capacity of saliva plays a critical role in helping restore a neutral pH at the tooth surface. Remineralization occurs when the dietary carbohydrate is removed and the pH of the biofilm is raised to approximately 7.0. Once the pH returns to higher than the critical point, demineralization is arrested and minerals can be added back to the partially dissolved enamel crystallites. The remineralization process is a natural repair mechanism to restore the minerals again, in ionic forms, to the hydroxyapatite (HAP) crystal lattice. It occurs under near-neutral physiological pH conditions whereby calcium and phosphate mineral ions are redeposited within the lesion from saliva and plaque fluid resulting in the formation of newer HAP crystals, which are larger and more resistant to acid dissolution. Numerous types of remineralizing agents have been researched and many of them are being used clinically, with significantly predictable positive results [24]. The recent researches on remineralization are based on biomimetic remineralization materials, having the capability to create apatite crystals within the completely demineralized collagen fibers. Synthesis of biomaterials are classified as top-down or bottom-up approaches. Top-down mineralization approach occurs by epitaxial growth over existing seed crystallites, which cannot occur by spontaneous nucleation of minerals on the organic matrix, such as demineralized dentine. In contrast to the top-down approach, the bottom-up approach starts with one or more defined molecular species, which undergoes certain processes that result in a higher-ordered and organized structure, such as self-assembly of amorphous nano-precursor particles and their subsequent mesoscopic transformation in biomineralization [25].

Dentin is a mineralized collagenous tissue and demineralized dentin does not significantly induce the deposition of calcium phosphate minerals in the remineralization solution. This is because there is no residual crystal on the surface of the demineralized dentin, which is detrimental to the deposition of calcium phosphate minerals. This top-down mineralization method does not depend on the spontaneous nucleation of minerals on organic substrates but on the epitaxial growth of residual apatite seeds ;thus, only loosely stacked plate crystals are present. In contrast, bottom-up methods assemble materials at nanoscale to form larger structures. The ideal approach to dentin remineralization is the “bottom-up” non-classical method. It is different from classical remineralization, which depends on pre-existing apatite crystals, but it is a biomimetic method mimicking the natural biological condition. Biomimetic non-collagen analogs and molecules are generally used for remineralization of dentin. The commonly used biomimetic molecules include casein phosphopeptide–amorphous calcium phosphate (CPP–ACP). [26]

Reynolds EC et al (2003) utilized microradiography to assess remineralization and found that CPP-ACP is better than a dentifrice with higher fluoride levels at promoting remineralization of subsurface lesions.[27] Kumar VL et al (2008) investigated the efficacy of CPP-ACP containing Tooth Mousse on the remineralization of enamel lesions and to compare its efficacy to that of a fluoride-containing toothpaste and found that CPP-ACP containing Tooth Mousse remineralized initial enamel lesions and it showed a higher remineralizing potential when applied as a topical coating after the use of a fluoridated tooth- paste. [28] An in vitro study by Raghu et al. evaluated the remineralization potential of CSP on demineralized enamel and found that toothmin increased microhardness of enamel and enhanced surface smoothness with significant remineralizing potential.[29] An in-vitro study conducted by Trishagni Chaudhury, Toothmin (Calcium Sucrose Phosphate CaSP), has got the best remineralization potential when compared to casein phosphopeptide-amorphous calcium phosphate with fluoride (CPP-ACPF), and bioactive glass on demineralized enamel.[30] In an in-vitro study by Abhishek Singh et al, they found that Aclaim on daily application will provide maximum protection against enamel demineralization, by the formation of new n-HAp layer which is insensitive to demineralization.[31] Another in-vitro study by Ritesh Kulal evaluated the remineralization potential of Nanohydroxyapatite toothpaste (ACLAIM) and found that Aclaim showed 97.62% occlusion of the tubules, enhancing the remineralization process when compared to Novamin and ProArgin.[32] Mohammad Chair Effendi et al (2020) evaluated the effectiveness of chitosan nanoparticles and microparticles and CPP-ACP in inhibiting the demineralization of tooth enamel and found that the Chitosan nanoparticles are significantly more effective than CPP-ACP and chitosan microparticles in inhibiting tooth enamel demineralization.[33] Tian et al (2012) in his invitro study of Induced synthesis of hydroxyapatite by chitosan for enamel remineralization which is based on the principles of biomineralization , spindle - shaped hydroxyapatites (HA) were synthesized through biomimetic method with chitosan as template under a controllable way in vitro and found that Chitosan-enhanced biomimetic remineralization.[34] In the present study ,the Toothmin group showed remineralization efficiency, which co-related with Renu et al, Gangrade et al, Menon et al and Raghu et al. Toothmin contains CaSP and inorganic calcium phosphate, in which 10% to 12% calcium and 8% to 10% phosphorus by weight are found. As it is completely soluble in water at all pH values, it is able to provide high concentrations of calcium and phosphate ions in the oral cavity without precipitation. As surface microhardness depends mainly on mineral content, especially the inorganic content of enamel, the reason for the above findings was Calcium Sucrose Phosphate's higher water solubility, which provides for higher concentrations of free calcium and phosphorus ions. It has been suggested that a 1% Toothmin solution contains about 30×10^{-3} M calcium and 10×10^{-4} M sucrose phosphate ions, while saliva contains only about 1.4×10^{-3} M calcium and 4×10^{-3} M phosphate ions. Therefore, the high concentrations of calcium and phosphate ions in Toothmin facilitates rapid remineralization of enamel.[35]

CONCLUSION:

Based on the results we conclude that Toothmin showed better remineralizing potential followed by Conybio ,Aclaim and GC tooth mousse.

CLINICAL SIGNIFICANCE:

1. Demineralization solution like cola drink can cause damage to the tooth structure even on short duration
2. Remineralization therapy is a contemporary approach for lesions detected at the earliest stage of the disease to render a noninvasive treatment approach that is crucial for dental profession in shifting their therapeutic approach to a new paradigm.
3. The present study showed that Toothmin had the potential to remineralize the artificial carious lesion.

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