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Research Article

COMPARATIVE EVALUATION OF THE EFFECT OF VARIOUS REMINERALIZING AGENTS ON BLEACHED ENAMEL SURFACE: AN *IN VITRO* STUDY

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ABSTRACT

The aim of this study is to comparative evaluate the remineralization potential of various newer remineralizing agents such as Fluoride enriched Casein Phospho-peptide Amorphous calcium phosphate, Beta Tri- calcium Phosphate and Hydroxyapatite based cream on bleached enamel surface using Surface hardness test. 40 freshly extracted intact human premolars extracted for orthodontic purpose were used to prepare 75 enamel sections from the buccal and palatal surfaces of t`he teeth. The teeth were divided into 5 groups of 15 specimens and subjected to surface treatment as follows: Grp 1: Intact tooth (Control group) - No Surface treatment, Grp 2: Bleaching with 35% Hydrogen peroxide (Pola- office), Group 3: CPP-ACPF (GC Tooth Mousse plus, GC Corp) for two applications, 4 mins each for 14 days, Group 4: β-TCP (3M ESPE ClinPro) for 4 min for 14 days, Group 5: Hydroxyapatite based cream (ReminPro, VOCO) for 30 minutes for 10 days. All the specimens were stored in artificial saliva in the intervals and throughout the study. The specimens were subjected to qualitative and quantitative evaluation using Surface Hardness test for Vickers Hardness Number. Enamel specimens treated with bleaching agent (Pola Office) showed a decrease in microhardness which was statistically significant when compared with unbleached enamel [Grp1]. Among the remineralising agents tested in the study Hydroxyapatite based cream was found to be marginally more effective then the CPP-ACPF and β-TCP which was not statistically different.

KEYWORDS: β-TCP, CPP-ACPF, HA, Pola Office, Surface Hardness.

INTRODUCTION

The most conservative and non invasive treatment for discoloured teeth is bleaching/ whitening therapy when compared with the other option of using resin-bonded composites, porcelain veneers, and/or crowns[1]. Currently available bleaching systems are composed of hydrogen peroxide (HP) or carbamide peroxide as one of its precursors. Although the appropriate mechanism of action is not relevantly clear, the superoxide free radical is the most likely cause for the oxidation of stained pigments[2,3].

Inspite of the favourable results of bleaching therapy, the documented possible side effects of the various bleaching agents used include alteration of the enamel surface morphology and mineralization level[4,5]. About 15-78% of patients reported experiencing hypersensitivity in the teeth after undergoing vital tooth bleaching[6]. This led to the present trend of advocating the application of remineralizing agents to control demineralisation and promote reminerlization following the bleaching procedures. Universally, the Remineralizing [RML] agents available contain calcium phosphate with or without fluoride which creates a supersaturated environment around the early lesion; thus, preventing mineral loss and forcing calcium and phosphate ions in the vacant areas[7].

Traditionally, fluoride based products were the chiefly available remineralizaing agents that have been extensively used and tested in the past. However, in the past decade several new therapeutic remineralizing agents based on various formulations such as Fluoride enriched Casein Phosphopeptide - Amorphous Calcium Phosphate[CPP-ACPF], Beta Tri-calcium Phosphate[β-TCP] and Hydroxyapatite [HA] have been introduced. Although, several studies have been conducted to evaluate the remineralization capacity of these newer agents on demineralized enamel; their comparative evaluation on bleached enamel is relatively unknown. In the present study, the effects of newer remineralizing agents such as Fluoride enriched Casein Phosphopeptide- Amorphous Calcium Phosphate, Beta Tri-calcium phosphate and a Hydroxyapatite based agent on the surface of bleached enamel were evaluated and compared using Surface hardness test.

MATERIAL AND METHOD

Freshly intact human premolars extracted for orthodontic purposes were collected from patients of age group 14-18 yrs. The collected teeth were disinfected and stored as per the recommendations and guidelines laid by Occupational Safety & Health Administration (OSHA)[8]. Teeth with white spot lesions, caries, restorations, erosions, surface discolorations were excluded.

A total 75 enamel specimens measuring4x4x2 mm were prepared using a diamond cutting disc and slow speed handpiece under water cooling. Except the front surface all the other

surfaces of teeth were covered using an impermeable surface coating material like nail polish.

The prepared specimens were randomly divided into 5 groups of 15 specimens each and designated as Group 1, 2, 3, 4 and 5.

Except for Group1 (Control), enamel surface of all the specimens in the Groups 2, 3, 4 and 5 were subjected to bleaching procedure with 35% Hydrogen Peroxide [H_2O_2] applied four times for 8 minutes [according to manufacturer's instructions].

The specimens were subject to surface treatment as follows:
Group 1: Intact tooth (Control group) - No Surface treatment.
Group 2: Bleaching with 35% Hydrogen peroxide (Pola- office)
Group 3: CPP-ACPF (GC Tooth Mousse plus, GC Corp) for 2 applications, 4 mins each for 14 days. [According to manufacturer's instruction]
Group 4: β-TCP (3M ESPE ClinPro) for 4 min for 14 days
Group 5: Hydroxyapatite based cream [ReminPro, VOCO] for 30 minutes for 10 days [According to manufacturers instruction]

All the specimens were stored in artificial saliva in the intervals and throughout the study.

The specimens were subjected to qualitative and quantitative evaluation using Surface Hardness test for Vickers Hardness Number [VHN]. Data obtained was compared and statistically analysed using One-Way ANOVA and Tukeys Post-hoc test

RESULTS

Estimation of microhardness of using surface hardness test

Group 1 (intact - control) showed highest microhardness value [328.27] while Group 2 (demineralised) showed lowest[301.93]. Amongst the experimental groups, Group 5 surface treated with HA based cream showed higher microhardness [314.27] compared to Group 4 surface treated with CPP-ACPF [311. 53] & Group 3 surface treated with β-TCP [311.2] respectively.
When comparing Group 1(control) and Group 2 (demineralised) statistical significant difference was found [Table 2].
When Group 1 (control) was compared with Group 3, 4 and 5 (surface treated with β-TCP, CPP-ACPF & HA) respectively no statistical significant difference was found [Table 2]; however, when Group 2 (demineralized) was compared the difference was statistically significant. [Table 2]
When Group 2 (demineralised) was compared with Group 3, 4 and 5 (surface treated with β-TCP, CPP-ACPF & HA) statistical significant difference was found [Table 2]

DISCUSSION

The effect of newer remineralizing agents such as Fluoride enriched Casein Phospho-peptide- Amorphous Calcium Phosphate [CPP - ACPF], Beta Tri- calcium Phosphate[β-TCP] and Hydroxyapatite [HA] based cream on bleached enamel surface was evaluated in this study, using Vicker's hardness test.

Amongst the remineralizing agents tested, the specimens treated with Hydroxyapatite based cream showed the highest surface hardness value followed by groups treated by CPP-ACPF and β-TCP respectively; while the specimens without any surface treatment after bleaching showed the lowest.

Amongst the control groups, Group 2 (without surface treatment after bleaching) showed lower surface hardness compared to Grp 1(intact non bleached surface), which was statistically significant and is in accordance with the results of several studies[9,10,11,31]. This can be attributed to the fact that during peroxide not only bleaches the enamel surface but also affects the interprismatic and intraprismatic portion by degrading some of the proteins around the enamel rods and crystallites. Thus, any mineral element associated with the enamel protein is also removed, which would explain the loss of calcium and phosphorous in these areas; thus, contributing to the occurrence of microstructural damage and possibly even to changes in microhardness [12, 13].

Enamel primarily consists of rods or prisms of highly organized and tightly packed mineral crystallites separated by organic material and water[14, 15]. Mature enamel comprises of different calcium phosphate salts (mostly hydroxyapatite) representing 88–90% of volume and 95–96% of its weight; while the organic matter accounts for approximately 1–1.5% of enamel weight. The presences of these small quantities of protein and water in enamel have an important softening or plasticizing effect[16, 17].

All the experimental groups surface treated with the different remineralizing agents showed a definite increase in surface hardness compared to the control group without surface treatment after bleaching [Grp 2]. This was statistically significant and is in agreement with the results of several studies[20, 24, 28]. The peroxide based bleaching agent caused a significant decrease in the enamel microhardness, when compared with the surface hardness of the intact unbleached group. However, subsequent treatment with different remineralizing agents showed a relative recovery in the microhardness values in comparison to the unbleached group.

The specimens in the study treated with Hydroxyapatite cream showed the highest values of surface microhardness, which was statistically significant in comparison to control (Group 2 - surface treated with bleaching) but not statistically significant when compared with Grp 4 [CPP-ACPF], Group 3 [β-TCP]. This is in agreement with a study by *Marchetti E et al*, which demonstrated a continuous increase in mineral gain and the return of the microhardness values to near baseline level when a HA based cream was used after bleaching procedure[18]. In several studies this has been attributed to the filling of the irregular enamel of the bleached surface with hydroxyapatite (HA) crystals and the formation of fluorapatite (FA) due to the high fluoride content of 1450 ppm fluoride, which is 61% higher than other brands available [900 ppm in CPP-ACPF & 950 ppm in β-TCP][19,20,21].

In this study the CPP-ACPF treated group showed the second highest microhardness recovery, which was statistically significant when compared with Grp 2 (surface treated with bleaching) but not with Grp 5 (HA) and Grp 3 (β-TCP). This is in agreement with studies by Hala M Abass et al, Se abo hammar et al, Sule Bayrak et al, Asmaa Alkhtib et al [22-25] and can be due to the fact that the peptides in the CPP–ACP may diffuse into the inter-crystallite spaces, (as the size of the peptide complex is small [~2 nm]) created by bleaching. It localises and increases the bioavailability of calcium and phosphate ions and may enhance the potential for remineralization and deposition of minerals around the "freshly cleaned" enamel crystallites, leading to an increase in hardness thus regaining the pre-bleaching enamel physical properties. Incorporation of fluoride into CPP-ACP as CPP-ACPF nanocomplex co-localizes fluoride ions along with calcium and phosphate, maintaining a state of super saturation on the tooth surface; hence, resulting in higher

levels of remineralisation and fluoride incorporation into the mineral phase[26].

A study done by Metz et al, stated that fluoride incorporation into the enamel crystal needs saturation condition that favours fluoride incorporation, also if enamel specimens were demineralised prior to a fluoride product application, then the driving force will allow incorporation of the fluoride ion into the enamel apatite to increase the surface hardness[27].

In this study, specimens treated with Beta Tri- calcium Phosphate [β-TCP] showed the lowest recovery in microhardness, which was statistically significant in comparison to Group2 (bleached enamel surface) but not statistically significant when compared with Group 5 (HA) and Group 4 (CPP-ACPF) which is in agreement with the studies done by Karlinsey et al and Balakrishnan et al[28, 29].

TCP is a precursor to hydroxyapatite formation, which is bioactive and manifests lattice defect that allow for crystal modification. During the manufacturing process, TCP fuses with sodium lauryl sulphate, to ensure that the calcium oxides are protected from undesirable interaction with fluoride, which could render both calcium and fluoride ineffective. A protective barrier is created around the calcium-phosphate ions allowing it to coexist with the fluoride ions, which acts as a bubble that transports and breaks down when it comes in contact with tooth surface to make the calcium, phosphate and fluoride readily available. The remineralisation efficacy of f-TCP group is due to the presence of fluoride compatible functionalized calcium phosphate ingredient that imparts superior remineralisation at the enamel surface, thereby boosting the enamel surface strength.

Kodaka *et al.* correlated the Vickers hardness with P, Ca concentration in enamel and reported that the outer enamel surface is harder than the inner surface. Considering the importance of the surface layer of enamel, the evaluation of changes in this region is relevant[30].

Surface micro hardness (SMH) measurement is a suitable technique for this purpose and is appropriate for a substrate like enamel; which has fine microstructure, non-homogenous surface & is prone to cracking. This method provides a relatively simple, non-destructive and rapid method in demineralization and remineralization studies[31].
Microhardness measurement can be estimated by three different parameters like Knoop's Hardness Number (KHN), Vicker's Hardness Number (VHN) and Brinnel's Hardness Number (BHN). In the present study Vicker's hardness method was chosen over Knoop's hardness because a square shape of indent obtained in VHN is easy and accurate to measure under a microscope. The minutest of changes in the square shape indent can be easily detected, whereas the rhomboid shape indentation with opposing surfaces parallel to each other obtained with the Knoop's hardness test makes it difficult to detect the error[32].

Artificial saliva, composed of NaCl, KCl, $MgCl_2$, K_2HPO_4, $CaCl_2$, CMC-NA and IEW has also been used in studies conducted by Zhang et al, Jayarajan et al to stimulate the salivary component in oral cavity[33,34]. The use of artificial saliva in this study cannot model remineralization and demineralization process in intraoral environment which is related to various biological factors, such as salivary pellicle and biofilm. Natural saliva offers a great protection than artificial saliva due to its ability to form salivary pellicle that restricts acid diffusion and transport of ions in and out of enamel surface.

The findings of this in-vitro study may not be fully representative of the in-vivo conditions; in which the oral cavity is continually bathed with saliva that contains various minerals (i.e., fluoride, calcium phosphate), lipids, carbohydrates and proteins. They also do not represent unfavourable conditions where the deficiency of saliva or poor oral hygiene might increase the caries risk or a greater acidogenic challenge that occurs in a shorter period of time.

Further investigations should be undertaken in order to evaluate the long-term effect of the application of these remineralizing agents on bleached enamel surface.

Table 1: One way anova test done to compare the 5 groups

		N	Mean	Std. Deviation	F/ Statistics	Mean Square/DF2	P Value
Micro Hardness	Control	15	328.27	10.833	10.644	1355.887	**<0.001**
	Bleached enamel	15	301.93	12.08			
	Treated with Clinpro	15	311.2	11.156			
	Treated with GC tooth mousse plus	15	311.53	10.439			
	Treated with Reminpro	15	314.27	11.841			
	Total	75	313.44	13.921			

Table 2: Posthoc Tukey test to compare intergroup comparison

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	P Value
Microhardness	Control	Bleached enamel	26.333*	**0.001**
		Treated with Clinpro	17.067*	**0.001**
		Treated with GC tooth mousse plus	16.733*	**0.001**
		Treated with Reminpro	14.000*	**0.01**
	Bleached Enamel	Treated with Clinpro	-13.623	0.038
		Treated with GC tooth mousse plus	-14.33	0.04
		Treated with Reminpro	-12.333*	**0.03**
	Treated with Clinpro	Treated with GC tooth mousse plus	-0.333	1
		Treated with Reminpro	-3.067	0.945
	Treated with Reminpro	Treated with GC tooth mousse plus	2.733	0.964

CONCLUSION

Within the limitation of this in vitro study, it can be concluded that bleaching results in statistically significant loss of minerals from enamel surface when compared to the intact sound enamel. All the remineralizing agents tested were found to be effective in remineralizing the bleached enamel surface in the following order: HA, CPP-ACPF and β-TCP. Among the remineralizing agents tested in the study, Hydroxyapatite based cream was found to be marginally more effective than the CPP-ACPF and β-TCP based products which was not statistically significant.

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