Evaluation of the Efficacy of Biomin Containing Tooth Remineralising Agent To Prevent Stain Absorption on Freshly Bleached Enamel: An In Vitro Study.

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ABSTRACT:-

Title of study: - Efficacy of BIOMIN containing tooth remineralising agent to prevent stain absorption on freshly bleached enamel: An in vitro study.

Background Knowledge: - Teeth when subjected to bleaching bring about the desiccation of the enamel, making it more susceptible to stain absorption. However, subjecting the freshly bleached enamel surface to various surface treatments of BIOMIN and Casein Phosphopeptide - Amorphous Calcium phosphate (CPP-ACP) brought about the reduction in stain absorption.

This capacity of these remineralising agents is assessed in this study.

Materials and Method: - Forty extracted human permanent maxillary central incisors were subjected to bleaching with 35%carbamide peroxide for four days. They were divided into four groups of 10 each. Group I was the control group. Group II was immersed in tea solution without surface treatment, while Group III and IV were immersed in tea solution with surface treatment of BIOMIN and CPP-ACP respectively. Spectrophotometer was used for colour analysis.

Results: - Surface treatment of teeth with BIOMIN and CPP-ACP on freshly bleached enamel surface, significantly reduced the stain absorption.

Conclusion: - Remineralizing agents reduce stain absorption after tooth bleaching.

Keywords:- Vital Bleaching, Remineralising Agents, Biomin, Extrinsic Staining.

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I. Historical Background

The history of dentistry is comprised of many efforts undertaken to achieve an effective toothwhitening method. Tooth bleaching began in 1848 with the use of chloride of lime (Dwinelle, 1850), and in 1864, Truman introduced the most effective technique for bleaching non - vital teeth, a method which used chlorine from a solution of calcium hydrochlorite and acetic acid (Kirk, 1889).

In the late nineteenth century, many other bleaching agents were also successfully used including cyanide of potassium (Kingsbury, 1861), oxalic acid (Bogue, 1872), sulfurous acid (Kirk, 1889), aluminum chloride (Harlan, 1891), sodium hypophosphate (Harlan, 1891), pyrozone (Atkinson, 1892), hydrogen dioxide (hydrogen peroxide or perhydrol), and sodium peroxide (Kirk, 1893).

All these substances were considered as either direct or indirect oxidizers acting on the organic portion of the tooth.

Furthermore, in the late 1960s, a successful home-bleaching technique was established when Dr. Bill Klusmier, an orthodontist, instructed his patients to use an "over-the-counter" oral antiseptic, Gly-Oxide (Marion Merrell Dow, Kansas City, MO, USA), which contained 10% carbamide peroxide delivered via a customfitting mouth tray at night. The "over-the-counter" (OTC) bleaching agents were first launched in the United States in the 1990s, containing lower concentrations of hydrogen peroxide or carbamide peroxide and sold directly to consumers for home use (Greenwall et al., 2001).

Finally, the current bleaching technique came into use, which typically uses different concentrations of hydrogen peroxide, between 15% and 40%, with or without light and in the presence of rubber dam isolation (Haywood, 2000; Ontiveros, 2011).

However, Bleaching can be inappropriate or dangerous when the surface, thickness and health of enamel has been compromised for any reason like microcracks permeating the deeper penetration of stain or thinned enamel as seen in many systemic disorders and in older age. It can sometimes cause some low-grade reversible pulpal inflammation and may lead to hard tooth structure damage.this is mainly due to the process of demineralisation of the surface enamel due to the acidic nature of the bleaching agent.

This disadvantage of bleaching can be overcome by the use of commercially available remineralising agents which claim to replenish the lost mineral content of enamel thus making it more stain resistant.

Tooth remineralisation is a naturally occurring process in the oral cavity. [2] It is defined as a process in which calcium and phosphateions are sourced to promote ion deposition into crystal voids in demineralised enamel. Remineralisation remains imperative towards the management of non-cavitated carious lesions and prevention of disease progression within the oral cavity. The process also has the ability to contribute towards restoring strength and function within tooth structure. [3] The effect of demineralisation can be reversed if there is sufficient time to allow remineralisation to occur to counteract the acids in the oral cavity. [5]

II. Introduction

Vital tooth bleaching with Carbamide Peroxide (CP) gels is becoming more and more popular. Although no macroscopically, clinically remarkable damages because of vital bleaching of the dental hard tissues have been described in literature, there are scientific reports which demonstrate alterations of the histological aspects and composition of bleached dental enamel. It is observed that bleaching with 10% CP may result in a decrease of the calcium and phosphate content and also of the fluoride amount in enamel. In a previous study, it was shown that the loss of micro hardness in bleached enamel could be outweighed by a remineralization period following the bleaching period. It may be speculated that in this case micro structural defects may be repaired by the absorption and precipitation of components of the saliva, such as calcium and phosphate.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been shown to prevent enamel demineralization and promote remineralization of enamel surface lesions in animal and human in situ caries models.

BioMin is a similar compound which releases fluoride, calcium and phosphate in essential proportions to create fluorapatite. An effective armour coating, which is twice as strong as the tooth's enamel. A constant supply of low levels of fluoride in biofilm / saliva / dental interface is considered beneficial in preventing dental decay.

It is conceivable that a dietary component, such as tea consumed during or just after the completion of bleaching treatment may lead to staining of the bleached enamel surface. [5,6]

It is further unknown whether an appropriate length of remineralization time should elapse after bleaching, and before consumption of tea.

Although numerous studies on bleaching have been reported, little research is available to evaluate the effect of tea, coffee, cola etc. on the color of freshly bleached enamel surface of human teeth. The literature review also does not show any study done to analyze the effect of various remineralizing agents such as Biomin and CPP-ACP applied over the freshly bleached enamel surface of human teeth, to prevent stain absorption.

It was thought if the enamel of freshly bleached tooth was surface treated, so that along with the effect of reduced sensitivity, it may reduce the absorption of stains and therefore maintain the effect of bleaching for a longer time. [7]

Hence, an attempt was made to evaluate the freshly bleached enamel surface of extracted human teeth for stain absorption subjected to varied surface treatment.

III. Materials And Method

SAMPLE SIZE:-Forty freshly extracted human permanent maxillary central incisors were selected for the study.

INCLUSION CRITERIA:-

Teeth free from dental caries.restorations and with normal crown anatomy were chosen.

STORAGE OF SAMPLES:-

The teeth were stored in 5% normal saline at room temperature.

PREPARATION OF SAMPLES FOR ANALYSIS:-

- 1. Each tooth was numbered on the root portion.
- 2. Tooth samples were embedded in white hard carving wax with approximately 10mm thickness.

The samples were then divided into four groups of 10 samples each.

GROUP –I Samples without placing in tea solution.

GROUP – II Samples placed in tea solution but without any surface treatment.

GROUP-III Samples treated with GC TOOTH MOUSSE PLUS and placed in tea solution.

GROUP- IV Samples treated with ELSENZ TOOTH CRÈME and placed in tea solution.

All the samples were analyzed under the spectrophotometer at the following stages in the study,

- Pre bleaching evaluation
- Post bleaching evaluation: -
- At the end of one hour of staining.
- At the end of 24 hours of staining.

Spectrophotometer used for this study was, "Spectrophotometer (Spectra flash 500) by data color international (Lawrenceville, NJ)". The aperture of the data-color spectrophotometer used in the study was 3.0mm in diameter. Readings were recorded in CIELAB color system in the form of L*, a*, b*. L* characterizes the lightness and can range between 0 (dark) to 100 (light). The value of a* defines a color on a red - green axis and b* defines a color on a yellow - blue axis.

The total change in color (dE*) of all the teeth samples was calculated by using following formula,

$$dE *= \sqrt{(dL *)^2 + (da *)^2 + (db *)^2}$$

 $dE *= \sqrt{(dL *)2 + (da *)2 + (db *)^{-2}}$ Where dL*, da*, and db* represent the difference in L*, a*, and b* values, respectively.

The spectrophotometer readings of all the samples were recorded before bleaching. Labial surface of each sample was coated with a single layer of 15% CP bleaching agent (ULTRAWHITE BLEACHING KIT) using micro brushBleaching Agent was left in place for 8 hours.

Samples were stored in artificial saliva for the next 16 hours and subjected to bleaching again. This cycle was continued for 8 successive daysAt the end of 8 days all the samples were subjected to spectrophotometric analysis for the post bleaching readings.

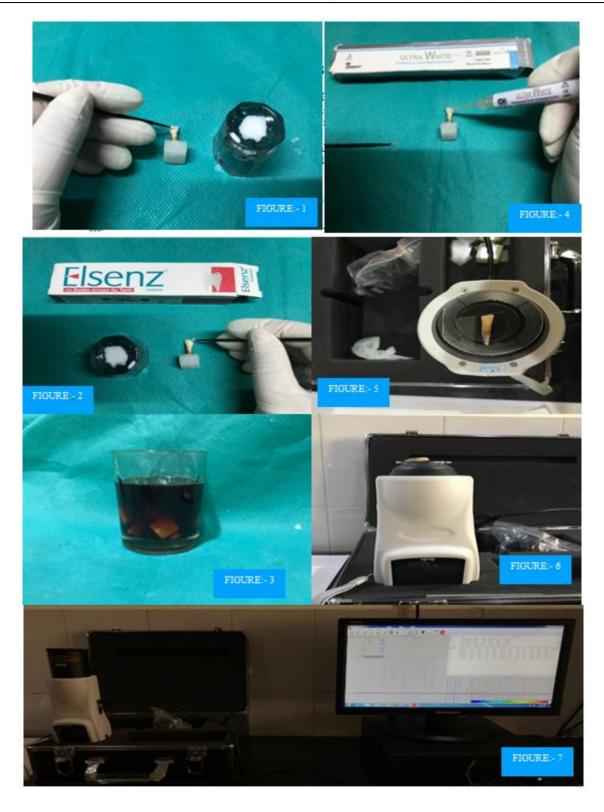
Group I - Samples were stored in artificial saliva without placing in tea solution. Group II - Samples were stored in artificial saliva after placing in tea solution, but without surface treatment. Group III - Casein Phosphopeptide - Amorphous Calcium phosphate (CPP-ACP; GC Tooth mousse remineralizing agent - Ultradent Products Inc.) was applied with a brush, over the dried labial surface of the samples and left for five minutes(figure 1). Group IV ELSENZ TOOTH CRÈME(containing BIOMIN) was applied with a brush, over the dried labial surface of the samples and left for five minutes (figure 2). After five minutes, gel over the enamel surface of group III and IV was gently wiped off with gauze and the teeth samples were stored in artificial sali-

After one hour, group II, III and IV were removed from the artificial saliva and immersed in freshly prepared tea solution for 10 minutes (FIGURE 3). The tea solution was prepared by boiling two grams of tea (Girnar CTC tea; Girnar Food and Beverages Pvt Ltd., Kurla, Mumbai, India) in

100ml. of distilled water for 5 minutes, and filtered through a strainer. Fresh tea solution was made each day for the study.

After 10 minutes, samples were removed from the tea solution, washed thoroughly, dried with gauze, mounted on wax blocks and color of each of the sample was evaluated. After which the samples were restored in artificial saliva for next 24 hours. At the end of 24 hours, the above samples were again removed from the artificial saliva, immersed In freshly prepared tea solution for 10 minutes and evaluated by using spectrophotometer (figures: -5,6,7).

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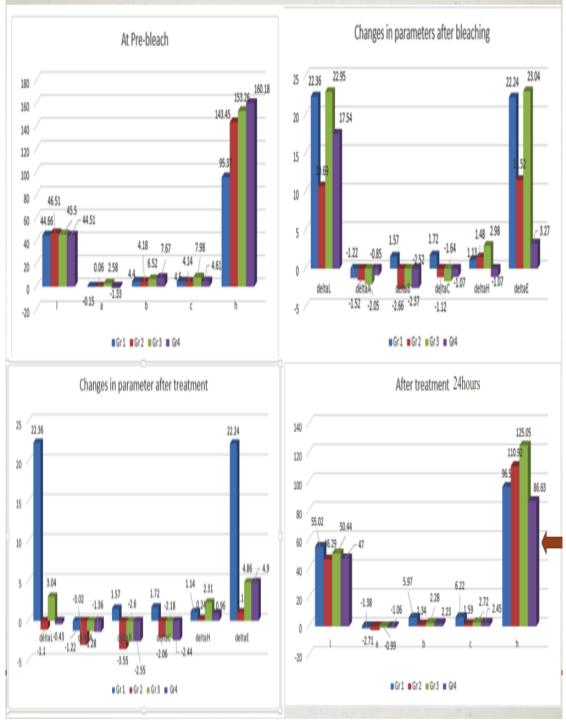
gr		I	а	b	С	h
1	Mean	55.02	-1.38	5.97	6.22	96.50
	N	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	30.62	0.92	1.23	1.40	1.52
2	Mean	46.29	-2.71	1.34	1.59	110.92
	N	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	1.51	1.83	1.49	1.47	3.23
3	Mean	50.44	-0.99	2.28	2.72	125.05
	N	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	2.12	0.64	1.32	0.99	21.17
4	Mean	47.00	-1.06	2.23	2.45	86.63
	N	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	3.48	0.87	1.23	1.06	40.33
Total	Mean	46.69	-1.54	2.96	3.24	104.78
	N	20.00	20.00	20.00	20.00	20.00
	Std. Deviation	14.63	1.28	2.19	2.14	25.73
P ^a value		0.063, NS	0.281, NS	0.012, S	0.012, S	0.017, S
Post hoc pairwise comparison ^b		-	-	1>3,4>2	1>3,4>2	3>2>1,4

^aKruskal Wallis test, Mann Whitney U test **TABLE 1:-** READINGS OF SPECTROPHOTOMETER ANALYSIS ONE HOUR AFTER BLEACHING.

gr		deltaL	deltaA	deltaB	deltaC	deltaH	deltaE
1	Mean	22.36	-1.22	1.57	1.72	1.14	22.24
	N	5.00	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	5.54	0.29	1.34	1.43	0.31	5.59
2	Mean	-1.10	-3.02	-3.55	-2.06	0.24	1.10
	N	5.00	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	1.51	1.82	1.49	3.19	1.49	1.70
3	Mean	3.04	-1.28	-2.60	-2.18	2.31	4.86
	N	5.00	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	2.12	0.64	1.32	0.99	0.73	0.88
4	Mean	-0.43	-1.36	-2.55	-2.44	0.96	4.90
	N	5.00	5.00	5.00	5.00	5.00	5.00
	Std. Deviation	3.55	0.87	1.26	1.06	1.51	2.82
Total	Mean	5.97	-1.72	-1.78	-1.24	1.16	8.27
	N	20.00	20.00	20.00	20.00	20.00	20.00
	Std. Deviation	10.36	1.25	2.38	2.47	1.29	8.94
P ^a value		0.003, S	0.224,	0.012, S	0.055,	0.080,	0.002, S
Double invisor			NS		NS	NS	
Post hoc pairwise comparison ^b		1>2,3,4	-	2>3,4>1	-	-	1>3,4>2

^aKruskal Wallis test, Mann Whitney U test

TABLE 2:- Readings of Spectrophotometer Analysis 24 Hours After Bleaching.



Graphical Representation Of Results

Statistical analysis was performed using KRUSKAL WALLIS TEST along with post hoc pairwise comparison by MANN WHITNEY U TEST. The level of statistical significance was set at 0.05. Group I Samples showed a very slight change towards the negative (darker) direction at the end of 24 hours (dE*).

Group II Samples showed a significant change in colour value. There was a statistically significant change in total colour (dE^*), at the end of 1 and 24 hours (TABLES 1 AND 2). Group III and Group IV:

- 1. Samples showed the change in total colour (dE*), at the end of 1 & 24 hours.
- 2. The stain absorption gradually increased from 1 to 24 hours (dE^*)but the rate of absorption of stain was least in the samples of group IV.

IV. Discussion

Bleaching restores normal colour to a tooth by decolourization of stain with a powerful oxidizing or reducing agent. ELSENZ TOOTH CRÈME enhances remineralisation of enamel surfaces by occluding the dentinal tubules, which results in reduced hypersensitivity as well as reduced stain absorption. The product is the result of an exclusive licencing agreement between BioMin Technologies of London and Group Pharmaceuticals whereby Group Pharmaceuticals will manufacture Elsenz toothpaste at its facility near Bangalore and bring the product to the Indian market. Elsenz, incorporates the new BioMinF toothpaste ingredient, providing a new tooth repair technology which will bring relief to the millions of adults and children in India who are prone to tooth decay and sensitivity, dental decay is the most prevalent disease worldwide and the majority of adults will also experience tooth sensitivity at some stage during their lives.

According to Professor Robert Hill, Chair of Dental Physical Sciences at Queen Mary, University of London, who led the team which developed BioMinF; "Using remineralising toothpaste makes teeth far more resistant to attack from acidic soft drinks like fruit juices and sodas. It is also much more effective than conventional toothpastes where the active ingredients, such as soluble fluoride, are washed away and become ineffective less than two hours after brushing".

This study compares the efficacy of ELSENZ against the commercially acclaimed GC TOOTH MOUSSE PLUS.

GC Tooth Mousse Plus is a topically applied, great tasting crème that delivers a powerful combination of two proven tooth protection and strengthening technologies – RECALDENT (CPP-ACP) and fluoride. RECALDENT (CPP-ACP) is a specific milk-derived protein casein phosphopeptide – amorphous calcium phosphate which binds calcium and phosphate so

it can be delivered to tooth surfaces in a soluble form. As such, RECALDENT (CPP-ACP) protects and strengthens teeth in a similar way to proteins in saliva, which we recognise are essential for strong teeth and a healthy oral environment. RECALDENT(CPP-ACP) is the end result of many years of research by the University of Melbourne into the anticariogenic properties of milk.

Storage in saliva was chosen, to simulate the oral environment. The Artificial Saliva was used instead of human saliva in order to standardize the conditions in the study.

The eight-hour period of bleaching was chosen to simulate the condition of wearing bleaching tray overnight. The whitening effects are primarily due to degradation of high molecular weight, complex organic molecules that reflect a specific wavelength of light and are responsible for the colour of the stain. The resulting degradation products formed after, dissociation of carbamide peroxide into hydrogen peroxide, are of lower molecular weights and are less complex molecules that reflect less light and result in a reduction or elimination of the discoloration (Flaitz and Hicks, 1996).

The colour of the teeth is influenced by a combination of their intrinsic colour and the presence of any extrinsic stains that may form on the tooth surface. Intrinsic tooth colour is associated with the light scattering and adsorption properties of the enamel and dentine, with the properties of dentine playing a major role in determining the overall tooth colour. Extrinsic stains tend to form in areas of the teeth that are less accessible to tooth brushing and the abrasive action of a toothpaste and is often promoted by smoking, dietary intake of tannin-rich foods (e.g. red wine, tea, coffee,etc) and the use of certain cationic agents such as chlorhexidine, or metal salts such as tin and iron.

The reason to use tea as a solution for staining of the teeth was, that tea has been proven to have a higher capacity to stain teeth by Attin et.al. in the year 2003.

V. Conclusion

It was observed that groups II, III, and IV showed the greater change in colour (dE*) after immersing in tea solution at the end of one hour, than that at the end of 24 hours.

Due to the remineralizing effect of Artificial Saliva, CPP-ACPF and BIOMIN which tend to prevent the stain absorption more effectively at the end of 24 hours after surface treatment.

Out of the various surface treatments, ELSENZ TOOTH CREME (group IV) showed the least total colour change after immersing in tea solution, compared to without surface treated teeth samples (group II).

These findings were due to the remineralizing capacity of BIOMIN agents present as the prime component of ELSENZ.

WITHIN THE LIMITATIONS OF THIS STUDY, THE FOLLOWING CONCLUSIONS WERE DRAWN:

- 1. Teeth when stored in artificial saliva with no surface treatment and without subjecting to staining showed no significant change in colour.
- 2. Stain absorption was found to be least when tooth was surface treated with ELSENZ TOOTH CRÈME.

This study provides a solution to reduce stain absorption by the application of BIOMIN containing ELSENZ TOOTH CRÈME & CPP-ACPF containing GC TOOTH MOUSSE PLUS, which along with reducing sensitivity would also reduce the absorption of stain in the period immediately after bleaching.

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