



## COMPARATIVE EVALUATION OF FOUR DIFFERENT REMINERALIZING AGENTS ON THE BLEACHED ENAMEL SURFACE BY USING SEM WITH EDX: AN IN VITRO STUDY

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### ABSTRACT

To evaluate the effect of 22% carbamide peroxide (CP) on the mineral content of enamel and to evaluate the remineralization of bleached enamel after treatment with four different remineralizing agents.

**Materials and methods:** About 40 extracted human maxillary incisors were cleaned using ultrasonic scaler. Analyzed using scanning electron microscopy with EDX (Energy Dispersive X ray Spectroscopy) to determine the initial mineral content. 22% CP was applied to the labial surfaces 8 h/day for 2-week. Then, samples were washed and stored in artificial saliva. Post-bleaching mineral content is analyzed by using scanning electron microscopy with EDX. The samples were randomly divided into four groups. Group A: GC tooth mousse plus. Group B: Toothmin, Group C: 6.5% (w/v) GSE solution. Group D: 6.5% (w/v) Wheat Grass solution. Remineralizing agents were gently agitated on the bleached enamel with an applicator tip for 10-min and cleaned thoroughly using deionized water. The mineral content of bleached enamel after the remineralization regimen was assessed using EDX. Statistical analysis was done using one-way ANOVA.

**Results:** Bleaching with 22% CP significantly decreased the mineral content of the enamel. Group B Toothmin showed the highest remineralization than Group A, C and D but was not statistically significant.

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## INTRODUCTION

The primary constituent of the human teeth is hydroxyapatite which is a highly mineralized crystal. There is always occurs a continuous cycle of demineralization and remineralisation in the dental hard tissues of our body<sup>1</sup>. The occurrence of dental caries is because of demineralization, which is due to a drop in the pH of our oral cavity. An increase in the oral pH leads to the deposition of Calcium, Phosphate and Fluoride ions<sup>2</sup>.

Bleaching has paved a new pathway in the aesthetic dentistry. It is most preferred conservative method which lightens the discoloured teeth. Carbamide peroxide (CP), H<sub>2</sub>O<sub>2</sub> are the main bleaching contents. A technique in which the patient wears a customized tray loaded with 10% Carbamide Peroxide at night, called the NIGHT GUARD BLEACHING TECHNIQUE is the most popular technique followed these days<sup>3</sup>. Carbamide peroxide is a combination of H<sub>2</sub>O<sub>2</sub> and Urea, which on application on to the tooth surfaces lead to the slow

release of H<sub>2</sub>O<sub>2</sub> which penetrate through the enamel, leading to the breakdown of the high molecular weight organic molecule to simpler low molecular weight with lesser colour reflectance, which ultimately leads to a colour change in enamel and dentine<sup>4</sup>.

At times because of the unintentional contact with the dentine in carious lesions and enamel defects there occurs some deleterious effects resulting in the increased demineralization and loss in the mineral content. Sensitivity of the tooth is yet another a major conflict of interest. A lot many cases of hypersensitivity were reported in the IN OFFICE bleaching procedure, which has lead to the discontinuation of the bleaching treatment. It's because of these disadvantages there is an increase in the clinical trials in the field of remineralisation treatment<sup>6</sup>.

Few of the remineralising agents increasing the micro hardness of bleached enamel surface are preparation like GC Tooth Mousse (Recaldent), Toothmin Tooth cream (Abbott Healthcare Pvt Ltd). Studies showing the effect of these remineralising agents are not available. Antioxidant property

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has shown to have an additive effect along with the remineralising property.<sup>7</sup>

In this view, Grape seed extract and Wheat grass which have the antioxidant property there were tried as surface remineralising agents. GSE is composed of monomeric catechin, epicatechin, Gallic acid, a rich source of proanthocyanidine and procyanidines. Confocal laser scanning microscope was employed to evaluate the efficacy of GSE as a remineralising agent and results have shown a positive effect on the remineralisation<sup>8</sup>.

Modern prospective caries studies require the measurement of small changes in a tooth's mineral content, especially in a single caries lesion. One recent technique is scanning electron microscopy with an energy dispersive X-ray analysis. It is a microanalytical technique that is employed to quantitatively estimate the amounts of mineral in a given tooth samples<sup>11</sup>.

Wheat grass is yet another nutraceutical product which is a good source of Vit A, Vit C, Vit E, Vit K, Thiamine, Riboflavin, Niacin, Vit B6, pantothenic acid, calcium, phosphorous, potassium, iron, zinc, copper, manganese and selenium. Wheatgrass has anti-inflammatory, antiaging and antioxidant properties<sup>9</sup>.

Hence, in my present study we are evaluating and comparing the efficacy of GC Tooth Mousse plus, Toothmin, GSE and wheat grass extract as remineralising agents on the bleached enamel surface using SEM with EDX.

## MATERIALS AND METHODS

- Preparation of 6.5%w/v of GSE solution in phosphate buffer solution ( pH 7.4 )

About 6.5 g of Grape seed extract (GSE) powder was dissolved in 100 ml of phosphate buffer solution.

- Preparation of 6.5% w/v of wheat grass solution in phosphate buffer solution ( pH 7.4)

About 6.5 g of wheat grass powder was dissolved in 100 ml of phosphate buffer solution.

About 40 extracted human maxillary single rooted non carious teeth were collected. Teeth were cleaned using ultrasonic scaler. Analyzed using scanning electron microscope with EDX to determine the initial mineralcontent. 22% carbamide peroxide (Philips zoom, m & m dental associates, Mumbai, India) was applied to the labial surfaces 8 h/day for 2-weeks. Then, samples were washed and stored in artificial saliva. Post-bleaching using scanning electron microscope with EDX analysis was done.

The samples were randomly divided into four groups (n = 10) based on the treatments as follows:

Group	Material used	Manufactured
A	Gc Tooth Mousse	Recaldent,(CPP-ACP),India
B	Toothmin	Abbott Healthcare Pvt., Ltd, Mumbai, India
C	6.5% GSE Solution	Zenith nutrition Resveratol,India
D	6.5 % Wheat Grass Solution	Medizen labs Pvt.ltd India

- Remineralizing agents were gently agitated on the bleached enamel with an applicator tip for 10-mins and cleaned thoroughly using deionized water.

- The mineral content of bleached enamel after the remineralization regimens was assessed using EDX. Statistical analysis was done using one-way Chi-square and KruskalWallistest.



Fig 1 Materials



Fig 2 WGS solution



Fig 3 GSE solution



Fig 4 application of CP



Fig 5 Gc tooth mousse application



Fig 6 Toothmin application



Fig 7 GSE application



Fig 8 wheat grass application

## RESULTS

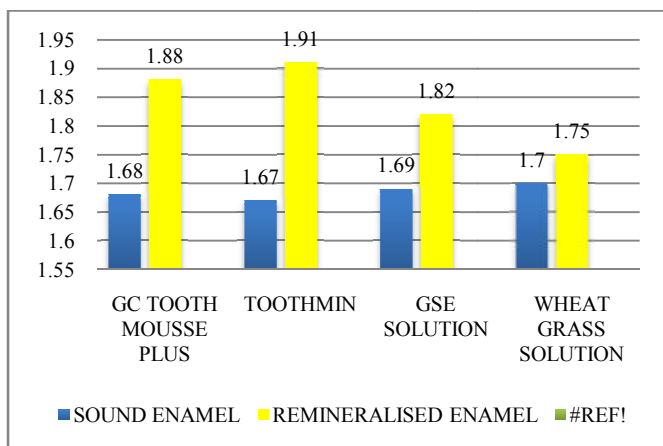
EDX was used to determine the calcium and phosphorus content in % weight of sound, bleached and remineralized enamel in each group<sup>7</sup>. The calcium (Ca) and phosphorus (P) content was converted into Ca/P ratios. Table 1 shows that there was a statistically significant difference in the mineral content of groups A, B, C and D when the sound and remineralized enamel were compared. Graph 1 represents the Ca/P ratio of bleached and remineralized samples analyzed by U-test. There was a significant increase in the mineral content after surface treatment with remineralizing agents in all the groups (P = 0.00). Chisquare was applied to compare the mean Ca/P ratios of the study groups after remineralization Group B (Graph 2) is showing better remineralizing properties compared to remaining all Group A, C, D.

**Table 1** Comparison of the mineral content of sound and remineralized enamel samples in study groups

Group	Procedure	Mineral content (Mean ±SD)	Z#	P
GC Tooth Mousse Plus (A)	Sound enamel	1.68±0.28	12.618	0.02*
	Remineralised enamel	1.88±0.72		
Toothmin(B)	Sound enamel	1.67±0.45	41.201	0.00*
	Remineralised enamel	1.91±0.34		
GSE solution(C)	Sound enamel	1.69±0.37	23.451	0.04*
	Remineralised enamel	1.82±0.22		
Wheat grass solution(D)	Sound enamel	1.70±0.18	32.981	0.05
	Remineralised enamel	1.75±0.16		

#Mann Whitney U test, \* Statistically significant, p<0.05.

Table 1 shows that there was a statistically significant difference in the mineral content of groups A, B, C and D when the sound and remineralized enamel were compared.



**Graph 1** Comparison of the mineral content of sound and remineralized enamel samples in study groups

## DISCUSSION

Bleaching procedure has been acknowledged to change the enamel matrix as a result of the pH and robust oxidizing impact of oxide<sup>10</sup>. Bleaching agents end up releasing free radicals that cause fragmentation of pigmented molecules into smaller species and alter the sunshine absorption and thereby crop or eliminate the stains. Hydrogen peroxide causes the discharge of free radicals, and therefore the impact may be intense by a decrease in pH of the bleaching agents, and thereby increase in dynamic changes in the micro hardness of enamel and dentin<sup>1,2</sup>.

Raman spectroscopical study (2008) showed that exposure to 10% CP for seven days resulted in significant decrease in phosphate concentration within the bleached enamel<sup>4</sup>. In vitro studies have evaluated the remineralization potential of various preparations on the bleached enamel hardness<sup>11</sup>.

Kaur *et al* (2015) conducted a study to evaluate the effect of GC Tooth Mousse and Toothmin Tooth Cream on microhardness of bleached enamel and concluded that Both GC Tooth Mousse (Recaldent) and Toothmin Tooth cream (Abbott Healthcare Pvt.Ltd) increase the microhardness of bleached enamel<sup>13</sup>.

Toothmin tooth cream could be a newly introduced remineralizing agent that's supported Anticay Technology. This distinctive technology has been commercialized by Australia based Biotechnology Company. Anticay could be a

mixture of metallic element disaccharide phosphates and inorganic metallic element phosphates consisting of 10–12% metallic element and 8–10% chemical element by weight<sup>6</sup>. In our study after remineralization the mineral content of tooth is increased from 1.67mm to 1.91mm by using Toothmin. Rubel *et al* (2016) stated that grape seeds and wheat grass are gaining more importance because the “Proanthocyanidins” present in them have excellent anticaries, remineralizing, and antibacterial properties. GSE and wheat grass showed greater preservation of tooth structure, prevention of lesion progression and inducing remineralization<sup>8, 9</sup>. The GSE employed in the current study shows the results of remineralisation increased from 1.69 to 1.82 and wheat grass showed remineralisation from 1.70 to 1.75.

According to the results obtained within the limitations of our study, all the 4 remineralizing agents considerably increased the mineral content of the bleached surface. Toothmin shows greater remineralisation properties compared to all the other three products with no statistically important distinction between all groups.

## CONCLUSION

Within the limitations of the present study, agents such as Toothmin, CPP-ACP, GSE, and wheat grass help in remineralizing the bleached surface and Toothmin showed highest remineralizing properties compared to all the other three groups.

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