Effect of 10% sodium ascorbate on the calcium: Phosphorus ratio of enamel bleached with 35% hydrogen peroxide: An *in vitro* quantitative energy-dispersive X-ray analysis

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Abstract

Objectives: The study assessed quantitatively the calcium and phosphorous loss from the enamel surface following bleaching with 35% hydrogen peroxide and reversal with 10% sodium ascorbate using energy-dispersive X-ray analysis (EDAX). **Materials and Methods:** Eight non-carious, freshly extracted human permanent maxillary central incisors without any visible defects were used. Each specimen was bleached with 35% hydrogen peroxide activated by light and reversed with sodium ascorbate antioxidant gel. The calcium and phosphorous content in weight percent of sound, bleached and reversed enamel was acquired using EDAX. The Ca/P ratio was calculated from the obtained data. One-way ANOVA followed by Post Hoc Tukey test was used for comparing the Ca/P ratio of sound, bleached and reversed enamel. **Results:** All the samples subjected to bleaching using 35% hydrogen peroxide showed a statistically significant decrease in the Ca/P ratio as compared with samples in which no bleaching procedure was performed (*P*-value < 0.01). The striking finding was that there was a significant increase in the Ca/P ratio on application of sodium ascorbate antioxidant gel when compared with the bleached enamel (*P*-value < 0.01). **Conclusion:** The authors concluded that 35% hydrogen peroxide causes a significant decrease in the Ca/P ratio. This decrease in the Ca/P ratio can be restored by the application of 10% sodium ascorbate antioxidant gel.

Keywords: Antioxidant gel, bond strength reversal, sodium ascorbate

Introduction

The advent of bleaching materials represented an important milestone in cosmetic dentistry.[1] The use of hydrogen peroxide was first reported by Harlan in 1884, which is currently the most effective bleaching material.[2] Bleaching is known to stimulate patients toward acceptance of additional esthetic dental procedures. With the increasing number of patients seeking esthetic correction, clinicians may face difficulties in bonding resin composites to previously bleached enamel. Several studies in the literature have shown that the bond strength of enamel decreased after bleaching with concentrated 35% hydrogen peroxide.[3] A period of up to 3 weeks is required for resin–enamel bond strengths to return to values obtained for unbleached enamel.[1]

This decrease in bond strength can also be reversed by applying a biocompatible and neutral antioxidant such as sodium ascorbate before resin–composite application.[4,5] Ascorbic acid and its sodium salt are potent antioxidants capable of quenching reactive free radicals in biological systems. Because Vitamin C and its salts are non-toxic and widely used in the food industry as antioxidants, it is unlikely that their use on enamel and dentin will create any adverse biological effect or clinical hazards.[3]

In the past decade, numerous studies evaluated the effects of peroxide-containing bleaching agents on tooth hard tissues. However, there are no reports in the literature regarding the effects of 10% sodium ascorbate on the enamel surface. The molecular constituents of dental hard tissues have been the subject of many studies using a variety of methods, including infrared spectroscopy, electronic microprobe, Raman spectroscopy and energy-dispersive X-ray analysis (EDAX).[6] X-ray microanalysis using EDAX is routinely used in the study of matrix elements like phosphate and calcium.[7]

Thus, the aim of this *in vitro* study was to evaluate quantitatively the calcium and phosphorous loss from the enamel surface following bleaching with 35% hydrogen peroxide and reversal with 10% sodium ascorbate using EDAX.

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Materials and Methods

A total of eight non-carious, freshly extracted human permanent maxillary central incisors without any visible defects were used in this study. After extraction, the teeth were cleaned of any residual tissue tags, pumiced and washed under running tap water. They were stored in distilled water at +4°C until needed for the study, a period not exceeding 1 week. The labial surfaces of the teeth were polished with fine grit silicon carbide paper on a water-irrigated metallurgical polishing wheel.

The specimens were stored in artificial saliva except during the bleaching, bonding and testing procedures. The samples were stored in artificial saliva as described in British Standard 7115, part 2, BSI London, 1988. The artificial saliva with an electrolyte composition similar to human saliva was prepared by mixing sodium chloride – 0.5 g, sodium bicarbonate – 4.2 g, sodium nitrate – 0.03 g and potassium chloride 0.2 g in 100 ml of distilled deionized water.

The initial mineral content of each sample was measured using EDAX. All the teeth were then mounted in an elastomeric impression material (Aquasil; Dentsply) for the purpose of applying the bleaching agent and antioxidant gel.

Bleaching procedure

The specimens were bleached with 35% hydrogen peroxide (Opalescence Extra), exposed to a fast halogen-curing light (3000 mW/cm^2) for 20 s and left standing for 15 min. The gel was washed away from the tooth surface. A fresh layer was reapplied, light activated, left standing for another 15 min and further washed away. The teeth were then stored in artificial saliva for 24 h at room temperature. The mineral content of each specimen after bleaching was measured using EDAX.

Preparation of antioxidant gel

Sodium ascorbate (sodium salt of ascorbic acid) was used for the antioxidant preparation. The antioxidant gel (2.5% [wt/wt]) containing sodium ascorbate (10%) was prepared by dispersing the carbopol 976P resin polymer in purified water containing sodium ascorbate under gentle mixing. The mixture was stirred until thickening occurred and then neutralized by dropwise addition of thriethanolamine until a transparent gel appeared. The quantity of thriethanolamine was adjusted to achieve a gel pH of 7.

Antioxidant application

The samples were then subjected to an application of antioxidant gel. Sodium ascorbate gel was placed on the enamel surfaces of the embedded teeth for 120 min. After the antioxidant treatment, the enamel surface was thoroughly rinsed with distilled water for 30 s. The mineral content of each specimen after reversal was measured using EDAX.

Quantitative evaluation

EDAX analysis

Three peak plots per specimen were taken:

1. The first was to evaluate the normal enamel surface prior to any procedure.
2. The second was to evaluate the surface change of the same specimen after bleaching with 35% hydrogen peroxide.
3. The third was to evaluate the surface change of the same specimen after reversal with 10% sodium ascorbate.

The calcium and phosphorous content in weight percent of sound, bleached and reversed enamel was tabulated and statistically analyzed. The calcium and phosphorous content was then converted into Ca/P ratio in each group and a range of Ca/P ratio was calculated from the obtained data.

Statistical analysis

Results of the present study were subjected to statistical analysis to interpret the significant differences between various Ca/P ratios of normal, bleached and reversed specimens. Statistical analysis was performed using SPSS, version 10. One-way ANOVA followed by Post Hoc Tukey test were used.

Results

The elements detected in one representative sample of sound, bleached and reversed enamel are shown in Figure 1, which shows a typical EDAX plot obtained from the enamel. Table 1 gives the calcium and phosphorous wt% in all eight specimens of sound, bleached and reversed enamel and their calcium:phosphorous ratio. Table 2 gives the mean calcium wt%, phosphorous wt% and mean Ca/P ratio of sound, bleached and reversed enamel. Table 3 gives the comparisons of Ca/P ratio in sound, bleached and reversed enamel.

The Ca/P ratio of sound enamel was 2.13 $\pm$ 0.17. In the bleached enamel, the Ca/P ratio decreased to 1.48 $\pm$ 0.11. This ratio kept on increasing to reach a value of 2.15 $\pm$ 0.08

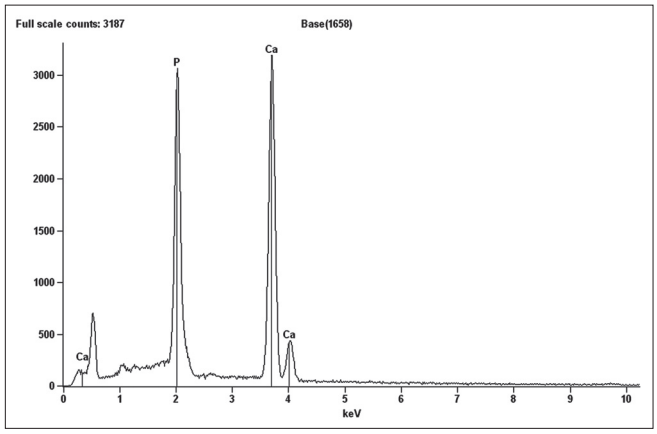

Figure 1: Energy-dispersive X-ray analysis plot obtained from the enamel

Table 1: Spectral calcium and phosphorous concentrations with their corresponding Ca/P ratios

Samples	Sound			Bleached			Reversed		
	Ca%	P%	Ca/P	Ca%	P%	Ca/P	Ca%	P%	Ca/P
1	37.63	17.94	2.09	42.4	29.37	1.44	37.82	17.84	2.12
2	36.22	16.54	2.19	41.02	28.45	1.44	37.32	17.43	2.14
3	38.53	18.33	2.1	43.65	29.12	1.5	38.43	18.14	2.12
4	37.56	17.23	2.18	42.1	28.98	1.45	39.12	18.54	2.11
5	35.56	16.54	2.15	43.01	28.55	1.5	37.62	17.32	2.17
6	38.94	18.92	2.05	44.21	29.98	1.47	38.67	17.98	2.15
7	38.02	18.01	2.11	41.89	27.99	1.5	38.65	17.67	2.19
8	37.98	17.56	2.16	44.93	29.02	1.55	38.17	17.21	2.22

Table 2: Mean calcium and phosphorous content and the Ca/P ratios for sound, bleached and reversed enamel

Group	Mean Calcium Wt%	Mean Phosphorous Wt%	Mean Ca/P
Sound enamel	37.56	17.63	2.13
Bleached enamel	42.90	28.93	1.48
Reversed enamel	38.22	17.76	2.15

Table 3: Comparison of the Ca/P ratios for sound, bleached and reversed enamel

Sound enamel vs. bleached enamel	$P < 0.01$
Bleached enamel vs. reversed enamel	$P < 0.01$
Sound enamel vs. reversed enamel	$P > 0.05$

on reversal with antioxidant gel. The results showed that all the samples subjected to bleaching using 35% hydrogen peroxide showed a statistically significant decrease in the Ca/P ratio as compared with samples in which no bleaching procedure was performed (*P*-value < 0.01). The striking finding was that there was a significant increase in the Ca/P ratio on application of sodium ascorbate antioxidant gel when compared with the bleached enamel (*P*-value < 0.01).

Discussion

Bleaching agents release free radicals as nascent oxygen and hydroxyl or peri-hydroxyl ions when they are applied to dental structures. These molecules react with electron-rich regions of pigments inside the dental structure, breaking down large pigmented molecules into smaller, less-pigmented ones. One of the possible adverse effects is that the enamel structure may be weakened by oxidation of the organic and inorganic elements. Hydrogen peroxide and the released free radicals could react with both the organic and the inorganic structures of the enamel. The reaction between peroxide and the organic materials on the surface or in the subsurface enamel can result in morphological alterations.[8] Ernst *et al*. evaluated four bleaching agents using scanning electron microscopy (SEM).[9] They found that the enamel underwent slight morphological alteration after bleaching. Tong *et al*. found that 30% hydrogen peroxide treatment with a bleaching light for 30 min caused no measurable loss in the enamel,[10] while another study reported a decrease in the Ca/P ratio on the enamel surface with the use of 30% hydrogen peroxide.[11]

On the other hand, this dissolved peroxide remnant is also deleterious to the bonding of resinous materials.[5] This may pose a problem in certain clinical conditions where bleaching may not be the only treatment of choice and requires additional bonding procedures. In such conditions, recommendations of waiting periods for the application of composite materials on to the bleached enamel surface ranges from 1 day to 6 weeks.[4,5]

However, in order to remove the dissolved peroxide remnants on the bleached enamel, it has been demonstrated that the application of a catalase or 10% sodium ascorbate on the bleached enamel and dentin immediately after the bleaching treatment makes the above-mentioned waiting periods unnecessary.[4] It is proposed that sodium ascorbate may remove the oxidative effect of the bleaching agent. It is also possible that, by restoring the altered redox potential of the oxidized bonding substrate, sodium ascorbate allows free radical polymerization of the adhesive to proceed without premature termination and hence reverses the compromised bonding.[5,12]

EDAX stands for energy dispersive X-ray analysis. It is sometimes also referred to as EDS or EDX analysis. During EDAX, the specimen is bombarded with an electron beam inside the SEM. The bombarding electrons collide with the specimen atoms' own electrons, knocking off some of them in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. To do so however the transferring electron must give up some energy by emitting an X-ray.[9]

The output of an EDX analysis is an EDX spectrum. The EDX spectrum is just a plot of how frequently an X-ray is received for each energy level. An EDX spectrum normally displays peaks corresponding to the energy levels for which most of the X-rays had been received. Each of these peaks is unique to the atom and, therefore, corresponds to a single element. The higher a peak in a spectrum, the more concentrated the element is in the specimen.[9]

Calcium and phosphorous are present in the hydroxyapatite crystal, which is the main building block of dental hard tissues. Changes in the calcium/phosphate ratio indicate alterations in the inorganic components of hydroxyapatite. Previous studies have shown that bleaching causes calcium and phosphate ion loss in the dental hard tissues, change in the calcium/phosphate ratio and surface alterations depending on their concentration.[6,13] In the present study, the Ca/P ratio was altered on the application of 35% hydrogen peroxide. There was a decrease in the Ca/P ratio on the enamel surface following an application of 35% hydrogen peroxide, which was consistent with earlier studies.[11] This suggested that the resistance of enamel surfaces decreased in these groups when there was an acid attack. Rotstein *et al*.[14] reported a loss of strength and higher solubility of enamel, dentin and cementum after bleaching. The change in the original ratio between the organic and the inorganic components of the tissues increased the solubility.[14]

The present study showed that there was an increase in the Ca/P ratio following the application of sodium ascorbate antioxidant gel, reaching a value equivalent to that of normal enamel. This increase in the Ca/P ratio may be either due to an increase in the phosphorous content or a decrease in the calcium content. It is well known that the antioxidant gel is not a source of phosphorous ions. Thus, this increase in the Ca/P ratio can be attributed to the further loss of calcium ions and, therefore, it is proposed from the present study that antioxidant application restores the normal Ca/P ratio of the tooth but causes a decrease in the actual calcium content of the tooth.

This study being a relative study, the actual calcium loss was not estimated. The findings of this *in vitro* study may not be representative of the *in vivo* condition in which the oral cavity is continuously bathed with saliva that contains various minerals, lipids, carbohydrates, proteins and other substances.

Conclusion

According to the results and within the limitations of this *in vitro* study, it can be concluded that 35% hydrogen peroxide causes a significant decrease in the Ca/P ratio. This decrease in the Ca/P ratio can be restored by the application of 10% sodium ascorbate antioxidant gel. Further studies are required to evaluate the actual calcium loss, the reactions of the antioxidant gel with tooth structure and the alterations of enamel surface caused by antioxidant application.

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