The effect of two bleaching agents on the phosphate concentration of the enamel evaluated by Raman spectroscopy: An ex vivo study

SOKKALINGAM MOTHILAL VENKATESAN, GOPAL SHANKAR NARAYAN, ANIL KUMAR RAMACHANDRAN, RAJAMANI INDIRA

Abstract

Aim: The aim of this ex vivo study was to evaluate the effect of in-office bleaching agents,–35% and 38% hydrogen peroxide containing bleaching agents, on the phosphate concentration of the enamel evaluated by Raman spectroscopy. Materials and Methods: Forty noncarious, craze-free human maxillary incisors, extracted for periodontal reasons, were used in this study. Baseline Raman spectra from each specimen were obtained before the application of the bleaching agent to assess the phosphate content present in the teeth. The teeth were divided into two groups: Group A – bleached with pola office bleach (35% hydrogen peroxide, potassium nitrate) (light activated). Group B – bleached with opalescence Xtra bleach (38% hydrogen peroxide potassium nitrate and fluoride) (chemical activated). After the bleaching procedure, the treated specimens were taken to obtain Raman spectra to assess the phosphate loss after bleaching treatment. Results: The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Conclusion: Within the limitations of this study, it can be concluded that the chemically activated bleaching agent showed minimal phosphate loss when compared to light activated bleaching agent. The chemically activated bleaching agent was better than the light activated bleaching agent when values were evaluated statistically.

Keywords: Bleaching, hydrogen peroxide, phosphate, Raman spectroscopy

Introduction

Esthetics of the teeth is of great importance to patients, including the tooth color. With the increasing demand for treatments to enhance aesthetic appearance, tooth bleaching is becoming a more common procedure in dental clinics. In 1877, Chappell first reported the bleaching technique. Even though a number of bleaching agents have been suggested, hydrogen peroxide still remains the most commonly used agent.

Vital tooth bleaching is an increasingly requested dental treatment. In contrast to more aggressive methods such as crowns or resin-bonded veneers, vital tooth bleaching is considered a more conservative approach to lightening teeth. In the past, a 10% concentration of carbamide peroxide was considered as the standard. In an attempt to increase the efficiency of the bleaching agents, higher concentrations were also used. However, most studies on tooth whitening relied solely on a subjective color matching technique to evaluate the outcome of bleaching procedures and only a few controlled clinical trials reported the effects of higher concentration agents.

The external bleaching of discolored tooth enamel in vivo is considered to be one of the most conservative of the restorative techniques. However, there is a lack of understanding of the mechanisms involved in bleaching at a molecular level. The molecular constitution of dental hard tissues has been studied using a variety of methods, including electronic microprobe, infrared (IR) spectroscopy, and Raman spectroscopy. The latter method is suitable for hard dental tissue analysis and has been used to quantify dentin and enamel modified by lactic acid and sodium hypochlorite.

The Raman spectroscopic technique allows vibrational spectra of minerals to be obtained by analyzing scattered light caused by visible or near-visible monochromatic laser excitation. This method possesses several advantages over IR absorption. However, there appears to be a paucity of information available in the literature regarding the phosphate concentration of enamel following the bleaching procedure with the latest bleaching material – 35% and 38% hydrogen peroxide. Being a nondestructive technique, the samples were studied before and after experimental procedures, allowing each sample to serve as its own control.

The aim of this ex vivo study was to evaluate the effect of in-office bleaching agents – 35% and 38% hydrogen peroxide
containing bleaching agents on the phosphate concentration of the enamel evaluated by Raman spectroscopy.

Materials and Methods

Forty noncarious, craze-free human maxillary incisors extracted for periodontal reasons were used in this study. All the teeth were cleaned of blood and debris using an ultrasonic scaler. After cleaning, the teeth were stored in saline until use which was changed daily. Prior to obtaining Raman spectra, each specimen was washed in distilled water and dried with a blotting paper. Baseline Raman spectra from each specimen were obtained before the application of the bleaching agent to assess the phosphate content present in the tooth [Figures 1–4].

The teeth were divided into two groups:

Group A: bleached with pola office bleach (35% hydrogen peroxide, potassium nitrate) (light activated).

Group B: bleached with opalescence Xtra bleach (38% hydrogen peroxide potassium nitrate and fluoride) (chemical activated).

All the teeth were aligned in rubber base putty impression material for the purpose of the bleaching procedure.

Group A was bleached with pola office bleach. Each tooth is exposed to a LED power bleach system for three cycles – 15 min each with 10 min resting time. The bleaching agent from the tooth surface is then removed with wet cotton at the end of every 10 min and reapplied again as before for the light activation. After the bleaching procedure, the treated specimens were taken to obtain Raman spectra to assess the phosphate loss after bleaching treatment.

Group B specimens were treated with chemically activated opalescence Xtra boost. The active ingredient in Opalescence Xtra boost is 38% hydrogen peroxide. The product is packaged as two syringes, one containing the liquid hydrogen peroxide and the other a dry, proprietary chemical activator.

The dry material is expressed from its syringe into the syringe containing the hydrogen peroxide. The resulting material is then expressed back and forth from one syringe to the other to thoroughly mix it. After mixing, the syringes are separated and the mixture is expressed directly onto the isolated tooth.

Figure 1: Light activated bleaching

Figure 2: Chemical activated bleaching

Figure 3: Raman spectroscopy

Figure 4: Phosphate concentration of enamel before and after bleaching
in a 0.5- to 1.0-mm-thick layer using a supplied disposable dispenser tip. The mixed bleaching agent is red in color to make it easier to visualize during use. After 15 min, the bleaching agent is removed from the teeth using wet cotton and rinsed. The process is repeated for three cycles with 10 min resting time. Opalescence Xtra boost has the advantages of not requiring a curing light, has a neutral pH that helps prevent post-treatment sensitivity, and maintains freshness because it is mixed immediately prior to use.

An intense broad band at 980 cm⁻¹ characteristic of the v₁ symmetric stretching mode of the tetrahedral phosphate (PO₄)₃ group, and representative of the mineral phase was often referred to as a chromophore. Heteroatoms, carbonyl, and phenyl rings in the conjugated system and were often included in compounds that possess extended conjugated chains. Materials in solution or on a surface are typically organic in nature. The color producing materials found within the tooth structures leading to a reduction in color. In general, the mechanism of bleaching by hydrogen peroxide was not well understood and can form a number of different active oxygen species depending on reaction conditions, including temperature, pH, light, and the presence of transition metals. Under alkaline conditions, hydrogen peroxide bleaching generally proceeds via the perhydroxyl anion (HO₂⁻). Under photochemically initiated reactions using light or lasers, the formation of hydroxyl radicals from hydrogen peroxide had been shown to increase.

As peroxide diffused into the tooth, it reacted with organic materials and formed hydroxyl radicals. BLEACHING AND DECOLORIZATION OF THE CHROMOPHORE COULD OCCUR BY DESTROYING ONE OR MORE OF THE DOUBLE BONDS IN THE CONJUGATED CHAIN, BY CLEAVING THE CONJUGATED CHAIN, OR BY OXIDATION OF OTHER CHEMICAL MOIETIES IN THE CONJUGATED CHAIN. HYDROGEN PEROXIDE OXIDIZED A WIDE VARIETY OF ORGANIC AND INORGANIC COMPOUNDS. THE MECHANISMS OF THESE REACTIONS WERE VARYING AND DEPENDENT ON THE SUBSTRATE, THE REACTION ENVIRONMENT, AND CATALYSIS.¹

When compared between the groups, the chemical activated bleaching agent showed less phosphate loss because of the presence of components such as potassium nitrate and fluoride. If the fluoride is incorporated into or adsorbed on the hydroxy apatite crystal, the crystal becomes more resistant to acid dissolution. This reaction explains the role of fluoride in enamel remineralization.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Bleaching results in the loss of phosphate groups from the surface enamel.

**Discussion**

The demand of the population regarding the dental appearance is greater than ever today. Discoloration of the teeth could be a physical handicap that impacts on persons' self-image, self-confidence, physical attractiveness, and employability. It cannot, therefore, be dismissed simply as a cosmetic problem.

Bleaching is a decolorization or whitening process that can occur either in solution or on a surface. The color producing materials in solution or on a surface are typically organic compounds that possess extended conjugated chains of alternating single or double bonds and often include heteroatoms, carbonyl, and phenyl rings in the conjugated system and were often referred to as a chromophore.

BLEACHING AND DECOLORIZATION OF THE CHROMOPHORE COULD OCCUR BY DESTROYING ONE OR MORE OF THE DOUBLE BONDS IN THE CONJUGATED CHAIN, BY CLEAVING THE CONJUGATED CHAIN, OR BY OXIDATION OF OTHER CHEMICAL MOIETIES IN THE CONJUGATED CHAIN. HYDROGEN PEROXIDE OXIDIZED A WIDE VARIETY OF ORGANIC AND INORGANIC COMPOUNDS. THE MECHANISMS OF THESE REACTIONS WERE VARYING AND DEPENDENT ON THE SUBSTRATE, THE REACTION ENVIRONMENT, AND CATALYSIS.¹

When compared between the groups, the chemical activated bleaching agent showed less phosphate loss because of the presence of components such as potassium nitrate and fluoride. If the fluoride is incorporated into or adsorbed on the hydroxy apatite crystal, the crystal becomes more resistant to acid dissolution. This reaction explains the role of fluoride in enamel remineralization.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Bleaching results in the loss of phosphate groups from the surface enamel.

**Results**

**Statistical analysis**

Statistical analysis was done using the paired ‘t’ test. Table 1 shows the mean value and SD for both the groups before and after the bleaching procedure.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Bleaching results in the loss of phosphate groups from the surface enamel.

**Discussion**

The demand of the population regarding the dental appearance is greater than ever today. Discoloration of the teeth could be a physical handicap that impacts on persons' self-image, self-confidence, physical attractiveness, and employability. It cannot, therefore, be dismissed simply as a cosmetic problem.

Bleaching is a decolorization or whitening process that can occur either in solution or on a surface. The color producing materials in solution or on a surface are typically organic compounds that possess extended conjugated chains of alternating single or double bonds and often include heteroatoms, carbonyl, and phenyl rings in the conjugated system and were often referred to as a chromophore.

BLEACHING AND DECOLORIZATION OF THE CHROMOPHORE COULD OCCUR BY DESTROYING ONE OR MORE OF THE DOUBLE BONDS IN THE CONJUGATED CHAIN, BY CLEAVING THE CONJUGATED CHAIN, OR BY OXIDATION OF OTHER CHEMICAL MOIETIES IN THE CONJUGATED CHAIN. HYDROGEN PEROXIDE OXIDIZED A WIDE VARIETY OF ORGANIC AND INORGANIC COMPOUNDS. THE MECHANISMS OF THESE REACTIONS WERE VARYING AND DEPENDENT ON THE SUBSTRATE, THE REACTION ENVIRONMENT, AND CATALYSIS.¹

When compared between the groups, the chemical activated bleaching agent showed less phosphate loss because of the presence of components such as potassium nitrate and fluoride. If the fluoride is incorporated into or adsorbed on the hydroxy apatite crystal, the crystal becomes more resistant to acid dissolution. This reaction explains the role of fluoride in enamel remineralization.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Bleaching results in the loss of phosphate groups from the surface enamel.

**Results**

**Statistical analysis**

Statistical analysis was done using the paired ‘t’ test. Table 1 shows the mean value and SD for both the groups before and after the bleaching procedure.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. Bleaching results in the loss of phosphate groups from the surface enamel.

**Discussion**

The demand of the population regarding the dental appearance is greater than ever today. Discoloration of the teeth could be a physical handicap that impacts on persons' self-image, self-confidence, physical attractiveness, and employability. It cannot, therefore, be dismissed simply as a cosmetic problem.

Bleaching is a decolorization or whitening process that can occur either in solution or on a surface. The color producing materials in solution or on a surface are typically organic compounds that possess extended conjugated chains of alternating single or double bonds and often include heteroatoms, carbonyl, and phenyl rings in the conjugated system and were often referred to as a chromophore.
bleaching material. Hence, the phosphate loss was more when compared to the chemical activated bleaching agent.

The results showed that the chemically activated bleaching agent showed less phosphate loss when compared with the light activated bleaching agent. This was because of Opalescence Xtra boost which is chemically activated gel that contains potassium nitrate and fluoride.[8]

In the clinical setting in vital teeth, there is a continuous outward movement of fluid through the dentinal tubules and porous enamel, which would tend to flush out any applied bleaching agent. The use of extracted teeth that were devoid of dentinal fluid probably allowed the bleaching agent to permeate the tooth more quickly than would be the case clinically.[2]

Tooth enamel is the most highly mineralized body tissue with minerals constituting 96% of the enamel, the rest being water and organic material. The primary mineral component is hydroxyapatite, with the basic formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, though other ions, such as fluorides, are usually incorporated. The organic portion of the enamel does not contain collagen, as dentin and bone do. Instead, it has two unique classes of proteins called amelogenins and enamelins. The role of these proteins is not understood, but is possibly involved in the development and the structural integrity of the enamel. The dissolution of the enamel in an acid occurs as a result of the interaction of hydrogen ions and hydroxyapatite.[2]

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 8\text{H}^+ \rightarrow 10\text{Ca}^{2+} + 6\text{HPO}_4^{2-} + 2\text{H}_2\text{O}$$

Hence, quantification of the phosphate group in hydroxyapatite is therefore a good indicator of the degree of mineralization of the enamel. In recent studies, the effect of bleaching agents on the surface microhardness and morphology of enamel specimens have been studied by storing in artificial saliva at 37 °C during the course of the study, although other studies did not use saliva as a storage medium. Artificial saliva contains phosphate, which has the potential effect of reversing the effects caused by the bleaching gels. However, the composition of the saliva was seldom given, and moreover, this introduced a variable. In such studies, both the effect of the bleaching gel on the enamel and the effect of saliva on bleached enamel were assessed at the same time.[2]

In this study, the salivary variable has been purposely eliminated so that the sole effect of a hydrogen peroxide gel on the molecular concentrations of enamel phosphate could be ascertained as a function of time. The specimens were therefore stored for the duration of the study in buffered saline.[2]

In this study, phosphate loss was assessed after the application of bleaching agents containing hydrogen peroxide (by Raman spectroscopy). Phosphate has been proved to exert cariostatic action, reduces enamel solubility, and acts as a buffer in neutralizing salivary, bacterial, plaque, and food pH values. Phosphates interfered with enzymatic processes on enamel surfaces to increase host resistance, decreases bacterial adhesion, and interferes with synthesis of extracellular polysaccharide formation. Finally, phosphate maintains plaque calcium and phosphorus levels.[6]

The results of this study were in accordance with Bistey et al. They showed changes in superficial enamel after hydrogen peroxide treatment, which affected the highly mineralized enamel even at low (10%) concentration. The alterations detected using the FT-IR method were proportional to the concentration. At higher concentration, more severe alteration was found in the IR spectra. It was not only the concentration of the peroxide solutions, but also the increase of treatment time that emphasized the spectral changes.[7]

The results were also in accordance with Chen et al. who evaluated the effects of fluoridated bleaching agents and postbleaching fluoridation treatment on the whitening efficiency and microhardness of bovine enamel. They found that the fluoridated bleaching agents produced less demineralization of surface morphology and microhardness. The addition of fluoride did not impede the whitening effect.[8]

Albers proposed a bleaching mechanism suggesting that whitening occurs owing to the diffusion of peroxide into enamel, and dentin and optimum whitening occurs when saturation of molecular bonding occurs. He further postulated that continued bleaching caused a progressive demineralization of the enamel with concomitant enamel matrix degradation of a layer probably only a few microns deep. This allowed an interpretation of the present study as follows. The application of chemical and light activated bleaching agents resulted in a decrease in the phosphate peak intensity, the largest decrease occurring with the light activated bleaching agent compared to chemical activated bleaching agent. This was to be expected, as the longer the samples were exposed to bleaching; the greater would be the alteration to both the inorganic and organic structures. The presence of fluoride in chemical activated bleaching agent had decreased the phosphate loss from the enamel when compared to light activated bleaching agent.[2]

However, both the inorganic and organic elements, albeit only a few microns deep, would have been degraded to an extent that washing the specimen, as per the experimental protocol, prior to obtaining Raman spectra, removed the altered superficial layer, exposing a less affected layer containing a higher phosphate group concentration.[2]

Further studies are required using the different type of bleaching agents, with different heat and light activating
sources, different time duration to investigate the molecular status of both organic and inorganic elements of the enamel and in vivo.

**Conclusion**

Within the limitations of this study, it can be concluded that chemically activated bleaching agents showed minimal phosphate loss when compared to light activated bleaching agents. Chemically activated bleaching agents were better than light activated bleaching agents when values were evaluated statistically.

Raman spectroscopy is a noninvasive technique that requires simple sample preparation, and this method is used to evaluate the level of phosphate content in the enamel. Consideration of all the parameters of this study proved that chemically activated bleaching agents have an edge over light activated bleaching agents, in maintaining the phosphate concentration of the enamel.

**References**

1. Joiner A. The Bleaching of teeth: A Review of Literature. J Dent 2006;34:412-9.
2. Santin A, Pulham CR, Rajab A, Ibbetson R. The effect of a 10% carbamide peroxide bleaching agent on the phosphate concentration of tooth enamel assessed by Raman spectroscopy. Dent Traumatol 2008;24:220-3.
3. Ten Cate AR. Oral Histology. Development, Structures, and Function. Chapter 11, 3rd ed. New Delhi: Jaypee Brothers; 1991. p. 218-35.
4. Greenwall L. Bleaching Techniques in Restorative Dentistry. An Illustrated Guide. Chapter 3. London: Martin Duntiz Ltd; 2002. p. 31-61.
5. Available from: http://www.ultradent.com/products/instructions/opal_boost.pdf. (accessed the webpage lastly on 21.1.12)
6. Peter S. Essentials of preventive and Community Dentistry. 1st ed Chapter 4. Arya (MED) Publishing house New Delhi 2002. p. 166-8.
7. Bistey T, Nagy IP, Simo A, Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J Dent 2007;35:325-30.
8. Chen HP, Chang CH, Liu JK, Chuang SF, Yang JY. Effect of fluoride containing bleaching agents on enamel surface properties. J Dent 2008;36:718-25.

**How to cite this article:** Venkatesan SM, Narayan GS, Ramachandran AK, Indira R. The effect of two bleaching agents on the phosphate concentration of the enamel evaluated by Raman spectroscopy: An ex vivo study. Contemp Clin Dent 2012;3:S172-6.

**Source of Support:** Nil. **Conflict of Interest:** None declared.