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The effect of a 10% carbamide peroxide bleaching agent on the phosphate concentration of tooth enamel assessed by Raman spectroscopy

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Abstract –The study assessed changes in phosphate concentrations of surface enamel treated with a proprietary bleaching agent ('*PEROXIDE*') containing 10% carbamide peroxide over a 28-day period using Raman spectroscopy. Six non-carious human molar teeth (age range 12–21 years), extracted for orthodontic reasons, were used. From the enamel face of each half tooth, a near flat enamel section, approximately 2 × 2 mm, was cut, providing 12 specimens. Each specimen was treated with 10% carbamide peroxide for 8 h day^{-1} for 28 consecutive days, with Raman spectra being obtained prior to bleaching and after 7, 14, 21 and 28 days. Raman spectra were acquired on a confocal LabRam 300 spectrometer fitted with an Olympus B microscope (Olympus, Middlesex, UK).The difference in the maximum peak values for phosphate group concentrations were tested using the Friedman test (non-parametric ANOVA) and Dunn's multiple comparison test. An intense broad band at 980 cm^{-1}, characteristic of phosphate groupings, was always observed. At 7 and 14 days, and again at 28 days, there was a significant decrease in the phosphate group concentration compared with base-line measurements ($P < 0.05$) but not at 21 days ($P > 0.05$). Ideally, bleaching should not be continued to a point where surface enamel is lost, and the present study suggests that a regime using 10% carbamide peroxide should not extend to 7 days.

The external bleaching of discolored tooth enamel *in vivo* is considered to be one of the most conservative of the restorative techniques, requiring no surgical intervention. The use of carbamide peroxide at 10% was first used by Haywood & Heyman (1989) (1). It breaks down to produce 3.6% hydrogen peroxide (2) and is considered both safe and effective (3). However, there is a lack of understanding of the mechanisms involved in bleaching at a molecular level. Previous investigations have studied microhardness and surface roughness of enamel surfaces (4, 5), microleakage around restorations (6, 7), pulpal responses (8–10) and sensitivity (11, 12), and the latter was attributed to the removal of the mineral content of enamel and dentine (13). Few studies have addressed the changes that occur in enamel at a molecular level.

The molecular constituents of dental hard tissues have been the subject of many studies using a variety of methods, including electronic microprobe (14), infrared (IR) spectroscopy (15, 16) and Raman spectroscopy (17–19). The latter method is suitable for hard dental tissue analysis and has been used to quantify dentin and enamel modified by lactic acid (20) and sodium hypochlorite (21).

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. The method possesses several advantages over IR absorption, including simple sample preparation, easy spectral/band analysis, and linear response to mineral and chemical concentrations. Being a non-destructive technique, samples can be studied before and after experimental procedures, allowing each sample to serve as its own control.

The aim of the present study was to assess changes in phosphate concentrations of surface enamel treated with a proprietary bleaching agent ('*PEROXIDE*'; SDI Ltd., Victoria, Australia) containing 10% carbamide peroxide over a 28-day period using Raman spectroscopy.

Materials and methods

Six non-carious human molar teeth (age range 12–21 years), extracted for orthodontic reasons, were used. The teeth were immediately cleaned of all blood and debris in an ultrasonic cleaner containing buffered saline solution, and within 1 h of extraction were sectioned in a mesio-distal plane. From the enamel face of each half tooth, a near flat enamel section, approximately 2 × 2 mm, was cut, providing 12 specimens. The specimens were placed in a sterilized multi-compartmental ice-cube tray containing buffered saline solution and numbered 1–12. They were kept for not more than

7 days prior to the commencement of the experimental procedure. For the duration of the study, the specimens were stored in buffered saline, which was changed daily.

Prior to obtaining Raman spectra, each specimen was washed in distilled water and dried with blotting paper. Base-line spectra from each specimen were obtained before the application of the bleaching agent. Subsequently, each specimen was treated with 10% carbamide peroxide, ('*PEROXIDE*') for 8 h day^{-1} for 28 consecutive days, with Raman spectra obtained at 7, 14, 21 and 28 days.

Raman spectra were acquired on a confocal LabRam 300 spectrometer (Horiba Jobin Yvon, Middlesex, UK) fitted with an Olympus B microscope (Olympus, Middlesex, UK). The excitation source was with monochromatic radiation emitted by a He/Ne laser operating at 632.8 nm (red) in combination with a slit of 150 um, confocal hole 250 um and 1800 mm g^{-1} holographic grating providing high resolution. The LabRam was connected to a Dell Optiplex Gn (Dell inc., Texas, TX, USA) using Microsoft Windows 95 (Microsoft corp., Washington, DC, USA) as an operating system. Prior to obtaining spectra, the machine was calibrated using a silicon specimen with a characteristic band at 520.8 cm^{-1}.

All measurements were systematically made under the same conditions, especially the same laser penetration depth (hole adjustment setting) in order to decrease the fluorescence phenomena resulting from organic components within the tissue (22).

Statistical analysis

The difference in the maximum peak values for phosphate group concentrations were tested using the Friedman test (non-parametric ANOVA) and Dunn's multiple comparison test.

Results

Figure 1 shows a typical Raman spectrum obtained from enamel. An intense broad band at 980 cm^{-1}, characteristic of the v_1 symmetric stretching mode of the tetrahedral phosphate (PO_4^{3-}) group, and representative of the mineral phase (carbonated hydroxyapatite) was always observed, and varied in peak intensity according to the experimental conditions. Table 1 gives the spectral peak intensity of the phosphate group in all 12 samples, relative to the baseline associated with each sample. Table 2 gives the comparisons of spectral peak intensities of phosphate groups in the control samples versus the experimental samples

All specimens showed a progressive decrease in the peak intensity of the v_1 band over the experimental period. At 7 and 14 days, and again at 28 days, there was a significant difference in the phosphate group concentration compared with the base-line measurements ($P < 0.05$) but not at 21 days ($P > 0.05$).

Discussion

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 $Ca_{10}(PO_4)_6(OH)_2$, though other ions, such as fluoride, are usually incorporated. The organic portion of 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 enamel. The dissolution of enamel in an acid occurs as a result of the interaction of hydrogen ions and hydroxyapatite as follows:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O$$

Quantification of the phosphate group in hydroxyapatite is therefore a good indicator of the degree of mineralization of enamel. In recent studies on the effect of bleaching agents on surface microhardness and morphology of enamel (5, 23, 24), specimens have been

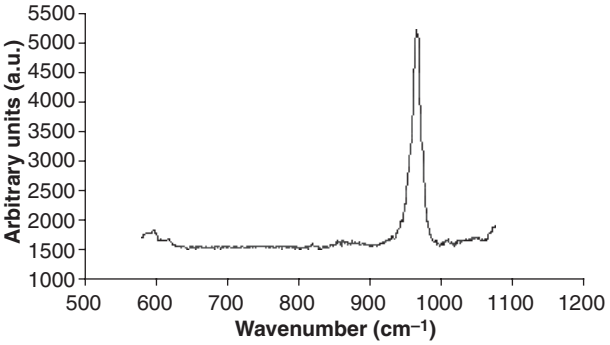

Fig 1. Raman sepctrum obtained from enamel.

Table 1. Spectral peak intensities of phosphate group

Sample	Control	7 days	14 days	21 days	28 days
1	2546	420	388	371	301
2	720	378	329	607	496
3	1134	518	505	534	517
4	1056	583	594	517	329
5	1006	392	303	435	350
6	1078	377	285	458	389
7	697	516	298	498	328
8	414	323	305	390	365
9	681	474	376	439	358
10	676	460	373	491	343
11	460	406	360	445	369
12	691	476	349	440	353

Table 2. Comparisons of spectral peak intensities of phosphate group

Control vs 7 days conditioning	$P < 0.05$
Control vs 14 days conditioning	$P < 0.001$
Control vs 21 days conditioning	$P > 0.05$
Control vs 28 days conditioning	$P < 0.001$

Dunn's multiple comparison test.

stored in artificial saliva at 37°C during the course of the study, although other studies did not use saliva as a storage medium (25–27).

Artificial saliva contains phosphate, which will have the potential effect of reversing the effect caused by the bleaching gels. However, the composition of the saliva is seldom given, and moreover, this introduces a variable. In such studies, both the effect of the bleaching gel on enamel and the effect of saliva on bleached enamel are assessed at the same time.

In the present study, the salivary variable has been purposely eliminated so that the sole effect of a 10% carbamide peroxide gel on the molecular concentrations of enamel phosphate can be ascertained as a function of time. The specimens were therefore stored for the duration of the study in buffered saline. Further studies on the salivary effects are being undertaken.

Carbamide peroxide breaks down to give 3.6% hydrogen peroxide and urea (2), which in turn breaks down producing water, oxygen, carbon dioxide and ammonia, and this results in a concomitant slight lowering of pH of the bleaching agent (28). This reduction in pH would affect the dissolution of the mineral content of enamel, though it is of interest to note that *in vivo*, a 10% carbamide peroxide solution caused a pH rise above 7, which would not cause breakdown of enamel.

These apparent contradictions illustrate the limited knowledge that exists on the exact mechanisms involved in tooth bleaching and highlight the need to investigate every aspect of these mechanisms and not the apparent overall clinical effect alone.

Confocal Raman spectrometry allows the molecular analysis of mineralized dental tissues. The output information is provided in the form of curves representing the intensity of the signal according to the frequency, and its mathematical exploitation permits comparative and quantitative analyses. By this process, the *in vitro* action of 10% carbamide peroxide on enamel from human permanent teeth was investigated.

In the present study, maximum peak intensity values of the phosphate groups were used. The intensity is measured in arbitrary units. Previous studies (20, 29) have used the total area under the band or the width at half the maximum peak value for sample comparison. However, in a recent study (30), it was shown that there was no difference in the results analyzed using maximum peak intensity, area under curve or half-width values. This is because the peak shape is highly symmetrical. It was therefore decided to use the maximum peak intensity values in this study. At 21 days, there was no significant difference between the base-line measurement, suggesting an increase in phosphate group concentration compared with that at 7 and 14 days. It is unlikely that the continued application of carbamide peroxide caused an increase in phosphate concentration.

Albers (31) proposed a bleaching mechanism suggesting that whitening occurs owing to the diffusion of peroxide into enamel, and dentine 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 micros deep. This hypothesis allows an interpretation of the present study as follows. The application of 10% carbamide peroxide resulted in a decrease in the phosphate peak intensity, the largest decrease occurring at 7 days, but continuing over the experimental period. This is to be expected as the longer the samples are exposed to bleaching, the greater will be the alteration to both the inorganic and organic structures (1). However, by 21 days, 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, removes the altered superficial layer, exposing a less affected layer containing a higher phosphate group concentration. Subsequent bleaching up to 28 days continued the effect seen at 7 and 14 days.

Further studies are required to investigate the molecular status of both the organic and inorganic elements of enamel at smaller time limits and to substantiate this hypothesis.

Clinical implications

Bleaching results in the loss of phosphate groups from surface enamel probably accompanied by enamel matrix degradation. Ideally, bleaching should not be continued to a point where surface enamel is lost, and the present study suggests that this means that the regime with 10% carbamide peroxide should not extend to 7 days, especially in patients whose salivary composition limits remineralization.

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