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Effects of pH Values of Hydrogen Peroxide Bleaching Agents on Enamel Surface Properties

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Clinical Relevance

The erosion of enamel was observed in acidic bleaching solutions and resulted in a slight whitening effect. However, the neutral or alkaline bleaching agent shows a more obvious whitening effect, without the evidence of enamel erosion.

SUMMARY

This study investigated the influence of pH values of bleaching agents on the properties of the enamel surface. Sixty freshly extracted premolars were embedded in epoxy resin and mesiodistally sectioned through the buccal aspect into two parts. The sectioned slabs were distributed among six groups (n=10) and treated using different solutions. Group HCl was treated with HCl solution (pH=3.0) and served as a positive control. Group DW, stored in distilled water (pH=7.0), served as a negative control. Four treatment groups were treated using 30% hydrogen peroxide solutions with different pH values: group HP3 (pH=3.0), group HP5 (pH=5.0), group HP7 (pH=7.0), and group HP8 (pH=8.0). The buccal slabs were subjected to spectrophotometric evaluations. Scanning electron microscopy investigation and Micro-Raman spectroscopy were used to evaluate enamel surface morphological and chemical composition alterations. pH value has a significant influence on the color changes after bleaching ($p<0.001$). Tukey's multiple comparisons revealed that the order of color changes was HP8, HP7>HP5, HP3>HCl>DW. No obvious morphological alterations were detected on the enamel surface in groups DW, HP7, and HP8. The enamel surface of groups HCl and HP3 showed significant alterations with an erosion appearance. No obvious chemical composition changes were detected with respect to Micro-Raman analysis. Within the limitations of this study, it was concluded that no obvious

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morphological or chemical composition alterations of enamel surface were detected in the neutral or alkaline bleaching solutions. Bleaching solutions with lower pH values could result in more significant erosion of enamel, which represented a slight whitening effect.

INTRODUCTION

The use of bleaching agents such as hydrogen peroxide (HP) or carbamide peroxide (CP), for esthetic purposes, has gained worldwide popularity because of their efficacy in whitening teeth at minimal expense and invasion of dental hard tissues.[1] Color alteration is the most noticeable evidence in a bleaching procedure; however, the mechanisms of the whitening effects are far from clear.[2,3] As understood thus far, two possible approaches of teeth bleaching have been proposed: chemical composition alterations and erosion of tooth hard tissue.[4,5] Chemical composition alterations of bleached teeth have been shown by several studies using energy dispersive spectroscopy,[6] x-ray diffraction,[7] or infrared spectroscopy.[8] It was considered to be the penetration effect that peroxide radicals, which are generated by the degradation of hydrogen peroxide on the enamel surface, penetrate the enamel/dentin and break down the pigment of the discolored dentin.[9] With respect to erosion of tooth hard tissue, however, general agreement has not been reached. Most scanning electron microscopy (SEM) studies of enamel bleached with CP or HP show little or no change in enamel morphology.[10] Shallow erosion,[11] increased porosity,[12] or more substantial changes in enamel structure have also been reported.[13,14] Micro computed tomography (Micro-CT) analysis has revealed that the density of enamel decreased after bleaching treatment, which was considered to be the dissolution or demineralization of enamel.[15,16] These studies indicated that erosion of enamel might occur during bleaching procedures. It should be noted that the bleaching agents used in these studies exhibited various pH values, which might be the possible explanation for these controversial results.

Nevertheless, there are few published articles regarding the effects of pH values on the bleaching procedure. Therefore, the aim of the present study was to investigate the effects of 30% hydrogen peroxide solutions with different pH values on enamel surface morphology, chemical composition, and color. The null hypothesis of this study was that pH values of bleaching agents have no significant influence on the outcomes of tooth-bleaching procedures.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the School and Hospital of Stomatology, Wuhan University. Sixty noncarious human premolars freshly extracted for orthodontic reasons and free from enamel defects were included in this study. Discolored teeth, such as tetracycline stained teeth or teeth with fluorosis, were excluded. Patients from whom teeth were being extracted were asked to read and sign a consent form prior to the extraction. The teeth were cleaned thoroughly and stored in 0.5% thymol at 4°C until required. All of the teeth were collected within two weeks and stored for no longer than three weeks.

Specimen Preparation

All of the teeth were embedded into translucent epoxy resin and longitudinally sectioned near the buccal surface from the mesiodistal direction using a diamond rotary cutting instrument (SP1600, Leica Microsystems GmbH, Wetzlar, Germany). One buccal enamel part and one major part were obtained from each tooth. To ensure the section plane was located within the enamel layer, the thickness of the buccal part was limited to less than 0.7 mm (Figure 1). Because of the destruction of some buccal parts, only 33 buccal parts were obtained. The sectioned surfaces were polished using 800-, 1200-, 2000-, and 2500-grit silicon carbide paper under water flushing to obtain standardized flat enamel surfaces.

General Study Design

Sixty major parts were subjected to spectrophotometric evaluations. Twenty-one buccal parts, randomly selected from the 33 buccal parts, were subjected to Micro-Raman spectroscopy analyzes and SEM investigations (Figure 1). The specimens were randomly distributed among six groups as follows:

- DW (negative control group): specimens were stored in 5 mL distilled water (pH=7.0, Milli-Q, Millipore Inc, France).
- HCl (positive control group): specimens were immersed in a 5-mL HCl solution (pH=3.0, Sinopharm Chemical Reagent Co Ltd, Shanghai, China).
- HP3, HP5, HP7, and HP8: specimens were immersed in 5-mL HP solutions with pH values of 3.0, 5.0, 7.0, and 8.0, respectively (Sinopharm Chemical Reagent Co Ltd, Shanghai, China).

pH values of the solutions were measured using a portable pH meter with a direct electrode, which was

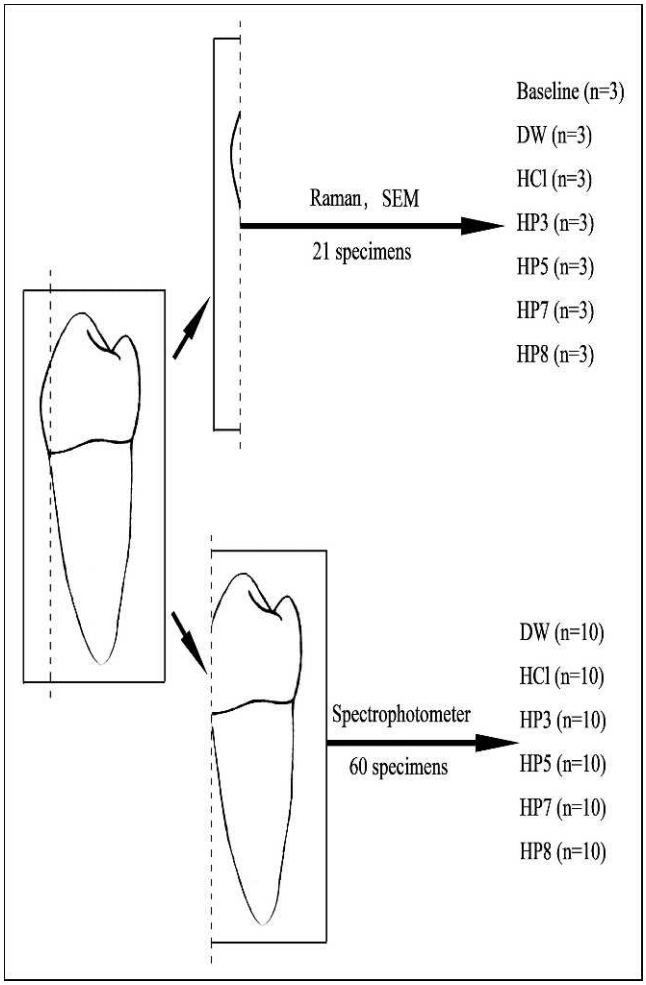

Figure 1. *Schematic diagram of specimen fabrication and grouping information.*

calibrated with standard buffer solutions at pH 4.0 and 7.0 prior to analysis (MA 235, Mettler Toledo Inc, Canton, MA, USA). 1 M HCl or 1 M NaOH solution was added into the HP solutions to adjust the pH to desired values. The concentrations of HP in the solutions were monitored using a testing kit for HP solutions (KGT018, Nanjing Keygen Biotech Inc, Nanjing, China).

The specimens were treated in the solutions for 30 minutes. The solutions were then refreshed and the treatments repeated for another 30 minutes.

Color Measurements

The colors of the specimens were measured using a spectrophotometer (PR-650 Spectra Scan, Photo Research Inc, Chatsworth, CA, USA), equipped with the MS-75 and SL-0.5 lens. The spectrophotometer and a standardized illumination source D65 (OL 53, Optronic Laboratories Inc, Orlando, FL, USA) provided an optical configuration of 0°/45° geometry, recommended for measuring the color of translucent materials.[17] The measurement aperture size was 1.5 mm in diameter. For all of the color measurements, the spectral reflectance was obtained from 380 to 780 nm, with a 2-nm interval, and expressed in terms of three coordinate values (L*, a*, and b*), which were established by Commission International de l'Eclairage.[18] Before measurements, the spectrophotometer was calibrated with a white reflectance standard tile supplied by the manufacturer. The color measurements were performed before the treatments (baseline) and at 30 minutes and 60 minutes after the treatments, respectively.

Color differences (ΔE) between the baseline and 30 minutes or 60 minutes after treatments were registered as ΔE_{30} or ΔE_{60} and determined by the equation $\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$. ΔL*, Δa*, and Δb* represent the varieties in lightness, green-red coordinate, and blue-yellow coordinate, respectively.

Micro-Raman Spectroscopy

The buccal slabs of enamel were analyzed using Micro-Raman spectroscopy (Smart Raman DXR, Thermo Fisher Scientific Inc) before the treatments (baseline) and after the 60-minute treatments. The excitation source was monochromatic radiation emitted by a Nd YAG laser operating at 532 nm (10 mW) in combination with a slit of 100 μm. Before the measurement session, the instrument calibration was performed using the Alignment/Calibration Tool applied by the manufacturer. Raman spectra were collected and recorded with a stigmatic Raman spectrograph, optimized for an air-cooled 1024×256 pixel CCD camera (grid with 900 lines/mm). The spectra were recorded between 50 and 3500 cm^{-1}, and the overall spectral resolution was about 2 cm^{-1}. Spectral data were visualized on a computer and processed using OMNIC software (Standard Packages, Thermo Fisher Scientific Inc). All measurements were systematically made under the same conditions, especially the same laser penetration depth, to decrease the fluorescence phenomena resulting from organic components within the tissue.[20]

SEM Analysis

After Micro-Raman spectroscopy, all specimens were thoroughly cleaned using distilled water and air dried, subsequently sputtered with gold, and investigated using SEM (JSM-5510LV, JEOL, Tokyo, Japan). Photomicrographs of representative areas

of the enamel surfaces were recorded at a magnification of 20k×. The SEM investigations were performed before the treatments (baseline) and at 60 minutes after the treatments.

Statistical Analysis

The means of ΔE_{60} were analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc comparisons were performed to evaluate the within-group factor of Treatment. All analyses were accomplished using the statistical software SPSS program (SPSS 13.0 for Windows, SPSS, Chicago, IL, USA), and the level of significance was established as α=0.05.

RESULTS

The concentrations of HP solutions are listed in Table 1. The concentrations of HP decreased while the pH values of the solution increased. After 30 minutes of bleaching treatment, the lowest HP concentration was less than 20% in group HP8.

Color Changes After Treatments

Means of ΔE and color coordinates (L*, a*, and b*) of each group are shown in Figure 2. In the first 30-minute treatment session, increased L* values and decreased b* values were detected in all groups (except group DW). In the second 30-minute treatment session, the L* and b* values in groups DW and HCl did not change. One-way ANOVA revealed that the factor of Treatment significantly influenced the ΔE_{60} ($p<0.001$; Table 2). The results of Tukey's post hoc comparisons are listed in Table 3. The order of color changes was HP8, HP7>HP5, HP3>HCl>DW. No statistical differences were found between groups HP3 and HP5 or HP7 and HP8.

Surface Morphology

After the treatments, the specimens showed different surface morphologies (Figure 3). No obvious morphological alterations were detected on the enamel surface in both groups DW and HP8. The enamel surface in groups HCl and HP3 showed significant alteration with erosion appearance. The surface morphological changes in groups HP5 and HP7 were less distinct than those of groups HCl and HP3.

Micro-Raman Analyses

The phosphate ν1 band, a characteristic of the ν1 symmetric stretching mode of the tetrahedral phosphate (PO_4^{3-}), was seen clearly in all specimens at 960 cm^{-1}. It is also evident for ν3 phosphate at 1028 cm^{-1}, ν4 at 591 cm^{-1}, and ν2 at 430 cm^{-1}, respectively. The typical symmetric stretching mode of Type B carbonate was presented at 1075 cm^{-1}. Representative spectrums are shown in Figure 4. No obvious alterations were detected after the treatments, except for the increased intensity of the band at 579 cm^{-1} in all of the bleaching treated groups. The typical Raman peak at 591 cm^{-1} was usually assigned as the ν4 phosphate. The untypical signals for apatite in the spectrum, at 579 cm^{-1}, in all the bleached groups indicated variations in the P-O bond lengths.

Table 1: *Measurements for the Concentration of HP in the Solutions With Different pH Values*

Group	HP Concentration, w/v%		
	Baseline	**Stored in Air for 30 min**	**Reacted With Teeth for 30 min**
HP3	28.30 ± 1.23	27.92 ± 1.35	25.32 ± 1.71
HP5	28.30 ± 1.23	27.84 ± 1.63	24.66 ± 2.16
HP7	28.30 ± 1.23	25.12 ± 1.81	23.84 ± 2.89
HP8	28.30 ± 1.23	24.48 ± 1.71	19.96 ± 2.97

DISCUSSION

Three possible degradation formulas for hydrogen peroxide are its acting as a strong oxidizing agent through the formation of free radicals,[21] reactive oxygen molecules, or hydrogen peroxide anions,[22] which are shown as the following formulas:

1. $H_2O_2 \rightarrow 2HO^{\bullet}$
   $HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet}$
   $HO_2^{\bullet} \leftarrow\rightarrow H^+ + O_2^{\bullet}$
2. $2H_2O_2 \leftarrow\rightarrow 2H_2O + 2O^{\bullet} \leftarrow\rightarrow 2H_2O + O_2$
3. $H_2O_2 \leftarrow\rightarrow H^+ + HOO^-$

It is noticed that hydrogen ions (H^+) are produced in the process of hydrogen peroxide break down (formulas a and c), which could produce a relatively acidic environment with the bleaching procedures and might affect the surface and subsurface integrity of dental tissues. In addition, many bleaching products are formulated at lower pH values to ensure the stability of hydrogen peroxide. Therefore, the pH value of the bleaching agents might be an important factor in the reactions of the bleaching process.

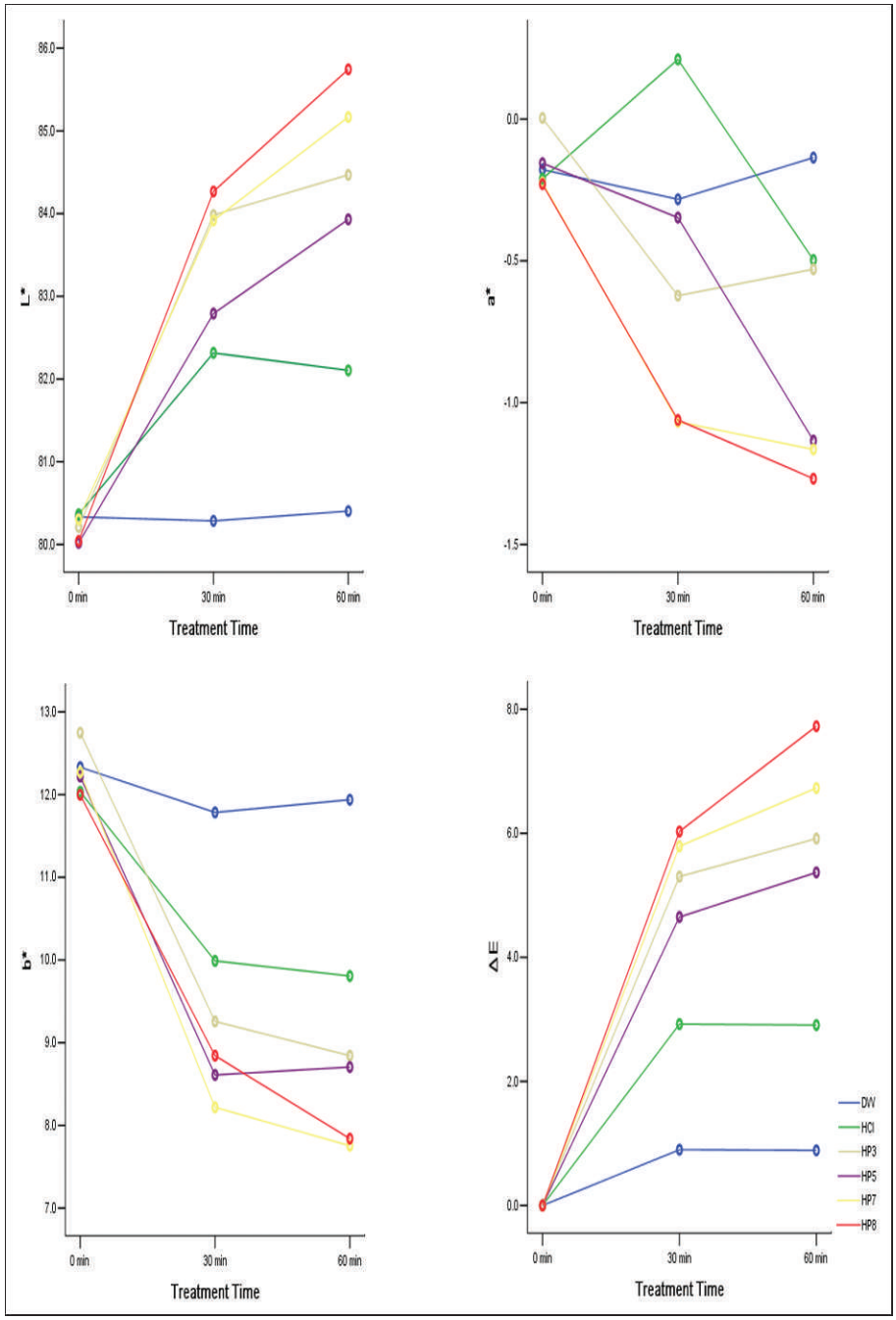

Figure 2. *Color coordinates (L*, a*, and b*) and total color changes (ΔE) in each group.*

The present study is part of ongoing research to develop a further understanding of the mechanisms of tooth bleaching. It is important to consider that both the bleaching matrix and peroxide may have a role in tooth tissue response to the bleaching procedures. The potential effects of the acidic ingredient in bleaching agents on the bleaching procedures are the focus of this study. Acidic treatment on the tooth surface would result in the erosion of enamel, which could reduce the translucency of enamel and ensure opaqueness of the subjacent dentin layer.[12,23-25] It is called the frosted-glass effect, representing a certain extent of the teeth-whitening effect. Whether enamel erosion occurs during the bleaching process has not received general agreement; however, the pH values of the bleaching products have attracted the attention of researchers. Sulieman and others[4] have reported

Table 2: *One-Way ANOVA Results of ΔE_{60} Values*

	Sum of Squares	df	Mean Square	F	*p*
Between groups	326.343	5	65.269	49.511	<0.001
Within groups	71.187	54	1.318		
Total	397.530	59			

Table 3: *Means and Tukey's Post Hoc Comparisons of ΔE_{60} Values*

Group	Means of ΔE_{60}	SD	Tukey's Intervals			
DW	0.89	0.60	a			
HCl	2.91	0.81		b		
HP3	5.91	1.34			c	
HP5	5.37	1.16			c	
HP7	6.73	1.64				d
HP8	7.73	1.63				d

that HP itself had no deleterious effects on enamel but that the pH of the bleaching materials might cause adverse effects during the bleaching procedure. Although some attempts have been made to provide a less acidic or neutral environment for the bleaching procedures, there are still a number of bleaching products with low pH values. Therefore, it is necessary to explore the potential interactions of free radicals released from HP and the acidic environment in the bleaching process.

Some reports have found that calcium was dissolved from human enamel treated with 10% CP commercial bleaching agents with a pH of 4.7 to 5.3.[1,26] In addition, decalcification has also been reported in enamel treated with CP with a pH of 6.7 to 6.8, which is higher than the critical pH.[15,27] Therefore, the bleaching solutions with pH values of 5.0 and 7.0 were adopted in this study. For the investigation of the effects of more acidic or alkaline bleaching solutions on enamel, pH values of 3.0 and 8.0 were also included. In the neutral or alkaline environments, HP solution was not stable and the degradation increased. Thus, the concentration of HP in the solutions was monitored in the preliminary study. After 30 minutes of exposure in the air, the lowest HP concentration was 24.48 ± 1.71% (in group HP8), which was slightly decreased from the original concentration (28.30 ± 1.23%). So, 30 minutes was set as the treatment interval for all groups.

In groups HP7 and HP8, three color coordinate alterations (ΔL*, Δa*, and Δb*) and total color

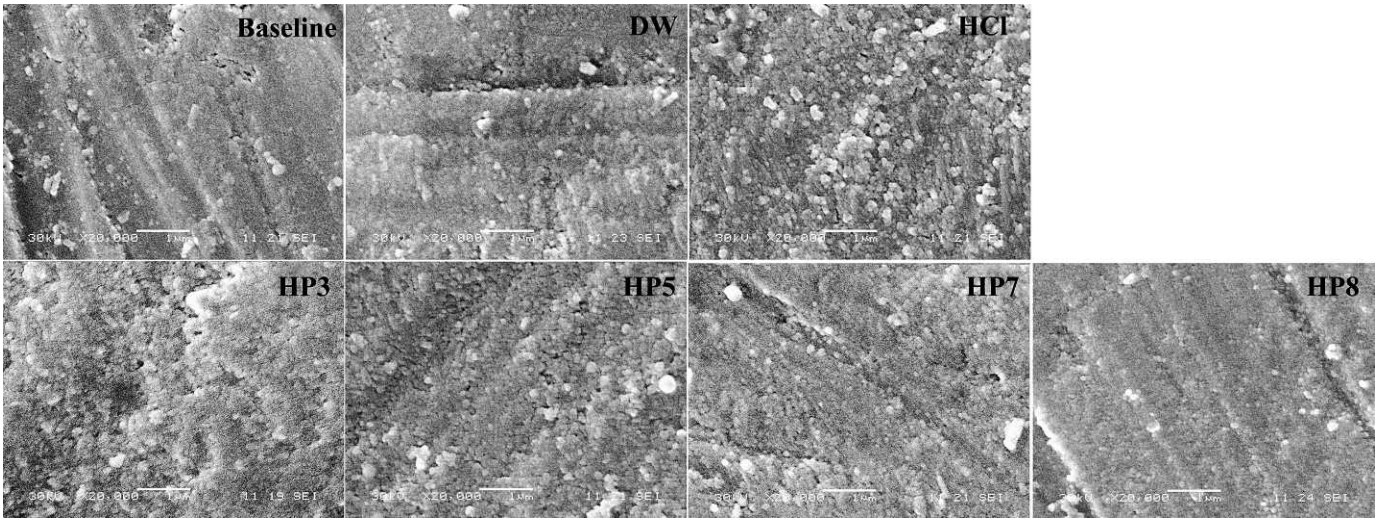

Figure 3. *Representative photomicrographs of enamel surface pretreatment and posttreatment (magnification of 20K×). Acidic erosion of enamel surface was detected in groups HCl, HP3, and HP5.*

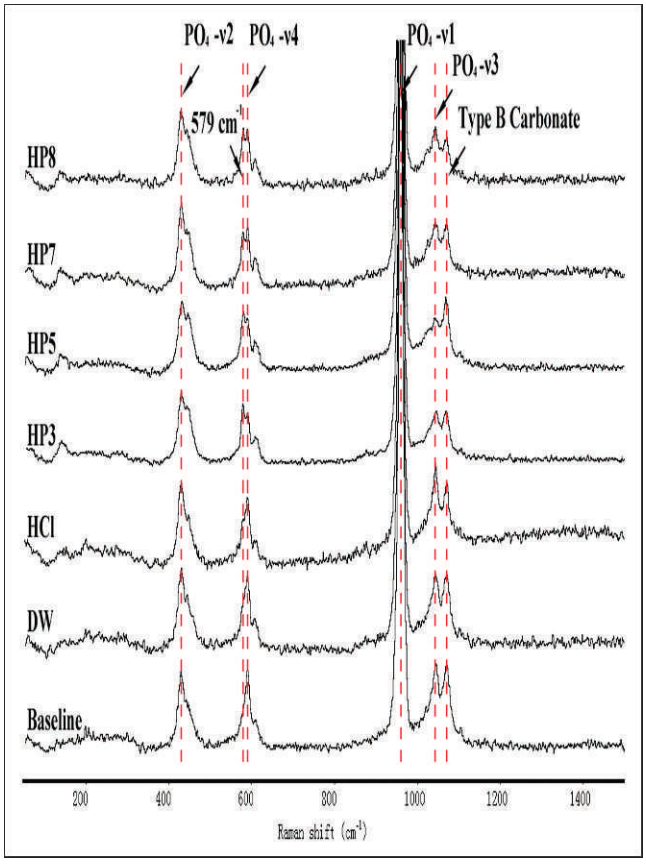

Figure 4. *Line scans of micro-Raman Spectra pretreatment and posttreatment (cutout region 200-1400 cm^{-1} peaks, which are sensitive to apatite integrity). No obvious changes of Raman specta were detected in most of the specimens.*

differences (ΔE_{60}) were significantly higher than in the other groups. This result is consistent with the high degradation of HP in these groups (Table 1). Although there was a certain extent of tooth-whitening effect in group HCl, the color alteration was statistically lower than in all the bleaching groups. In the second 30-minute treatment session, no obvious color changes were observed in both groups DW and HCl. This indicated that enamel erosion inducing color alteration was limited and that an acid solution with a pH value of 3.0 could not affect the deeper tooth tissue in a 30-minute exposure.

SEM microphotographs detected obvious enamel erosion in HCl groups HP3 and HP5. The pH values in these groups were less than the critical pH value of enamel dissolving (pH=5.5). There was a slight morphological change in group HP7, however, which has a higher pH value than the critical pH for enamel dissolution. This might indicate that the enamel erosion was possibly induced by the H+ brought out in the HP breakdown process. The results were consistent with some studies that reported the decalcification of enamel with CP with a pH of 6.7 to 6.8. From a typical SEM photograph from group HP8, it is thought that bleaching does not alter the enamel surface topography as reported by various researchers. Generally, the present study supported the idea that the peroxide agents did not induce the erosion of the enamel surface.

The available literature shows that both enamel and dentin are permeable by HP and CP agents. Limited data regarding passage of active oxidizing components into dental hard tissues suggested that the peroxide can diffuse into the enamel-dentin complex and even reach the pulp chamber.[28,29] The bleaching-induced chemical changes of dentin have been proven by several prior studies. However, in this study, Raman spectroscopy was used only to investigate the potential enamel surface alterations after bleaching treatment. Therefore, all of the Raman peaks represented the compositions of the hydroxyapatite in enamel.[30,31] No obvious chemical alterations in the enamel surface were detected from the results of the Raman spectrum. However, in the bleaching groups, the presence of the peak intensity at 579 cm^{-1} might indicate the tiny alteration of enamel tissue after bleaching treatments. With respect to the Raman spectrum and SEM investigations, no obvious changes were found in group HP8, which indicated that there was no evidence of the oxidation or erosion of the enamel surface in the alkaline HP bleaching solution. On the other hand, significant whitening effects were detected in group HP8 using a spectrophotometer. This might indicate that the whitening effects were mainly conducted by the oxidation within the dentin layer.

Obviously, the at-home bleaching systems using lower concentrations of HP or CP are more popular; however, these systems commonly require a continual application for one month, in which the demineralization and remineralization procedures will interact. Therefore, a higher concentration of HP solution was selected in this study, and a routine in-office bleaching procedure was performed to simplify the bleaching procedure and differentiate demonstrably the phases of bleaching and postbleaching. However, in *in vivo* trials, the remineralization effects of saliva might lessen the erosion of acidic bleaching agents. Therefore, remineralization effects should be involved in future studies.

CONCLUSIONS

Within the limitations of this study, the following conclusions were drawn: There was no evidence of

enamel erosion in alkaline bleaching solutions with the SEM and Micro-Raman investigations. Acidic treatment could impact the extent of the bleaching effect; however, oxidation reactions might play more important roles in teeth-bleaching procedures.

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