Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence

Tao Jiang
Wuhan University
Key Laboratory for Oral Biomedical Engineering
Ministry of Education
School and Hospital of Stomatology
237 Luoyu Road
Wuhan 430079, China
and
Institute of Analytical and Biomedical Science
Luojia Hill
Wuhan 430072, China

Xiao Ma
Yining Wang
Wuhan University
Key Laboratory for Oral Biomedical Engineering
Ministry of Education
School and Hospital of Stomatology
237 Luoyu Road
Wuhan 430079, China

Hua Tong
Xinyu Shen
Wuhan University
Institute of Analytical and Biomedical Science
Luojia Hill
Wuhan 430072, China

Yaogai Hu
Wuhan University
College of Electronic Information
and
Institute of Analytical and Biomedical Science
Luojia Hill
Wuhan 430072, China

Jiming Hu
Wuhan University
Institute of Analytical and Biomedical Science
Luojia Hill, Wuhan 430072, China

Abstract. The safety of tooth bleaching, which is based upon hydrogen peroxide (HP) as the active agent, has been questioned. Our aim was to investigate the effects of 30% HP on human tooth enamel. The specimens were divided randomly into three groups and treated with distilled water, HCl, and HP, respectively. Raman scattering and laser-induced fluorescence of enamel were determined before and after treatment. Microhardness testing and scanning electron microscopy were also used. The results of Raman scattering showed that the Raman relative intensity of enamel changed significantly after HP and HCl treatment. These findings were consistent with the results of microhardness testing and morphological observations. In addition, a small band at 876 cm^{-1} due to O–O stretching of HP became pronounced during HP treatment, which provided direct evidence that HP has the ability to penetrate enamel. Meanwhile, the results of laser-induced fluorescence revealed that HP caused the greatest fluorescence reduction. This suggested that the organic matter in enamel might be greatly affected by HP, which was also supported by the results of microhardness. It can be concluded, therefore, that the 30% HP may have adverse effects on the mineral and the organic matter of human tooth enamel. [DOI: 10.1117/1.2870114]

Keywords: Raman scattering; laser-induced fluorescence; enamel; hydrogen peroxide.

Paper 07184RR received May 22, 2007; revised manuscript received Oct. 25, 2007; accepted for publication Oct. 30, 2007; published online Feb. 28, 2008.

1 Introduction

The color of teeth is of particular concern to a large number of people seeking dental treatment. It has been reported that 28% of adults in the United Kingdom and 34% in the United States are dissatisfied with the appearance or color of their teeth.[1]

Tooth bleaching is a well-accepted method of treating discolored tooth. It refers to the clinical application of a chemical solution to a tooth surface in order to achieve a lightening effect. The method is based upon hydrogen peroxide (HP) as the active agent.[2] HP can be applied directly or produced in a chemical reaction from carbamide peroxide. It acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules, and hydrogen peroxide anions.[2] These reactive molecules attack the long-chained, dark-colored chromophore molecules and split them into smaller, less colored, and more diffusible molecules.[2]

Address all correspondence to: Jiming Hu, Institute of Analytical and Biomedical Science, Wuhan University, Luojia Hill, Wuhan 430072, P R China; Tel: +86 27 68752439; Fax: +86 27 68754067; E-mail: jmhu@whu.edu.cn, and Yining Wang, Key Laboratory for Oral Biomedical Engineering, Ministry of Education, School and Hospital of Stomatology, Wuhan University, 237 Luoyu Road, Wuhan 430079, P R China; Tel: +86 27 87646351; Fax: +86 27 87873260; E-mail: wang.yn@whu.edu.cn

1083-3668/2008/13(1)/014019/9/$25.00 © 2008 SPIE

Although there is little question about the efficacy of tooth bleaching, the safety of this technique has been questioned.[2] A primary concern is that the structure of sound enamel may be weakened by the bleaching agent. Numerous studies have evaluated the effects of the peroxide-containing bleaching agents on the surface morphology of enamel. Some reported no or little topographic alterations of bleached enamel,[3–6] but others have found increased porosity, pitting, erosion, and demineralization of enamel prism peripheries.[7–9] Additionally, surface hardness and wear resistance of enamel have also been investigated, and the results have varied from no effect to significant decrease in hardness and fracture resistance of enamel.[10–13] Several studies have evaluated the effects of bleaching agents on the chemical composition of enamel using energy dispersive spectroscopy,[14] x-ray diffraction, and infrared spectroscopy.[15,16] The limitation of the above-mentioned analytical techniques is that they are invasive to the enamel sample. Moreover, these techniques provided very limited information about the organic matter that comprises only a minor part of enamel, and they fail to reach general agreement.

This paper is an application of laser Raman spectroscopy to the study of the structure of human tooth enamel. This technique was selected because it is a very powerful, noninvasive analytical method and involves only minimal sample preparation. It has been proven to be of great value for studying the molecular structure of enamel mineral.[17–21] The vibration modes of PO_4^{3-}, CO_3^{2-}, and OH^- groups can be studied by Raman spectroscopy.[21] The frequency and the band shape of the most prominent ν_1 PO_4^{3-} depend on the local mineral environment and therefore change with ionic incorporation and crystallinity.[21,22] Meanwhile, the intensity of ν_1 PO_4^{3-} is linearly proportional to phosphate group concentration within the hydroxyapatite molecule, and it thus can be used to analyze changes in the phosphate group concentration.[17,23]

Laser-induced fluorescence, which is usually regarded as the interference of Raman scattering, can also be measured by a Raman spectrometer.[24–26] It is well known that laser-induced fluorescence is one of the most striking features of enamel.[27,28] Dental caries can be measured as a change in fluorescence of the tooth substance when exposed to laser light.[27,28] Based on recent studies,[26,29] it is highly possible that the laser-induced fluorescence of sound enamel originates from the organic matter in enamel. Thus, the laser-induced fluorescence during Raman measurement may provide important information about the organic matter.

The aim of the study was to explore the effects of 30% HP on sound tooth enamel by applying Raman scattering and laser-induced fluorescence. In addition, microhardness testing and scanning electron microscopy (SEM) were also used.

2 Materials and Methods

The study protocol was reviewed and approved by the Ethics Committee of the School and Hospital of Stomatology, Wuhan University. Patients from whom teeth were being extracted were asked to read and sign a consent form prior to the extraction.

2.1 *Sample Preparation*

Intact human premolars, extracted for orthodontic reasons, were selected. Buccal and lingual surfaces were devoid of stain, enamel cracks or fractures, caries, or other defects. The teeth were cleaned thoroughly and stored in 0.5% thymol at 4 °C until required. Dental blocks (2 mm × 3 mm × 4 mm) were obtained from middle 1/3 of the buccal or lingual halves of each tooth by a low-speed saw (Isomet, Buehler Ltd., Lake Bluff, Illinois) under water-cooling. The dental blocks were individually embedded in colorless translucent acrylic resin with the enamel surface exposed for treatment. The specimens were serially polished by means of 600- to 3000-grit SiC papers with water as a cooler to obtain flat standardized enamel surfaces. Subsequently, they were polished with diamond spray (1 μm, 0.5 μm) and polishing cloths. Then the specimens were ultrasonicated for 5 min with deionized water (DW, Milli-Q water).

2.2 *Treatment Procedure*

All specimens were washed under running distilled water for 30 s and dried by compressed air for 3 s before treatment started. Three specimens prepared from each tooth were randomly divided into three groups and treated as follows:

- Group DW (n=20): The specimens were immersed in 2 mL DW for 60 min.
- Group HCl (n=20): The specimens were immersed in a 2-mL HCl solution (pH≈3.2, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 60 min.
- Group HP (n=20): The specimens were immersed in a 2-mL HP solution (pH≈3.2, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 60 min.

All experiments were carried out at 24 °C.

2.3 *Raman Spectroscopic Detection*

Raman/fluorescence spectra were recorded with a confocal micro-Raman spectrometer (LabRam HR800, Jobin Yvon Horiba, France) equipped with a 20-mW He-Ne laser (632.8-nm wavelength). A dry Olympus 100× objective (NA=0.9) in combination with an adjustable confocal hole was used. Raman scattering was detected by using an air cooled 1024 × 256 pixels CCD camera. Peak frequencies and rapid checking of instrumental performance were calibrated with the silicon phonon line at 520 cm^{-1}.

Ten specimens in each group were used for Raman spectroscopy. The specimens were marked and washed in running DW for 30 s before the measurements. The Raman/fluorescence spectra were acquired at six points near the marker. Three points were measured using a confocal hole of 100 μm. For each spectrum, an average of three acquisitions of 30 s was used. The other three were measured using a confocal hole of 1000 μm. An average of three acquisitions of 10 s was used. The specimens were measured repeatedly at the same location at 0, 15, 30, and 60 min after treatment, and the white light images were recorded. The Raman spectrum of 30% HP solution was also acquired.

Spectral data were visualized on a computer and processed using LabSpec 5 spectroscopic software. Profile data were then imported into Origin 7.0 software (Origin-Lab Corporation, Northampton, Massachusetts). Raman absolute intensity (RAI), Raman relative intensity (RRI), and fluorescence in-

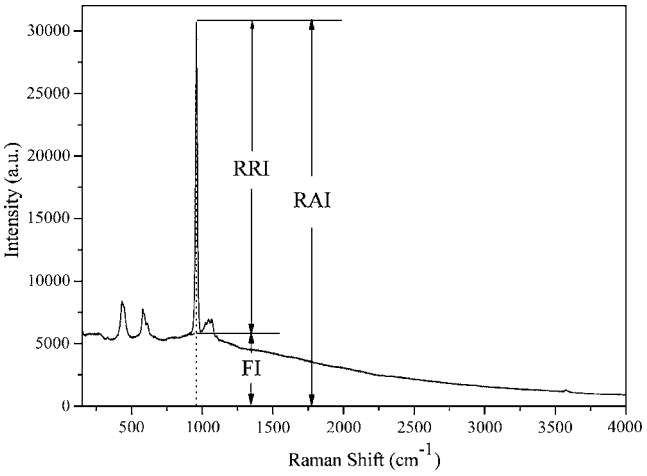

Fig. 1 Raman absolute intensity (RAI), Raman relative intensity (RRI), and fluorescence intensity (FI) at 960 cm^{-1} of the representative Raman/fluorescence spectra. The spectra were recorded with a 100× objective, and the confocal hole was set to 1000 μm. An average of three acquisitions of 10 s was used.

tensity (FI) at 960 cm^{-1} of the Raman/fluorescence spectra were calculated (Fig. 1). The RAI is the intensity of the Raman peak at 960 cm^{-1} before the spectrum is baselined. The RRI is the intensity of the same peak after the spectrum is baselined between 990 and 930 cm^{-1}. The FI is equal to RAI minus RRI. All values were transformed to percentage values, where the values of the baseline were 100% and the changed values were calculated as a percentage of the baseline.

2.4 *Microhardness Testing*

Microhardness testing was performed at 0, 15, 30, and 60 min after treatment. Ten specimens in each group were used for the testing. Microhardness was measured with a hardness tester (HXD-1000TMC/LCD, Taiming, Inc., Shanghai, China) with a load of 200 g for 15 s. Five Vickers hardness indentations were performed on each specimen and averaged before treatment. The indentations were made in five widely separated locations. The measurements at 0 min were denoted as the start values and served as a baseline. The measurements at 15, 30, and 60 min after treatment were made near the baseline indentations (space=100 μm). The microhardness values were recorded and subsequently transformed to percentage microhardness, where the microhardness of the baseline was 100% and the changed microhardness was calculated as a percentage of the baseline.

2.5 *SEM Analysis*

Two specimens from each group were selected for SEM analysis after the final microhardness testing. The specimens were desiccated and sputter-coated with gold in a vacuum evaporator. Images of central areas in the enamel were taken using a scanning electron microscope (Fei QUANTA-200, Eindhoven, The Netherlands).

2.6 *Statistical Analysis*

Statistical analysis was performed by using SIGMASTAT software. The percentage RRI, FI, and microhardness were analyzed by one-way repeated-measures (RM) ANOVA (or Friedman RM ANOVA on ranks) in each group for the time factor. One-way ANOVA (or one-way ANOVA on ranks) was used for the comparison of data among groups at the same time point. *Post hoc* pairwise comparisons were obtained with a Student-Newman-Keuls (SNK) test. The results are presented as mean ± SE. The level of significance was set at a P value of 0.05.

3 Results

3.1 *Raman Scattering and Laser-Induced Fluorescence of Enamel*

The main features of the Raman scattering of enamel were well consistent with previous results[17,18] (Fig. 1). The strongest band at 960 cm^{-1} arose from ν_1 PO_4^{3-}. The bands at 1045 and 1024 cm^{-1} were assigned to ν_3 PO_4^{3-}, 610 and 580 cm^{-1} to ν_4 PO_4^{3-}, and 430 cm^{-1} to ν_2 PO_4^{3-}. The bands at 1068 and 3573 cm^{-1} were attributed to ν_3 $CO_3^{2-}$and OH^- stretch, respectively. The laser-induced fluorescence of enamel appeared as a featureless background in the Raman/fluorescence spectra (Fig. 1).

Both RRI and FI of enamel increased greatly with the opening of the confocal hole from 100 to 500 μm, but they showed little variations with the opening of the confocal hole from 500 to 1000 μm [Fig. 2(a)]. When the enamel was illuminated by the laser of the Raman spectrometer, the RRI remained almost unchanged with increasing time, while the FI decreased considerably [Fig. 2(b)].

3.2 *Effects of Treatments on the Raman Scattering of Enamel*

In all groups, the frequency of Raman bands, Raman bandwidth at 960 cm^{-1}, and carbonate phosphate intensity ratio showed little variation after treatment. However, significant changes in RRI were found in groups HP and HCl (Figs. 3 and 4).

Using a 100-μm hole, the one-way RM ANOVAs (or Friedman RM ANOVAs on ranks) revealed that the percentage RRI remained unchanged in group DW ($P=0.730$), but changed over time in groups HP and HCl ($P=0.016$ and $P=0.011$). Unlike the continual decrease in group HCl, the percentage RRI of group HP increased significantly in the first 30 min and then decreased at 60 min. The one-way ANOVAs (or one-way ANOVAs on ranks) showed that there was no significant difference among three groups at 15 min after treatment ($P=0.680$) but that there were significant differences at 30 min and 60 min after treatment ($P=0.013$ and $P=0.003$). The percentage RRI of group HCl was significantly lower than that of group HP at 30 min, and it was significantly lower than those of groups HP and DW at 60 min after treatment.

Using a 1000-μm hole, the results suggested that the percentage RRI remained unchanged in groups DW and HP ($P=0.694$ and $P=0.410$) but changed over time in group HCl ($P<0.001$). The percentage RRI of group HCl increased significantly at 15 min and then decreased continually at 30 min and 60 min. There was no significant difference among the three groups at 15 min and 30 min after treatment ($P=0.052$ and $P=0.705$), but there were significant differences

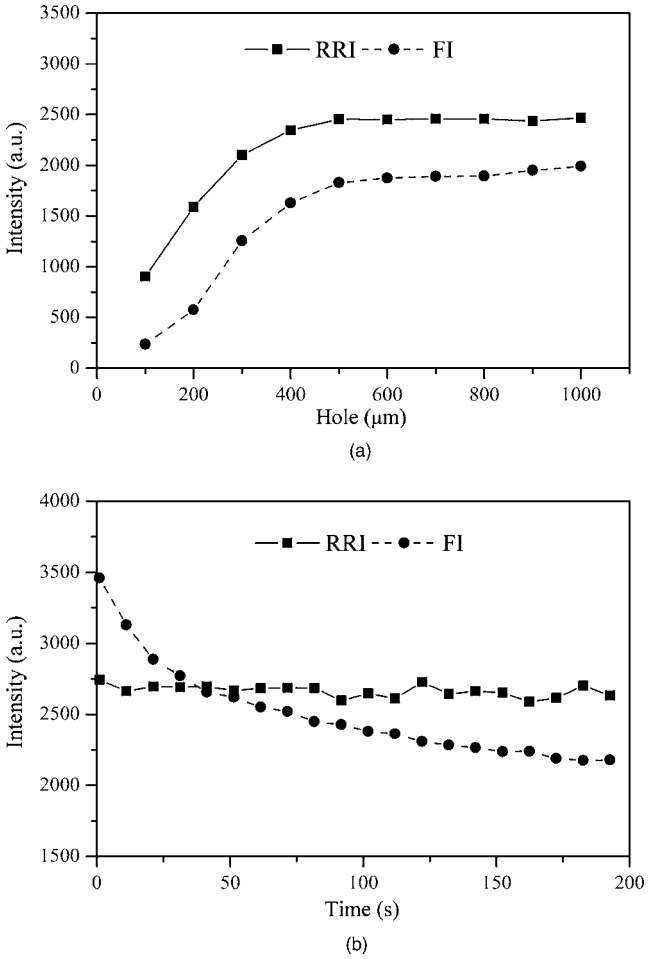

Fig. 2 Effects of confocal hole (a) and laser illumination (b) on the RRI and FI. The spectra were recorded with a 100× objective. An average of three acquisitions of 1 s was used.

at 60 min after treatment (P=0.002). The percentage RRI of group HCl was significantly lower than those of groups HP and DW at 60 min after treatment.

In group HP, a small band at 876 cm^{-1} became pronounced over time (Fig. 3). It was identified as O–O stretching of HP when compared with the Raman spectrum of 30% HP solution (data not shown). This weak band disappeared quickly when the specimens were removed from the HP solution (data not shown).

3.3 *Effects of Treatments on the Laser-Induced Fluorescence of Enamel*

The results suggest that the percentage FI changed over time in all groups (all $P<0.001$) using both 100- and 1000-μm holes (Figs. 3 and 4). There were significant differences among three groups at 15 min, 30 min, and 60 min after treatment (all $P<0.001$). The percentage FI of group HP was significantly lower than those of groups DW and HCl at 15 min, 30 min, and 60 min after treatment. There were no significant differences between groups DW and HCl at all time points.

3.4 *Microhardness Testing*

The one-way RM ANOVAs revealed that the percentage microhardness remained unchanged in group DW (P=0.761) but changed over time in groups HP and HCl (both $P<0.001$; Fig. 5). There was no significant difference among the three groups at 15 min after treatment (P=0.127), but there were significant differences at 30 min and 60 min after treatment (P=0.029 and P=0.002). Both the percentage microhardness of group HCl and that of group HP were significantly lower than that of group DW at 30 min and 60 min after treatment. And the percentage microhardness of group HCl was significantly lower than that of group HCl at 30 min and 60 min after treatment.

3.5 *Morphological Observations*

White light images of the enamel surface in different groups presented different variations (Fig. 6). The enamel surface of group DW remained almost unchanged during the treatment period. Those of group HCl and HP showed obvious morphological changes after treatment. However, the change of the enamel surface in group HP was milder than that in group HCl at each time point. In both groups, the morphological changes became more pronounced as the treatment time increased.

The SEM images revealed that the enamel surface in group DW was smooth, flat, and polished (Fig. 7). The surfaces in groups HCl and HP showed distinct structures of enamel, which included enamel rods and narrow interrods. Under higher magnification, the nanocrystals in the rods and interrods became distinguishable from each other.

4 Discussion

In the present study, we monitored the changes of enamel subjected to different treatments in Raman scattering, laser-induced fluorescence, microhardness testing, and surface morphology. Although the results of morphological observations and microhardness testing suggested that both HP and HCl caused changes in the enamel structure, the chemical information obtained from these tests was indirect and limited. The results of Raman scattering and laser-induced fluorescence provided new insights into the chemical changes in the enamel structure. Based on these results, we propose models for the changes in the enamel structure caused by HP and HCl (Fig. 8).

An important finding in the study is that significant changes in RRI were found using a 100-μm hole in both group HCl and group HP. This indicated that the phosphate group concentration within the enamel surface had changed. It is very interesting that the change of RRI in group HCl showed similar trends to that of microhardness. The results are not unexpected, because phosphate group concentration within the enamel is a good indicator of the degree of mineralization,[23,30] while microhardness determination can provide indirect evidence of mineral loss or gain in the dental hard tissues.[31]

The different trends of RRI changes between groups HCl and HP using a 100-μm hole indicated that HCl resulted in stronger demineralization of enamel than HP did, although they had the same pH value. It is somewhat unexpected to

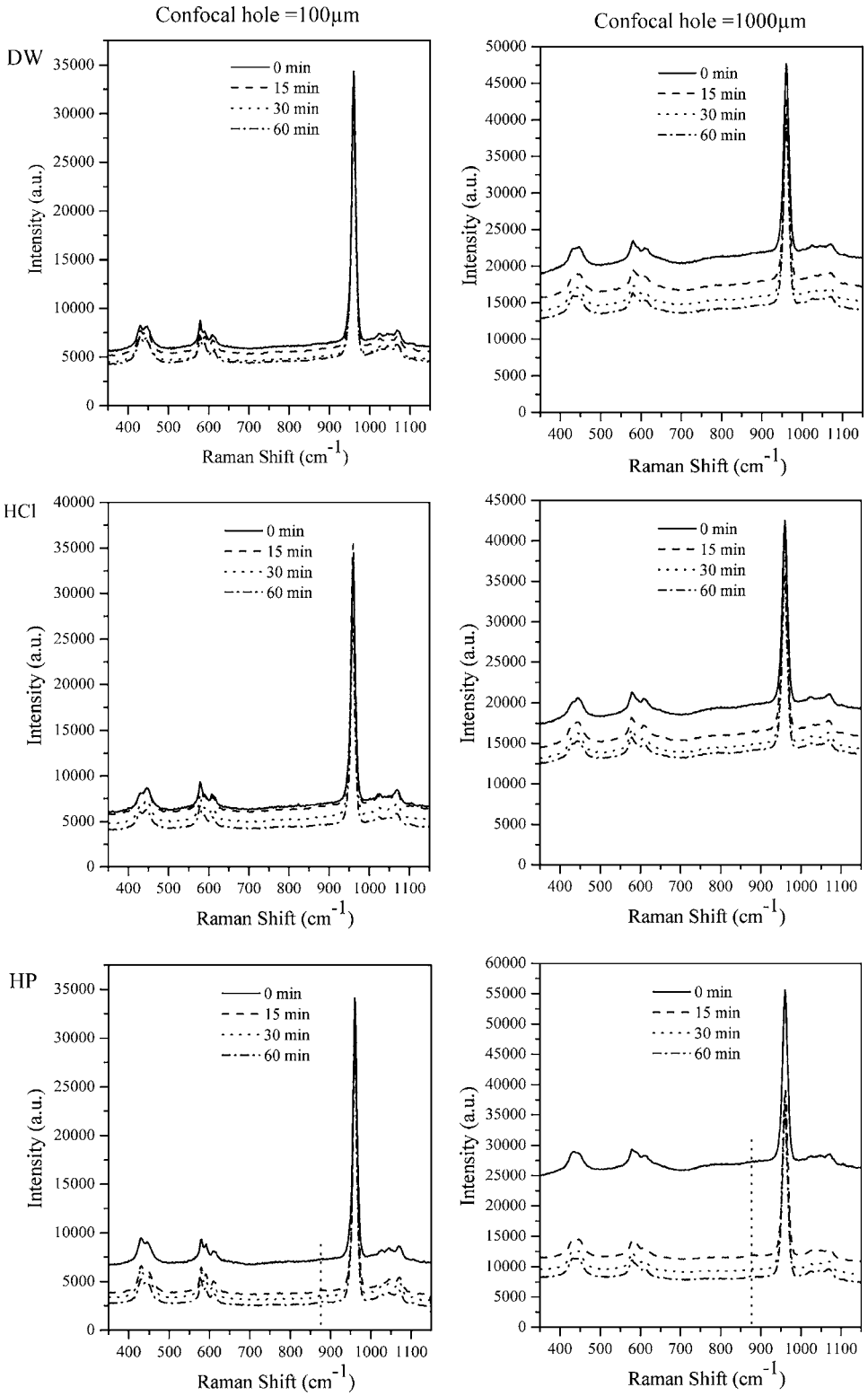

Fig. 3 Raman/fluorescence spectra of enamel at each time point in different groups. The spectra were recorded with a 100× objective. An average of three acquisitions of 30 s (confocal hole=100 μm) or 10 s (confocal hole=1000 μm) was used.

observe that the RRI of enamel in group HP increased significantly from 0 to 30 min. A recent study has shown that the polishing procedure could produce a smear layer about 280-nm thick on the enamel surface.[9] The increase of RRI could be explained by assuming that the phosphate group concentration in the smear layer is lower than that of the underlying enamel. Since the white light images suggested that the smear layer was slowly removed during the first 30 min, the

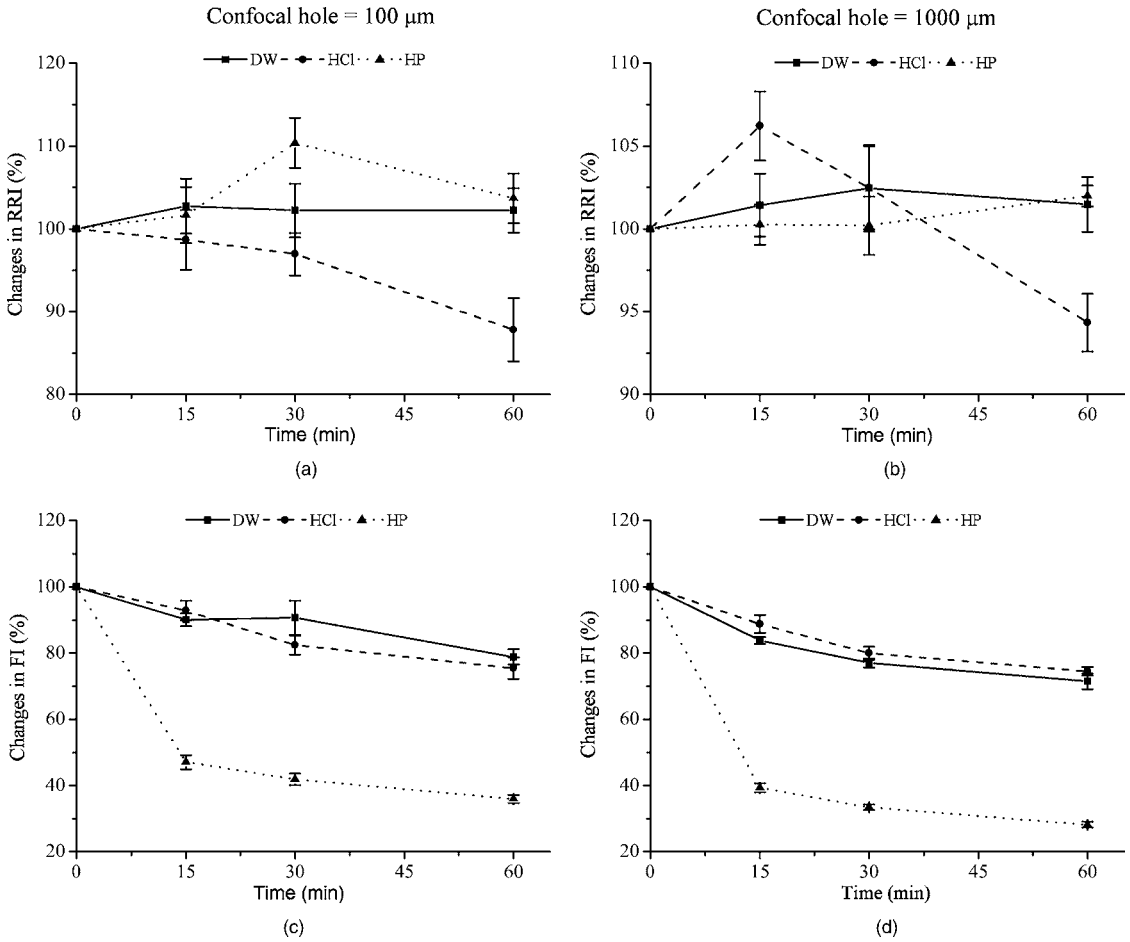

Fig. 4 Percentage RRI and FI changes of different groups using a confocal hole of either 100 μm [(a) and (c)] or 1000 μm [(b) and (d)].

exposure of the underlying enamel results in the increase in RRI at 30 min. After then, the RRI of the enamel in group HP decreased significantly, which indicated that the freshly exposed underlying enamel might be demineralized markedly from 30 min to 60 min. Unlike HP, the HCl might have removed the smear layer completely and demineralized the exposed underlying enamel greatly during the first 15 min. Thus, the increase of RRI was not observed in group HCl.

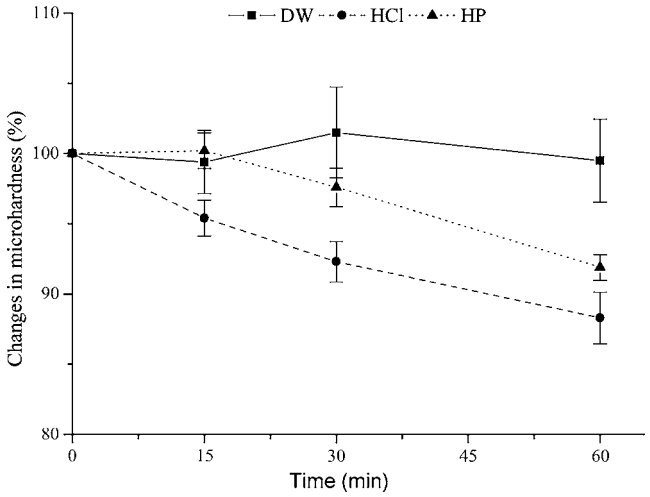

Fig. 5 Percentage microhardness changes of enamel in different groups.

The RRI changes using 1000- and 100-μm holes showed different trends in both group HCl and group HP. This is mainly because the confocal Raman spectrometer has different depth resolutions with different confocal holes. When spectra are acquired with the 100× objective, the depth resolutions determined with a silicon wafer were about 2 μm using a confocal hole of 100 μm and 8 μm using a 1000-μm hole (as reported by the vendor). Because the optical property of dental enamel is different from silicon wafer, the resolutions of the confocal Raman spectrometer should be changed when the enamel was measured. Although the precise depth resolutions could not be determined in present study, it is quite certain that a Raman spectrometer with a 1000-μm hole has a much larger analyzed depth than that with a 100-μm hole. This means that the analyzed volume with the 100-μm hole is more representative of the surface than that with a 1000-μm hole.

The significant increase in RRI at 15 min in group HP using a 1000-μm hole also indicated that deeper enamel might have higher phosphate group concentration. And the RRI of the exposed enamel should be even higher without the demineralization of the superficial enamel. Unlike that of

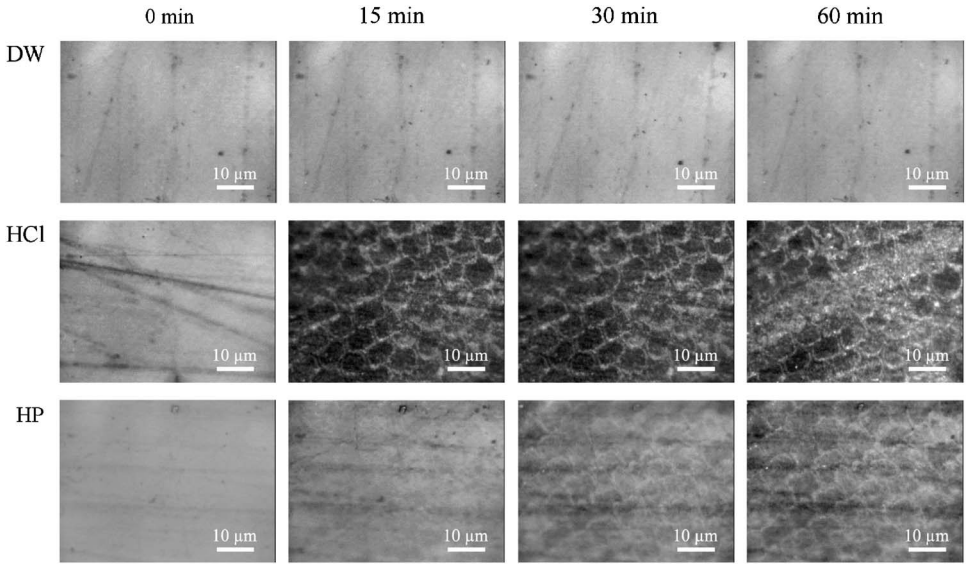

Fig. 6 White light images of enamel surface at each time point in different groups.

group HCl, the RRI of group HP using a 1000-μm hole remained unchanged. This suggested that the demineralization caused by HP was likely limited to the enamel surface.

The results of Raman scattering also provided direct evidence that HP can penetrate the enamel. A weak band at 876 cm^{-1}, which became pronounced after treatment in the Raman/fluorescence spectra of group HP, is due to O–O stretching of HP. This band was also observed in dentin subjected to HP but with much stronger intensity (data not shown). The difference in band intensity between enamel and dentin is probably because enamel is more compact than dentin. After the specimens were removed from the HP, the 876 cm^{-1} band in both enamel and dentin disappeared quickly.

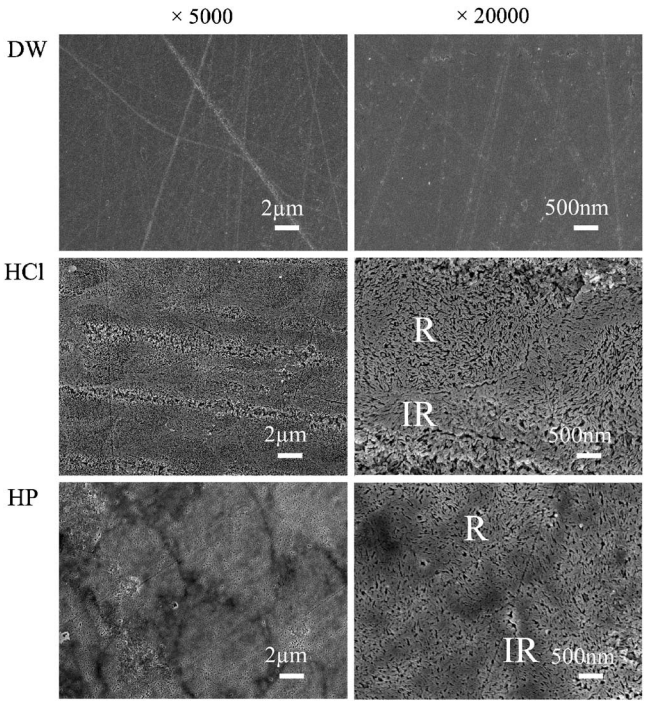

Fig. 7 SEM images of enamel surface after treatment in different groups. R: enamel rods; IR: enamel interrods.

Another important finding in this study is that the FI decreased dramatically in group HP using both 100- and 1000-μm holes, compared with those in groups DW and HCl. This indicated that the matter that causes the laser-induced fluorescence might be changed greatly during the treatment. It has been suggested that fluorescence excited by different wavelength light may have different originations.[27] Likely fluorophore candidates range from dityrosine and cross-linked chains of structure proteins to protoporphyrins produced by bacteria.[27] Hibst and Paulus suggested that fluorescence of sound teeth induced by red laser light (655 nm) might be the result of combining the inorganic matrix with a low concentration of absorbing organic molecules.[29] Recently, a study by Fattibene et al. suggested that the organic materials responsible for laser-induced fluorescence were likely to be electron paramagnetic resonance (EPR) active, while the paramagnetic centers associated with the EPR native signal were located in the protein–mineral interface.[26]

To understand the origin of laser-induced fluorescence better, the enamel structure should be explained. It is well known that dental enamel is the most highly mineralized and hardest biological tissue. It is comprised of approximately 96% mineral, 3% water, and 1% organic matter (noncollagenous protein) by weight.[32] Although the protein comprises only a minor part of enamel, it is contained in the spaces between mineral crystals, where it serves as a "glue" between crystallites.[33] According to the model proposed for enamel by Brik et al.,[34] the organic component of enamel can be described with two subsystems, conventionally called "organic-1" and "organic-2." The first of these subsystems disintegrates under heating in the temperature range 160 to 250°C, and the temperature range of disintegration of the second is 300 to 550°C. "Organic-1" fills the space between the enamel rods and is 10- to 20-nm thick, while "organic-

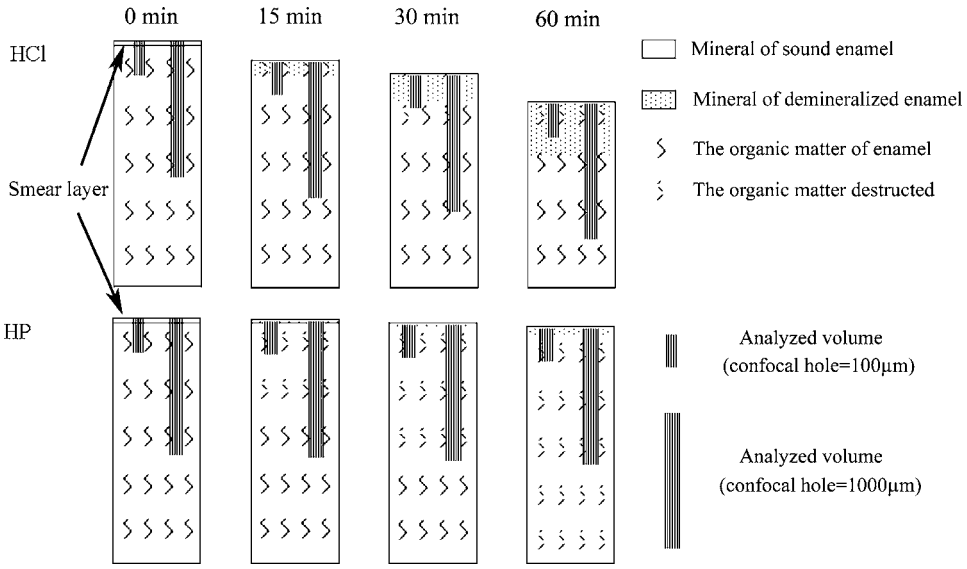

Fig. 8 Proposed models for the changes in the enamel structure caused by HP and HCl.

2"covers the hydroxyapatite nanocrystals and is 2- to 5-nm thick. Fattibene et al. modified Brik's model and pointed out that the protein and mineral components of calcified tissues should not be considered as separate phases, but rather as an ensemble, where proteins and mineral crystals are chemically linked.[26] They proposed that dentin and enamel could be modeled as made up of three phases: a bulk mineral, a bulk organic, and a protein–mineral interface. The latter phase was identified as the "organic-2" component, which is responsible for laser-induced fluorescence.

Weak but significant FI reduction was observed in group DW using both 100- and 1000-μm holes. This is probably due to the photobleaching because the specimens were repeatedly measured at the same place during the treatment period. The FI reduction in group HCl is likely due to the combination of photobleaching and demineralization. Since HCl removed the outer enamel layer, which was most affected by the laser, the FI reduction in group HCl should be less than that in group DW if there are no other factors. However, the FI reduction of group HCl did not show significant difference from that of group DW at each time point. This is probably because HCl can remove the organic matter in the outer enamel surface by demineralization.

The greatest and significant FI reduction in group HP should be mainly attributed to the strong oxidizing ability of HP. HP is a strong oxidant, and it is acidic. Demineralization of enamel by HP may result in some FI reduction, but the effect should be very weak because the demineralization of enamel in group HP was milder than that of group HCl. We suggest that the great reduction in FI results from the fact that HP can penetrate the enamel through the boundaries between nanocrystals, and it may attack the organic matter not only in the outer enamel but also in the inner enamel during the penetration.

The microhardness loss of enamel in group HP also provided indirect evidence for the destruction of organic matter. The enamel in group HP showed higher microhardness loss at 60 min compared with that in group HCl at 15 min. However, both the morphological observation and Raman spectroscopy suggested that enamel surface in group HP was much less demineralized. The possible explanation is that the microhardness loss is due to the combined effects of demineralization and destruction of organic matter by HP. Several other studies have demonstrated significant decrease in hardness and fracture resistance of bleached enamel.[12,13] The authors also hypothesized that HP or carbamide peroxide might cause changes to the organic component of enamel and dentin by altering of the organic matrix or protein oxidation.

This study is related to the use of HP in tooth bleaching. In a clinical situation, both HP and carbamide peroxide can be used as bleaching agents. It should be pointed out that some commercially available bleaching products have a pH value close to neutral. The demineralization effect of these agents should be minimal; however, the oxidative effect is still there. As the free radical reaction is not specific, the destruction of the organic matter in enamel is likely unavoidable. Since the little organic matter plays an important role in the integrity of enamel, the clinical application of HP-containing agents should be performed with caution. The effects of destruction of organic matter on the structure and function of the enamel need to be further investigated.

5 Conclusions

In this study, Raman scattering and laser-induced fluorescence provided new insights into the changes in the enamel structure induced by HP. The 30% HP caused significant change in the RRI in surface enamel and reduced the FI of enamel greatly and significantly. Therefore, the 30% HP may have adverse effects on both the mineral and the organic matter of human tooth enamel, and it should be used with caution.

Acknowledgments

This work was supported by Grant Nos. 30400507, 60701020, and 30772058 from the National Natural Science Foundation of China.

References

1. A. Joiner, "The bleaching of teeth: a review of the literature," *J. Dent.* **34**(7), 412–419 (2006).
2. J. E. Dahl and U. Pallesen, "Tooth bleaching—a critical review of the biological aspects," *Crit. Rev. Oral Biol. Med.* **14**(4), 292–304 (2003).
3. V. B. Haywood, T. Leech, H. O. Heymann, D. Crumpler, and K. Bruggers, "Nightguard vital bleaching: effects on enamel surface texture and diffusion," *Quintessence Int.* **21**(10), 801–804 (1990).
4. C. P. Ernst, B. B. Marroquin, and B. Willershausen-Zonnchen, "Effects of hydrogen peroxide–containing bleaching agents on the morphology of human enamel," *Quintessence Int.* **27**(1), 53–56 (1996).
5. R. H. Leonard, Jr., J. C. Eagle, G. E. Garland, K. P. Matthews, A. L. Rudd, and C. Phillips, "Nightguard vital bleaching and its effect on enamel surface morphology," *J. Esthet. Restor. Dent.* **13**(2), 132–139 (2001).
6. F. K. Cobankara, N. Unlu, H. C. Altinoz, and O. Fusun, "Effect of home bleaching agents on the roughness and surface morphology of human enamel and dentine," *Int. Dent. J.* **54**(4), 211–218 (2004).
7. N. C. Bitter, "A scanning electron microscopy study of the effect of bleaching agents on enamel: a preliminary report," *J. Prosthet. Dent.* **67**(6), 852–855 (1992).
8. C. M. Flaitz and M. J. Hicks, "Effects of carbamide peroxide whitening agents on enamel surfaces and caries-like lesion formation: an SEM and polarized light microscopic *in vitro* study," *ASDC J. Dent. Child* **63**(4), 249–256 (1996).
9. B. Fu, W. Hoth-Hannig, and M. Hannig, "Effects of dental bleaching on micro- and nano-morphological alterations of the enamel surface," *Am. J. Dent.* **20**(1), 35–40 (2007).
10. M. Sulieman, M. Addy, E. Macdonald, and J. S. Rees, "A safety study *in vitro* for the effects of an in-office bleaching system on the integrity of enamel and dentine," *J. Dent.* **32**(7), 581–590 (2004).
11. N. Unlu, F. K. Cobankara, C. Altinoz, and F. Ozer, "Effect of home bleaching agents on the microhardness of human enamel and dentin," *J. Oral Rehabil.* **31**(1), 57–61 (2004).
12. R. R. Seghi and I. Denry, "Effects of external bleaching on indentation and abrasion characteristics of human enamel *in vitro*," *J. Dent. Res.* **71**(6), 1340–1344 (1992).
13. V. Cavalli, M. Giannini, and R. M. Carvalho, "Effect of carbamide peroxide bleaching agents on tensile strength of human enamel," *Dent. Mater.* **20**(8), 733–739 (2004).
14. I. Rotstein, E. Dankner, A. Goldman, I. Heling, A. Stabholz, and M. Zalkind, "Histochemical analysis of dental hard tissues following bleaching," *J. Endod.* **22**(1), 23–25 (1996).
15. U. Oltu and S. Gurgan, "Effects of three concentrations of carbamide peroxide on the structure of enamel," *J. Oral Rehabil.* **27**(4), 332–340 (2000).
16. H. Cimilli and C. H. Pameijer, "Effect of carbamide peroxide bleaching agents on the physical properties and chemical composition of enamel," *Am. J. Dent.* **14**(2), 63–66 (2001).
17. H. Tsuda and J. Arends, "Raman spectroscopy in dental research: a short review of recent studies," *Adv. Dent. Res.* **11**(4), 539–547 (1997).
18. E. Wentrup-Byrne, C. A. Armstrong, R. S. Armstrong, and B. M. Collins, "Fourier transform Raman microscopic mapping of the molecular components in a human tooth," *J. Raman Spectrosc.* **28**(2–3), 151–158 (1997).
19. A. Carden and M. D. Morris, "Application of vibrational spectroscopy to the study of mineralized tissues (review)," *J. Biomed. Opt.* **5**(3), 259–268 (2000).
20. P. Tramini, B. Pelissier, J. Valcarcel, B. Bonnet, and L. Maury, "A Raman spectroscopic investigation of dentin and enamel structures modified by lactic acid," *Caries Res.* **34**(3), 233–240 (2000).
21. G. Penel, G. Leroy, C. Rey, and E. Bres, "Micro-Raman spectral study of the PO4 and CO3 vibrational modes in synthetic and biological apatites," *Calcif. Tissue Int.* **63**(6), 475–481 (1998).
22. C. P. Tarnowski, M. A. Ignelzi, Jr., and M. D. Morris, "Mineralization of developing mouse calvaria as revealed by Raman microspectroscopy," *J. Bone Miner. Res.* **17**(6), 1118–1126 (2002).
23. F. Gilchrist, A. Santini, K. Harley, and C. Deery, "The use of micro-Raman spectroscopy to differentiate between sound and eroded primary enamel," *Int. J. Paediatr. Dent.* **17**(4), 274–280 (2007).
24. W. Hill and V. Petrou, "Caries detection by diode laser Raman spectroscopy," *Appl. Spectrosc.* **54**(6), 795–799 (2000).
25. T. Izawa and M. Wakaki, "Application of laser Raman spectroscopy to dental diagnosis," *Proc. SPIE* **5687**, 1–8 (2005).
26. P. Fattibene, A. Carosi, V. De Coste, A. Sacchetti, A. Nucara, P. Postorino, and P. Dore, "A comparative EPR, infrared, and Raman study of natural and deproteinated tooth enamel and dentin," *Phys. Med. Biol.* **50**(6), 1095–1108 (2005).
27. G. K. Stookey, "Quantitative light fluorescence: a technology for early monitoring of the caries process," *Dent. Clin. North Am.* **49**(4), 753–770 (2005).
28. B. T. Amaechi and S. M. Higham, "Quantitative light-induced fluorescence: a potential tool for general dental assessment," *J. Biomed. Opt.* **7**(1), 7–13 (2002).
29. R. Hibst and R. Paulus, "Caries detection by red excited fluorescence: investigations on fluorophores," *Caries Res.* **33**(4), 295 (1999).
30. C. Hannig, A. Hamkens, K. Becker, R. Attin, and T. Attin, "Erosive effects of different acids on bovine enamel: release of calcium and phosphate *in vitro*," *Arch. Oral Biol.* **50**(6), 541–552 (2005).
31. J. Arends and J. J. Ten Bosch, "Demineralization and remineralization evaluation techniques," *J. Dent. Res.* **71**(Spec), 924–928 (1992).
32. M. Deakins and J. F. Volker, "Amount of organic matter in enamel from several types of human teeth," *J. Dent. Res.* **20**(2), 117–121 (1941).
33. M. C. Maas and E. R. Dumont, "Built to last: the structure, function, and evolution of primate dental enamel," *Evol. Anthropol. Issues News Rev.* **8**(4), 133–152 (1999).
34. A. Brik, E. Haskell, V. Brik, O. Scherbina, and O. Atamanenko, "Anisotropy effects of EPR signals and mechanisms of mass transfer in tooth enamel and bones," *Appl. Radiat. Isot.* **52**(5), 1077–1083 (2000).