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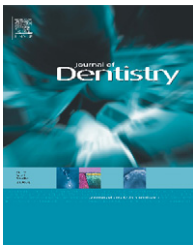

Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide

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ABSTRACT

Objectives: To investigate the effects of acidic and neutral 30% hydrogen peroxide (HP) on human tooth enamel in terms of chemical structure, mechanical property, surface morphology and tooth colour.

Methods: Twenty-seven human dental blocks were obtained from premolars and randomly divided into three groups ($n = 9$): Group acidic HP (30% HP, pH ≈ 3.6), Group neutral HP (30% HP, pH ≈ 7.0) and Group DW (distilled water, pH ≈ 6.8). Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), Raman spectroscopy, atomic force microscopy (AFM) investigation, microhardness test and colour measurements were carried out before and after treatments. ATR-FTIR and Raman spectroscopy were analysed and then the carbonate:mineral ratio (C:M), Raman absolute intensity (RAI), Raman relative intensity (RRI), and laser-induced fluorescence intensity (FI) were obtained for evaluation.

Results: The C:M, percentage microhardness and percentage RRI of group acidic HP decreased more significantly than those of group neutral HP ($P = 0.02$, $P = 0.001$, $P < 0.001$, respectively) and group DW ($P = 0.01$, $P = 0.008$, $P < 0.001$, respectively). Whilst group neutral HP and group DW had no statistical difference in above terms ($P = 0.818$, $P = 0.528$, $P = 0.158$, respectively). Significant morphological alterations were observed in group acidic HP. Group acidic HP and neutral HP had no significant difference in percentage FI ($P = 0.652$) and ΔE ($P = 0.906$).

Conclusions: This study suggested that neutral 30% HP had the same efficiency in tooth bleaching and it caused less deleterious effects on enamel than acidic 30% HP.

1. Introduction

Vital tooth bleaching is a conservative and effective method for treating discoloured teeth.[1] There are three fundamental approaches, namely, in-office bleaching, at-home bleaching and over the counter (OTC) whitening products.[2,3] As an important technique of vital bleaching, in-office bleaching is in clinical demand when patients strongly request the immediate whitening results, do not wear bleaching trays, or lack the compliance. In-office approach is mainly based upon HP as the active agent, with relatively high concentrations ranging from 25% to 35%.[4] Generally, the bleaching agent is applied to the teeth after the complete isolation of the soft tissues with rubber dam.

The efficacy of tooth bleaching has been validated by numerous *in vitro* and *in vivo* studies. However, a primary concern is that the enamel structure may be weakened by bleaching agents. Several studies reported that tooth bleach-

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ing treatment could result in alterations of surface morphology,[5–8] variation in chemical components,[9–14] decrease in microhardness, nanohardness and fracture toughness.[6,11,15–20] Therefore, it is still necessary to minimize the potential adverse effects during the bleaching treatment.

The possible negative effects during tooth bleaching could be associated with the pH value, oxidative effect and compositions of bleaching agents.[19] It has been suggested that the acidity may be a main reason for the side effects of HP with lower pH value.[21,22] However, few studies did attempt to investigate the effects of pH value on tooth structure and components, although majority of previous studies focused on the concentrations, product formats, application modes and times.[1,4,21,23]

In a previous study,[24] it was demonstrated that the combination of HP and hydroxyapatite (HA) was effective in tooth bleaching. HA could significantly reduce the microhardness loss of enamel caused by 30% HP and make little change on the morphology of enamel surface. One of the possible mechanisms is that HA is an alkaline salt, which can increase the pH value of HP solution and made it less acidic. The other is that HA particles adhere evenly to the enamel surface and form a protective layer for the underlying enamel, which lessen the direct contact of HP with the enamel surface. Based on the results of the previous study, it was therefore hypothesised that the potential adverse effects of acidic HP could be minimized by directly adjusting the pH value of HP solution.

The purpose of this study was to evaluate the effects of acidic and neutral 30% HP solutions on human tooth enamel in terms of chemical structure, mechanical property, surface morphology and tooth colour by use of noninvasive methods, including attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), Raman spectroscopy, atomic force microscopy (AFM), microhardness test and tooth colour measurement.

2. Materials and methods

2.1. Tooth selection

Twenty-seven premolars, freshly extracted for orthodontic reasons, were selected. All of them were devoid of stain, enamel cracks or fractures, caries and other defects on the buccal and lingual surfaces. They were cleaned thoroughly and stored in 0.5% thymol at 4 °C until required.

2.2. Sample preparation

A dental block (3 mm × 4 mm × 4 mm) was obtained from middle 1/3 of buccal halves of each tooth by a low-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) with the cutting precision of ±0.1 mm under water cooling. Dental blocks were individually embedded in colourless translucent acrylic resin, keeping the enamel surfaces unsealed for treatment. To obtain flat standardized enamel surfaces, the surfaces were serially polished by means of 600- to 3000-grit silicon carbide papers with water as a cooler. Subsequently, they were polished with 0.5 μm aluminium oxide slurry and polishing cloths, followed by rinsing with running water to get rid of debris layers. Then the specimens were ultrasonicated for 5 min with distilled water to remove smear layer. Prior to the experiment, prepared specimens were stored in the artificial saliva[25] at a 37 °C incubator for 7 d, and the solution was renewed every day.

2.3. Treatment procedure

All specimens were rinsed with running distilled water for 30 s and dried by compressed air for 15 s before treatment. They were randomly divided into three groups as follows:

- Group acidic HP ($n = 9$): The specimens were immersed in 4 ml 30% HP solution (pH ≈ 3.6, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 4 h.
- Group neutral HP ($n = 9$): The specimens were immersed in the neutral 30% HP (pH ≈ 7.0) for 4 h, which was a mix of 4 ml 30% HP solution and 5 μl saturated NaOH solution.
- Group DW ($n = 9$): The specimens were immersed in 4 ml distilled water (pH ≈ 6.8) for 4 h.

All above treatments were carried out at room temperature, and the solutions were replaced each hour. The pH values of solutions were measured with a digital pH metre (Sartorius, Göttingen, Germany).

2.4. ATR-FTIR spectroscopy

ATR-FTIR spectrometric investigations were performed with a Thermo Nicolet 5700 infrared spectrometer (Nicolet, Madison, WI, USA) and the smart iTR sampling accessory with the diamond crystal as an internal reflection element. ATR spectra were recorded in the range from 650 to 4000 cm^{-1} at 4 cm^{-1} resolution, with 128 scans co-addition.

To keep measurement at the same place before and after treatment, the reverse side of each specimen was marked in three different areas by means of a water-cooled highspeed handpiece with a small round bur. Each specimen was put onto the face of diamond crystal of the smart iTR sampler accessory, with the marked surface up, and the pointed tip of the standard pressure tower pressed onto the marker.

ATR spectra of water were obtained[26] and subtracted from the samples by OMNIC 7.0 software (Nicolet, Madison, WI, USA). After baseline correction and normalization, the carbonate:-mineral ratio (C:M) was examined, which was the ratio of the integrated areas of CO_3^{2-} ν_2 contour to the PO_4^{3-} ν_1, ν_3 contour.

2.5. Raman spectroscopic detection

The specimens were marked and washed in running DW for 30 s before the Raman spectroscopic detection. Raman/fluorescence spectra were recorded by a Raman Spectrometer (i-Raman Portable Raman Spectrometer, B&W TEK Inc., Delaware, USA) with a microscope (B&W TEK Inc., Delaware, USA). The spectra were acquired at three points near the marker on each specimen. Each spectrum was made under the following conditions: 0–3200 cm^{-1} spectrum range, 7000 ms integration time, 5 times for the average and room temperature.

Spectral data were visualized on a computer and processed using BWSpec 3.26 spectroscopic software (BWSpec, B&W TEK

Inc., Delaware, USA). Profile data were transformed and then imported into Origin 8.0 software (OriginLab Corporation, Northampton, MA). Raman absolute intensity (RAI), Raman relative intensity (RRI), and laser-induced fluorescence intensity (FI) at 960 cm^{-1} of the typical Raman spectrum were calculated as the previous research.[6] 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.

2.6. AFM investigation

Three specimens from each group were selected randomly for the investigation of AFM after treatment. The surface morphology of enamel can be observed.[27] Micrographs of five different areas per specimen were taken using an atomic force microscope (SPM-9500J3, Shimadzu Corp., Japan) with the size of 10 μm × 10 μm images. AFM software of SPM-Offline 2.30 (SPM-Offline, Shimadzu Corp., Japan) was used to obtain the average of the square height difference between surface peaks and valleys (root mean square of the heights).

2.7. Microhardness test

Microhardness was measured by a microhardness tester (HXD-1000TMC/LCD, Taiming Inc., Shanghai, China) with a load of 200 g for 15 s. Five Vickers indentations were performed on every specimen and the measurements after treatment were made near the baseline indentations (100 μm space). The average of five indentations was recorded as the microhardness value of each specimen, 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.

2.8. Colour measurement

A spectrophotometer (PR-650 Spectra Scan, Photo Research Inc., California, USA) was applied to measure the colour of teeth based on the CIE $L^*a^*b^*$ colour space system (0-degree observer and illuminant D65[28]). Before each measurement session, the spectrophotometer was calibrated with a white reflectance standard according to the manufacturer's protocol. The middle region of each specimen was measured in a circular area with 1.5 mm in diameter. For insuring that the same area of each sample was surveyed every time, a custom sample holder was used to position the specimens.

Baseline and final colour measurements were taken before and after treatments. The differences between L^*, a^* and b^* were expressed as ΔL^*, Δa^* and Δb^*, where $\Delta L^* = L^*_{\text{post-treatment}} - L^*_{\text{baseline}}$, $\Delta b^* = b^*_{\text{post-treatment}} - b^*_{\text{baseline}}$, $\Delta a^* = a^*_{\text{post-treatment}} - a^*_{\text{baseline}}$. The overall colour differences were calculated by the following expression: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

2.9. Statistical analysis

Statistical analyses were performed with SPSS 16.0 (SPSS, Chicago, IL, USA) for WINDOWS. The results were presented as mean ± SD. The differences of the percentage of RRI and FI, the percentage of microhardness, ΔL^*, Δa^*, Δb^* and ΔE in each group were analysed by one-way ANOVA followed by Tukey's multiple comparison test. The carbonate:mineral ratio (C:M) were analysed by two-way repeated-measures ANOVA and post hoc comparisons was obtained with Tukey's multiple comparison test. A P-value of <0.05 was considered to represent statistical significance.

3. Results

3.1. ATR-FTIR spectroscopic analysis

The representative ATR-FTIR spectra of enamel after baseline correction and normalization are showed in Fig. 1. Great alterations were found in group acidic HP after treatment, whilst little changes were detected in the group neutral HP and group DW. The carbonate:mineral ratio (C:M) was calculated and analysed (Fig. 2). The decreasing of C:M in group acidic HP was significant different from others ($P = 0.02$, $P = 0.01$, respectively). Group neutral HP and group DW had little change in the C:M after treatment and had no statistical difference ($P = 0.818$).

3.2. Raman spectroscopy

The main features of the Raman spectroscopy of enamel were showed in Fig. 3. The strongest band at 960 cm^{-1} arose from PO_4^{3-} ν_1, and the band at 1045 and 1024 cm^{-1} was assigned to

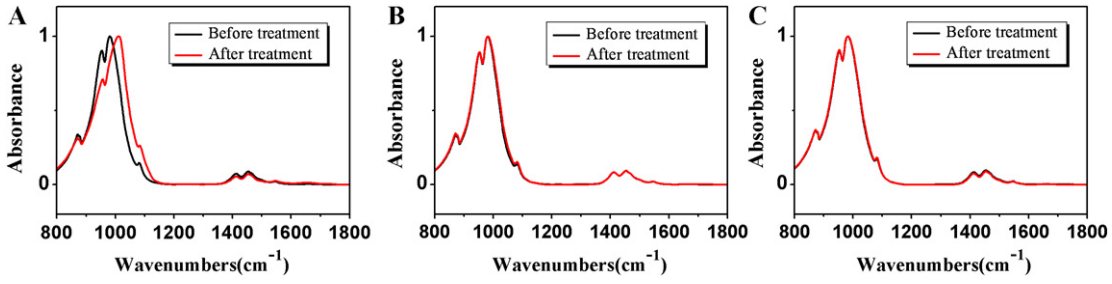

Fig. 1 – A representative series of ATR-FTIR spectra (A–C) recording the treated enamel surface were marked in different colours for different time points. (A) Group acidic HP, (B) Group neutral HP, (C) Group DW. The spectra were normalized by phosphate band intensity (885–1100 cm^{-1}).

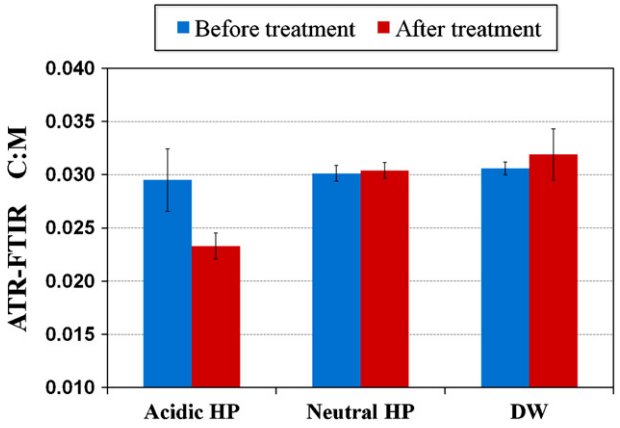

Fig. 2 – The variation of carbonate:mineral ratio (C:M) for three groups before and after treatment.

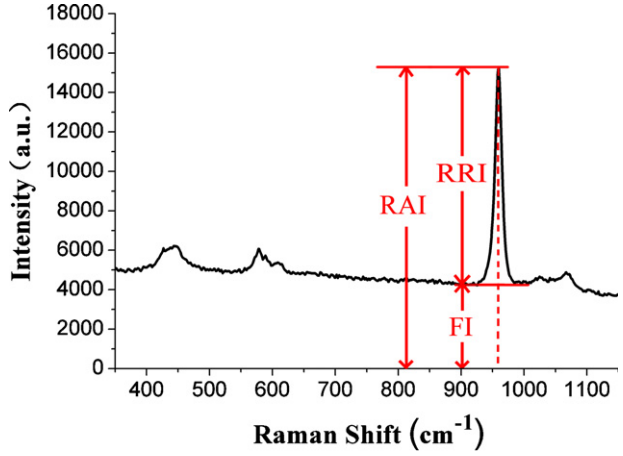

Fig. 3 – A typical Raman spectrum of human tooth enamel was recorded under the condition of 7000 ms integration time and 5 times for the average. Raman absolute intensity (RAI), Raman relative intensity (RRI), and laser-induced fluorescence intensity (FI) at 960 cm^{-1} of the Raman spectrum were emphasised with red mark.

PO_4^{3-} ν_3, 610 and 580 cm^{-1} to PO_4^{3-} ν_4, 430 cm^{-1} to PO_4^{3-} ν_2, and 1068 cm^{-1} to CO_3^{2-} ν_3. The FI of enamel appeared as a featureless background in Raman spectra.

After treatment, the significant decreasing in RRI was found in group acidic HP. However, the spectra of group neutral HP and group DW showed little change. For the percentage RRI, group acidic HP showed lower RRI than others ($P < 0.001$, $P < 0.001$, respectively), whilst group neutral HP and group DW had no statistically significant difference ($P = 0.158$) (Fig. 4). The percentage FI in two HP groups were greatly different from group DW ($P < 0.001$, $P < 0.001$, respectively), whilst group acidic HP and group neutral HP had no significant difference ($P = 0.652$) (Fig. 4).

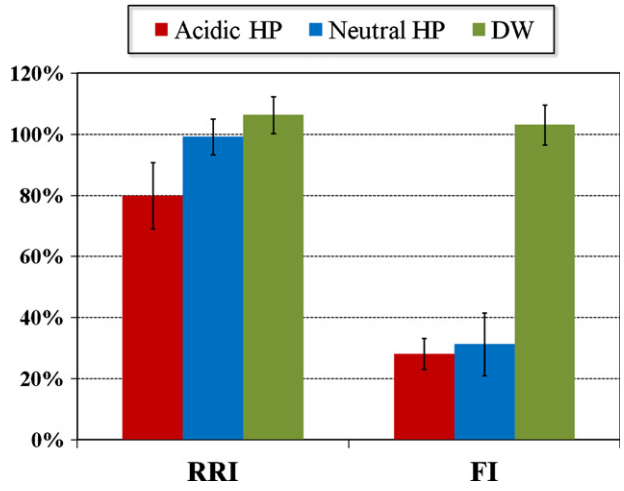

Fig. 4 – Percentage RRI and percentage FI changes of different groups after treatment.

3.3. *AFM investigation*

The morphological alterations of enamel surface for three groups were obtained by means of AFM (Fig. 5A–C). The enamel surface treated by acidic HP had obvious morphological changes after treatment and it was rather irregular and rough. However, the images revealed that the enamel surface in group neutral HP and group DW was smooth, flat, and polished, and remained almost unchanged during the treatment period.

3.4. *Microhardness test*

There was no difference on the baseline values of microhardness amongst three groups ($P = 0.411$). After treatment, the percentage microhardness of group acidic HP decreased and was significantly lower than others ($P = 0.001$, $P = 0.008$, respectively), however, no significant difference on micro-

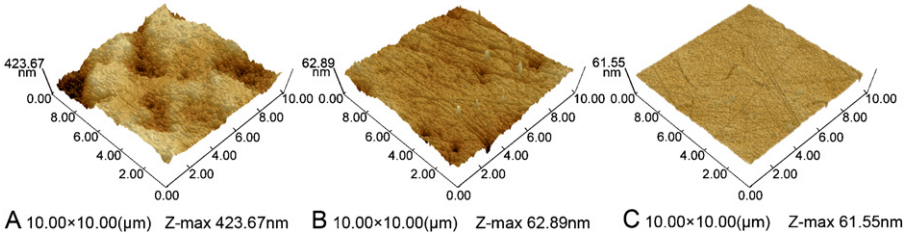

Fig. 5 – Three-dimension AFM images of tooth enamel surface for three groups after treatment (A–C). (A) Group acidic HP, (B) Group neutral HP, (C) Group DW. The "Z-max" presents the morphological changes after treatment.

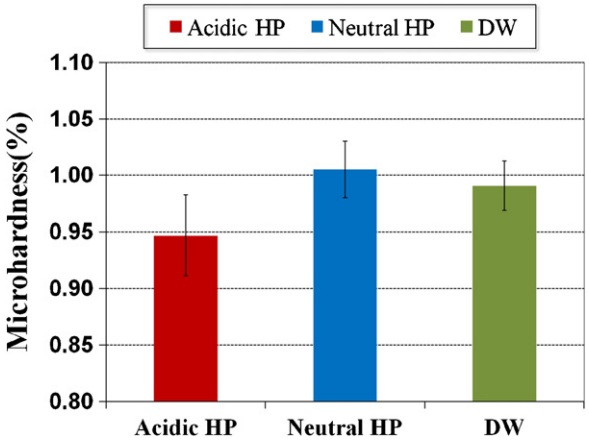

Fig. 6 – Percentage microhardness changes of tooth enamel for each group.

hardness was found in group neutral HP and group DW ($P = 0.528$) (Fig. 6).

3.5. *Colour measurement*

There was no significant difference in L^*, a^* and b^* values amongst the three groups at the baseline ($P = 0.455$, $P = 0.698$, $P = 0.915$, respectively). After treatment, Δa^*, ΔL^*, Δb^* and ΔE value was observed except in group DW (Fig. 7). The ΔE of group acidic HP and group neutral HP were significantly higher than that of group DW ($P = 0.001$, $P = 0.002$, respectively). Group acidic HP and neutral HP had no significant difference in Δa^*, ΔL^*, Δb^* and ΔE values ($P = 0.776$, $P = 0.998$, $P = 0.772$, $P = 0.906$, respectively).

4. Discussion

Bleaching agents containing highly concentrated HP, especially 30% HP, are widely used in in-office tooth bleaching. To prevent the influences of interference factors as much as possible, the 30% HP solution was commonly employed in previous *in vitro* studies.[6,12,24,29] It has been reported that the greater the peroxide concentration, the more acidic the pH of the bleaching product.[30] Bistey et al.[12] found that the alterations in enamel were directly proportional to the peroxide concentration and treatment time according to the FT-IR spectroscopy. Therefore, 30% HP was chosen as the typical percentage in the present study, in order to investigate the protective effect of neutral pH under relatively rigorous condition.

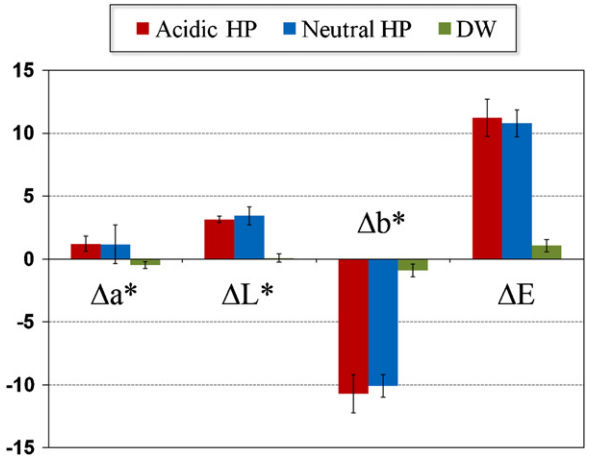

Fig. 7 – Tooth colour change of three groups after treatment.

In this study, the results of colour measurement indicated that whitening effect of neutral 30% HP was similar to that of acidic 30% HP. Meanwhile, neutral 30% HP showed less negative effects on enamel surface than those of acidic 30% HP.

The outcomes of microhardness test and AFM observation demonstrated that acidic 30% HP could result in a significant microhardness loss and morphological change of enamel, which were in accordance with the previous studies.[6,24] Since neutral HP had little effects on the microhardness and morphology of enamel, it suggested that acidic HP could cause microhardness loss and morphological change of enamel due to the demineralization effect of acidic solution.[22,31]

The important finding detected by ATR-FTIR was that acidic HP could change the components of enamel surface. The significant decrease in C:M ratios indicated that the mineral components of enamel were affected by acidic HP. These results were consistent with the previous study, which found that acidic HP had similar effects on the mineral components of dentine.[29] In addition, no significant changes of C:M ratios were found in groups of neutral HP and DW. Without the acidity, neutral HP might result in little componential alterations of enamel surface.

To describe the changes of tooth enamel, phosphate group concentration within enamel is a good indicator of the degree of mineralization.[32] Moreover, the intensity of PO_4^{3-} ν_1 in Raman spectroscopy is linearly proportional to phosphate group concentration within the hydroxyapatite molecule.[33,34] The Raman spectroscopy showed that RRI of enamel subjected to acidic HP decreased dramatically, which indicated that the phosphate group concentration within the enamel surface had reduced. Nevertheless, neutral HP and DW had little change in RRI, which indicated that the phosphate group concentration within the enamel surface had rarely changed. Based on the outcomes of both Raman spectroscopy and ATR-FTIR, it was demonstrated that neutral HP had little demineralization effect on the enamel surface. The demineralization effects are most likely caused by acidic erosion processes rather than by peroxide per se.

From the results of this study, it was of clinical significance that neutral 30% HP resulted in less deleterious effects than acidic HP and also had the same efficiency of tooth bleaching. However, the majority of frequently applied commercial HP products are highly acidic and concentrated to maintain long-term stability.[21] Thus, neutral HP should be recommended to manufacturers for the development of less destructive bleaching products. In clinical practice, acidic HP could be adjusted into a neutral one at the time of application to teeth.

As a limitation of *in vitro* study, it is unavoidable to partly or completely remove the aprismatic surface layer for a flat

enamel surface to test. Therefore, the polished enamel surface might be a little different from the upper aprismatic surface layer, which is generally more highly mineralized than the subsurface and thus more resistant to demineralization.[24,35–37] Another limitation is that most of commercial bleaching agents are gels, which may contain some added ingredients of carbomer, glycerin, fluoride, metal salts and flavours.[18,38] Consequently, it is necessary to involve these factors in the future studies to investigate the effects of neutral HP under these conditions.

A concern is the prevention of irritation to the soft tissues in clinical practice owing to the caustic and oxidizing nature of the highly concentrated HP. In-office bleaching procedures require extensive tissue isolation by use of a rubber dam and/or a resin barrier. As a caustic and oxidizing agent, highly concentrated HP may cause burns and bleaching of gingivae and mucous membranes and even produce localized oral toxicity following sustained exposure if mishandled.[1,39,40] Without the acidity, neutral HP has less adverse effect on teeth. Nevertheless, further *in vivo* and *in vitro* investigations are needed to confirm whether neutral HP has less effect on soft tissues or not.

5. Conclusions

Within the limitation of this *in vitro* study, the following conclusions can be drawn:

1 Neutral 30% HP solution resulted in no significant variations on human tooth enemal in terms of chemical structure, mechanical property and surface morphology except the required colour change.
2 The acidity might be a most likely reason for the negative effects of HP with lower pH value. Neutral HP could be recommended to tooth bleaching for the purpose of reducing deleterious effects on tooth enamel.

Acknowledgements

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