In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel

Tamas Bistey a,*, István P. Nagyb, Anett Simó a, Csaba Hegedus a

a University of Debrecen, Medical and Health Science Center, Faculty of Dentistry, Department of Prosthetic Dentistry, P.O. Box 13 H-4012 Debrecen, Hungary
b University of Debrecen, Faculty of Science, Department of Physical Chemistry, P.O. Box 7 H-4010 Debrecen, Hungary

A R T I C L E   I N F O

Article history:
Received 14 June 2006
Received in revised form
11 October 2006
Accepted 14 October 2006

Keywords:
Hydrogen peroxide
Tooth whitening
Human enamel
Infrared spectroscopy

A B S T R A C T

Objectives: The aim of the present study was to determine the alteration in human enamel after hydrogen peroxide treatment using FT-IR spectroscopy. It is hypothesized that infrared spectroscopy is capable of showing alterations in human enamel after peroxide treatment and the alteration in enamel is proportional to peroxide concentration.

Methods: The effects of 10, 20 and 30% hydrogen peroxide solutions on human enamel were tested. Thirty non-carious human teeth, extracted for periodontal reasons, were used in this study. They were divided into 3 groups of 10, according to the peroxide concentration, sectioned, and the specimens were embedded in resin for infrared spectroscopic analysis. The total treatment time was 120 min. Spectra of the specimens were taken before treatment and 30, 60 and 120 min after it. Another spectrum was taken in a week.

Results: Infrared spectroscopic analysis showed two distinct bands (biological PO_4^−_1 and PO_4^−_2) that were capable of describing the alterations in enamel structure. On comparing the infrared spectra of non-treated and treated specimens, structural changes were detected in the superficial enamel. The alteration in enamel was proportional to treatment time and hydrogen peroxide concentration. Higher concentration and longer treatment time resulted in more severe alterations. The numerical analysis of the spectra revealed that on using concentrated hydrogen peroxide solutions the alterations of the IR spectra were more pronounced. The spectra taken in 1 week after treatment did not show spontaneous reversibility in enamel structure.

Conclusion: At-home and in-office peroxide-containing bleaching agents are capable of causing alteration in enamel at low and high concentrations as well. According to the results of this study it is recommended to perform tooth whitening using low concentration of hydrogen and/or carbamide peroxide, and shorten treatment time to reduce the possible destruction but reach the required change in color.

© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

At-home and in-office peroxide-containing bleaching regimens have become more and more popular in dentistry. The possible causes of the revolutionary demand of this esthetic treatment can be explained by the huge number of the available whitening products and also, an increasing need for the more conservative treatment of discolored teeth.

From the first description of at-home whitening in 1989 until today, numerous studies investigated the effects of
hydrogen and carbamide peroxide on and in tooth tissues. Although the majority of the studies did not mention side effects on the structure of the teeth, opposite results were also obtained. Among the inorganic constituents, a significant decrease in the calcium content and the calcium/phosphorus ratio (Ca/P ratio) was demonstrated after 7 days of treatment with 30% hydrogen peroxide. Arwill et al. studied the dental hard tissues and reported increased porosity of enamel treated with 30% hydrogen peroxide for 6 h. Attin et al. investigated the hardness of bleached enamel. Their study showed decreased microhardness after the interventions.

Other studies have investigated the outer surface of enamel after whitening. Hegeduš et al. described surface changes using atomic force microscopy after 28 h of bleaching with 10% carbamide peroxide and 30% hydrogen peroxide. As a result, the enamel surface became more irregular and surface grooves became deeper and rougher after treatment. Bitter described similar surface grooves on enamel with scanning electron microscopy (SEM) and showed changes in the enamel surface after 30 h of treatment. In their study, Tiley et al. investigated the effect of concentrated hydrogen peroxide solutions on enamel and found precipitation on enamel surface after immersion into 35% hydrogen peroxide for 60 min. McCuickin et al. compared the effects of different types of bleaching products and found increased surface roughness and waviness through profilometric analysis on enamel surface. Goldberg et al. and Arends et al. examined the effects of urea solutions on human enamel. These studies showed that both the inorganic and organic phases played an important role in structural changes in the enamel after bleaching.

The investigation of the highly mineralized enamel is difficult but FT-IR spectroscopy, which requires minimal specimen preparation, can give additional information on the changes of enamel. The facts mentioned above and the results of the investigations of tooth whitening agents on tooth hard tissues are still controversial, further research on tooth hard tissues treated with peroxides is required.

The aim of the present study was to describe the alterations of enamel after treatment with hydrogen peroxide solutions of different concentration (10–30%) using infrared spectroscopy in vitro. The working hypotheses of this work were: (1) infrared spectroscopy is capable to show alterations in human enamel after peroxide treatment and (2) the alteration in enamel is proportional to hydrogen peroxide concentration.

2. Materials and methods

Thirty teeth (10 molars, 15 premolars, 5 incisors) were used in this study. The teeth were caries-free and extracted for periodontal and orthodontic reasons. The teeth originated from 10 people, (three teeth per person). Only the crowns of the teeth were used in the investigation. The roots were cut with a high-speed rotary instrument using water–air cooling spray, and the pulp was removed from the pulp chamber. The specimens were stored in a freezer at -25 °C until use. After removal from the freezer, the specimens were embedded into Araldite (Ciba, Basel, Switzerland) and the surface of the block that covered the buccal side of the tooth was prepared with a rotary instrument to produce a flat enamel surface for the sample holder.

The specimens in groups 1, 2 and 3 were treated with 10%, 20% and 30% hydrogen peroxide solutions (Sigma Chemical Corp. Product Code: H1009), respectively. The pH of the hydrogen peroxide was 7.2 (measured in our laboratory). Treatment was performed at room temperature in closed dishes while the specimens were flooded entirely by the solution. Since infrared spectra were taken of all specimens before treatment, they all served as their own negative control. The total treatment time was 120 min. The spectra were taken at 30, 60 min after the beginning of the treatment. A third spectrum was taken at the end of the 120 min treatment. The specimens were removed from the hydrogen peroxide solution and washed with isotonic salt solution before taking the spectra. The spectra of the hydrogen peroxide and of the Araldite were also taken as controls to notice their presence on the prepared surface of the embedded enamel specimens. After storage in isotonic salt solution in closed dishes in sterile conditions for a week, the spectra of the enamel specimens were taken again to examine the reversibility of the changes.

FT-IR spectrometric investigations were performed with a SPECTRUM-ONE infrared spectrometer (Perkin-Elmer Inc., Wellesley, MA, USA) equipped with an Universal ATR unit (3x bounce diamond crystal ATR). The instrument was operated under the following conditions: 4000–650 cm⁻¹ range, 4 cm⁻¹ resolution, 4 scans co-addition, and room temperature. The investigated surface was positioned against the diamond crystal of the ATR unit, and was pressed with a force gauge at a pressure to make the necessary contact to yield a characteristic spectrum. Pressure was set to 145–150 arbitrary units of the device’s pressure meter, standard for this model of Perkin-Elmer Instruments.

The quantitative analysis of the results was made by calculating the area of the spectra between the selected wavelengths.

3. Results

Human enamel specimens showed a characteristic infrared spectrum with two distinct peaks, representing the hydroxyapatite structure: biological PO₄ ν₁ at 996 cm⁻¹ and biological PO₄ ν₂ between 1410 and 1460 cm⁻¹ wavelength before hydrogen peroxide treatment. At 886 cm⁻¹ a secondary peak was seen, which represented the carbonate apatite (ν₂ CO₃) phase of enamel. The characteristic shape of the biological PO₄ ν₂ was doubled at 1410 and 1460 cm⁻¹.

Alterations in the IR spectra of superficial enamel of the specimens were found after hydrogen peroxide treatment in all cases (i.e. peroxide concentration and treatment time). Fig. 1A shows the typical changes of IR spectra of the enamel after treatment with hydrogen peroxide of three different concentrations for 120 min. The biological PO₄ ν₁ became wider and distorted after treatment. These changes were directly proportional to the hydrogen peroxide concentration. The distortion of the biological PO₄ ν₂ could also be observed in Fig. 1A. The biological PO₄ ν₂ had been distorted to such a degree after the treatment with 20% hydrogen peroxide that
only a part of the band could be seen at 1460 cm\(^{-1}\) (spectrum c). At 30% hydrogen peroxide concentration, the biological PO\(_4\) \(^{\text{n2}}\) was missing (spectrum d).

The effects of treatment time or the kinetics of hydrogen peroxide on enamel are shown in Fig. 1B. The kinetics can be best demonstrated at 10% hydrogen peroxide concentration because at 20% of concentration the biological PO\(_4\) \(^{\text{n2}}\) is dissolved. The biological PO\(_4\) \(^{\text{v1}}\) becomes wider and distorted. The characteristic shape of the biological PO\(_4\) \(^{\text{v2}}\) can be seen after 30 min of treatment (spectrum b), but only a single peak can be observed after 60 min, and it becomes indistinguishable after 120 min.

The quantitative description of the effects of hydrogen peroxide on enamel can be made by calculating the area of the biological PO\(_4\) \(^{\text{v1}}\) (Fig. 2). Table 1 shows the reliability of the spectral integral of the biological PO\(_4\) peaks. \(^{\text{v1}}\) and \(^{\text{v2}}\) were significantly easier to notice in 23 specimens (78%) and 22 specimens (74%), respectively. It means that both biological PO\(_4\) \(^{\text{v1}}\) and \(^{\text{v2}}\) can describe the severity of destruction in enamel after peroxide treatment. Nevertheless the biological PO\(_4\) \(^{\text{v1}}\) was used for the quantitation of the destruction since the biological PO\(_4\) \(^{\text{v2}}\) is not only distorted but missing at 30% hydrogen peroxide.

After storing the enamel samples in isotonic salt solution for a week, no spontaneous reverse effects were found in the
4. Discussion

IR spectroscopy can function as a non-destructive method in the analysis of calcified tissues and revealed alterations in enamel after hydrogen peroxide treatments. Besides the whitening effect of peroxides, side effects can also take place during or after bleaching treatment. The wide use of peroxide-containing tooth whitening materials requires further investigation of these materials. Hydrogen peroxide is the only active ingredient in the most frequently applied bleaching products. That is why specifically the effects of this substance were studied in this paper.

Our results showed changes in superficial enamel after hydrogen peroxide treatment, which affected the highly mineralized enamel even at low (10%) concentration. The alterations detected using the FT-IR method, were proportional to the concentration. At higher concentration, more severe alteration was found in the infrared spectra. It was not only the concentration of the peroxide solutions but also the increase of treatment time that emphasized the spectral changes. The biological PO$_4$ v1 and biological PO$_4$ v2 exhibited severe alteration. The biological PO$_4$ v1 became wider when the treatment time or concentration were increased. The biological PO$_4$ v2 distorted at 20% hydrogen peroxide concentration. Although spectral changes revealed certain destruction of the hydroxy-apatite structure, it is hard to define its extent at chemical level. The most important question is the chemical interaction between the peroxide and the apatite structure. It has been an established fact that peroxides and free radicals penetrate the enamel and other tooth hard tissues during bleaching. Their inner position or place in the apatite crystals is not known. The presence of hydrogen peroxide in our study was established on the basis of the IR spectra. Hydrogen peroxide was present in the IR spectra between 3500 and 4000 cm$^{-1}$.

It is hypothesized that hydrogen peroxide, which can form a so-called diperoxo (H$_4$O$_4$) format, is capable of changing the apatite structure and the PO$_4$ is replaced with diperoxo ligands forming a new complex. The other substitution of the coordinated ligands in hydroxy-apatite can happen when the complex is formed through metal ions (e.g. ferrum) or when the CO$_3$ is replaced by the *OH released from H$_2$O$_2$. Between 3000 and 3700 cm$^{-1}$ the OH band can be found in the IR spectra. This region was variable with very little consistency. It is supposed that enamel is hydrated during peroxide treatment or bleaching. The interpretation of this inconsistency can be in the possible alteration of OH binding in the crystal. This binding can become weaker and stronger. In other words, the crystal can release the OH or bind in a stronger way. A change in OH binding can also take place in the possible substitution. It is supposed that these substitutions are weak and the process can be reversed by applying fluoride. The treatment with desensitizing fluoride gels or preventive pastes can induce the build-in of fluoride. If this ‘remineralization’ is fast (high concentration of F$^-$) the penetration of fluoride is probably not possible into the deep enamel and the peroxide effect takes place for a long period of time.

To describe the changes in enamel a quantitative analysis was performed by calculating the area of the biological PO$_4$ v1.

\[
\text{Area of biological PO}_4 \text{ v1 (1%)} = 5.6362x - 1444.4
\]

Fig. 4 – Area of the biological PO$_4$ v1 plotted against the treatment time. Changes in the area (severity of destruction) are most expressed between the 30 and 60-min treatment.

Infrared spectra even after the lowest tested concentration of hydrogen peroxide (10%).

<table>
<thead>
<tr>
<th>Biological PO$_4$ v1, 996 cm$^{-1}$</th>
<th>Biological PO$_4$ v2, 1460–1410 cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearly noticeable</td>
<td>23 samples</td>
</tr>
<tr>
<td>Noticeable</td>
<td>4 samples</td>
</tr>
<tr>
<td>Invaluable</td>
<td>3 samples</td>
</tr>
<tr>
<td>Total</td>
<td>30 samples</td>
</tr>
</tbody>
</table>

Table 1 – Reliability of the quantitative analysis of biological PO$_4$ v1 peak. The biological PO$_4$ v1 and v2 could be noticed in the spectra of 27 samples out of 30. Accordingly, both peaks can describe the severity of destruction in the enamel.

Please cite this article in press as: Bistey T, et al., In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel, Journal of Dentistry (2006), doi:10.1016/j.jdent.2006.10.004
McCracken and Haywood found significant Ca loss of enamel after 6 h of treatment with 10% carbamide peroxide solution. In another study, Oltu and Gürgan, using infrared absorption spectroscopy and X-ray diffraction analysis, revealed the role of high concentration of carbamide peroxide in the changes of enamel, and they found that hydrogen peroxide at a lower (10–16%) concentration, did not have a major effect on the enamel structure. Lewinstein et al. came to a similar conclusion. They found that carbamide peroxide could cause demineralization in enamel at low and high concentrations alike, but remineralization could take place using fluorides at low concentration. In their study, Efeoglua et al. demonstrated the demineralization of enamel samples using µCT evaluation after 10 carbamide peroxide treatment sessions. Such demineralization took place in a depth of 50 μm.

Controversially, Seghi and Denry demonstrated no significant decrease in enamel microhardness values after 12 h of treatment with low concentration of carbamide peroxide. Potocnik et al. reported that 10% carbamide peroxide did not alter enamel microhardness.

Another important factor in tooth whitening is the reversibility of the inner or surface alterations in tooth hard tissues. The second half of our study examined the spontaneous reversibility of changes in enamel after peroxide treatment. The specimens were stored in isotonic salt solution at room temperature between the spectra were taken. IR spectral analysis did not show a reverse process in enamel after 1 week neither at the biological PO₄ ν₁ nor at the biological PO₄ ν₂ bands. According to the above, it can be stated that hydrogen peroxide causes an irreversible alteration in enamel after 120 min, even if it is not observed clinically. On the other hand, clinicians can whiten the teeth without causing any kind of alterations. To achieve this, the selection of the appropriate concentration and treatment time is the prerequisite of finding such a safe method.

According to our results it is recommended that tooth whitening should not take longer than 1 h and low-concentration peroxide-containing agents should be applied. In esthetic dentistry when peroxide-containing bleaching gels are used not only the esthetic result(s) but the possible side effects should be consider especially when the treatment time and/or concentration is determined. Our results indicate that a lower peroxide concentration can be safer than a higher one (Figs. 3 and 4). On the other hand, above a ‘critical’ concentration, the destruction in enamel is not increased. But with the use of higher concentration of bleaching gels, soft tissue irritation can be more expressed. So it can be concluded that lower peroxide concentrations can be safer with respect to tooth hard tissue destruction. Nevertheless, further studies are required to estimate the effects of whitening peroxides on enamel and other tooth hard tissues.

5. Conclusion

FT-IR spectroscopy resulted in severe morphological alteration in superficial human enamel after treatment with 10–30% hydrogen peroxide in vitro. The changes in enamel were directly proportional to the treatment time and peroxide concentration. The biological PO₄ ν₁ and ν₂ band distorted after peroxide treatment. The distortion of biological PO₄ ν₁ was linear in time and increase with the hydrogen peroxide concentration. The biological PO₄ ν₂ was distorted up to 20% concentration and missing after treatment with hydrogen peroxide at a concentration of 30%. Spontaneous reversibility of the changes was not detected after storing the enamel specimens in sterile isotonic salt solution.

Acknowledgements

The authors thank Gyula Szőör Ph.D., DCS, and full professor at the Department of Mineralogy University of Debrecen for his critical reading of this paper and advice in understanding the hydroxyapatite structure. This investigation was supported by the project no. 006/2004 of the National Research and Technology, Hungary.

References