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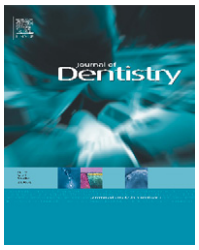

In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel

Tamas Bistey [a,*], *István P. Nagy* [b], *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*

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ABSTRACT

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 $\nu 1$ and $\nu 2$) 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.

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[1] until today, numerous studies investigated the effects of

* *Corresponding author*. Tel.: +36 52 342 224; fax: +36 52 342 224.
E-mail address: bistey@jaguar.dote.hu (T. Bistey).

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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,[2–4] opposite results were also obtained.[5–9]

Among the inorganic constituents, a significant decrease in the calcium content and the calcium/phosphorous ratio (Ca/P ratio) was demonstrated after 7 days of treatment with 30% hydrogen peroxide.[5,6] Arwill et al.[10] studied the dental hard tissues and reported increased porosity of enamel treated with 30% hydrogen peroxide for 6 h. Attin et al.[11] 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. Hegedűs et al.[12] 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[13] 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, Titley et al.[14] 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. McGuckin et al.[15] 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.[16] and Arends et al.[17] 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 (3× 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 PO_4 $\nu 1$ at 996 cm^{-1} and biological PO_4 $\nu 2$ 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 (ν_2 CO_3) phase of enamel. The characteristic shape of the biological PO_4 $\nu 2$ 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 PO_4 $\nu 1$ became wider and distorted after treatment. These changes were directly proportional to the hydrogen peroxide concentration. The distortion of the biological PO_4 $\nu 2$ could also be observed in Fig. 1A. The biological PO_4 $\nu 2$ had been distorted to such a degree after the treatment with 20% hydrogen peroxide that