New approach to determine the morphological and structural changes in the enamel as consequence of dental bleaching

I. Izquierdo-Barba, C. Torres-Rodríguez, E. Matesanz, M. Vallet-Regí

Department of Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain

Network Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBV, Madrid, Spain

Department of Salud Oral, Facultad de Odontología, Universidad Nacional de Colombia, Sede Bogotá, Colombia

Department of Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain

ABSTRACT

Nowadays, there are a number of methods very effective for the dental bleaching, which are typically strong oxidizing agents, as the hydrogen peroxide, applied directly to the tooth surface. After bleaching, several research studies have been carried out to evaluate the efficacy of bleaching agents on teeth, there being a great controversy concerning the techniques used and their pre-treatment requirements derived, which could alter the final results. In fact, there is a strong necessity to develop different approach to determine the real consequences of bleaching treatment by using an unchangeable and entire tooth. Herein, to evaluate the effects of 35% (v/v) hydrogen peroxide treatment onto morphological, chemical and structural features in the human enamel and dentin, environmental scanning electron microscopy, electron probe micro analyzer and X-ray diffraction techniques have been used. Although such effects have been widely investigated with several techniques, including XRD and SEM, the novelty of this study lies on the techniques and methodology used to characterize the human teeth after bleaching treatment. This approach allows carrying out the analyses without any previous pretreatment, such as powdering, dried or metal sputtering, and its study in the same tooth piece before and after bleaching, which avoids the possible intrinsic differences derived from the use of different pieces. The obtained results display that neither the structural nor the chemical features of both enamel and dentin are altered after bleaching treatment. However, the morphology of the enamel is notably altered, appearing pronounced pores which could affect to the possible bacterial colonization. These findings put an end to the controversies on the different obtained results in the literature of the bleaching effects in the enamel and set standards for future studies.

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1. Introduction

Dental bleaching is commonly carried out to correct discolouration of anterior teeth. Most bleaching agents are strong oxidizing agents and the most popular bleaching agent includes hydrogen peroxide [1]. Although this bleaching agent is highly effective in lightening tooth colour, concerns have been expressed regarding to associated post-bleaching complications including alteration in the surface morphology of enamel and dentin, change in its chemical composition, increase in its permeability, and notable changes in its mechanical properties [23]. However, there is much controversy between the methodologies used for these studies of bleaching effect, which can affect the intrinsic features of tooth [45]. While some authors did no observe adverse effects, others claimed reduction in calcium phosphate ratio and loss of organic components from treated enamel surfaces. Nonetheless, it is highly probable that low pH and hydrogen peroxide oxidation could lead to structural changes in dentin during internal dental bleaching [3,4]. The purpose of the current study has been to determine the effects of dental bleaching with H2O2 35% (w/v) during 20 min on the morphological, chemical and structural features of human molar teeth by using environmental scanning electron microscopy (ESEM), electron probe micro analyzer (EPMA) and X-ray diffraction (XRD). These techniques are excellent tools for determining the morphological, structural and chemical changes as a function of intratooth localization by using the same piece before and after bleaching and avoiding any preconditioning treatment. X ray powder diffraction studies have been already carried out on bulk samples of grinded enamel showing no differences between bleached and unbleached samples [6], but this sample preparation procedure could

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prevent from finding out the possible enamel alterations happening in
the tooth surface compared with the deeper enamel or even the
dentin. By probing the non-ground enamel tooth both in surface and
in depth, we expect to detect any possible structural change resulting
from the bleaching treatment. Moreover, the morphological features
in the bleaching effect have been also conducted using multiple
traditional scanning electron microscopies (SEM) [2,7,8], which
requires a previous specimen preparation, allowing the study on
dehydrated teeth and affecting to real microstructural features with
respect to fresh teeth. In the present study the environmental
scanning electron microscopy (ESEM) has been used because is an
instrument which allows the examination of the surfaces of hydrated,
unfixed specimens with depth of field and resolution and magnifica-
tion equivalent to that typically afforded by SEM. Furthermore, we
have selected different areas of the tooth from shallower areas of
enamel to deeper areas of the dentin, in order to determine the range
of action of this bleaching agent with respect to morphological
changes in the different selected areas.

2. Experimental

For this study different third human molar from different individ-
ual aged 18 to 23 years obtained for orthodontic indication, prior
informed consent and informative book. this study protocol was
reviewed and approved by the Local Ethics Committee of the Faculty
of Dentistry at the National University of Colombia. Extraction,
disinfection and storage of the samples were carried out according
to Tooth Bank protocol [9]. For ESEM and XRD studies, the whole
tooth were sagittally cut at amelocemental junction (longitudinal
section) with a diamond blade on two halves. Then, one of half of
the crowns was subjected to treatment with 38% $H_2O_2$ bleaching gel
for 20 min on the surface and another half was untreated (Fig. 1). Not
that treatment was carried out onto the most superficial part,
avoiding treatment in the cutting area. After that the teeth were
gently rinsed and dried. ESEM and XRD results shown in this man-
uscript derive to one tooth which is the most representative result.
ESEM was performed in a FEI QUANTA 200 at an accelerating voltage
of 30 kV, a low vacuum of 0.7 Torr, and at a working distance of
10 mm, with X-ray energy dispersive (EDS) spectrometer Oxford
detector. X-ray powder diffraction scans were measured on a Pan-
alytical Empyrean diffractometer with Cu tube operated at 40 mA and
45 kV. Point focus collimated to 1 mm x 1 mm was used for incident
beam optics and a Pixel 2D position sensitive detector in the diffracted
beam optics. All scans were measured in reflection mode.
We got XRD scans for two spots in the surface of the bleached and
unbleached enamel and two equivalent spots in the deeper enamel
exposed in the tooth section. To complete the study, we measured
one additional dentin spot.

The changes of the chemical composition surface of bulk enamel
were also determined by using electron probe microanalyzer (EPMA)
in a JEDL Superprobe JXA-8900. The analyses were carried out on five
whole teeth, which were mounted in resin leaving exposed the
surface enamel, and coating with graphite and analyses by EPMA.
Then, these same fragments were bleached and analyzed again by
EPMA, attempting to analyze in the same area that before treatment.
Data are expressed as mean ± standard deviation of five specimens
and 15 different analyses of different area. Statistical analysis was
performed using the Statistical Package for the Social Sciences (SPSS)
version 19 software using an analysis of variance (ANOVA) with post-
hoc Scheffe's test. p < 0.05 was considered significant.

3. Results and discussion

Fig. 2 shows the tooth bleaching effects on morphological proper-
ies of human enamel. SEM micrographs show notable changes

Fig. 1. Scheme displaying the manipulation of samples to determine the effect of
bleaching with $H_2O_2$ directly onto human molar teeth by XRD and ESEM-EDS
studies. The low magnification indicates the different parts of the teeth (enamel
dentin) which were deeply studied with these techniques.

Concerning the enamel morphology of the bleached-enamel surface
(left side) compared with the unbleached surface (right side). Typical
enamel structure prism is displayed in the untreated sample (Fig. 2,
left) [5,10]. However, after bleaching, a total loss of this typical
morphology is observed, appearing deep longitudinal cavities through
the enamel structure to a depth of about 200 μm, being less striking
until 400 μm. High magnification ESEM micrographs corresponding
to the outer part of the enamel show notable decrease of the contrast
which evidences the abrasive effect of bleaching agent, according to
previous reported results [11]. These changes in the porosity in the
shallower areas of enamel are been previously reported, showing that
bleaching agents increased enamel porosity owing to the disruption
of the matrix protein, likely through free radical-induced oxidation
could affect seriously to posterior bacteria colonizaton [12,13].
However, these studies have also pointed to a serious modification in the
dentin area, which has been not evidence in this study (Fig. 3), despite its
higher content of organic matter with respect to enamel area. These
results demonstrate that treatment with hydrogen peroxide at 38% for
20 min produces significant morphological changes in the most
superficial parts of the enamel without altering the dentin area.
Similar results have been reported for other modern bleaching agent
based on cold light effects, widely used in office treatment, due to
high efficiency and low side effects [14].

Fig. 4 shows the X-ray powder diffraction scans obtained. Scans
(a) and (b) correspond to the treated and untreated enamel surface

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and both of them show a highly textured hydroxyapatite pattern with the 0 0 2 reflection enhanced as expected [14]. No significant differences can be appreciated between these two scans. Scans (c) and (d) were measured in the enamel in depth spots and once again no significant differences between the bleached and unbleached halves are shown up. The different aspect of these in depth enamel diffractograms with respect to those measured in the surface is a consequence of the strong texture and the different sample orientation during the data collection, but it is not attributable to any structural differences. These results are coincident with those obtained in synthetic hydroxyapatite [15] and with ground enamel hydroxyapatite [6]. And finally, the scan (e) from the tooth dentin shows the typical broad peaks expected for this substance [16]. In agreement with results EPMA data are displayed in Table 1. No significant differences in the chemical composition were evidenced in the enamel surface before and after bleaching treatment, which is consistent by other research groups [17].

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Fig. 3. Dentin morphology study. ESEM micrographs of a longitudinal section of molar human teeth showing enamel-dentin interface and dentin. The left and right sides correspond to non-treated and bleached parts of the molar teeth, respectively.

Fig. 4. X-ray diffraction study. XRD patterns measured on (a) enamel surface unbleached, (b) Enamel surface bleached, (c) Enamel in tooth section unbleached, (d) Enamel in tooth section bleached, and (e) dentin in tooth section. Indexing based in hydroxiapatite PDF card 01-098-4605 (International Centre for Diffraction Data PDF-4 file).

Table 1

<table>
<thead>
<tr>
<th>Sample/elements</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
<th>Cl</th>
<th>F</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non treated</td>
<td>61.0±3.0</td>
<td>35.7±2.7</td>
<td>0.6±0.2</td>
<td>1.25±0.7</td>
<td>0.63±0.3</td>
<td>0.82±0.8</td>
</tr>
<tr>
<td>Bleached</td>
<td>61.0±3.5</td>
<td>35.0±2.6</td>
<td>0.7±0.3</td>
<td>1.14±0.8</td>
<td>0.70±0.5</td>
<td>1.40±0.7</td>
</tr>
</tbody>
</table>

4. Conclusions

A new approach to determine the morphological, structural and chemical features of human tooth after bleaching on the human tooth has been carried. For this purpose, ESEM EPMA and XRD techniques, which permit to study the different constituents of the teeth (enamel and dentin) without needing previous pretreatment, such as powdering, dried or metal sputtering, which could alter the obtained results has been performed. The results derived from the current research indicate that neither the structural nor the chemical features of enamel and dentin are altered after bleaching treatment. Nonetheless, the morphology of the enamel is notably altered, with the appearance of channels with very pronounced pores. These channels could make enamel more susceptible to bacterial attack. The major scientific relevance of this study relies on the techniques and methodology used to deeply characterize the teeth after bleaching treatment, putting an end to the controversies on the different effects found in the literature, and setting novel standard protocols for future studies.

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