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

I. Izquierdo-Barba [a,b], C. Torres-Rodríguez [c], E. Matesanz [d], M. Vallet-Regí [a,b,*]

[a] Departmento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain
[b] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Madrid, Spain
[c] Departamento de Salud Oral, Facultad de Odontología, Universidad Nacional de Colombia, Sede Bogotá, Colombia
[d] CAI de Difracción de Rayos X, Universidad Complutense, Madrid, Spain

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ABSTRACT

Nowadays, there are a number of methods very effectives 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 38% (p/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.

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 [2,3]. However, there is much controversy between the methodologies used for these studies of bleaching effect, which can affect the intrinsic features of tooth [4,5]. 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 H_2O_2 38% (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

* Corresponding author at: Departmento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain.
Tel.: +34 91 394 1861; fax: +34 394 1786.
E-mail address: vallet@ucm.es (M. Vallet-Regí).

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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-grinded 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 magnification 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 individual 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 sagitally 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). Note 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 manuscript 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 Panalytical Empyrean diffractometer with Cu tube operated at 40 mA and 45 kV. Point focus collimated to 1 mm × 1 mm was used for incident beam optics and a Pixcel 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 JEOL 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 Scheffé's test. $p < 0.05$ was considered significant.

3. Results and discussion

Fig. 2 shows the tooth bleaching effects on morphological properties of human enamel. SEM micrographs show notable changes 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 with 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 colonization [12,13]. However, these studies have also pointed to a serious modification in the dentin area, which has been not evidenced 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].

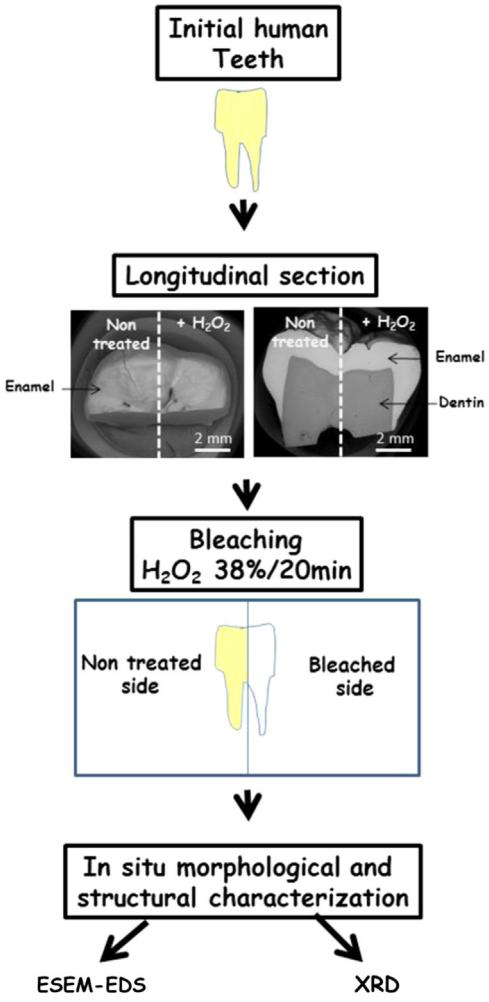

Fig. 1. Scheme displaying the manipulation of samples to determine the effect of bleaching with H_2O_2 directly onto human molar teeth by XRD and ESEM-EDS studies. The low magnification indicates the different parts of the teeth (enamel and dentin) which were deeply studied with these techniques.

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