Archives of Oral Biology 65 (2016) 52–58

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Graded changes in enamel component volumes resulted from a short tooth bleaching procedure

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 2 November 2015
Accepted 27 January 2016

Keywords:
Enamel
Tooth bleaching
Hydrogen peroxide
Histology
Biochemistry

ABSTRACT

Aim: To test the hypothesis that changes in enamel component volumes (mineral, organic, and water volumes, and permeability) are graded from outer to inner enamel after a short bleaching procedure.
Materials and methods: Extracted unerupted human third molars had half of their crowns bleached (single bleaching session, 3 × 15 min), and tooth shade changes in bleached parts were analyzed with a spectrophotometer. Ground sections were prepared, component volumes and permeability were quantified at histological points located at varying distances from the enamel surface ($n = 10$ points/location), representing conditions before and after bleaching.
Results: Tooth shade changes were significant ($p < 0.001$; 95% CI = −1/−8; power = 99%), and most of the enamel layer was unaffected after bleaching, except at the outer layers. Multiple analysis of covariances revealed that most of the variance of the change in enamel composition after bleaching was explained by the combination of the set of types of component volume (in decreasing order of relevance: mineral loss, organic gain, water gain, and decrease in permeability) with the set of distances from the enamel surface (graded from the enamel surface inward) (canonical $R^2 = 0.97$; $p < 0.0001$; power > 99%).
Conclusions: Changes in enamel composition after a short bleaching procedure followed a gradient within component volumes (mineral loss > organic gain > water gain > decrease in permeability) and decreased from the enamel surface inward.

1. Introduction

Tooth bleaching of the crown can be described as the combined increase in the lightness and reduction in yellowness of the tooth. Most of the yellowness of the tooth crown comes from the interaction of incident light with dentine after light interacts with the enamel layer (first inward and then outward) and then returns to the eye of the observer (Ten Bosch & Coops, 1995). Reduced translucency of enamel contribute to tooth lightness (Ten Bosch & Coops, 1995), and it has been shown that part of the tooth bleaching effect of bleaching agents is due to a reduction in the translucency of enamel (Vieira, Arakaki, & Caneppele, 2008; Ma et al., 2009, 2011). The reduced translucency of enamel decreases the amount of light that reaches subjacent dentine, which, in turn, reduces the amount of dentine color reflected to the human eye, thus contributing to the lightening and reduction of yellowness of the tooth crown. It has been recently reported that most of the bleaching effect of bleaching agents in the color of the tooth crown is resulted from alterations in the optical properties of enamel, not in those of dentine (Ma et al., 2011). This observation puts enamel in the center of the debate on the mechanism of tooth bleaching, opening questions on which enamel component would undergo more pronounced changes when a tooth crown is bleached. There is evidence indicating that the reduced enamel translucency caused by bleaching agents is due to the oxidation of the organic matter, but not to any decrease in either organic or mineral contents (Eimar et al., 2012).

Considering that the amount of organic matter in enamel is much lower than that of dentine, it is not possible to explain the higher contribution of enamel in tooth bleaching compared to dentine's contribution when only the oxidation of organic matter is

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http://dx.doi.org/10.1016/j.archoralbio.2016.01.016
0003-9969/

considered. Probably the mineral component, which is the most abundant in enamel, plays a role in tooth bleaching. Some studies reported demineralization of enamel associated with tooth bleaching (Al-Salehi, Wood, & Hatton, 2007; Wiegand, Schreier, & Attin, 2007; Cakir, Korkmaz, Firat, Oztas, & Gurgan, 2011; Mondelli et al., 2015). This is consistent with the fact that hydrogen peroxide, even at neutral pH, breaks down producing hydrogen ions (Xu, Li, & Wang, 2011) that might cause demineralization in enamel. It is important to note that increased pore volume in enamel is accompanied by an increase in water volume, which, in turn, has a refractive index (1.33) different from that of the enamel mineral (1.62), thus contributing to increase light scattering (Van Der Veen, Ando, Stookey, & De Joselin de Jong, 2002) and reduce tooth yellowness. It must be emphasized that increased light scattering of the tooth crown due to enamel demineralization does not require that the entire thickness of the enamel layer is demineralized. This is supported by the fact that the use of 37% phosphoric acid for 30 s to etch normal human enamel results in an opaque appearance (reduced yellowness) of enamel and the associated mean depth of demineralized enamel is 7 μm (Hannig, Bock, Bott, & Hoth-Hannig, 2002). The report of changes in the optical properties of the whole enamel layer resulted from a long application time (1–4 weeks; 8 h/day) of the bleaching agent, the contributions of enamel and dentine to the bleaching effect were analyzed separately, but it was not known if optical changes in enamel after shorter application times would involve the entire enamel layer or just part of it (Ma et al., 2011). The report of changes in the oxidation of enamel organic matter also resulted from long application time (4 days; 24 h/day), and the contributions of enamel and dentine to the bleaching effect were not analyzed separately, so that it was known the proportion of enamel layer that was affected by oxidation of the organic matter (Eimar et al., 2012).

The transport of bleaching agents in enamel occurs by diffusion. As the bleaching agent diffuses into enamel, the fastest diffusion rate is expected to be along prisms sheaths (interprismatic enamel) (Shellis & Dibdin, 2003), and the changes in enamel components are expected to occur sequentially from outer to inner enamel. One important research question is what changes in the amount of enamel components occur as soon as the first signs of tooth bleaching are observed. In order to shed some light on this problem, we tested the hypothesis that alterations in the volumes of the main components of enamel (mineral, organic, and water volumes) and permeability are graded from outer to inner enamel after using a bleaching agent for a short application time that results in clinically relevant tooth bleaching.

2. Materials and methods

2.1. Sample size calculation and samples

Considering a predicted effect size "*r*" of 0.75 (correlation coefficient between mineral loss versus distance from the enamel surface), power of 80%, and two-tailed significance level of 5%, the calculated sample size was 10 per group (bleached and non-bleached groups) (Cohen, 1988). Extracted unerupted sound mature permanent third molars ($n = 10$) were collected. All teeth were donated by volunteers who provided signed consent, according to the protocol approved by the Ethical Committee on Research in Humans of the Federal University of Pernambuco (Protocol 348.061). The teeth were stored in 0.1% thymol solution, cleaned with pumice paste to remove organic coatings, dried and examined under the stereomicroscope (magnification of 20×) in order to exclude those with crack lines and opacities. All teeth were sectioned longitudinally at the center of the buccal surfaces in order to separate the halves into control (not submitted to bleaching; $n = 10$) and experimental (submitted to bleaching; $n = 10$) groups.

2.2. Bleaching procedure

Prior to the bleaching procedure, all experimental halves of the buccal surfaces were dried for 10 s and then had their tooth shades assessed using the Easy shade spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany). Subsequently, the experimental halves received three applications of 15 min of a self-activated bleaching gel with 35% hydrogen peroxide and pH 6.5 (Whiteness HP Maxx, FGM, Brazil), at room temperature (25° C), comprising only one bleaching session. The bleached halves were then cleaned with water/air spray and stored in distilled water. Seven days after the bleaching procedure, the bleached halves were dried with compressed air for 10 s and the tooth shade was assessed using the same spectrophotometer described above. After the bleaching procedure, the component volumes of dental enamel were quantified in all paired samples at selected histological points located at various distances from the enamel surface.

2.3. Quantification of mineral volume

All tooth halves were cut transversally to the tooth axis at a standardized distance from the enamel-cementum junction for each pair of control and experimental halves obtained from the same tooth. The cut slices were ground manually with the aid of a lapping jig and silicon carbide paper, resulting in undemineralized ground sections (~100 μm thick). All sections were microradiographed in high resolution X-ray film plates (AGHD photoplates, Microchrome, San Jose, California, USA) using a X-ray source with Tungsten cathode (PCBA Inspector, General Electric, Germany), and submitted to quantification of mineral volume using the Angmar formula, as described recently (Macena et al., 2014). Measurements were based on a mineral density of 2.99 g cm^{-3} (Elliott, 1997) and were performed at histological sites (area of 15 × 15 μm) located at the following distances from the enamel surface: 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 μm. Those points were located at a transversal line traced from the enamel surface inward, following the prism paths. For each pair of control and experimental halves obtained from a same tooth, the regions with the transversal lines presented the same thickness of the enamel layer, and the transversal lines were up to 1.5 mm apart from each other.

2.4. Quantification of water and organic volumes

The total water and the organic volumes were quantified at the same histological points where mineral volume was obtained. Phase retardances under water immersion (24 h of immersion in distilled water) were measured (mean of 5 measurements) in a transmitted light polarizing microscope (Axioskop 40, Carl Zeiss, Germany) equipped with a 0–5 orders Berek compensator and a green interference filter (546 nm; bandwidth of 10 nm). Phase retardance was divided by the sample thickness yielding the observed birefringence that was interpreted using the mathematical approach of Sousa et al. (2006) as described in various previous reports (Macena et al., 2014; Sousa, Vianna, & Santos-Magalhães, 2009; Barbosa de Sousa, Soares, & Vianna, 2013). The mineral volume measured from microradiography was used in the calculations. The water (α) and organic (β) volumes thus obtained were used to compute the volume more easily available for diffusion (α_d) (Barbosa de Sousa et al., 2013):

$$\alpha_d = \frac{\alpha^2}{\alpha + \beta} \quad (1)$$