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Biological action of bleaching agents on tooth structure: a review

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Abstract

The use of bleaching agents to remove stains is one of the main dental procedures to improve the aesthetics of teeth. This review presents the main agents used for tooth whitening, existing clinical protocols, and the structural changes that may occur through their use. The main bleaching agents consist of hydrogen peroxide and carbamide peroxide, which are used in bleaching techniques for vital teeth. These techniques can be performed in the office by a professional or by the individual in a home environment under professional guidance. Bleaching agents come in a variety of concentrations and there are over-the-counter products available on the market with lower concentrations of

hydrogen peroxide. Due to the chemical characteristics of the agents, changes in the organic and inorganic content of the tooth structure can be observed. These changes are related to morphological changes characterized by increased permeability and surface roughness, such changes compromise the mechanical resistance of the tooth. Furthermore, bleaching agents can promote molecular changes after reaching the dental pulp, resulting in oxidative stress of pulp cells and the release of pro-inflammatory mediators. Despite the bleaching effectiveness, tooth sensitivity is considered the main side effect of use. Therefore, among the heterogeneity of protocols, those that used the bleaching agent for a prolonged time and in lower concentrations presented more harmful effects on the tooth structure.

Keywords: dental bleaching, carbamide peroxide, hydrogen peroxide, enamel, dentin, dental pulp

1. Introduction

Dental bleaching is one of the most common procedures in dental practice to promote the color change of teeth aiming at improving esthetics and individual satisfaction (Blatz *et al.*, 2019). The efficacy of bleaching depends on the type of bleaching agent, pH of the oral microenvironment, application protocol, and the type of tooth discoloration, among other factors (Rodríguez-Martínez *et al.*, 2019).

Color changes may derive from genetic factors, such as the use of antibiotics during tooth formation (tetracycline-stains), and high fluoride levels (fluorosis) configuring intrinsic dental staining (Epple *et al.*, 2019). On the other hand, the adsorption of pigments on the dental surface resulting from frequent tobacco use, consumption of stained foods and drinks, and poor oral hygiene with biofilm accumulation can cause extrinsic dental staining (Joiner *et al.*, 2008; Epple *et al.*, 2019).

Pigments adsorbed on the dental structure are called chromophores, and they absorb light in the visible range, reflecting only wavelengths with a yellowish or brownish color (Carey, 2014). The organic compounds in these pigments are small molecules resulting from consumption, such as tea, coffee, and red wine. These molecules maintain double bonds between carbonyl or aromatic groups and often adhere to calculus or biofilm (Carey, 2014; Epple *et al.*, 2019).

The process of removing chromophores occurs through the oxidation of organic compounds by oxidizing agents such as hydrogen peroxide (H_2O_2), which is presented as the active agent in most bleaching systems (Alkahtani *et al.*, 2020). This agent is highly soluble in water and presents an acid pH in a solution. Due to unpaired electrons in its structure, it becomes reactive with a robust oxidizing capacity for organic and inorganic molecules (Kwon *et al.*, 2015).

This is why the main bleaching techniques use hydrogen peroxide as an oxidizing agent, in varied concentrations depending on the type of application protocol (Joiner *et al.*, 2008). In-office bleaching

uses hydrogen peroxide at higher concentrations, ranging from 30% to 40%, and the application is carried out under professional supervision. This protocol is performed in 2-3 sessions with time intervals determined by factors such as the concentration of the bleaching agent, clinical history of the individual, and degree of tooth color saturation (Hafez *et al.*, 2010; Alkahtani *et al.*, 2020).

At-home bleaching is another dentist-supervised protocol. Customized bleaching trays are fabricated to carry the bleaching material that remains in contact with the patient's dental structure for a few hours, which is determined by the professional (Alqahtani, 2014). In this at-home bleaching technique, the most used bleaching agent is carbamide peroxide, which is present in concentrations ranging from 10% to 22% (Epple *et al.*, 2019). This structural compound reacts with water decomposing into urea and hydrogen peroxide. Hydrogen peroxide decomposes once more into water and reactive oxygen species (ROS) that are responsible for the oxidation of organic components of the tooth structure, which increases light scattering and promotes increased tooth whiteness (Alkahtani *et al.*, 2020) (Fig. 1).

In addition to these dentist-supervised techniques, there are over-the-counter (OTC) bleaching products, that can be used without supervision and usually contain low concentrate hydrogen peroxide (1-6%) (Demarco *et al.*, 2009). This OTC system includes whitening toothpastes, mouth rinses, strips, and bleaching gels. Compared with dentist-supervised protocols, these products have a low bleaching potential (Karadas and Duymus, 2015).

The inappropriate use of bleaching systems by the professional or the patient can damage the dental structure by exacerbating the oxidation of molecules, leading to ultrastructural changes in the dental structure (Vilhena *et al.*, 2019). Therefore, this review addresses the main agents used for dental bleaching, existing clinical protocols, and the structural changes that may occur through their use.

2. Changes in dental enamel

2.1 Structure and composition

Enamel is an acellular mineralized tissue that protects the teeth from external damage and is completely formed during amelogenesis, before dental eruption (Gil-Bona and Bidlack, 2020). Enamel consists of hydroxyapatite (HAp) crystals (~ 95% by volume), water (~2-4%), and organic compounds (~1-2%) (Lacruz *et al.*, 2017). HAp crystals are grouped into clusters with hexagonal cross-sections forming enamel prisms that are approximately 1-2 nm thick and extend from the enamel-dentin junction to the outer surface. Such structures are interspersed with interprismatic enamel and HAp crests (Fattibene and Callens, 2010) (Fig. 2).

During the process of enamel formation called amelogenesis, specialized cells (ameloblasts), derived from the embryonic ectoderm promote the secretion of a protein matrix and water. This matrix comprises amelogenin and non-amelogenin proteins (Lacruz *et al.*, 2017). In addition, amelogenesis can be divided into three stages: pre-secretory, secretory, and maturation (Bartlett, 2013). The pre-secretory stage is characterized by morphological changes in epithelial cells that differentiate into ameloblasts (Bartlett, 2013). During secretion, ameloblasts provide the protein and mineral framework for enamel formation. The constant release of calcium ions results in the precipitation of calcium phosphate in the extracellular matrix composed mainly of amelogenins (Josephsen *et al.*, 2010; Bronckers *et al.*, 2015). In the maturation stage, the enamel layer is already completely formed. However, the HAp crystals increase in thickness and width due to the deposition of calcium ions and reduction in protein matrix and water (Lacruz *et al.*, 2013).

2.2 Physico-chemical changes

Dental enamel is the first structure that comes into contact with the bleaching agent, which undergoes a chemical oxidation process. Therefore, the permeability of dental enamel allows the diffusion of hydrogen peroxide through the interprismatic spaces (Kwon *et al.*, 2012). Some factors can increase the penetration of hydrogen peroxide, such as higher concentrations (Gökay *et al.*, 2004; Palo *et al.*, 2010), longer application times, and previous changes in the enamel structure such as cracks and porosities (Kwon and Wertz, 2015).

ROS generated by the hydrogen peroxide decomposition interacts with the enamel's organic compounds, resulting in single-bond molecules that present changes with different optical properties, changing the observed dental color. Such molecules become polar and have a lower molecular weight, facilitating their elimination through the tooth structure (Kwon and Wertz, 2015).

Apart from the oxidation of organic compounds, reactions can also occur with inorganic enamel components (Rodríguez-Martínez *et al.*, 2019). The mineral phase of enamel typically consists of HAp crests ($Ca_{10}(PO_4)6(OH)2$) that may have carbonate ions (CO_3^{-2}) adsorbed as substituents for phosphate (PO_4^{3-}) or OH (Lacruz *et al.*, 2017).

The crystallinity profile of enamel can be evaluated using an X-ray diffractometer (XRD), and a study showed that bleaching with 35% hydrogen peroxide for 30 minutes promoted a significant decrease in the main peak of HAp, indicating changes in the crystallinity in bovine teeth (Son *et al.*, 2012). Such crystallographic changes may be associated with the tooth's optical properties, where the HAp crystals' size is inversely proportional to the luminosity and hue of the tooth (Vargas-Koudriavtsev *et al.*, 2021). Thus, tooth shade is associated with HAp crystal size, tooth chroma is associated with HAp carbonization, and luminosity is associated with HAp crystal size and degree of HAp carbonization (Eimar *et al.*, 2011). Furthermore, the size of the HAp crystals along the c-axis is

related to the hardness of the enamel, which is responsible for the increased wear and fracture resistance of the structure (Eimar *et al.*, 2012).

Regarding the type of bleaching agent, a study showed that 16% carbamide peroxide used under a 21-day home protocol with daily applications of five hours was more harmful to the crystalline structure with a consequent reduction in crystal size compared with the in-office bleaching protocol that used 37.5% hydrogen peroxide for 24 minutes (Vargas-Koudriavtsev *et al.*, 2021). This finding agrees with other studies that showed changes in phosphate, titanium, and carbonate ions after prolonged bleaching (Bistey *et al.*, 2007; Venkatesan *et al.*, 2012; Vargas-Koudriavtsev, 2017, 2018).

The mineral loss was evidenced in some studies that performed bleaching with hydrogen peroxide. The energy-dispersive micro-X-ray fluorescence spectrometry analysis showed a loss of calcium and phosphorus (Tezel *et al.*, 2007; Paula Sde *et al.*, 2010; Pessanha *et al.*, 2017; Pinelli *et al.*, 2019; Ozdemir and Surmelioglu, 2021). In addition, high concentrations of carbamide peroxide can also affect mineral content with a reduction in the Ca/P ratio (Oltu and Gürgan, 2000; Bistey *et al.*, 2007). However, there is evidence that hydrogen peroxide bleaching possibly promotes greater mineral loss from tooth enamel than carbamide peroxide bleaching (Llena *et al.*, 2017). Moreover, this spectroscopic technique observed that the depth of mineral loss using an over-the-counter 44% carbamide peroxide agent could be as high as 30 μm (Pessanha *et al.*, 2017) (Fig. 3).

FT-Raman spectroscopy can detect the concentration of ions in sound and after-bleaching enamel, considering the vibrational peaks of PO_4^{3-} and CO_3^{-2} (Cavalli *et al.*, 2018). In the analysis of the Raman spectrum, an increase in the organic component, a decrease in the apatite component (Son *et al.*, 2012), and reduced proportions of the inorganic content (calcium and phosphorus) were observed (Souza *et al.*, 2010; Son *et al.*, 2012).

One study that used a 44% carbamide peroxide OTC product showed an increase in demineralization by assessing the PO_4^{3-} (~959cm^{-1}) band in the Raman spectrum (Silveira *et al.*, 2018). Another study evaluated PO_4^{3-} concentrations for 28 days with 10% carbamide peroxide. Raman spectroscopic results revealed that after 7, 14, and 28 days of bleaching, there was a reduction in PO_4^{3-} concentration (Santini *et al.*, 2008). On the other hand, another study showed that daily use of 14% and 9.5% hydrogen peroxide and 38% carbamide peroxide for several weeks caused loss of CO_3^{-2} (Vargas-Koudriavtsev, 2017).

The presence of oxygen in the enamel structure could be identified by confocal Raman microscopy by assessing the peak at 1552 cm^{-1} after bleaching with 38% hydrogen peroxide (Silveira *et al.*, 2012). This finding is relevant because it allows a better characterization of the mineral content associated with oxygen, which interferes with the adhesion of restorative and resin materials to dental structures (Dishman *et al.*, 1994).

The change in the bleaching agent's pH is another factor leading to HAp changes. More acidic pH, such as those of some hydrogen peroxide gels, can promote a progressive demineralization of the enamel, partly due to the excessive use of the bleaching agent beyond the manufacturer's recommendation (Castro *et al.*, 2016). Due to the more basic pH of carbamide peroxide gels, HAp dissolution does not occur but favors the oxidation of organic enamel compounds (Babot-Marquillas *et al.*, 2022). The by-products of carbamide peroxide decomposition (urea and ammonia) are responsible for breaking hydrogen bonds in proteins, which promotes the weakening of HAp crystals' support structure (Goldberg *et al.*, 2010).

Thus, changes in mineral content may be due to the diffusion of the oxidizing agent into the enamel structure and its subsequent decomposition into reactive oxygen species that react with the inorganic components present in HAp crystals, promoting their gradual dissolution (Cavalli *et al.*, 2018).

2.3 Morphological and mechanical changes

Most studies that evaluated enamel morphology used profilometric surface analysis (Götz*, et al.*, 2007a; Engle *et al.*, 2010; Abouassi *et al.*, 2011; Attia *et al.*, 2015; Babot-Marquillas *et al.*, 2020). One of the parameters analyzed by profilometry is surface roughness, which indicates the nature of a surface and reveals shape deviations (Mullan *et al.*, 2017). Therefore, this analysis allows the quantification of height deviations of a surface in 2D with only one profile but it can also be measured in 3D from the total surface (Field *et al.*, 2010).

The increase in roughness occurs by the reorganization of enamel prisms resulting from the oxidative effects of the bleaching agent at acidic pH. Therefore, bleaching with 35% hydrogen peroxide increased enamel roughness after an *in vitro* protocol of two sessions with intervals, each with three applications of 15 minutes (Ferreira *et al.*, 2021). Another study showed increased enamel surface roughness after using an OTC product with a pH of 6.0 (Kwon *et al.*, 2015). On the other hand, some studies did not show changes in enamel roughness (Faraoni-Romano *et al.*, 2007; Mielczarek *et al.*, 2008; Mondelli *et al.*, 2009; Kwon *et al.*, 2013).

The scanning electron microscopy (SEM) technique is used to characterize morphological changes on the enamel surface (Coceska *et al.*, 2016; Vilhena *et al.*, 2019) (Fig. 4). One study investigated the effect of bleaching agent concentration on in-office and at-home protocols. The authors observed through SEM that 10% and 22% carbamide peroxide for the at-home technique (4 hours daily for 7 days), increased the number and diameter of the pores on the enamel surface (Karimi *et al.*, 2021). Similar results were found for the 35% and 40% hydrogen peroxide for in-office bleaching (Karimi *et al.*, 2021).

On the other hand, low concentrations of hydrogen peroxide were also able to promote morphological changes, as shown in a study that observed the post-bleaching effects of hydrogen peroxide at concentrations below 6% (Lilaj *et al.*, 2019). The findings were evaluated in the at-home and in-office techniques, where small changes in enamel surface morphology were observed. Such changes seem to be associated with the pH of the bleaching agent, where an acidic pH favors the degradation of the organic and inorganic structure of the enamel, in addition to the degree of saturation, chelating properties, and viscosity of the bleaching agent (Sun *et al.*, 2011; Aykut-Yetkiner *et al.*, 2013; Pimenta-Dutra *et al.*, 2017). In addition, most bleaching gels have high concentrations of sodium and chloride that can generate undersaturation in relation to HAp (Magalhães *et al.*, 2012).

The morphological alterations of the enamel surface present characteristics of tissue loss by erosion due to the acid content of the solution associated with the oxidative effects on the organic and inorganic components (Joiner, 2007). Studies that investigated the morphology of the enamel surface showed varying evidence, ranging from no change (Götz*, et al.*, 2007b; Kwon *et al.*, 2015; Farawati *et al.*, 2019) to mild or moderate erosion with significant porosity on the surface of the enamel (Yeh *et al.*, 2005; Sun *et al.*, 2011; D'Amario *et al.*, 2012). The appearance of enamel erosion is indicated by the shearing of the enamel rods with changes in the interprismatic spaces (Coceska *et al.*, 2016).

In addition to these morphological changes, other studies investigated the mechanical properties of enamel through Vickers or Knoop microhardness tests. These methods can characterize enamel fracture toughness and evaluate the property of resisting plastic deformation, which is directly related to a loss or gain of a mineral component (Oyen, 2006; Joiner, 2007; Elfallah *et al.*, 2015). An *in vitro* study revealed that at-home bleaching with a 10% hydrogen peroxide protocol, when it exceeds the eight-week treatment period, increased the roughness and reduced the hardness and modulus of elasticity (De Miranda *et al.*, 2020). After eight weeks of treatment, a disorganized prismatic pattern with areas of enamel loss and a smoother surface was observed (De Miranda *et al.*, 2020).

Other studies have also shown changes in microhardness after exposure to hydrogen peroxide (Abouassi *et al.*, 2011; Klarić *et al.*, 2011; Magalhães *et al.*, 2012; Klarić *et al.*, 2013; Jurema *et al.*, 2018; Ferreira *et al.*, 2021). Using 10% carbamide peroxide for 3 ½ hours reduced microhardness after a single at-home bleaching application (Mushashe *et al.*, 2018). Another study showed that bleaching with carbamide peroxide reduced microhardness in previously demineralized enamel, reinforcing the need to apply remineralizing agents before or after bleaching, especially in teeth with previous signs of demineralized areas on the enamel (Ghanbarzadeh *et al.*, 2015). A comparison of polished (a common practice for VHN indentation procedure) and unpolished enamel samples submitted to 16% carbamide peroxide dentist-supervised nightguard bleaching for 14 days revealed that

the polished samples were more affected by the demineralization action of bleaching product, determined by an increased depolarization ration of the symmetric stretching band of phosphate (Pessanha *et al.*, 2020).

OTC products, which have low concentrations of peroxide (10% and 6%), also showed unfavorable results for enamel integrity characterized by reduced microhardness after a 14-day bleaching protocol (Majeed *et al.*, 2011; Yildirim *et al.*, 2022). On the other hand, whitening mouth rinses after bleaching intensify enamel damage, reducing microhardness and increasing roughness (Favaro *et al.*, 2019). Other studies that showed mechanical changes on enamel consider that these findings may have resulted from the oxidative processes of the bleaching gel associated with the acid content of bleaching mouth rinses (Bolay *et al.*, 2012; de Araújo *et al.*, 2013; Özkan *et al.*, 2013; Melo *et al.*, 2014; Vieira-Junior *et al.*, 2019; Santana Jorge *et al.*, 2022).

Morphological changes in enamel microtopography can also be evaluated using Atomic Force Microscopy (AFM). A study comparing enamel surfaces before and after bleaching with 35% hydrogen peroxide showed a considerable increase in the power spectral density (PSD) used to quantify the surface texture (de Freitas *et al.*, 2010).

3. Changes in dentin

3.1 Structure and composition

Dentin is a mineralized connective tissue, composed of a mineral phase (70% HAp), organic matrix (proteins, mainly collagen), and water, and this tissue is a physiological barrier that protects the dental pulp from harmful exogenous stimuli (Yumoto *et al.*, 2018; Epple *et al.*, 2019). The specialized cells that produce this tissue are called odontoblasts and are located in the dental pulp from where cytoplasmic projections originate and enter the dentin layer remaining in surrounding mineralized structures called dentinal tubules (Kawashima and Okiji, 2016) (Fig. 2).

Dentin has particularities about its structure and properties, mainly the presence of dentin tubules. These tubules have variations in density and dimensions with multiple functions, including dental hydration, transduction of physical signals, sensory responses, and fluid movements (Epple *et al.*, 2019). The tubules' location and orientation are fundamental to dentin's mechanical behavior. Likewise, the inorganic mineral component (calcium phosphate in the form of HAp) combined with an organic matrix interact chemically and structurally, which favors the hardness and strength of the dental structure (Arola *et al.*, 2017).

In addition, dentin plays an important role in dental color, which results from combining individual dentin and enamel colors and their optical characteristics (Joiner, 2006). Being more chromatic, dentin is the main dental tissue determining the overall dental color, which is influenced by a

combination of its intrinsic color and the presence of any extrinsic stains on enamel (Kwon and Wertz, 2015).

3.2 Mechanisms of bleaching agents in dentin

Hydrogen peroxide penetrates dentin and interacts with phosphoproteins or, more specifically, oxidizes the benzene ring into aromatic amino acid complexes (Carey, 2014). However, hydrogen peroxide interacts significantly with the organic as well as inorganic components of dentin. This interaction occurs after the diffusion of oxidizing components of the bleaching gel, which are moved by a concentration gradient (Ubaldini *et al.*, 2013).

In addition to carbamide peroxide and hydrogen peroxide used in vital dental bleaching techniques, sodium perborate ($NaBO_3$) can also be used for non-vital bleaching (Plotino *et al.*, 2008). This consists of applying the bleaching agent to the pulp chamber located in the coronal portion of the tooth and sealing the pulp chamber access with a temporary restorative material (Zimmerli *et al.*, 2010). This technique is used in non-vital teeth after endodontic treatment and root canal obliteration in patients without symptoms such as pain and tooth sensitivity (Zimmerli *et al.*, 2010).

Application protocols have varied over the decades with the application of sodium perborate associated with hydrogen peroxide (Ari and Ungör, 2002) or carbamide peroxide (Yui *et al.*, 2008). In addition to sodium perborate, high concentrations of hydrogen peroxide or carbamide peroxide can be used alone (Frank *et al.*, 2022).

Due to its low molecular weight, the bleaching agent can penetrate the dentin, releasing reactive oxygen species, and breaking the double bonds of organic and inorganic compounds within the dentinal tubules (Pallarés-Serrano *et al.*, 2021).

After external and internal bleaching, color changes may occur along the dentin. The treatment of dentin samples with 10% carbamide peroxide and 5.3% and 6% hydrogen peroxide demonstrated a significant increase in whiteness (Cavalli *et al.*, 2019).

3.3 Effects of bleaching on dentin morphology and structure

Bleaching agents affect the chemical and morphological structure of dentinal tissue. Although bleaching is a complex process, the main reaction is oxidation of the organic and inorganic dentin structure, which leads to morphological changes. Some studies have evaluated the effects of bleaching agents on micromorphology, showing that dentin is permeable to hydrogen peroxide and carbamide peroxide (Lewinstein *et al.*, 2004; Sasaki *et al.*, 2009; Llena *et al.*, 2018).

SEM has been widely used for surface morphological examinations after bleaching (Fig. 5). Studies have reported an increased surface roughness and porosity after bleaching treatment (Lewinstein *et al.*, 2004; Sasaki *et al.*, 2009; Demarco *et al.*, 2011). However, another study reported

similar surface roughness before and after bleaching, and a trend toward a smoother surface after bleaching (de Carvalho *et al.*, 2020). Changes in the microstructure of the dentin surface with slight modifications in the dentinal tubules, without loss of surface structure, were observed in another study after the bleaching with hydrogen peroxide (25% and 38%) in three 15-min applications (Klarić *et al.*, 2013).

In the evaluation of mineral components, studies using atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) showed that morphological changes in the dentin are mainly due to partial organic matrix protein lysis (Chng *et al.*, 2005). In addition, significant increases in the proteolytic activities of cathepsin B and matrix metalloproteinase were demonstrated after tooth bleaching, suggesting a dynamic change within the dental structure (Chng *et al.*, 2005; Orilisi *et al.*, 2021). Furthermore, FTIR analysis revealed that pH treatment induces the loss of dentin carbonate and proteins and changes in biological bands representative of HAp (Sato *et al.*, 2013). Analysis of the dentin infrared spectra revealed remarkable changes in the absorbance of the amide bond (1550 cm^{-1}) with tooth bleaching, thus indicating a possible loss or denaturation of proteins (Zimmerman *et al.*, 2010). Protein concentration was investigated as a potential contributor, revealing a loss or denaturing of collagen. Thus, this decrease in amide bonds was associated with reduced organic matter (Zimmerman *et al.*, 2010).

The influence of bleaching on the microchemical composition observed in Raman spectroscopy did not demonstrate deleterious effects (Götz*, et al.*, 2007a). These results confirmed that controlled concentrations of hydrogen peroxide at 11.7 and 14% do not produce changes in surface/subsurface histomorphology, surface microhardness, or microchemical mineral composition of teeth (Götz*, et al.*, 2007a). Energy dispersive X-ray spectrometer (EDX) analysis suggested that hydrogen peroxide and hydroxyl radicals do not influence the inorganic component of dentin, but influence organic tissue (Kawamoto and Tsujimoto, 2004). This bleaching effect could result from modifying the polypeptide chain in the organic substance and not from the interaction of the bleach with the pigments. Although studies with peroxide-based materials have shown that these agents do not influence dentin chemistry beyond clinical relevance (Arcari *et al.*, 2005; Rodrigues *et al.*, 2007; Kwon *et al.*, 2015), others have indicated significant changes in the Ca/P ratio, indicating that the inorganic components of HAp are altered (Jiang *et al.*, 2007; DR *et al.*, 2011; Andrade *et al.*, 2021).

The morphological properties and histomorphological effects evaluated by profilometry, surface confocal laser scanning microscopy (CLSM), and variable pressure scanning electron microscopy (VP-SEM) showed that the surfaces of root dentin did not change with bleaching (Götz *et al.*, 2007a). Micromorphological assessments by CLSM demonstrated normal tissue appearance for surface and subsurface dentin after bleaching (Götz *et al.*, 2007a). When evaluating changes in micromorphology

and the composition of Ca and P in dentin by CLSM and EDX, respectively, after the application of 37.5% hydrogen peroxide for 45 minutes and 35% carbamide peroxide for 90 minutes, no morphological change in the dentin was observed with both products. Ca and P decreased in dentin, with no significant differences between them or concerning the control (Cakir *et al.*, 2011).

3.4 Effects of bleaching on dentin hardness and strength

Variations in mechanical properties are influenced by age, location, or tooth type. If aggressive bleaching agents are applied in high concentrations, they will also alter teeth' nanomechanical behavior and damage dentin's organic matrix (Vieira *et al.*, 2012). Exposure of dentin to acidic environments reduces its resistance to fatigue and reduces tooth durability (Soares *et al.*, 2016; Arola *et al.*, 2017). This could lead to a mechanical weakening of the tooth due to a decreasing integration of Ca and P ions (Soares *et al.*, 2016; Arola *et al.*, 2017). Both bleaching agents (HP and CP) at different concentrations (25% and 38%) were shown to be able to cause a significant reduction in surface microhardness and a significant increase in dentin roughness after evaluation by AFM (Zimmerman *et al.*, 2010). The impact of bleaching agents on possible side effects also depends on the pH of the agent, as well as the quality of the dental hard tissues. Bleaching agents with higher acidity can produce more changes in the dentin structure and reduce its microhardness (Alkahtani *et al.*, 2020; de Carvalho *et al.*, 2020).

4. Changes in the dental pulp

4.1 Dental pulp structure

The dental pulp is made up of loose connective tissue, similar to other tissues of the human organism (Fig. 2). However, the pulp is surrounded by dentinal tissue. Specialized cells called odontoblasts are on the periphery of the pulp, and they are responsible for dentin formation. The close relationship between these two tissues allows them to be called the dentin-pulp or dentin-pulp complex (Yu and Abbott, 2007; Goldberg *et al.*, 2015). Histologically, the pulp is divided into four zones: (1) odontoblastic layer in the periphery of the pulp; (2) acellular layer; (3) layer rich in cells with high cell density; (4) pulp center, characterized by larger pulp vessels and nerves (Galler *et al.*, 2021).

The pulp tissue is formed by an extracellular matrix composed of proteoglycans and glycoproteins, intertwined with collagen fiber bundles, acting as a barrier to the spread of irritating agents (Semple and Dorin, 2012). The main pulp cells are fibroblasts, undifferentiated ectomesenchymal cells, and odontoblasts (Galler *et al.*, 2021). T lymphocytes are the most frequently encountered defense cells and are located close to blood vessels, producing cytokines, and interacting with other

defense cells when necessary (Gaudin *et al.*, 2015). In addition, macrophages participate in phagocytosis of cellular debris and pulp irritants, produce cytokines, growth factors, and act as antigen presenters for other defense cells (Weber *et al.*, 2018).

4.2 Penetration of peroxide into the pulp chamber

Dental sensitivity is considered the main adverse effect of tooth bleaching (Rezende *et al.*, 2016). The main explanation for post-bleaching sensitivity is the high penetrability of hydrogen peroxide in enamel and dentin, reaching the pulp chamber (Costa *et al.*, 2010) up to 15 minutes after its application (Cooper *et al.*, 1992). Hydrogen peroxide and its by-products can cause oxidative stresses in the pulp cells, promoting the release of inflammatory mediators (Soares *et al.*, 2014) (Fig. 6).

More recent studies showed that the amount of peroxide reaching the pulp chamber was lower when neutral or alkaline pH gels were used, regardless of the application technique (Mena-Serrano *et al.*, 2015; Balladares *et al.*, 2019). This can be explained by the greater morphological change, and loss of microhardness due to enamel demineralization generated by the acidic pH bleaching gel compared with neutral or alkaline gels (Sa *et al.*, 2013). This damage on the surface of the teeth results in an increase in enamel porosities and, consequently, greater passage of peroxides towards the pulp chamber (Pinto *et al.*, 2004). In parallel with this, randomized clinical trials have shown a lower percentage of patients reporting post-operative sensitivity using neutral or alkaline pH bleaching gels compared with acidic pH bleaching gels (Loguercio *et al.*, 2017; Martins *et al.*, 2018).

Furthermore, a study showed that when the acid gel is removed after 15-20 minutes of application, it prevents further changes in the enamel surface and decreases the passage of peroxides. Through these data, we can conclude that shorter times of gel application can lead to less deleterious effects on the tissues and, consequently, less pain (Balladares *et al.*, 2019). In addition, an interesting recent study showed that high-concentration bleaching gels stimulate the dental pulp more, increasing the likelihood of developing tooth sensitivity. Therefore, the clinician should opt for less concentrated bleaching agents with neutral or alkaline pH and short applications to generate fewer adverse effects (Chen *et al.*, 2021).

4.3 Molecular changes

The mechanisms associated with post-bleaching sensitivity due to peroxide penetration into the pulp remain unclear. However, it has been suggested that post-bleaching sensitivity is related to the penetration of peroxides through the enamel and dentin microstructure reaching the pulp, leading to the production of ROS. These decrease cellularity and cellular metabolism, alter vascular permeability, and even cause tissue necrosis (Soares *et al.*, 2015; Benetti *et al.*, 2017). The presence of ROS in

the pulp chamber has been associated with morphological changes, such as decreased mitochondrial respiration rates in odontoblasts and, consequently, severe damage to the pulp (Roderjan *et al.*, 2015).

Cell damage caused by ROS in the pulp chamber induces the synthesis and release of biochemical mediators involved in the inflammatory process, such as histamine, bradykinin, and prostaglandins (Cintra *et al.*, 2013; Soares *et al.*, 2014). The presence of these mediators increases vasodilation and permeability in the vascular and pulp chamber (Soares *et al.*, 2014), which mechanically stimulates peripheral nerve fibers (Otsuka and Yoshioka, 1993). These nerve structures respond to stimulation by producing and releasing peptide neurotransmitters, including substance P and calcitonin gene-related peptide (Caviedes-Bucheli *et al.*, 2008). These neuropeptides excite the transmitter nerves, thus promoting the emission of pain signals (Harrison and Geppetti, 2001).

However, recent findings have shown that peroxide-induced sensitivity is associated with transient ankyrin receptor 1 (TRPA1) potential (Trevisan *et al.*, 2014; Wang *et al.*, 2018). TRPA1 is a non-selective cation channel mainly expressed in nociceptive neurons and can be activated by ROS, and mechanical and thermal stimuli (Sun *et al.*, 2016; Viana, 2016). Increasingly, evidence points to TRPA1 being activated by various oxidizing compounds, including hydrogen peroxide (Andersson *et al.*, 2008; Trevisan *et al.*, 2014). These findings strongly relate to ROS and TRPA1 on the activation of nociceptors under high concentrations of peroxides.

An important study showed that in-office bleaching with 38% hydrogen peroxide resulted in more intense inflammation, greater macrophage migration, and greater pulp damage than at-home bleaching with 15% carbamide peroxide. However, these bleaching techniques did not induce mast cell migration and increased the number of blood vessels (Vaz *et al.*, 2016). Another study showed that similar concentrations of hydrogen peroxide and carbamide peroxide in various bleaching products exhibited different responses in dental pulp cells and tissue, suggesting that bleaching products contain unknown components that may influence their toxicity (Llena *et al.*, 2019).

Thus, more *in vitro*, *in vivo*, and randomized clinical studies are needed to investigate the effects of peroxides at different concentrations, pHs, and times and their adverse impact on the dental pulp.

5. Conclusion

The harmful effects of bleaching on tooth structure depend on the concentration and pH of the bleaching agent, application time, characteristics of the tooth structure, and chemical interactions. Therefore, several protocols in the literature show evidence of mild to moderate alterations in the physical-chemical aspect, with mineral loss resulting from structural alterations of the HAp crystals, reduction of the Ca/P ratio, and changes in the organic configuration of enamel and dentin. These changes can lead to morphological changes with increased surface roughness and porosity of enamel and dentin. Such findings are complemented by evidence of mechanical changes with a reduction in

microhardness and fracture toughness. On the other hand, the bleaching agent can promote damage to the dental pulp with the activation of oxidative stress mechanisms and a reversible or irreversible inflammatory process. Furthermore, synthesizing the findings of the reviewed literature, it was identified that most studies used point out that, for tooth whitening to generate fewer harmful effects, a clinical protocol must be adopted with a shorter period of application and an adequate concentration that favors the effectiveness and minimizes structural damage to the tooth.

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Figure 1. Schematic illustration of the decomposition process of carbamide peroxide ($CH_6N_2O_3$) into hydrogen peroxide (H_2O_2) and urea ($CH_4\ N_2\ O$). This in turn decomposes into ammonia (NH_3) and carbon dioxide (CO_2). Hydrogen peroxide decomposes into water and reactive oxygen species such as hydroxyl anion (OH^-) and reactive oxygen (O^*). These molecules promote the oxidation of the chromogens present in enamel and dentin and are then eliminated by diffusion.

Figure 2. (A) Schematic illustration of the tooth structure composed of enamel with its configuration of longitudinally arranged prisms, dentin with the presence of dentinal tubules, and the dental pulp composed of cells, blood vessels, and nerve endings. (B) Stages of enamel formation. HAp: hydroxyapatite.

Figure 3. (A) Line scans were obtained with micro-EDXRF for Ti (from the stabilizing putty), P, and Ca from the sample. (B) Plot of the derivative of the counts per position obtained for Ti, P, and Ca. (C) Comparison of

the plot of the derivative of the line scan obtained for P before and after treatment, with reference to the beginning of the tooth. (D) Comparison of the plot of the derivative of the line scan obtained for Ca, before and after treatment, with reference to the beginning of the tooth (Pessanha *et al.*, 2017).

Figure 4. The effect of bleaching with 10% carbamide peroxide used for the recommended period on the bovine enamel surface (14 days). (A) Unbleached enamel (control) with no changes on the surface. (B) Bleached enamel with partial loss of the aprismatic layer and irregular surface. Scale bar: 10 μm.

Figure 5. Changes in the surface structure of human dentin after bleaching with 16% (A) and 22% carbamide peroxide (B) applied one hour a day for six months. Scale bar: 20 μm.

Figure 6. Schematic illustration of the dental pulp and its main biological events resulting from tooth bleaching. (A) Photomicrograph of the dental pulp of third molars after bleaching treatment with 35% hydrogen peroxide three times (40 min sessions). Note the presence of vacuoles in the extracellular matrix and blood vessels with hyperemia. (B) Hydrogen peroxide generates a pulp response consisting of increased release of pro-inflammatory cytokines and reactive oxygen species (ROS). (C) There is a change in mitochondrial activity in odontoblasts. (D) Presence of inflammatory mediators and ROS increase vasodilation with mechanical stimulation of nerve endings. (E) Symptoms experienced by the individual include pain and tooth sensitivity.

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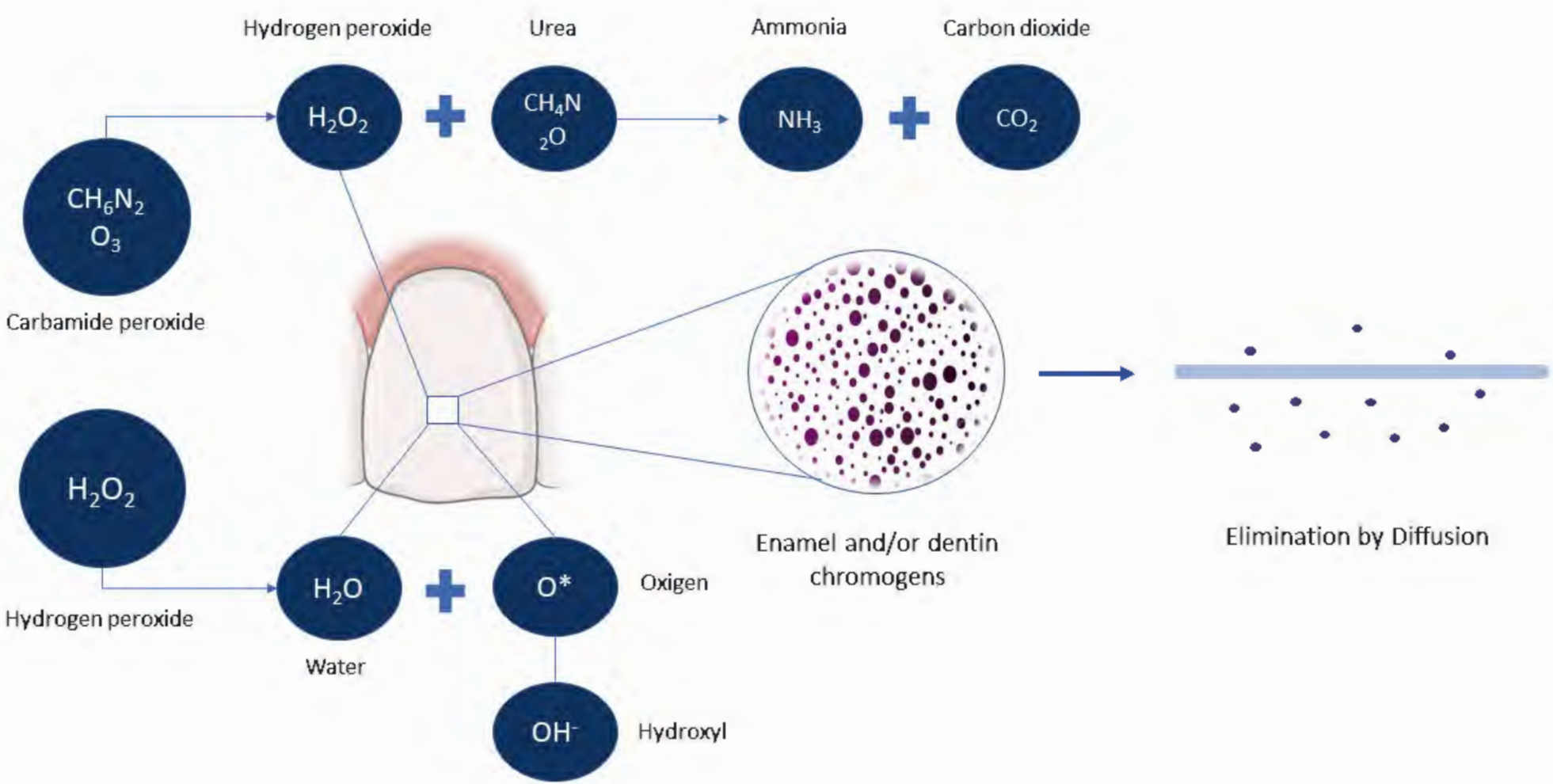
Hydrogen peroxide
Urea
Ammonia
Carbon dioxide
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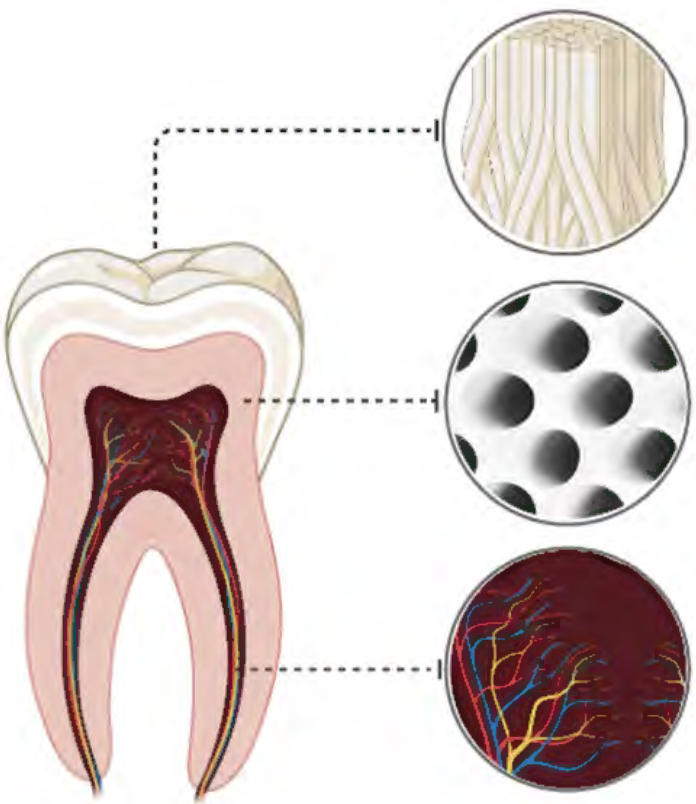
CH_4N_2O
NH_3
CO_2
$CH_6N_2O_3$
Carbamide peroxide
H_2O_2
Hydrogen peroxide
H_2O
Water
O*
Oxigen
OH^-
Hydroxyl
Enamel and/or dentin chromogens
Elimination by Diffusion

A



Enamel

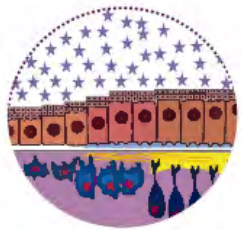
Dentin

Dental pulp

B

Amelogenesis

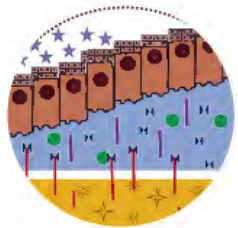
Pre-secretory stage



Morfological changes

Cell differentiation

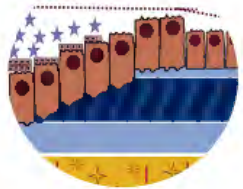
Secretory stage



Mineralization

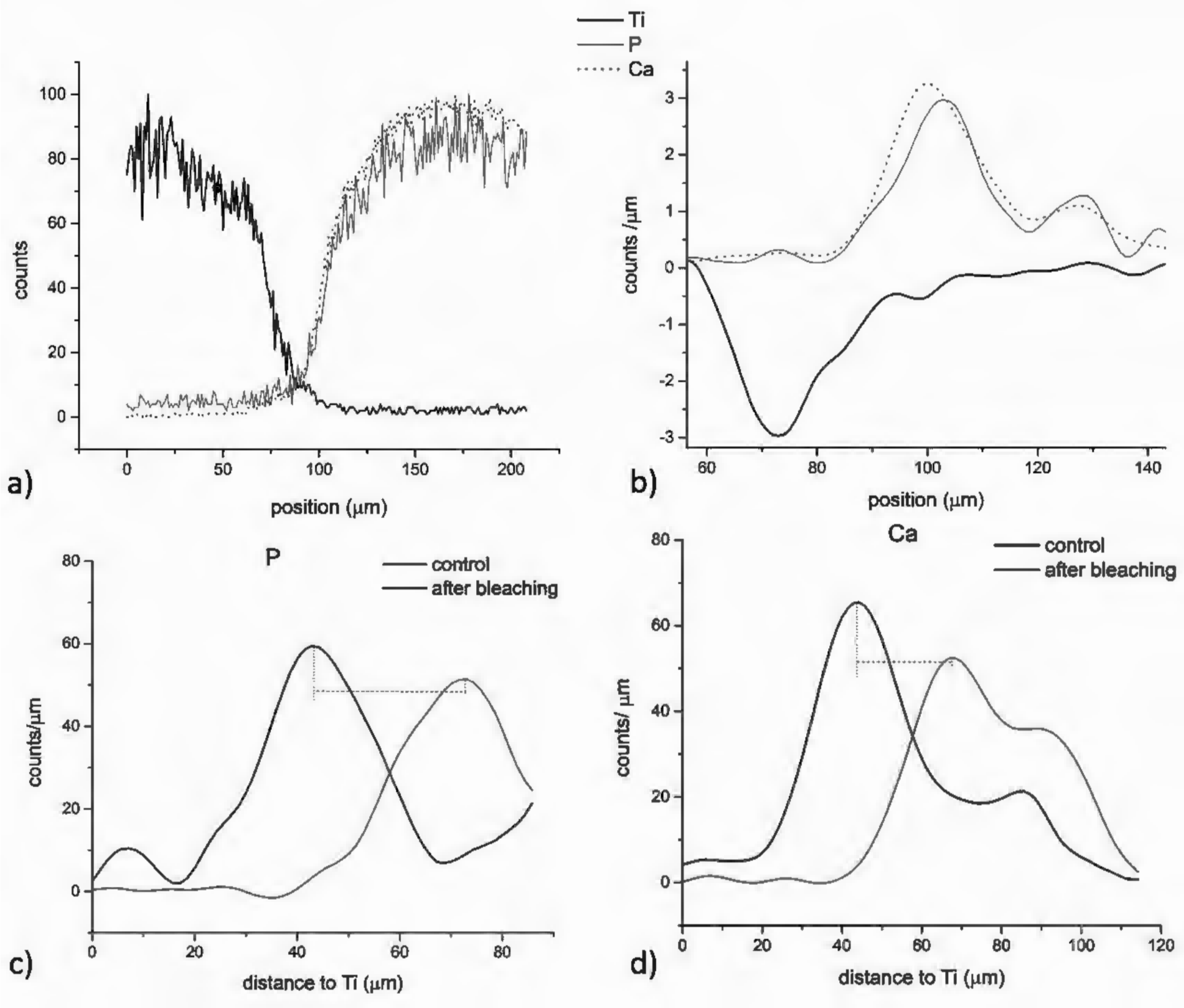
Calcium ion deposition

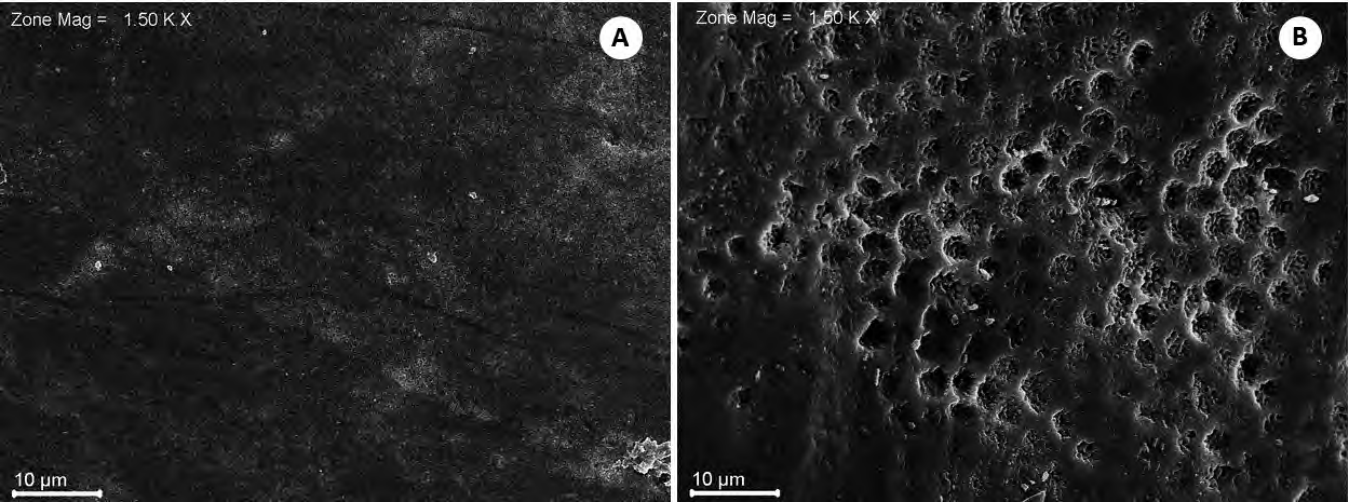
Maturation stage

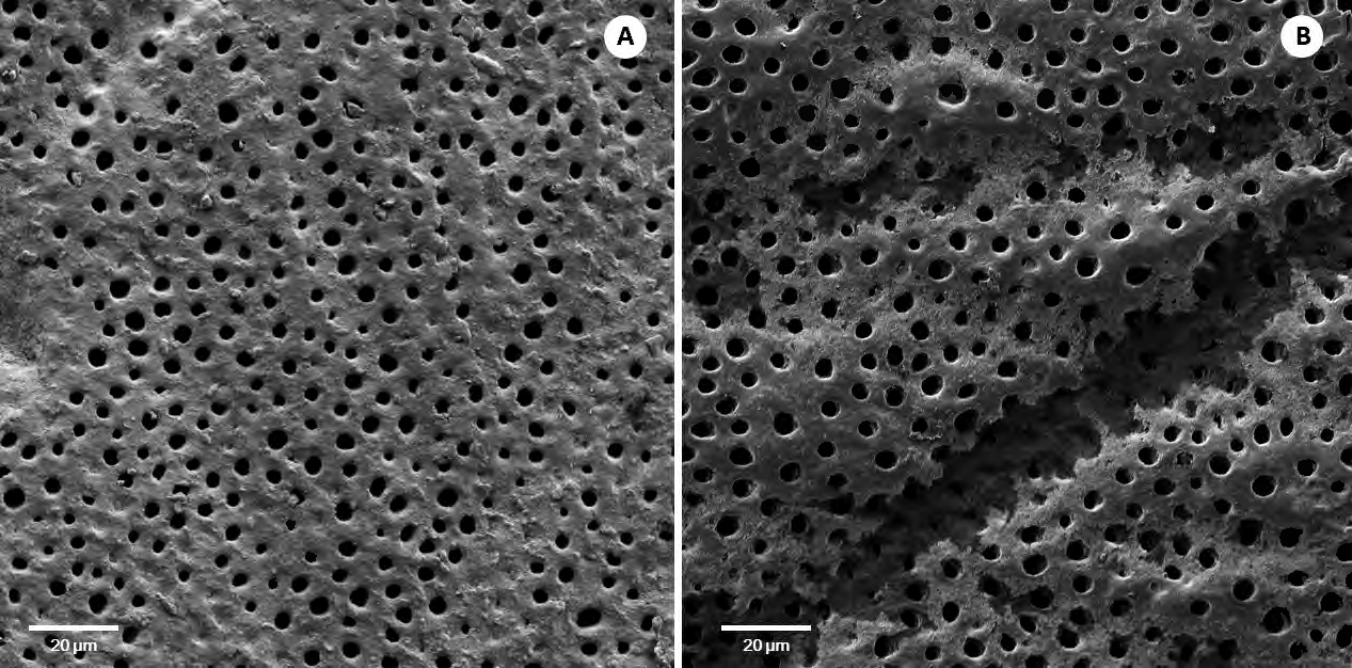


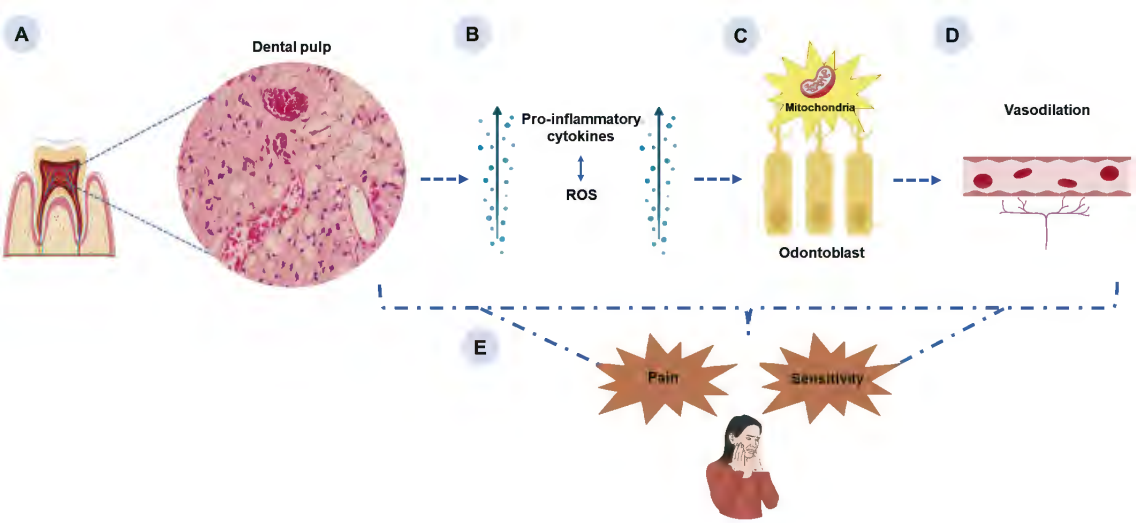
Increased HAp crystals

Reduction of organic matrix and water


a)
counts
position (μm)
b)
Ti
P
Ca
counts /μm
position (μm)
c)
P
control
after bleaching
counts/μm
distance to Ti (μm)
d)
Ca
control
after bleaching
counts/ μm
distance to Ti (μm)


Zone Mag = 1.50 K X
A
10 µm
Zone Mag = 1.50 K X
B
10 µm


A
20 µm
B
20 µm


A
Dental pulp
B
Pro-inflammatory cytokines
ROS
C
Mitochondria
Odontoblast
D
Vasodilation
E
Pain
Sensitivity