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MORPHOLOGY DYNAMICS OF THE PULP TISSUE AFTER REPEATED WHITENING THERAPY

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ABSTRACT

The aim of the study is to identify lesional dynamics of the alterations in the pulp tissue compounds after repeated whitening treatment. The study included 20 patients aged between 15 and 46. The teeth (upper first premolars) with indication of extraction in orthodontic purposes, were submitted to successive bleaching with Belagel whitening gel (20% carbamide peroxide). The whitening procedure was performed by the in office technique. The gingival tissue of the upper teeth was isolated with a rubber dam and the whitening gel was applied on the buccal surfaces of the first premolars for 30 minutes. The whitening procedure was repeated twice a week for three weeks. The period between one bleaching and another was four days. After that we removed the dental pulps and extracted the first upper premolars in orthodontic purposes. The pulpal biopsies were processed by the usual histological technique. For the electronmicroscopic study we used the Lehner technique in processing the biopsies and examination in transmission electron microscopy (TEM) with Philips microscope (University of Medicine Timisoara). The lesions of the pulp tissue undergo successive levels of severity, with the initial implication of the vascular dynamics at the peripheral subodontoblastic plexus and continuing with the activation of fibroblasts and debut of fibrillogenesis.The degradation of the pulp tissue is progressive after repeated whitening therapy with 20% concentration of the whitening gel.The vascular dynamics is implied in an inflammatory reactive context, with vascular thrombosis, variable degree of fibrosis, lineal calcifications, depending on the individual reactivity of the patients. Therefore overtreatment (frequently repeated whitening) is not advisable.

KEY WORDS: *pulp tissue, whitening therapy, premolars, cellular lesions, electron microscopic evaluation*

INTRODUCTION

Tooth's whitening is a relatively simple and conservative option, which has gained popularity in the past years. The whitening procedure can be applied as a dentist – supervised

night guard bleaching, the in office bleaching and OTC bleaching (over the counter products) (Mohan *et al.,* 2016).

The OTC bleaching products are available in form of gels, mouth rinses, paint on brush, chewing gums or tooth pastes. The active agents in tooth whitening products are hydrogen peroxide, carbamide peroxide, sodium perborate, chlorite. Whitening products are used alone or in combination with activators to increase their efficiency. In Europe tooth whitening is not very popular because of the concerns about its safety (Mohan *et al.,* 2016).

Haywood and Heymann first described "night guard vital bleaching" in 1989 (Haywood & Heymann, 1989) and in 1990 Croll used "custom tray dental bleaching" for children and teens (Croll & Donly, 2014).

In principal all whitening techniques described in literature are based on the direct use of hydrogen peroxide (H_2O_2) or its precursor carbamide peroxide (Feliz-Matos *et al.,* 2014). There are several studies (Bowles *et. al.,* 1987, Cooper *et al.,* 1992, Gökay *et al.,* 2000) that examine the penetration of carbamide peroxide and hydrogen peroxide into the pulp chamber. The studies show that this really happens with whitening agents. The central metabolic processes that appear in the pulp are glucose metabolism and collagen synthesis. Histological findings show moderate vasodilatation and aspiration of odontoblast nuclei into the dental tubules (Perchyonok & Grobler, 2015). Other studies evaluated the effects of hydrogen peroxide on dental pulp cells and found a mild inflammatory reaction of the pulp tissue and pulp necrosis (Cartagena *et al.,* 2015).

For the at-home whitening technique patients use an intraoral device – a tray filled with the whitening gel (carbamide peroxide or hydrogen peroxide) at low concentration. The in office whitening technique is performed by a professional and uses higher concentrations of whitening gel. The results appear more rapidly (Cartagena *et al.,* 2015).

Dental bleaching became nowadays a very popular and demanded treatment procedure and is highly performed in daily practice. Therefore the aim of this study was to see if any cell alterations appear in the pulp tissue compounds, after repeated whitening treatment and to identify them.

MATERIALS AND METHODS

Our study included 20 patients aged between 15 and 46 years. The teeth (first upper premolars) with indication of extraction in orthodontic purposes were submitted to successive bleaching with Belagel 20% carbamide peroxide.

The whitening procedure was performed by the in office technique. The dentition was professionally cleaned and the patients reinstructed in proper oral hygiene (Bass technique). The gingival tissue of the upper teeth was isolated with a rubber dam and the whitening gel was applied on the buccal surfaces of the first premolars for 30 minutes. The whitening procedure was repeated two times a week for three weeks. The period between one bleaching and another was four days. After that we removed the dental pulps and extracted the first upper premolars in orthodontic purposes. The pulp biopsies were fixed in buffed formaldehyde 10% for 48 hours, then paraffinized, sectioned at 3-5 μ and stained with topographic, HE and trichrome stains by the usual histological technique.

The extracted teeth were decalcified with an acid mixture during 1 to 3 weeks, then inclusionated in paraffin, sectioned at 5 – 7 μ, stained with usual methods and

immunohistochemical - $LSAB_2$ technique for actin – straight muscle and vimentin, DAB visualization.

For the electron microscopic study we used the Lehner technique for processing the biopsies after reimmersing the paraffin block pieces in Epon, postfixation in packed glutaraldehyde, micro sectioned at 0,5 μ, contrasting with lead citrate and uranyl acetate and examination in transmission electron microscopy (TEM) with Philips microscope (University of Medicine Timisoara).

RESULTS AND DISCUSSIONS

The lesions of the pulp tissue undergo successive levels of severity, with the initial implication of the vascular dynamics at the peripheral subodontoblastic plexus and continuing with the activation of fibroblasts and debut of fibrillogenesis contouring the aspects of a reactional pulp tissue.

The first target of the toxic action of the whitening gel was the vascular network at the level of the peripheral subodontoblastic plexus, continuing with the activation of fibroblasts, shown in Figures 1 and 2.

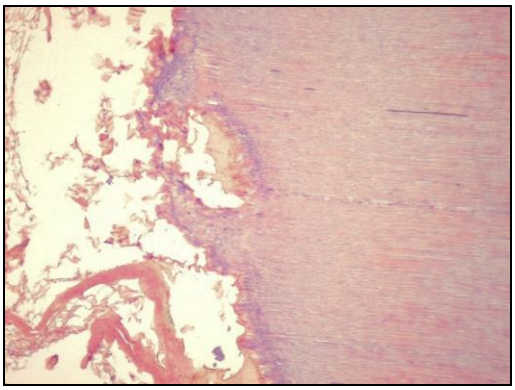

FIGURE 1. Decalcified tooth in optic microscopy examination. Circumpulpar dentin with large aries of matriceal lysis, some dentinal tubuli are enlarged and obliterated with an amorf bazofil material, large aries of pulpal necrosis (HE Stain x 200).

The examination under transmission electron microscopy reveals a marked collagenous fibrillogenesis, fibroclasia aspects suggesting active matrix reshuffling, areas of dystrophic decalcification which are shown in Figures 3 and 4.

Tooth whitening is considered safe and when it is used correctly, it does not cause irreversible changes in the tooth structure. Overtreatment can be dangerous. During whitening a degradation of the extracellular matrix takes place and the oxidation of chromophores in the dentin and enamel (Croll & Donly, 2014).

Peroxides can diffuse through the pulp. This depends on the concentration of the whitening gel and the time of action on the tooth surface (Feliz-Matos *et al.*, 2014).

Carbamide peroxide is frequently used in tooth whitening. It decomposes into hydrogen peroxide and urea in presence of saliva. Hydrogen peroxide has a low molecular weight, so it can penetrate enamel and dentin. It decomposes into oxygen and water. Urea decomposes into ammonia and carbon dioxide and is able to remove enamel proteins and minerals, weakening the tooth structure. According to some studies severe pulp reactions

appeared after using hydrogen peroxide 35% in whitening: reduced or absent odontoblast layer, missing predentine, inflammatory cells (Goldberg *et al.*, 2010).

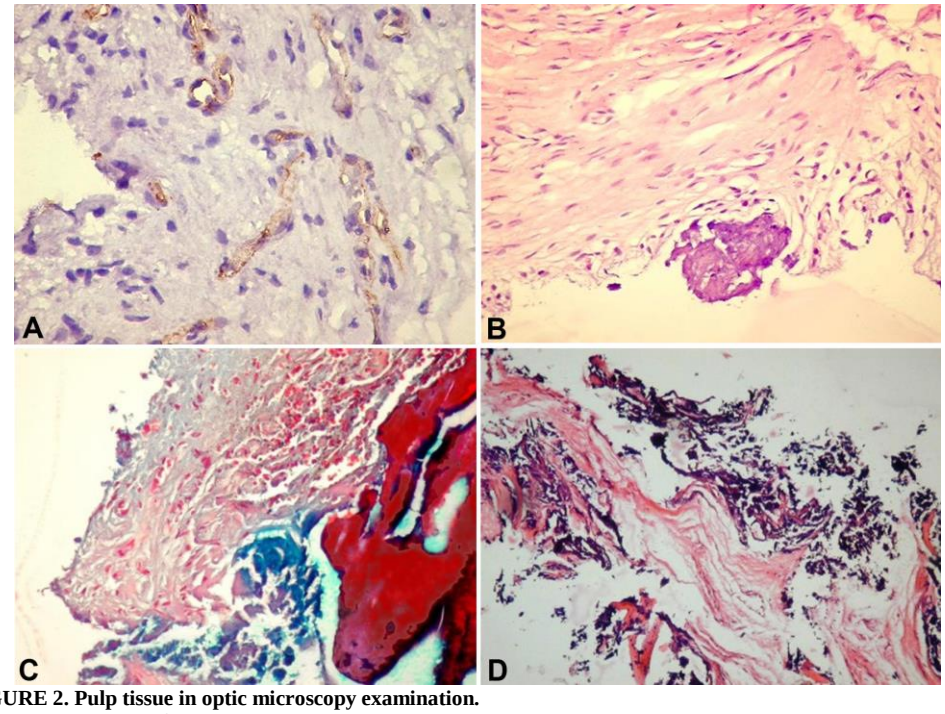

FIGURE 2. Pulp tissue in optic microscopy examination.
A: Immunostaining of the pulp vessels with antibodies anti CD34, peroxidase – antiperoxidase technique x 400. Positive immunoreaction of the vascular endothelium in the axial vessels of the pulp, also the reactivity of the subodotoblastic plexus. All the vessels are enlarged proving the vascular hiperreactivity in the pulp belong to the first stage of the pulp response (HE Stain x 400).
B: Nodular spherical pulp lithiasis in the peripheric pulp zone with lesions of acute serosal pulpitis, interstitial edema, rich fibroblastic reaction and partial disorganization of the odontoblastic layer (HE Stain x 400).
C: Lineal pulp lithiasis extended in the center of the pulp and fibrosis of the peripheric pulp zone with disappearance of the odontoblastic layer. Arteriovenular vessels are colapsed between the collagen fibers, some of them with lesional walls, haematic suffusions, old haemosiderinic pigment deposition (Trichrome Stain x 400).
D: Lineal and diffuse pulp lithiasis, collagen transformation of the pulp tissue, severe reduction of the cell population, degeneration of the vessels and nervous endings (HE Stain x 200).

Fixing the pulp requires the presence of macrophages, lymphocytes, mast cells, shown by our research and also a subodontoblastic cellular layer for creating a restorative dentine.

In our study we found a large number of lymphocytes, less granulocytes, mast cells with granular shape and fibroblasts implied in matrix reshuffling.

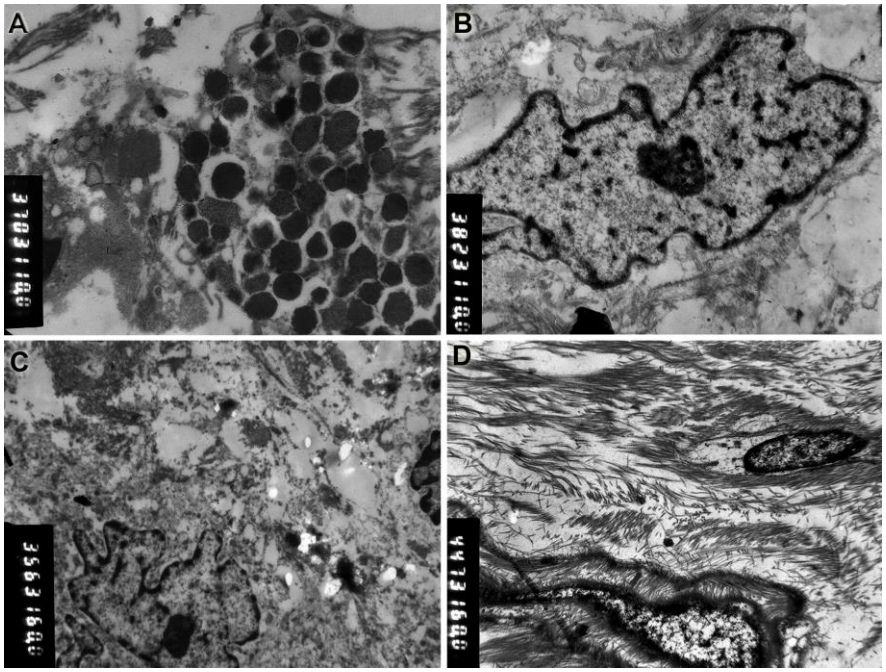

FIGURE 3. Pulp tissue in electron microscopy examination.
A: Pulp mast cell with degranulation in edematous tisular matrix (TEM x 10.000).
B: An active fibroblast participating in the collagen synthesis and in matrix reshuffling. Many glycogen deposits in the cytoplasm and collagen fibers engufted at the periphery of the cell (TEM x 10.000).
C: Fibroblast with proeminent nucleolus, having a role in active reshuffling (TEM x 10.000).
D: Active fibroblast in collagen synthesis; collagen fibers in the matrix; collagen lysis; perivascular edema and dissociated collagen fibers of the pulp matrix (TEM x 10.000).

CONCLUSIONS

The degradation of the pulp tissue is progressive after repeated whitening therapy with 20% concentration of the whitening gel. The vascular dynamics is implied in an inflammatory reactive context, with vascular thrombosis, variable degree of fibrosis, lineal calcifications, depending on the individual reactivity of the patients. Whitening therapy is a frequently used cosmetic protocol in daily clinical practice. Increased tooth sensitivity and mucosal irritation can be temporary. The effects on dental hard tissues and pulpal tissues are sometimes reversible and sometimes irreversible. Therefore overtreatment (frequently repeated whitening) is not advisable.

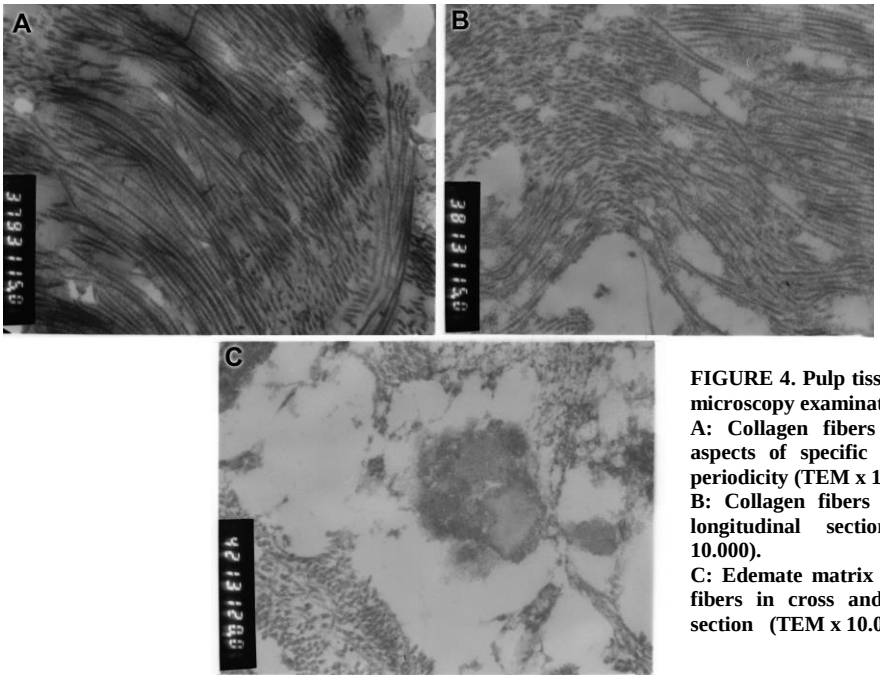

FIGURE 4. Pulp tissue in electron microscopy examination.
A: Collagen fibers with partial aspects of specific 64 Angström periodicity (TEM x 10.000).
B: Collagen fibers in cross and longitudinal section (TEM x 10.000).
C: Edemate matrix with collagen fibers in cross and longitudinal section (TEM x 10.000).

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