ABSTRACT

Aim: This study was designed to evaluate the histological and the ultrastructural changes in human dental pulp after in vivo bleaching with hydrogen peroxide (5 minutes speed whitening gel).

Design: Twelve patients between 18 and 24 years of age with caries-free upper and lower first premolars scheduled for orthodontic extraction were selected. First premolars of one side of all patients were whitened for two weeks using (5 minutes speed whitening gel plus white Products). The first premolars of the other side were left without whitening and served as control. After treatment, Patients were divided into two groups 6 patients each. Group (1): The whitened First premolars of this group were extracted by the end of the second week of treatment. Group (2) The whitened first premolars were left for another two weeks without treatment then they were extracted. Immediately after extraction, 1/3 of the apical portion of each root was sectioned off. Then the extracted teeth were prepared for histological and ultrastructural evaluation.

Results: Slight pulpal changes were histologically detected in group(I) which was confirmed by the ultrastrucrural level. The affected cells were the odontoblasts and the fibroblasts which appeared quiescent with regression of their cytoplasmic organells. The blood vesels were dilated with engorged RBCes, denoting mild reversible pulpitis. Pulp of the second group appeared histologically and ultrastructurally close to that of the control group.

Conclusion: The findings from this study demonstrated that at home bleaching procedures using 10% hydrogen peroxide might cause initial mild, localized pulp reactions. However, the changes observed did not affect the overall health of the pulp tissue and were reversible within two weeks post-treatment. Therefore, two weeks of treatment with 10% hydrogen peroxide used for at home bleaching could be considered safe for dental pulp.
INTRODUCTION

Tooth discoloration varies in etiology, appearance, localization, severity, and adherence to tooth structure. It may be classified as intrinsic, extrinsic, and a combination of both. After eruption of the tooth, aging, pulp necrosis, and iatrogenesis are the main causes of intrinsic discoloration. Coffee, tea, red wine, carrots, oranges, and tobacco give rise to extrinsic stain.

The challenge to enhance the cosmetic appearance of teeth has led to the launch of a multitude of improved toothpastes, in-office or home prescribed professional bleaching kits, and mass market technologies for tooth whitening. These products often contain hydrogen peroxide or carbamide peroxide (a source of hydrogen peroxide) as whitening agent. The whitening mode of action of hydrogen peroxide involves the diffusion of peroxide through enamel-dentin junction and dentin areas. This makes the tooth appear whiter and less yellow.

Researchers have extensively evaluated the safety of products containing hydrogen peroxide or carbamide peroxide. A large number of studies have focused on the effects of these products on the integrity of the tooth tissues, where some studies have reported some changes in hardness, whereas others have not.

Hydrogen peroxide acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules, and hydrogen peroxide anions. These reactive molecules attack the long-chained, dark-colored chromophore molecules and split them into smaller, less colored and more diffusible molecules. The outcome of the bleaching procedure depends mainly on the concentration of the bleaching agent, the ability of the agent to reach the chromophore molecules, and the duration and number of times the agent is in contact with chromophore molecules.

The few histological studies of pulps taken from teeth exposed to hydrogen peroxide have reported either a mild reversible inflammatory response or no inflammatory response. Up to our knowledge, no ultrastructural study has been carried out to evaluate the effect of hydrogen peroxide bleaching agent on the pulp in vivo, which is the aim of this study.

MATERIALS AND METHODS

Twelve young adult male patients having upper and lower first premolars teeth scheduled for routine orthodontic extraction were chosen for this study. The chosen teeth were free of detectable caries, without restoration, free of any visible defects, gum disease, gingivitis, dental braces, or severely receding gums. All patient were informed about the possible sensitivity that might occur during the early period of tooth bleaching. The selected teeth where bleached using Plus White 5 minute speed whitening (fig.1) (CCA Industries, Inc., East Rutherford, NJ ) ** 5 minutes per day for 14 days as follow:

** Ingredients: Pre-Whitening Rinse: WATER, GLYCERIN, POLYSORBATE 80, SODIUM BENZOATE, FLAVOR, SODIUM SACCHARIN, METHYL SALICYLATE, CITRIC ACID, BLUE 1 5 Minutes Whitening Gel: WATER (AQUA), POLOXAMER 407, GLYCERIN, 10% HYDROGEN PEROXIDE, METHYL SALICYLATE, SODIUM SACCHARIN, PHOSPHORIC ACID.
Directions

One teaspoon of the concentrated Pre-Whitening Rinse was mixed with three teaspoons of lukewarm water. Swished in mouth for 2-3 seconds and rinsed out. A small, continuous line of Plus White Speed Whitening Gel was squeezed into the mouth tray. To whiten anterior and posterior teeth, the mouth tray was filled completely from one end and the other end left empty in order to free the premolars on the other side away from the bleaching gel. Carefully the tray was inserted into the mouth, positioned over teeth, any excess gel was wiped off. The tray was left in for 5 minutes for whitening then it was removed and mouth was rinsed several times with lukewarm water, thoroughly flushing any remaining gel from mouth and gum area. These steps were repeated once daily for two weeks.

After treatment, patients were divided into two groups, six patients each. Upper and lower first premolars of group one were extracted at the day 14 from the beginning of the experiment. Teeth of group two (II) were extracted after two more weeks following the end of the experiment i.e., the day 30 from the beginning of the experiment. Teeth were extracted with minimal trauma under local anesthesia. The apical 1/3 of all extracted teeth were removed using sharp pliers to ensure proper pulp fixation. Teeth were immediately fixed in glutaraldehyde solution overnight at 4°C. Teeth were then washed in 7.4% phosphate buffer. After fixation was completed, the tissues were then transferred into EDTA solution for decalcification. The decalcifying agent was changed daily to enhance decalcification. After sufficient demineralization (8 weeks, as determined by radiographic examination), the specimens were then dehydrated in alcohol, cleared in xylol and embedded in wax. Paraffin cross sections (6 μm. thickness) were cut and prepared for Haematoxylin and Eosin stain.

RESULTS

Histological results

Control group

The coronal pulp of the control group showed the peripheral arrangement of the odontoblasts along the dentin, with the predentin layer in between. The odontogenic zone was demonstrated; it included the odontoblasts, and a zone of high density, the cell rich zone. The rest of the pulpal tissue revealed fibroblasts and collagen fibers (Fig. 2).

FIG. (2) A photomicrograph of the pulp from the control group showing: (OB) odontoblasts, (arrows) predentin, (OZ) odontogenic zone, (H&E X100).

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**Group I**

The specimens extracted directly in the day 14 after bleaching application revealed dilatation in the pulp vessels, collection of edema fluid, and actual extravasations of the red blood cells. Slight inflammatory cell infiltrate was present. The odontoblastic layer was still present, however, some of them revealed degeneration and vacuolization. Interestingly, the predentin layer was absent in few specimens while others did not (Figs. 3&4).

**Group II**

The teeth extracted at the day thirty showed resolution of the inflammatory response. Odontoblastic layer appeared to be returning to its normal functional state, although they had not returned to their normal size and shape. The hemorrhage and evidence of vascular damage had disappeared (Fig. 5).

**Transmission electron microscopic results**

**Control group**

The odontoblasts had a large nucleus in their basal part and Golgi apparatus in their distal part. Abundant rough endoplasmic reticulum (rer), and numerous mitochondria were scattered throughout the cell body. The process arises from the odontoblast at the predentin level. The nucleus contained peripherally dispersed chromatin (fig.6). The fibroblasts were the most numerous cells in the pulp. Each fibroblast had a large oval centrally located nucleus, and multiple processes. Higher magnification of the fibroblast illustrated Golgi apparatus, adjacent abundant (rer) and mitochondria. Transverse and longitudinal sections of collagen fibers existed in the extracellular matrix which surrounded the cells (Fig.7).
**Group I:**

Narrower odontoblasts with their nuclei displaced from their basal extremity or even placed sufficiently distally to create a prominent infranuclear region. The amount of (rer) were reduced and located around the nucleus, the supranuclear region was devoid of organelles except for few mitochondria (Fig.8). The ultrastructural image of the fibroblasts reflected their functional state. They appeared flattened, spindle shaped with closed faced nucleus. Occasionally some fibroblasts exhibited large lysosomes which appeared as large clear areas in the cytoplasm. On the other hand, multivesicular bodies and few mitochondria were also present (Fig.9A&B).

Other pulpal cells included Schwan cells associated with the pulp nerves, which included a mixture of mylinated and non-mylinated nerves. Macrophages which appeared as denticular cells were clearly demonstrated. Few, less organized collagen fibers were loosely dispersed among the pulpal cells (Fig. 10A&B).
FIG. (9) (A&B) An electron micrographs of group I showing: (A) The fibroblasts which appeared flattened, spindle shaped with closed faced nucleus. (B) some fibroblasts exhibited large lysosomes (thin arrows), multivesicular bodies (thick arrows), mitochondria(m) X5200

FIG. (10) (A&B) Electron micrographs of group I showing: (A) Schwann cells (thick arrows), myelinated nerves (thin arrows), non- myelinated nerves (arrow heads).X 3600. (B) macrophage (arrows) X5200
**Group II**

The pulp of the bleached teeth in group II showed almost similarity between that in the control group. Ultrastructural picture of both odontoblasts and fibroblasts reflected their functional activity to being resumed. Their cytoplasm was found to contain a full complement of organelles required for synthesis and secretion of extracellular material. Large cisternae of (rer), several saccules of Golgi apparatus (Fig.11 A&B).

**DISCUSSION**

Tooth whitening treatment has become very popular in the recent years. Before deciding to proceed with treatment, patients using home whitening should be informed of its potential risks and benefits. An agent commonly used for home whitening is the 5 minute speed whitening bleaching gel which is used in the present study with a hydrogen peroxide concentration 10%.

This research was directed throughout along line different than that previously explored. Instead of investigating classical histological changes in the pulp after bleaching, the focus of this work was detecting the ultrastructural events occurring in the pulp after application of the 5 minute speed whitening system.

All principles of inflammation that apply to any other body organ apply to the dental pulp. In addition, dental pulp has some unique features that make it unusually fragile and sensitive. First, it is encased by hard tissues that does not allow for the usual swelling associated with the exudates of the acute inflammatory process. Second, there is no collateral circulation to maintain vitality when the primary blood supply is compromised. (26)

One commonly reported potential side effect of home-using whitening system is tooth sensitivity.(12) In an attempt to translate and correlate previous laboratory studies, the histological results of group I of the present study revealed signs of pulpitis. These signs included dilatation of the blood vessels, edema fluid collection and extravasations of the red blood cells. The latter histological features were referred to as focal reversible pulpitis. (26) A frequent sequela of focal reversible pulpitis is the acute pulpitis, which is characterized by entire odontoblastic degeneration.(26) Acute pulpitis could not be referred to the pulpal condition of group I of the present research since the degeneration and vacuolization of the odontoblasts were observed in some focal areas and did not involve the entire layer of odontoblasts. Similar histopathological results were also revealed after 10% carbamide peroxide gel for

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**FIG. (11) (A&B) An electron micrographs of group II showing:** (A) odontoblast (OB), dentin (d), (B) Fibroblasts (F), both pulpal cells revealed large cisternae of (rer), mitochondria (m). X5200
two weeks.\(^{(14)}\) Though, some authors considered the histological evaluation to be the most reliable method to evaluate pulpal reactions to clinical procedures.\(^{(20)}\) Other authors attributed the vacuolization of the odontoblastic layer to be artifacts due to inadequate fixation of the pulp.\(^{(14)}\)

In the herein study, the ultrastructural picture of the pulpal cells in group I confirmed the histopathological results. Remarkable regression in the cytoplasmic organelles, together with the presence of lysosomes, autophagic vacuoles and multivesicular bodies were revealed in both odontoblasts and fibroblasts. Moreover, the closed faced nucleus contained peripheral electron dense chromatin denoting a decline in the cellular activity.

It is argued that higher hydrogen peroxide concentration causes higher pulpal peroxide penetration.\(^{(4,6)}\) Tse et al (1991) reported that cytotoxicity of hydrogen peroxide was influenced by both the concentration of the peroxide, and the length of time it was in contact with the cells. In the ongoing study, the hydrogen peroxide concentration in the bleaching agent was 10%, it was applied to the teeth for 5 minutes, for 14 consecutive days of treatments. This was a total of application time of 70 minutes. Seale et al (1981) used a 35% hydrogen peroxide concentration for 30 minutes to bleach the teeth in dogs. The pulpal reaction showed a marked inflammatory response, flattening and obliteration of the odontoblastic layer, vascular damage and even odontoclastic activity. The authors attributed the obliteration of the odontoblastic layer to the caustic nature of 35% hydrogen peroxide, and the odontoclasts and internal resorption to severe irritation.

The vitro studies may be limited to simulate the clinical condition. The current research was carried out on vital pulp. Matthews et al (1992 and 1994) reported that in vital pulp, the pulpal fluid pressure is capable of reducing inward diffusion of chemicals. However, Tse et al 1991 added that there are sufficient mechanisms in the pulp that protect the tissue from radicals generated from the reaction of hydrogen peroxide, and defense mechanism of the pulp would significantly reduce available levels of hydrogen peroxide.

Histopathological and ultrastructural results of group II of the ongoing study revealed regression in the inflammatory infiltrate, all pulpal cells seemed to be resuming their functional activity reflected in the presence of the full complement of the cell organelles. Robertson and Melfi (1980) and Seale et al. (1981) all suggested that pulp tissue possesses marvelous recovery ability. One possible mechanism by which the pulp may protect itself from damage by Hydrogen peroxide is by enzymatic breakdown of the molecule. Hydrogen peroxide may be degraded by two classes of enzymes: peroxidase and catalase. Peroxidase uses hydrogen peroxide to oxidize some other substrate. While catalase breaks down hydrogen peroxide to water and oxygen.

It could thus be concluded that at home bleaching procedures using 10% hydrogen peroxide might cause initial mild, localized pulp reactions. However, the changes observed did not affect the overall health of the pulp tissue and were reversible within two weeks post-treatment. Therefore, two weeks of treatment with 10% hydrogen peroxide used for at home bleaching could be considered safe for dental pulp.

**REFERENCES**


