Sequential Ultrastructural Investigation of Pulp Tissue Responses to Rabbit`S Teeth Bleaching

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Abstract: Teeth bleaching have been documented for teeth whitening by oxidizing agent are associated with morphological alterations of the enamel surface. To investigate the sequential changes of the pulp tissue structures, using transmission electron microscopy (TEM), after enamel bleaching utilizing light enhanced bleaching, this study was performed. Rabbit's teeth were randomly divided into six groups (Gs) as follows: control group (G1) was received no treatment, while test groups included (G2: 24 hours following treatment, G3: one week following treatment, G4: two weeks following treatment, G5: one month following treatment, and G6: two months following treatment). After termination of each period, the rabbits were sacrificed, the teeth were carefully dissected and removed. Each tooth was split open with chisel and hammer and the pulp was removed with a sharp excavator, and prepared for TEM investigation. The results revealed that bleaching effects on pulp tissue elements were of various degrees cellular alterations. The observed pattern of tissue changes fell into four phases: a) mild to moderate pulp tissue injuries may be produced as an early response of teeth bleaching, b) development of localized regions of severe cellular injuries and/or necrosis, c) regression in the signs of degeneration accompanied initial recovery of cellular elements of pulp tissue, and d) complete recovery and reassuming of various tissue elements of the pulp.
[Heba Ahmed Adawy, Mohamed Mohamed Fekry Khedr and Mohamed Gomaa Attia-Zouair **Sequential ultrastructural investigation of pulp tissue responses to rabbit`s teeth bleaching.** Life Science Journal 2011; 8(4):10261033]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 129

Key Words: bleaching, pulp, TEM, ultrastructural

1. Introduction:

There are a number of methods and approaches that have been described in the literature for the bleaching of vital teeth with the common active ingredient being an oxidizing agent that acts on the organic matrix within the teeth [1-10]. Accelerating peroxide bleaching with simultaneous illumination with various sources having arrange of wave lengths and spectral power has such as halogen curing lights, plasma arc lamps, lasers and light emitting diodes [2,3,11,12]. A number of approaches of evaluating pulp tissue changes following tooth whitening exist, each with their evidence about a penetration of bleaching materials into the pulp tissue has been found [11-17]. While the central region of both the coronal and the radicular pulp contains large nerve trunks and blood vessels, the peripheral zone is circumscribed by specialized odontogenic region composed of odontoblasts, cell free zone and cell rich zone.[18] pulp inflammation could be seen in various features[19]. It has been reported that adversely affect pulp tissue structures could be found [20-22]. Weather the pulp tissues could be regenerated after exposing to harmful effects was a matter of discussion [23,24]. Ultrastructural study about cellular structure of pulp as a response of enamel bleaching, have only been previously investigated in a very limited study.[25] In this regard, the ultimate goal of the current study was to obtain more information about cellular structure of pulp as a response of rabbit`s enamel bleaching by transmission electron microscopy (TEM).

Material and Methods:

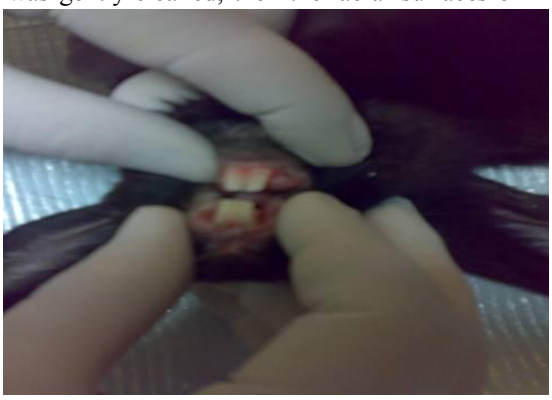
A total of forty two male New Zealand rabbits were selected from a reputable supply, the rabbit unit at the Faculty of Agriculture, Cairo University. The animals were divided into six groups, while a control group receiving no treatment (group 1: normal), the experimental groups were of five intervals (24 hours: (group 2) and one week: (group 3), two weeks: (group 4), one month: (group 5) and two months: (group 6). All animals were previously vaccinated and treated against scabies, coccidiosis and enteritis (viral hemorrhagic diseases). The animal`s weight ranged between 2.5 to 3 Kg with an age of 4-4.5 months. Two rabbits were kept in a separate cage, fed and maintained during the time of the study at a private animal housing unit. The rabbits were fed on a specific diet *ad labium* (about 150 g per day) and water. Rabbits were left for a few days before the procedure to settle down.

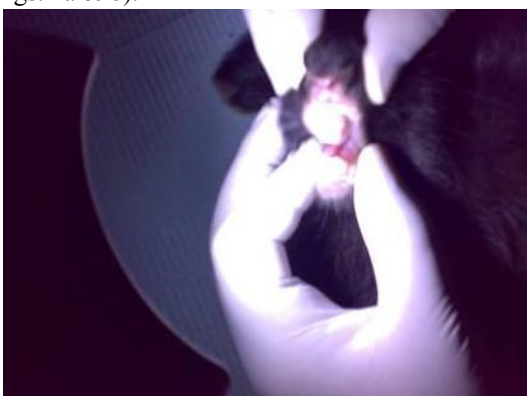
Anesthesia:

Rabbits were given an intramuscularly injection of tranquilizer Xylazine HCl (Xylaject®, The Egyptian Co. of Chemicals & Pharmaceuticalas {ADWIA}, 10th of Ramadan City, Cairo, Egypt) and anesthetic solution Ketamine HCl (Ketamar, Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt). 10%. Xylazine HCl was injected at a dose of 0.5 ml (2-3 mg / Kg) animal body weight. After ten minutes the second injection of Ketamine HCl was given at a dose of 50 mg per Kg animal body weight. This combination allowed an anesthetic duration of 20-30 minutes. The same doses were repeated when needed.

Procedures:

Prior to starting bleaching, the surface of each tooth was gently cleaned, then the labial surfaces of the rabbits` upper and lower anterior teeth were painted with a layer approximately 1 mm thick of a mixture of a 35% hydrogen peroxide solution and a bleaching agent according to the manufacture instruction. Then, exposed to a bleaching light source (Wave light, Schein, Melville, NY, USA) that contains a plasma arc lamp. The distance between the emitting tip of the light source and the tooth surface was at 15 mm. All the upper and lower incisors were exposed to the light source 3 s, then left standing for 5 min, and then exposed to the light source again for another 3 s. After they were left standing for another 2 min, each tooth was then exposed to the light source for another 3 s of final exposure. Then, the mixture that had been applied to the teeth was removed. This procedure was repeated three times (Figs. 1a & b).





Figs (1 A & B). Photomicrographs of rabbit`s teeth before and during the exposure to a bleaching light source that contains a plasma arc lamp.

Methods of Evaluation:

I. Clinical Inspection:

Animals were observed during the experimental periods; the teeth surfaces were inspected regularly before and after application of bleaching procedures.

II. Preparation of the specimens for transmission electron microscopy (TEM) examination:

Preparation and fixation:

After each period of investigation the rabbits were sacrificed, the teeth were carefully dissected and removed. The teeth were then placed in jars labeled by animal number and investigation duration. Specimens for TEM examination were prepared according to Bancroft and Stevens [26]. Each tooth was split open with chisel and hammer. The pulp was removed with a sharp excavator and rapidly immersed in a mixture of 2.5% glutaraldehyde and 10% formaldehyde (F/G solution) for 24-48 hours (Dard *et al.*) [27] . Each fixed pulp was placed on a glass slab and cut into three parts (superficial, middle and deep) using a sharp razor blade. From each part, cross sections (1 mm thick) were cut and washed several times in phosphate buffer solution with pH 7.2-7.4. The specimens were post-fixed in 1% osmium tetroxide for one hour, and washed again in phosphate buffer. The specimens were loaded in ascending concentrations of ethyl alcohol. After complete dehydration, the specimens were embedded in (EPON 812). Flat rubber moulds were used to obtain the specimen blocks. Semithin sections were cut with a diamond knife, mounted on glass slides and stained with 1.0% toluidine blue for light microscopic examination. The area of interest was selected for ultra-thin sectioning. The cut sections were stained with uranyl acetate and lead citrate to be examined with transmission electron microscope (Japan Electron Microscope 1010).

3. Results

Group 1 (control):

TEM revealed that odontoblastic layers were peripherally arranged in palisading pattern outlined the central part of the pulp. They appeared ovoid or columnar in shape with well-defined cell membrane. Odontoblastic nuclei are oval and most of them were

basally situated, while others occupied the whole cell. The nuclei showed normal chromatin distribution and dominance of euchromatin. There was distinct boundary between condensed and loose chromatin. The nuclei were surrounded by clear regular double nuclear membrane. Well developed strands of rough endoplasmic reticulum were arranged around the nuclei in the cytoplasm of odontoblasts. The electron dense strands of RER became are parallel to the long axis of the cell in the areas further from the nucleus (the supranuclear zone). Abundant mitochondria were detected in the odontoblastic cytoplasm especially in the apical region of the cells. Although they had a variety of shapes, each of them preserved its typical structure, which is a double membrane with internal cristae. Numerous junctions were seen subjacent to odontoblasts including tight junctions (Zonula occludens), belt desmosome (Zonula adherens) and gap junctions (nexus or hemidesmosome type) (Figs. 2&3). The central part of the pulp showed great numbers of fibroblasts that were immersed in a loose fibrillar connective tissue matrix. The nucleus of a fibroblast had elongated or fusiform apperance with normal chromatin. Moreover, the cytoplasmic extensions of fibroblasts were clearly apparent (Fig. 4). Vascular components of pulp tissue showed normal endotheliocytes lining the vascular lumen of blood vessel (Fig. 5).

Group 2 (24 hours-period):

Comparing this group to the previous one (control group), electron micrographs of pulpal tissue revealed minimal loss of orientation of odontoblastic cell layers associated with wide extracellular compartments. The cell membranes remained intact but the junctions between cell membranes were almost lost. Most of the odontoblasts appeared with regular nuclear membrane accompanied by slight peripheral or central chromatin clumping. Cell organelles within the cytoplasm were normally found and most of the RER and mitochondria were preserved (Fig. 6). Fibroblasts appeared normal in most of the specimens. The Blood vessels showed obvious hyperemia and widening of the lumen. Slight flattening of vascular endothelial cells was observed (Fig. 7). Some mylinated nerve endings showed hyalinization and loss of the stratification structure of myelin sheath (Fig 8).

Group 3 (one week-period):

Electron micrograph of pulpal tissue showed serious ultrastructural findings. The odontoblasts revealed an irregular nuclear membrane associated with severe peripheral and central chromatin clumping. The odontoblastic cytoplasm showed extensive vacuolization, swollen mitochondria with loss of their internal cristae and disrupted RER accompanied by much loss of ribosomes. The cell membrane was ill-defined in some samples. Complete loss of cell junctions between odontoblastic layer and wide extracellular matrix was noticed (Fig. 9). The fibroblasts showed intracytoplasmic vacuoles and irregularities in their nuclear membranes in a form of moderate peripheral and central chromatin condensation (Fig. 10). Severe hyperemia had showed up in the blood vessels of pulp tissue, increasing the width of the lumen of blood vessels. The endothelial cells were obviously affected; they showed vacuolization, irregular nuclear membranes and swollen mitochondria. Collagen degradation and hyalinization was observed throughout the ground substance (Fig. 11). Most of the mylinated nerves had lost their striations, expressed extensive hyalinization and vocalization (Fig. 12).

Group 4 (two weeks-period):

TEM revealed that the odontoblastic layers have minimal alterations. Most of the odontoblasts were shown to be preserved their ovoid regular outline of their nuclei with regular nuclear membrane and slight peripheral chromatin condensation. Swollen mitochondria were detected in the apical and paranuclear parts of the cell. RER showed moderate dilatation and loss of ribosomes. The cell membranes were preserved intact. The intercellular junctions were still lost and wide extracellular compartments were detected (Fig. 13). Few fibroblasts showed intracellular vacuolization and irregular nuclear membrane, while most of them showed normal ultrastructural findings, compared to the control group (Fig. 14). Hyperemia within blood vessels was seen to be decreased. However, the endothelial cells showed some vacuolization and irregularities in their nuclear membranes. Ultrastructural changes of mylinated nerves were less severe compared to that shown in the previous group (group 3; one week-period). Hyalinization and collagen fibrils degradation in the ground substance was still existed.

Group 5 (one month-period):

TEM, revealed that the odontoblastic layers have normal pattern on a whole although the intercellular wide compartments were still present (Fig. 15). Fibroblasts expressed nearly regular morphological features as in group 1 (Fig. 16). Most of the mylinated nerves were recovered on the whole, preserving the stratification of the myelin sheath. Hypremia within blood vessels was shown to be decreased and hyalinization of collagen fibrils was limited.

Group 6 (two months-period)

TEM examination of this group revealed nearly the same ultrastructural findings as those of group 1 , regarding the odontoblastic cell layer and the loose fibrillar connective tissue.

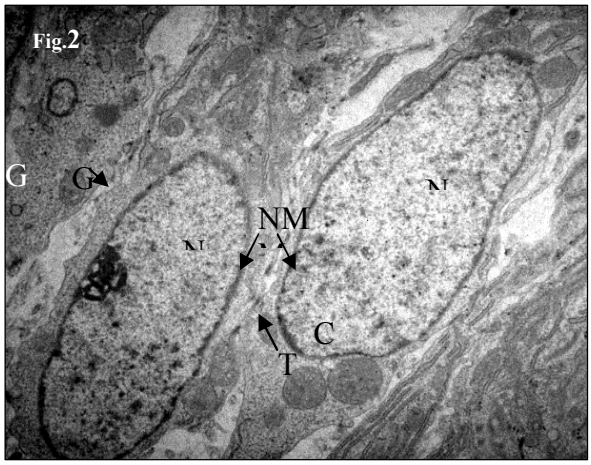


Fig. 2. Electron micrograph of group 1 showing; normal palisading odontoblasts with open faced nucleus (N), regular nuclear membrane (NM) and normal chromatin distribution (C). Notice tight (T) and gap junctions (G). X5000

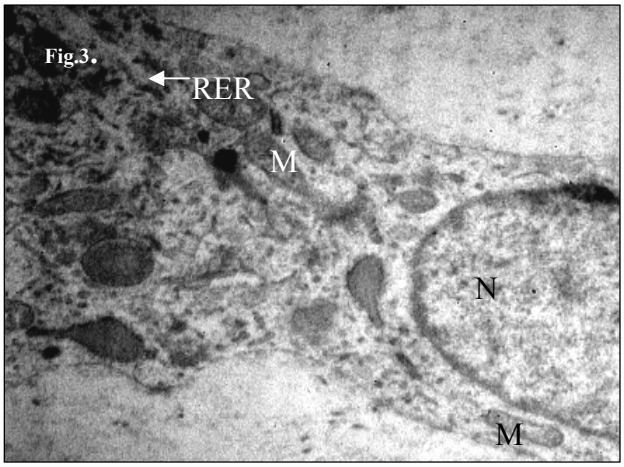


Fig. 3. Electron micrograph of mature odontoblast with abundant apical mitocondria and normal rough endoplasmic reticulum. X15000

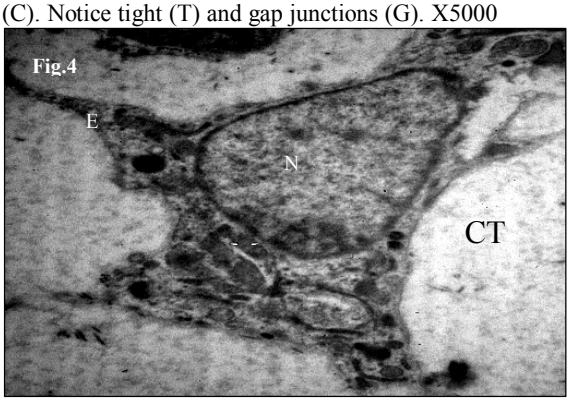


Fig. 4. Electron micrograph of normal fibroblasts immersed in a loose fibril connective tissue matrix (CT). Notice the cytoplasmic extensions of the cells (E) (X12000).

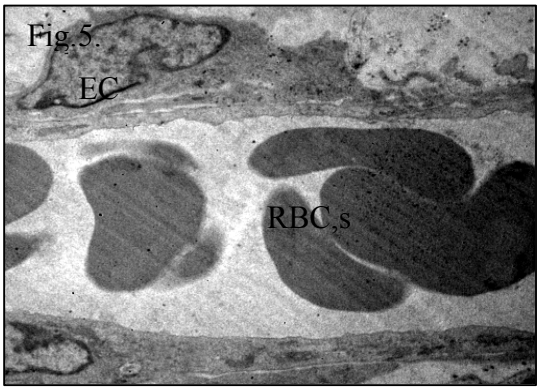


Fig. 5. Electron micrograph of normal blood vessel lined with endothelial cells (EC), and RBC,s in its lumen. (X8000).

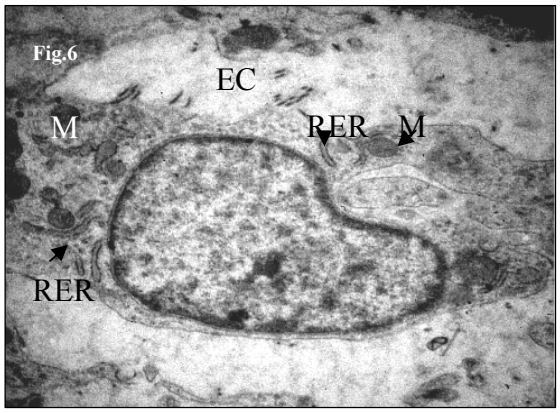


Fig 6. Electron micrograph of group 2 showing minimal loss of odontoblastic cell layer orientation with wide extracellular compartments (EC). Most of the RER and mitochondria (M) were preserved but the cell Junctions were nearly lost (X15000).

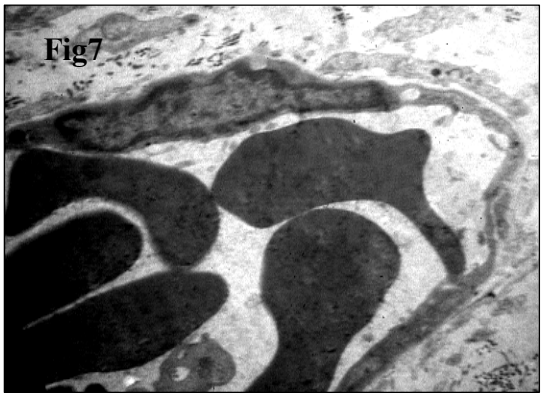


Fig. 7. Electron micrograph of group ll showing hyperemia and widening of the lumen of the blood vessels. Notice slight flattening of vascular endothelial cells (EC) (X10000,X4000).

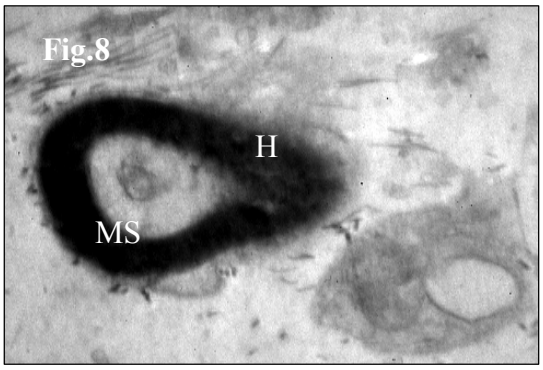


Fig. 8. Electron micrograph of group 2 showing endodontic nerve endings with moderate degree of hyalinization (H) and loss of the stratification structure of myelin sheath (MS) (X15000).

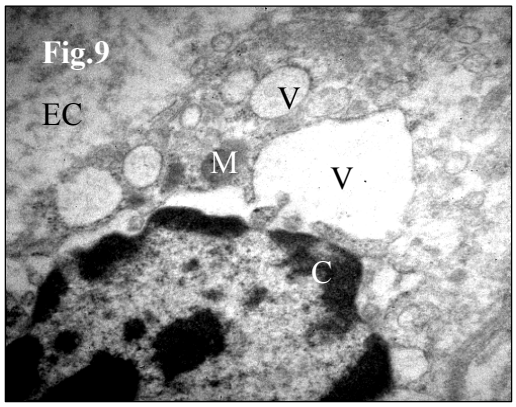


Fig. 9. Electron micrograph of group 3 showing serious ultra structural changes. The odontoblasts showed an irregular nuclear membrane (NM) with severe peripheral and central chromatin clumping (C). The cytoplasm showed severe vacuolization (V), swollen mitochondria (M). Notice the complete loss of cell junctions and wide extracellular compartments (EC), (X30000).

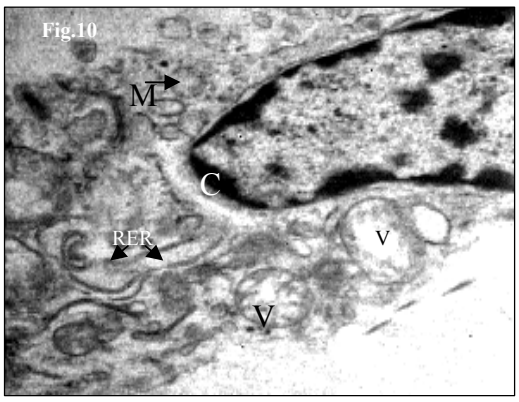


Fig. 10. Electron micrograph of group 3 showing some intracytoplasmic vacuoles (V), irregularity in the nuclear membranes(NM) , nuclear chromatin clumping (C)and swollen mitochondria (M) of fibroblasts. Notice areas of hyalinization in ground substance (H) and strands of RER (X6000, X15000).

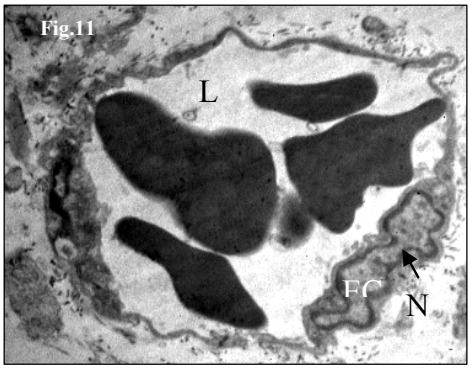


Fig. 11. Electron micrograph of group 3 showing severe hyperemia and increased width of the lumen (L) of blood vessels. The endothelial cells (EC) showed corrugated nuclear membranes (NM) (X8000).

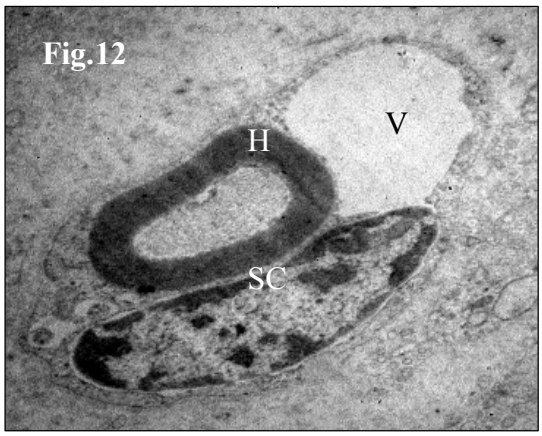


Fig. 12. Electron micrograph of group 3 showing loss of striations, hyalinization (H) and vocalization (V) of endodontic nerve endings. Notice Schwann cell (X15000).

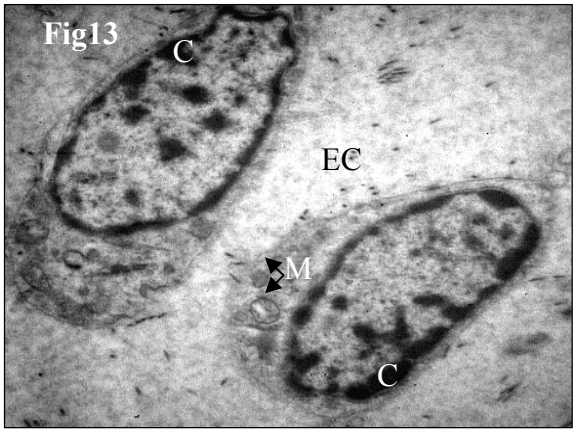


Fig. 13. Electron micrograph of group 4 showing odontoblastic cells with slight peripheral nuclear chromatin condensation (C) and swollen mitochondria (M). Notice loss of intercellular Junctions and wide extracellular compartments (EC) (X15000).

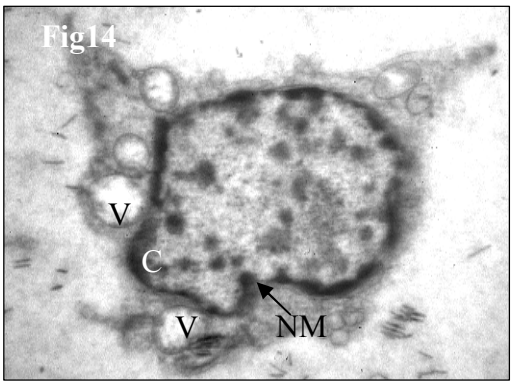


Fig. 14. Electron micrograph of group 4 of fibroblasts showing slight nuclear chromatin condensation (C) and irregularity in nuclear membrane (NM) and cytoplasmic vacuolization (V) (X26000).

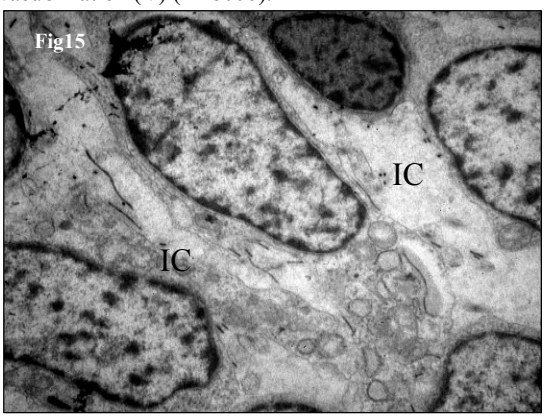


Fig. 15. Electron micrograph of group 5 showing normal odontoblastic layer on a whole except the wide intercellular compartments (IC) (X12000)

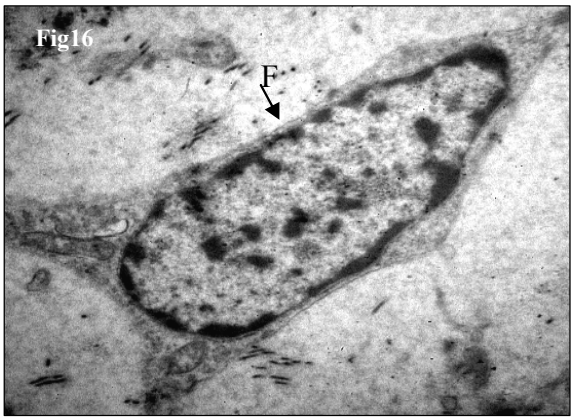


Fig. 16. Electron micrograph of group 5 normal fibroblast (F) in ground substance (X20000)

4. Discussion

To allow the observation of details not visible by the light microscope, magnified image of a specimen was performed, by transmission electron microscopy (TEM), to obtain more information about cellular structure of pulp as a response of enamel bleaching. The present study showed that male Germany rabbits were a successful animal model for this experiment. The plasma cell of odontoblasts seen in the low power electron micrograph displayed many of the main cytoplasmic components which are commonly encountered in the human odontoblasts. Moreover, at a high magnification micrograph, details of membranous and various cellular components and structures of stromal pulp tissues revealed similar results as in the human [18]. The present TEM results revealed that hydrogen peroxide in a combination with heat generation caused early alterations in pulp tissue elements at both the 24 hours and one week (groups 2&3) following the enamel bleaching application indicated that the pulp tissue did not remain healthy. Evidence of affection of the odontoblasts could be manifested as a loss of their orientation, in addition to chromatin clumping and RER affection. The primary degenerative changes of cellular organelles of odontoblasts, were in consistence with that reported by other researchers as a sequel of acute pulpitis[23], suggesting that the immediate and/or early effect of enamel bleaching may participate in the death of the severely damaged odontoblasts. This may be due to the penetration of the bleaching agent into the odontoblasts through the dentinal tubules as a second step to enamel and dentin demineralization [8,9]. At two weeks (group 3), in contrast to that shown by Essawy and Korany[25], pulpal blood vessels and nerve endings showed obvious alteration with loss of the stratification structure of myelin sheath. The severity of the changes was extended to include complete loss of cell junctions between odontoblastic layer Moreover, deeper pulp tissue structures showed pathological alterations. These included irregularities of the nuclear membranes of fibroblasts, severe vascular hyperemia, loss of nerve striations, swollen mitochondria and collaginolysis of ground substance accompanied hyalinization. This indicates that the light energy made the harsh peroxides penetrating deeper towards pulp tissue elements including odontoblasts, blood vessels and nerve tissues. The contradictory results compared to the aforementioned results can be attributed as, in their study, the bleach of preference for at-home teeth whitening is slower acting carbamide peroxide, which breaks down into hydrogen peroxide. Carbamide peroxide has about a third of the strength of hydrogen peroxide. This was in consistence with that reported by other researchers [20,21,23]. To answer how peroxide products can cause adverse effect on teeth enamel and pulp tissue elements: When peroxide-based oxidizing agents are used, a chemical reaction takes place which allows fluids to move through enamel defects into the

dentinal tubules directly towards the pulp tissue elements. Hydrogen peroxide (H_2O_2) can easily be broken down to hydroxyl (OH-) and per hydroxyl radicals (highly reactive molecules) [4]. Interestingly, at one month (group 4), electron micrograph revealed that the odontoblastic layer showed minimal alterations compared to the aforementioned groups. Most of the odontoblastic cells have preserved their ovoid regular outline of their nuclei with regular nuclear membrane, although, slight peripheral chromatin condensation was noticed. Moreover, RER showed moderate dilatation and the cell membranes were seen to be intact. While most fibroblasts showed normal ultrastructural finding, hyperemia within blood vessels was shown to be decreased and changes of endodontic nerves were less severe than that seen in the previous groups. Comparing the enamel bleaching effect of immediate response (24 hours & one week) of pulp tissue structures versus that of delay (one week), suggested that bleaching has only a modest positive impact on overall efficacy. Thus, pulp tissue affection appears to be over all limited and/or reversible. This can be attributed as: in vital pulp, there are sufficient mechanisms 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 [23] . It has been reported that the pulp may protect itself from damage by hydrogen peroxide to oxidize some other substrate, while catalase breaks down hydrogen peroxide to water and oxygen by enzymatic breakdown of the molecule. Hydrogen peroxide may be degraded by two classes of enzymes: peroxidase and catalase. The present results revealed a positive relationship between the intervals following bleaching and the magnitude of pulp response following enamel bleaching. This indicated that pulpal cells appeared to be resuming their functional activity.

At two months (group 5) the results revealed that almost all pulpal cellular elements seemed to be resuming their functional activity represented by the the appearance of pulp tissue normal ultrastructrual findings. odontoblastic layer almost appeared normal on a whole although the intercellular compartments were still wide. However, hypremia within blood vessels was shown to be decreased. Also, hyalinization and collagenolysis of the ground substance were limited. Moreover, most of the endodontic nerves were shown to be recovered, preserving the stratification of the myelin sheath. This indicated that the pulpal tissue structures appeared to be reversed. Similar results were reported by others [16,23]. Regeneration of pulp tissue elements can be explained as follow: stimuli that induce degeneration and/or necrosis in some cells can trigger the activation of replication pathways in others; recruited inflammatory cells not only clean up the necrotic debris but also elaborate mediators that drive the synthesis of new extracellular matrix (ECM). A collagen matrix acts as a molecular grid with channels and pores, which changes the original dense collagen structure into a sponge-like structure allowing cell migration. The remaining healthy pulp tissue structure, is a promising, allows cellular migration. Moreover, cell proliferation can be stimulated by intrinsic growth factors, injury, cell death or even mechanical deformation of tissues. Proliferating cells progress through a series of checkpoints (cell cycle). At three months (group 6) revealed that appearance of pulp tissue almost mimic normal ultrastructural findings. This can be explained as the ECM is much more than a space filler around cells; its various roles include: mechanical support for cell anchorage, determination of cell orientation, control of cell grpwth, maintainance of cell differentiation, scaffolding for tissue renewall, establishment of tissue microenvioenmentals and storage and presentation of regulatory molecules.[24]

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