

Ultrastructure of the Cellular Response of Rabbits' Gingivae to the Adverse Effects of Light Enhanced Bleaching

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Abstract: A total of 30 rabbits were selected. The animals were divided into equal 6 groups, 5 animals each. While a control group received no treatment (G1: normal), the animals of experimental groups (G2 to G6) were anesthetized and the labial gingivae of the upper and lower anterior teeth were painted with a layer of a mixture of a 35% hydrogen peroxide solution and a bleaching agent during the application enamel bleaching utilizing a plasma arc lamp for three intervals, 20 minutes each. The animals were sacrificed after five intervals: (24 hours: G2, one week: G3, two weeks: G4, one month: G5 and two months: G6) subsequently. After each period of investigation, the gingiva of the rabbits were carefully dissected and prepared for transmission electron microscopy examination. The results revealed that bleaching effects on gingival tissue elements were of various degrees cellular and nuclear affections. Moderate to severe cellular and nuclear injuries may be produced as an early response to the bleaching effect. Subsequently, tissue injuries were of various degrees involving the different gingival tissue elements.

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1. Introduction

Since bleaching has become a popular procedure, the side effects of peroxides was of great interest in research.⁽¹⁻⁵⁾ Moreover, studies have been performed in order to evaluate the adverse effects produced during and after bleaching procedures on soft tissues.⁽⁶⁻¹⁰⁾ The side/adverse effects with the various bleaching regimens studied included those of night guard vital bleaching and internal bleaching of endodontically treated teeth.⁽¹¹⁻¹³⁾ Damage of cellular and nuclear proteins and lipids was seen.^(14,15) Potential adverse outcomes including co-carcinogenic effects with tobacco, and free radical generation and release have been reported.⁽¹⁶⁾ Moreover, several studies have shown the involvement of reactive oxygen species including hydroxyl radical and hydrogen peroxide in colon cancer, breast tumors and stroke.⁽¹⁷⁻¹⁹⁾ However, Cellular response to peroxides has been investigated in various studies.⁽¹²⁻²⁸⁾ Ultrastructurally, gingival tissues have the characteristics of keratinized epithelium.⁽²⁹⁾ Since there was no reports about the ultrastructural response of cellular structures of gingiva during bleaching, the current study seemed of interest. The ultimate goal of the current study was to gain insight into the effect of bleaching on rabbits' gingivae, by transmission electron microscopy (TEM), following enamel bleaching.

2. Materials and Methods

A total of thirty male New Zealand rabbits were selected from a reputable supply, the rabbit unit at the Faculty of Agriculture, Cairo University. The animals' weight ranged between 2.5-3 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 libitum (about 150 gm per day) and water. Rabbits were left for a few days before the procedure to settle down. All animals were previously vaccinated and treated against scabies, coccidiosis and enteritis (viral hemorrhage diseases).

Treatment schedule;

The thirty animals were randomly divided into control and five experimental groups, 5 animals each. Animals in group (1) were received no further treatment. Animals in groups (2-6) were received the same treatment but sacrificed at different intervals (G2: 24 hours-treatment, G3: one week-treatment, G4: two weeks-treatment, G5: one month-treatment, and G6: two months-treatment). The labial gingivae of upper and lower anterior teeth of the rabbits in the experimental groups 2 to 6 were painted, using a brush, with a mixture of 35% hydrogen peroxide solution and the bleaching agent during the application of enamel bleaching according to the manufacturer's instruction. The mixture on the tooth surface and gingiva was the exposed to a bleaching light source (Wave light, Schein, Melville, NY, USA) that contains a plasma arc

lamp. All the upper and lower incisors teeth and labial gingivae were exposed to light enhanced bleaching three times, 20 minutes each according to the technique described in previous studies.^(4,5) Animals were inspected regularly before and after the application of the bleaching procedures and throughout the experimental periods (Fig.1). The animals were sacrificed at the termination of the aforementioned periods.

Preparation of the specimens for TEM examination

After each period of investigation, the rabbits were sacrificed, the gingiva of the rabbits were carefully dissected with a sharp scalpel No 15 and removed (Figs. 3 and 4). The gingival samples were cut into very small cubes nearly 1mm x 1mm and rapidly immersed in labeled jars of a mixture of 2.5% glutaraldehyde and 10% formaldehyde (F/G solution). Specimens were kept in FG solution for 24-48 hours in dried cool place. Specimens were then 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 for dehydration. After complete dehydration, the specimens were embedded in (EPON 812) using flat rubber moulds. Curing of EPON 812 moulds were done to obtain the specimen blocks. Semi-thin 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 TEM (Electron Microscope 1010, Japan).

3. Results

Control group

Semi-thin section examination of toluidine blue stained gingival specimens of this group, revealed the configuration of both epithelial and connective tissue cell layers. The gingival epithelium was appeared as stratified, squamous keratinizing epithelium, which consists of a basal layer, a spinous layer, a granular layer and a superficial cornified layer (Fig.6).

Ultrastructural features, using transmission electron microscope (TEM) revealed that the basal cells are columnar in shape with well-defined cell membrane. Their nuclei were elongated, centrally located, with homogenous chromatin distribution and condensation near the nuclear membrane. The nuclei were surrounded by clear regular double nuclear membrane. The rough endoplasmic reticulum (RER) was well developed, with flattened and parallel cisternae and arranged in the perinuclear region of the basal cell layer. Abundant mitochondria were detected showing their typical structure, which is a double membrane with internal cristae. They appeared

spherical and/or elongated in the basal cell cytoplasm. Some of them appeared swollen and lost their internal cristae. Numerous junctions were seen between adjacent basal cells mostly desmosomes. The spinosum cells were arranged above the basal cells and attained polyhedral outline. Their nuclei were voluminous, with irregular nuclear membrane and peripheral chromatin distribution. Nucleolus was very evident in this layer. Their cytoplasm contained some mitochondria, RER and small electron dense granules. The next overlying layer is the granulosum cell layer. It was characterized by the presence of flattened elongated cells with a wide centrally located nucleus. Superficially, the cornified layer was seen as flattened layer of cells with relatively narrow intercellular spaces.

The junction between a basal cell and the connective tissue of the underlying lamina propria was studded with numerous hemidesmosomes (HD) and is connected to it by a basal lamina (BL). The basal lamina appeared intact, consisted of an electron-dense layer, the lamina densa (LD) and an electron-lucent layer, the lamina lucida (LL). Anchoring fibrils (AF) extended from the undersurface of the lamina densa into the lamina propria. Densely packed groups of transversal and longitudinal collagen fibers were seen. A large collagen fiber (CF) consisting of hundreds of collagen fibrils is outlined. Fibroblasts were seen scattered throughout the connective tissue. They were irregularly shaped cell. Their nuclei were elongated and/or fusiform with normal chromatin distribution. Moreover, the cytoplasmic extensions of fibroblasts were clearly apparent and connected with each other by intercellular junctions. Some fibroblasts had intracellular vacuoles containing collagen fibrils. Vascular components revealed that blood vessels are lined with endotheliocytes and red blood corpuscles (RBCs) are seen in the vascular lumen (Figs. 7-10).

Group 2

By comparing this group with the control group, serious ultrastructural changes had occurred throughout the gingival tissue cell layers. The stratified epithelium cell layers were markedly altered. The arrangement of the basal cell layer was more or less clear. However, obvious destruction of intercellular cell junctions in basal cell layer and wide extracellular matrix were observed. Intracellularly, the basal cells showed varied degrees of cellular activity. Most of them showed some degree of pleomorphism with irregular cell membranes. The cytoplasm of the basal cells showed severe vacuolization and hyalinization. Mitochondria appeared rounded and swollen with wide spread of cristolysis. Also, RER had become disrupted, dilated with much loss of their ribosomes. The nucleus appeared enlarged with irregular nuclear membrane. Severe peripheral and central nuclear chromatin clumping was detected. In some cells the nuclear

membrane became ill-defined with severe chromatin condensation. Nuclear mitosis and activity could be seen clearly in basal epithelial cells of this group.

The spinosum cells were preserved their polyhedral shape but with less volume and wide extracellular matrix. The nucleus appeared irregular with central chromatin condensation. The cytoplasm showed vacuolization and many scattered swollen mitochondria. The granulosum layer had the same characteristic outline as seen in the control group but with wide extracellular matrix. Vacuolization and numerous swollen mitochondria were detected in the cytoplasm. The nuclear membrane was irregular with marked peripheral and central chromatin condensation. Superficially, the cornified layers were partially separated from each other and form the granular cell layer. A clear wide cleft was detected between the keratin layer and the granular cell layer in many areas of this group samples. The basal lamina in this group was markedly interrupted along its course. Also the anchoring fibers were not detected clearly in samples of this group. The ultrastructural alterations had progressed already to the subepithelial connective tissue. Such alterations were represented by clear areas of hyalinization and vacuolization with the presence of inflammatory cells in between the transversal and longitudinal collagen fibers. Fibroblasts appeared shrunken, with marked reduction in cytoplasmic and nuclear volume. Numerous swollen mitochondria were condensed in the cytoplasm. Partial loss of the intercellular junctions between the fibroblastic cell processes (Figs. 11-14).

Group 3

Various grades of ultrastructural alterations had expressed in different gingival layers of this group samples. The basal cell layer of gingival epithelium showed obvious intracellular changes. Mitochondria appeared large, swollen with loss of its internal crescent. The RER showed clear hyalinization and loss of their ribosomes. The nucleus appeared moderate in size, with irregular nuclear membrane with central and peripheral chromatin condensation. Nuclear mitosis was very dominant in this group sample. Wide intercellular cell spaces were very apparent between the basal cell layers. The spinosum and granulosum cell layers preserve their main characteristic orientation in this group, however they had noticeable wide extracellular matrix. Intracellularly, the cytoplasmic vacuolization and mitochondrial enlargement were common feature in these cell layers. The nuclear alterations such as peripheral chromatin condensation and irregular nuclear membrane were seen clearly in both spinosum and granulosum cell layers. Some parts of the cornified cell layer were completely lost, other parts appeared intact without evidence of superficial separation. The basal lamina was detected in favorable condition with its lamina lucida and lamina densa

layers. However, it expressed some minute sporadic areas of interruptions which were detected only on higher magnifications. The anchoring fibers were well defined in the underlying connective tissue (C.T). The lamina propria showed noticeable dilatation in blood vessels. No sign of hyalinization was detected in the lamina propria of this group. Some vacuolization could be seen between the numerous collagen bundles. Fibroblasts appeared scattered in the lamina propria, they showed small irregular nuclei with peripheral chromatin condensation. Their cytoplasmic organelles appeared more or less of regular appearance (Figs. 15-19).

Group 4

In this group, the gingival epithelium had different ultrastructural features than that of the previous groups. The basal cell layer of the gingival epithelium exhibited nearly regular orientation. They showed irregular cell membranes and enlarged nuclear-cytoplasmic ratio. Their nuclei expressed irregular nuclear membrane, central and peripheral chromatin condensation. No signs of nuclear mitosis were detected in this sample. Mitochondria showed hyalinization and loss their internal cristae. RER appeared with detached ribosomes. The intercellular spaces are still wide and little desmosomes could be detected between the basal cells. The spinosum, granulosum and the cornified cell layers expressed almost the same as those of group 3, while the deeper layer of the cornified part retained their nuclei. The basal lamina appeared intact along its course, with clear lamina lucida and lamina densa. The anchoring fibers could be seen clearly in such group sample. The lamina propria showed mild vasodilatation and hyalinization. Numerous fibroblasts had somewhat preserved cytoplasmic organelles (Figs. 20 and 21).

Groups 5 and 6

The last two groups were almost with similar features in various cell layers with noticeable increase in the epithelial thickness compared to that observed in the previous groups. The basal cell layer of gingival epithelium had regular orientation. Their cell membrane showed more or less regular and smooth outline. The nuclei appeared clear, open faced with regular nuclear membrane and chromatin distribution. Intercellular cell junctions, mostly desmosomes, were numerous and dominant especially between the basal cells. The spinosum cells retained their polyhedral shape. The intercellular spaces appeared edematous and vacuolated. Their cytoplasm expressed less vacuolization than that of the previous group. Their nuclei showed regular nuclear membrane and peripheral nuclear chromatin condensation. The granular cell layer showed regular orientation. However, the extracellular spaces were still evident. The cornified layer appeared regular and their basal part retained some nuclei. No separation could be seen

between the cornified layer and the underlying granular cell layer. The basal lamina of the gingival epithelial layer was intact and definite, neither perforations nor interruptions were noticed in both lamina densa and lucida layers for these samples. The lamina propria of these samples appeared in much favorable condition than the previous samples regarding tissue hyalinization and vasodilatation. Fibroblasts were

numerous and clear in these samples. Their cytoplasmic contents appeared more preserved. The nucleus appeared elongated, open faced with regular nuclear membrane and peripheral chromatin condensation. The intercellular junctions between their cytoplasmic processes could not be detected clearly in these samples (Figs. 22 - 29).



Fig. 1

Fig. 1: Photomicrograph of rabbit's anterior teeth and gingiva during exposure to a bleaching light source that contains a plasma arc lamp .



Fig. 2



Fig. 3

Figs. 2 & 3: Photomicrographs of rabbit's gingival tissue after exposure to the bleaching light source. Gingival tissue appeared edematous and inflamed (arrows).



Fig. 4



Fig. 5

Fig. 4 & 5: Photomicrographs of rabbit's gingiva (arrows) following mucoalveolar flap just prior to gingival tissue excision.

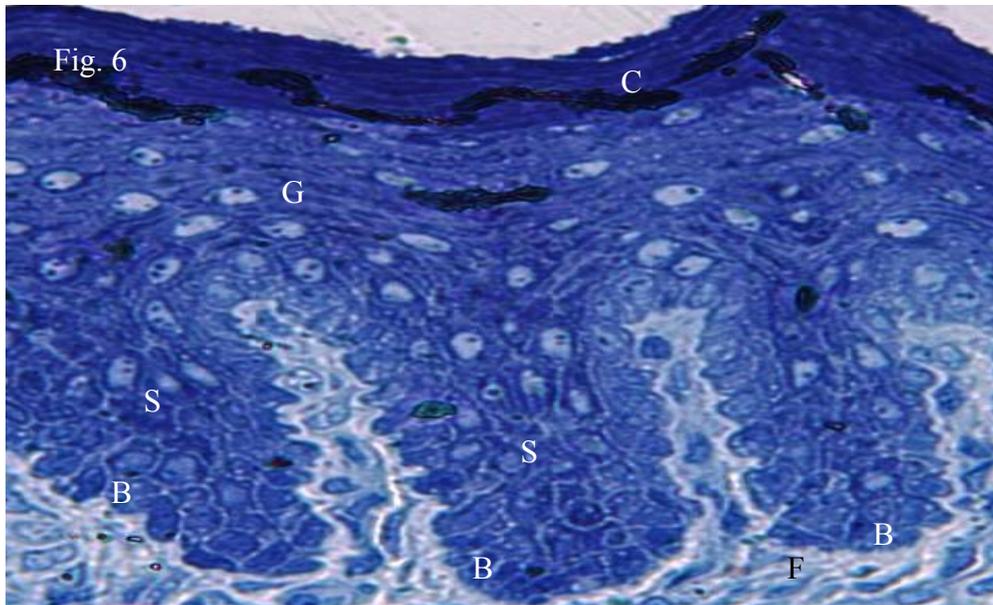


Fig. 6: Semi-thin section through rabbit's normal gingiva (group 1) showing basal cells (B), spinous cells (S), granular cells (G) and a superficial cornified layer (C). Note fibrous connective tissue containing spindle-shaped fibroblasts (F). (Toullidin blue stain X 400 original magnification).

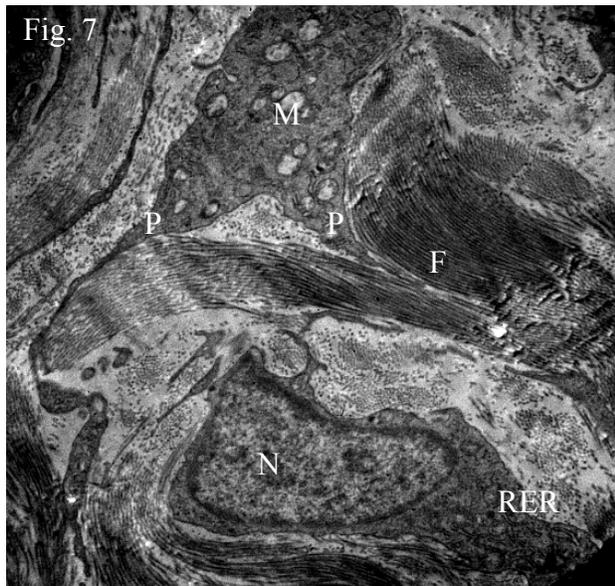


Fig. 7: Electronmicrograph of group 1, showing lamina propria of rat gingival tissue. (N) nuclus of fibroblast, (M) mitochondria, (P) cytoplasmic process, (RER) rough endoplasmic reticulum and (F) collagen fibers. X 8000 original magnification.

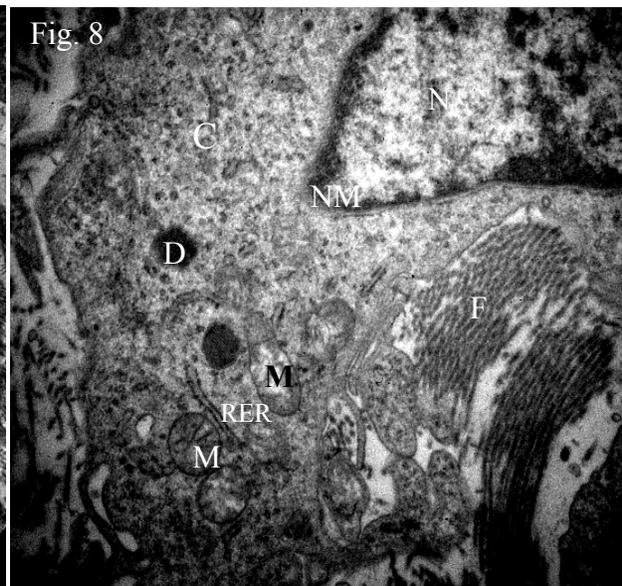


Fig.8: Electronmicrograph of group 1, showing fibroblastic cell. (N) nuclus, (M) mitochondria, (RER) rough endoplasmic reticulum, (D) electron dense spherical body containing fibers and (F) collagen fibers extracellularly. X 20000 Original magnification.

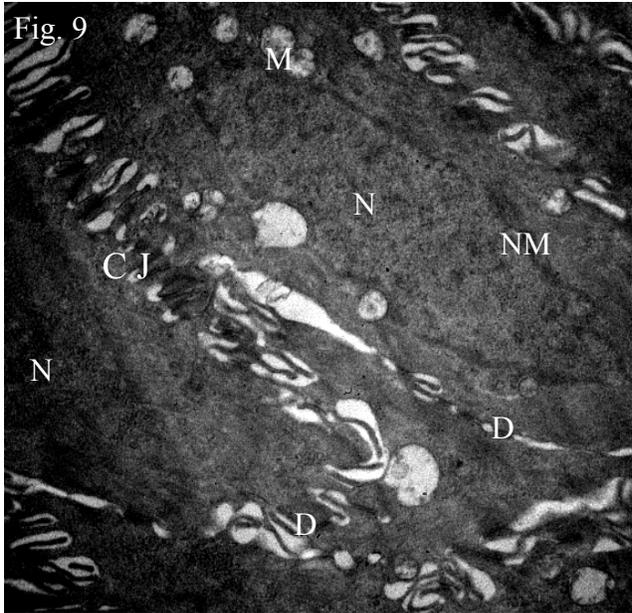


Fig. 9: Electronmicrograph of group 1, showing the basal cells of rat gingival epithelium. (N) basal cell nucleus, (NM) its nuclear membrane, (CJ) cell junctions and (D) desmosomes between basal cells. X 12000 original magnification.



Fig. 10: Electronmicrograph of group 1, showing basal cell with high magnification. (N) its nucleus, (NM) its nuclear membrane and (CJ) cell junctions. X 30000 original magnification.

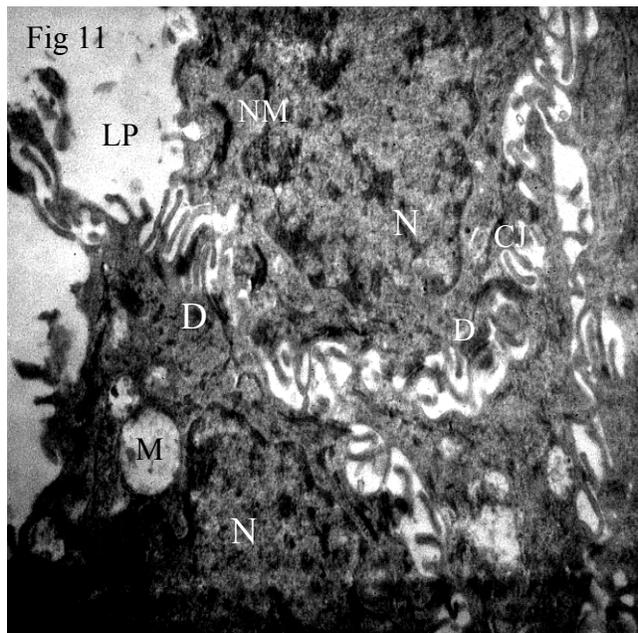


Fig. 11: Electronmicrograph of group 2, showing basal cells of gingival epithelium. (N) nucleus, (M) mitochondria, (D) desmosomes and (CJ) cell junctions. X 15000 original magnification.

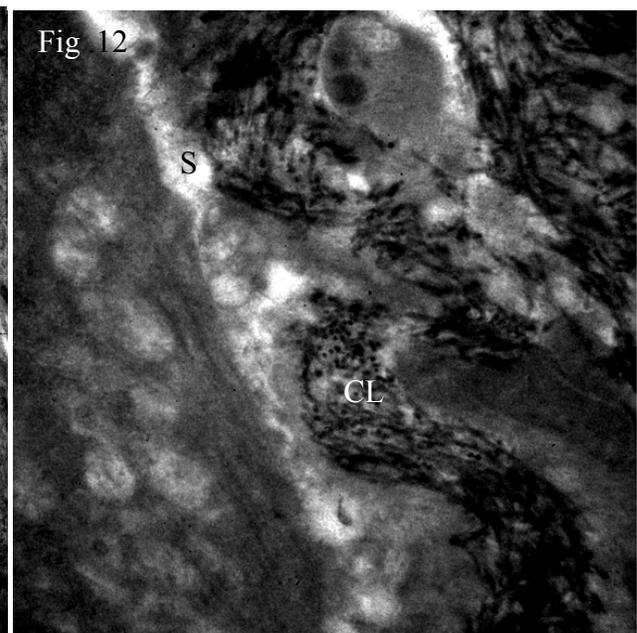


Fig. 12: Electronmicrograph of group 2, showing superficial cornified layer of gingival epithelium. (CL) cornified layer and (S) separation area. X 20000 original magnification.

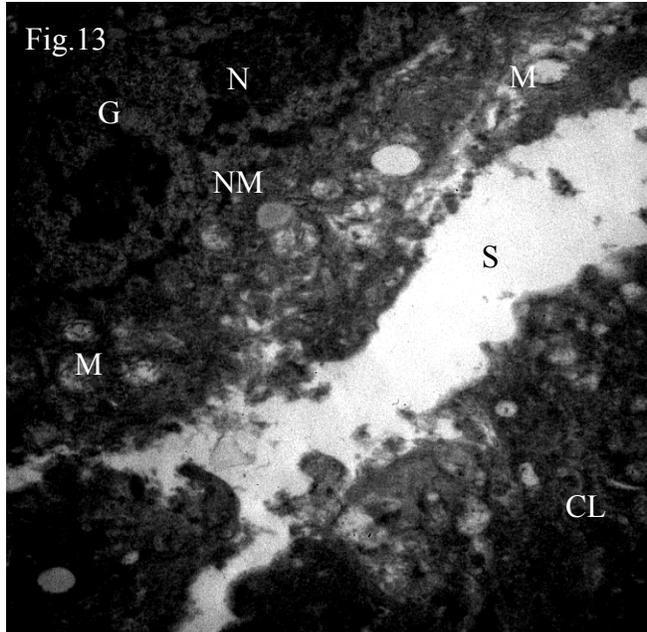


Fig.13: Electronmicrograph of group 2, showing the separation cleft in the superficial part of gingival epithelium. (G) granulosum layer, (N) nucleus, (NM) nuclear membrane, (M) mitochondria and (CL) cornified layer. X 15000 original magnification.

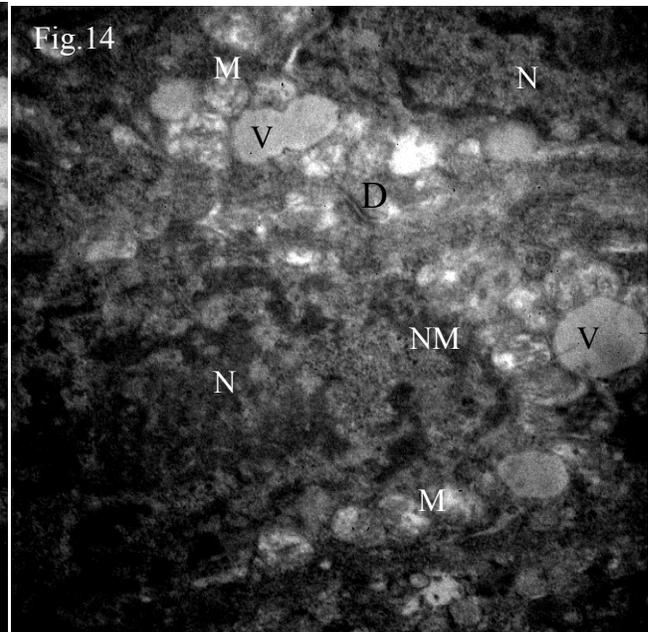


Fig.14: Electronmicrograph of group 2, showing the granular cell layer. (N) nucleus, (NM) nuclear membrane and (M) mitochondria, (V) intracellular vacuole and (D) desmosome. X 15000 original magnification.

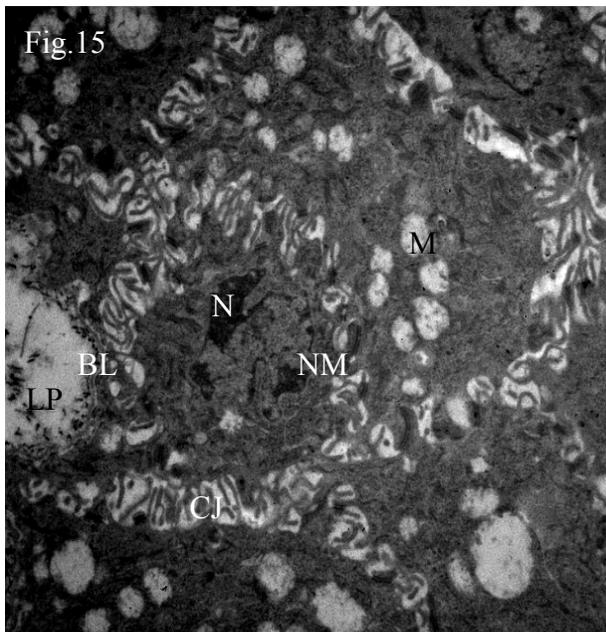


Fig.15: Electronmicrograph of group 3, showing basal cells of gingival epithelium. (N) nucleus, (NM) irregular nuclear membrane, (M) mitochondria, (CJ) cell junctions, (BL) basal lamina, and (LP) underlying lamina propria. X 8000 original magnification.

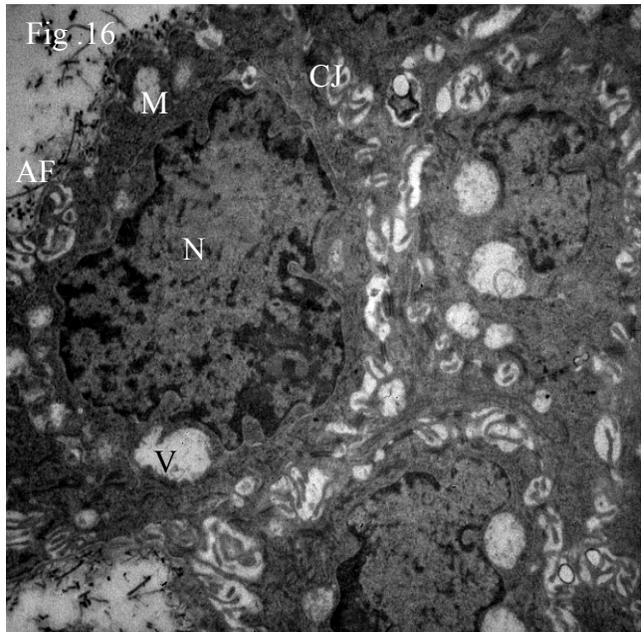


Fig.16: Electronmicrograph of group 3, showing higher magnification of basal cells. (N) nucleus, (V) intracellular vacuole, (CJ) cell junctions, (D) desmosomes, (M) mitochondria and (AF) anchoring fibers. X 15000 original magnification.

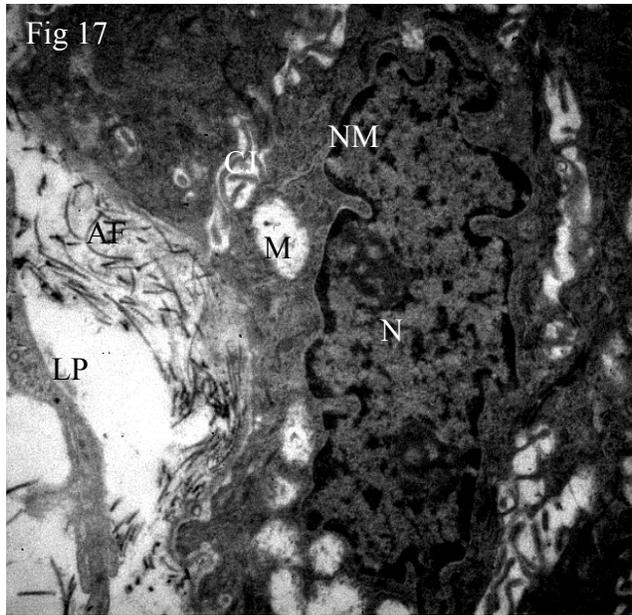


Fig. 17: Electronmicrograph of group 3, showing basal cell. (N) nucleus, (NM) nuclear membrane, (M) mitochondria, (CJ) cell junctions and (AF) anchoring fibers. X 15000 original magnification.

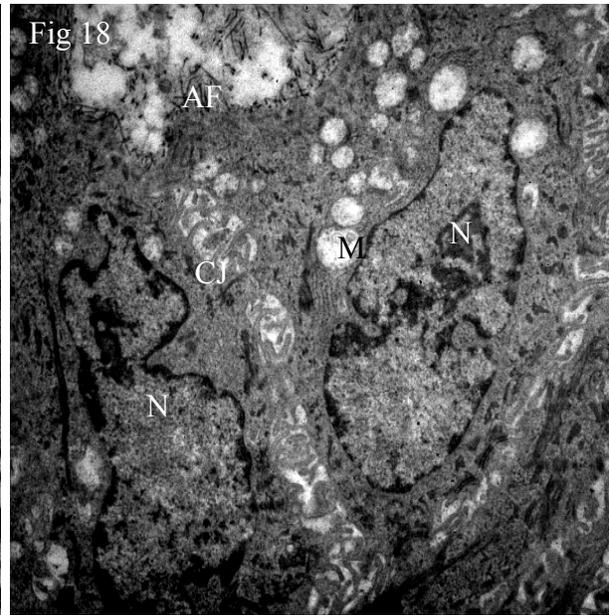


Fig.18: Electronmicrograph of group 3, showing nuclear mitosis in basal cells. (N) nucleus with central constriction, (M) mitochondria, (CJ) cell junctions and (AF) anchoring fibers. X 10000 original magnification.

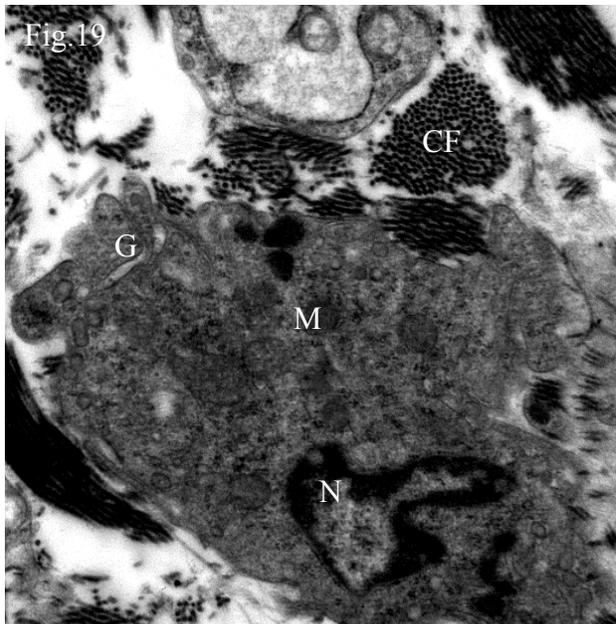


Fig. 19: Electronmicrograph of group 3, showing fibroblastic cell in gingival lamina propria. (N) nucleus, (M) mitochondria, (G) golgi and (CF) collagen fibers. X15000 original magnification.

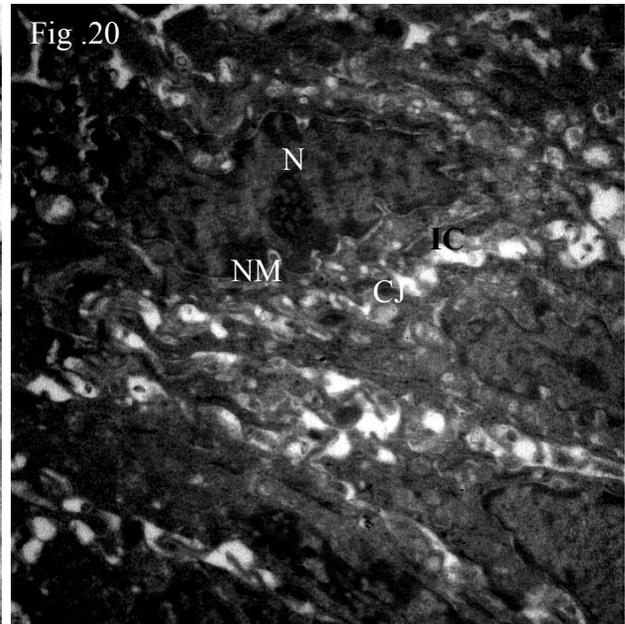


Fig. 20: Electronmicrograph of group 4, showing basal cell layer of gingival epithelium. (N) nucleus, (NM) nuclear membrane, (CJ) cell junctions and (IC) intercellular spaces. X 12000 original magnification.

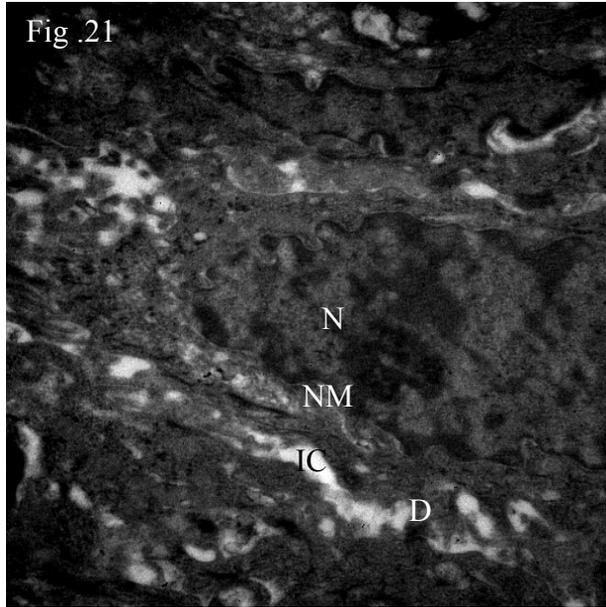


Fig. 21: Electronmicrograph of group 4, showing basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane, (IC) intercellular spaces and (D) desmosomes. X 15000 original magnification.

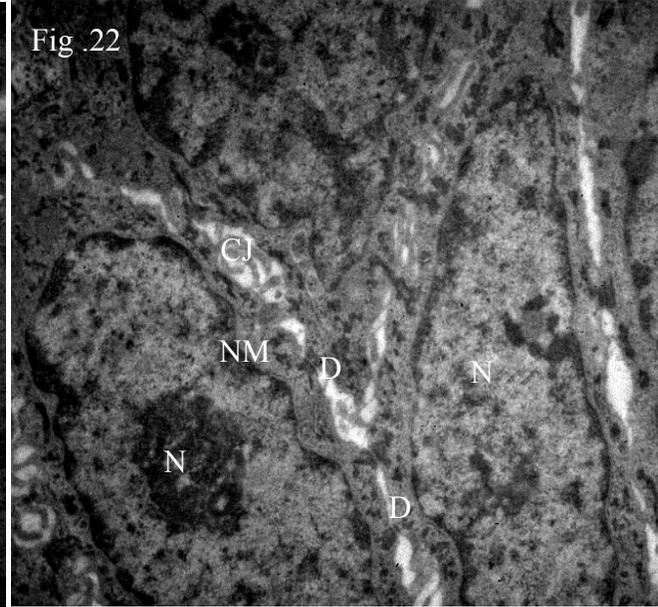


Fig. 22: Electronmicrograph of group 5, showing basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane, (CJ) cell junctions and (D) desmosomes. X 15000 original magnification.

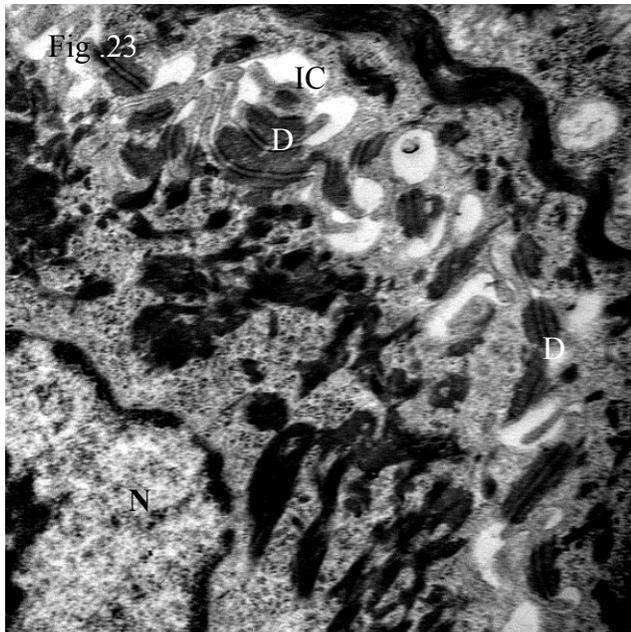


Fig. 23: Electronmicrograph of group 5, showing higher magnification of basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane and (D) desmosomes. X 25000 original magnification.

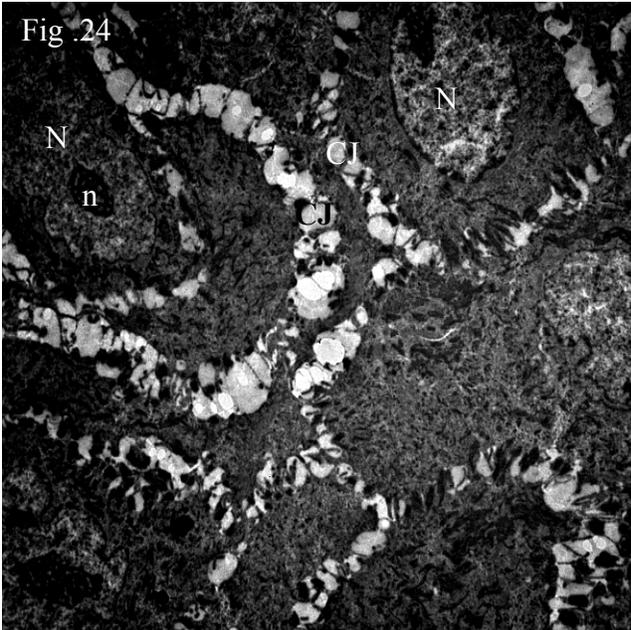


Fig. 24: Electronmicrograph of group 5, showing spinosum cell layer of gingival epithelium. (N) nucleus, (n) nucleolus and cell junction (CJ). X 8000 original magnification.

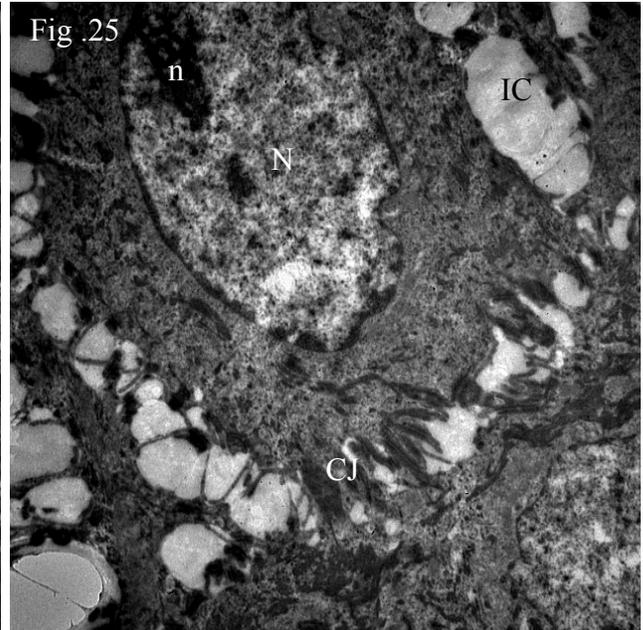


Fig. 25: Electronmicrograph of group 5, showing higher magnification of spinosum cells. (N) nucleus, (n) nucleolus, (CJ) cell junctions and (IC) intercellular spaces. X 15000 original magnification.

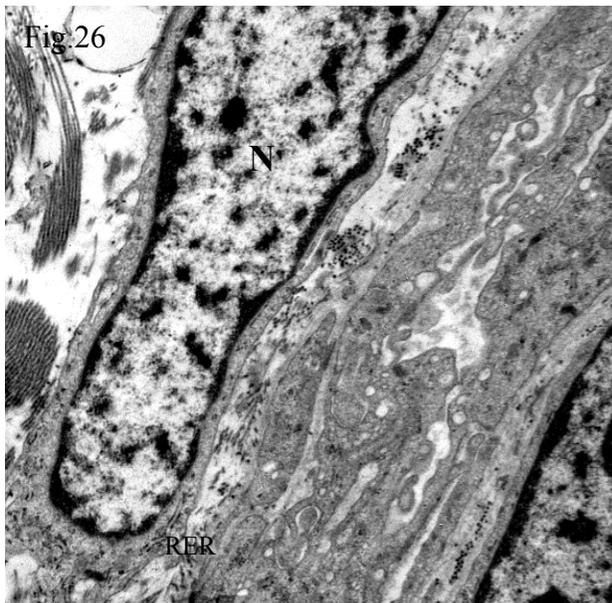


Fig.26: Electronmicrograph of group 5, showing fibroblastic cells of gingival lamina propria. (N) nucleus and (RER) rough endoplasmic reticulum. X 20000 original magnification.

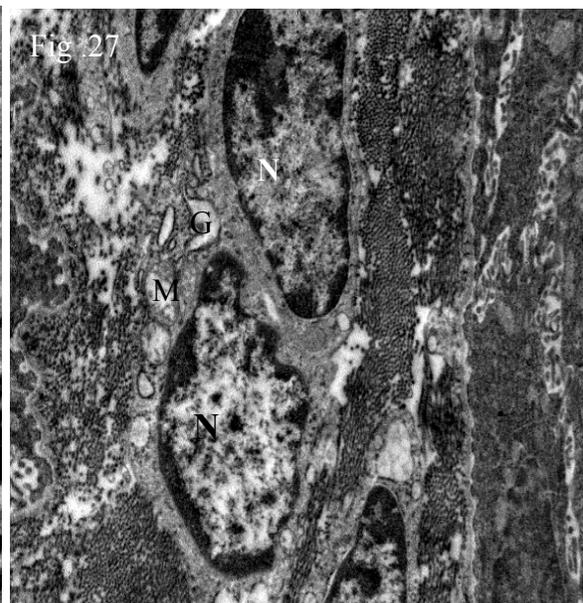


Fig. 27: Electronmicrograph of group 5, showing fibroblasts. (N) nucleus, (M) mitochondria and (G) golgi. X 15000 original magnification.

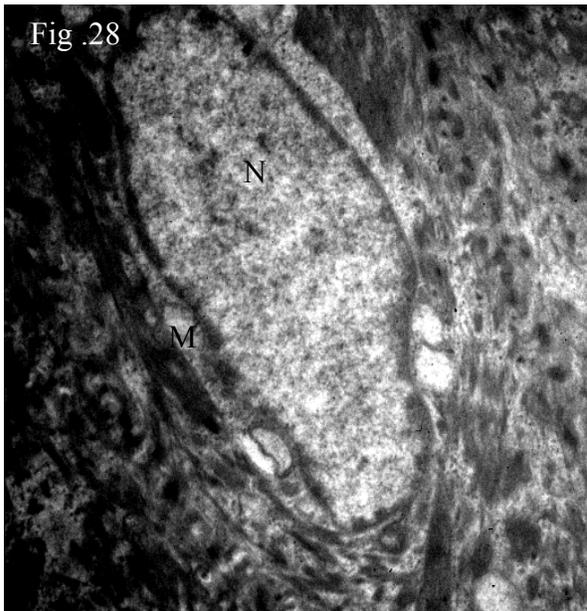


Fig. 28: Electronmicrograph of group 6, showing basal cells of gingival epithelium. (N) nucleus and (M) mitochondria. X 20000 original magnification.

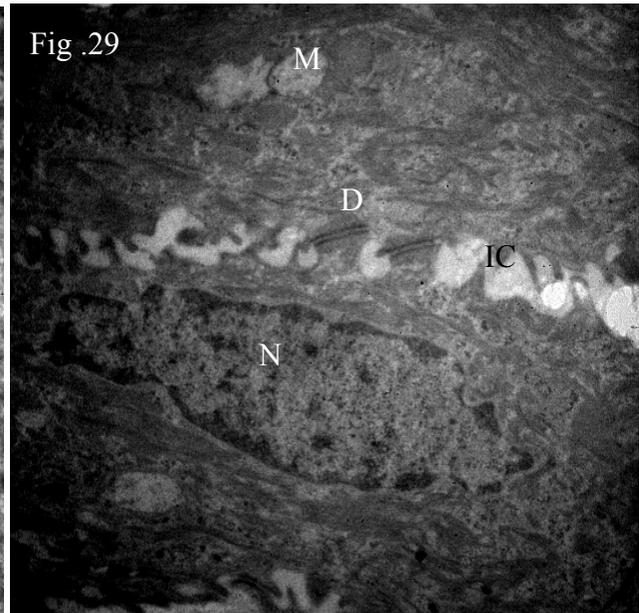


Fig. 29: Electronmicrograph of group 6, showing basal cell of gingival epithelium. (N) nucleus, (M) mitochondria, (D)desmosomes and (IC) intercellular spaces. X 15000 original magnification.

4. Discussion

Much debate still surrounds many of the noted tooth bleaching side effects/adverse effects. Qualifiers having been outlined, it has found that the most published side effects fall into various categories including tooth sensitivity, gastrointestinal mucosal irritation, changes to enamel and dentin hardness and surface structure, changes associated with internal bleaching. Gingival irritation is another common side effect that is experienced during a tooth whitening treatment. Gingival irritation may be chemically or physically induced teeth whitening problem.

In the present study, almost similar cellular morphological findings of the gingiva of the male Germany rabbits to that reported in human gingiva. The pattern of mucosa of the gingiva (epithelium and subepithelial connective tissues) seen in the low power displayed similar features that are commonly encountered in the human gingiva. Moreover, at a high magnification micrograph, details of membranous and various cellular components and structures revealed similar findings as in the human.⁽²⁹⁾ These results indicate that the rabbits' gingiva is a successful model for this experiment.

The present study revealed that the effects of bleaching agents on soft tissues in the mouth may adversely affect not only the gingival epithelium but

also the subepithelial tissue elements if they gain access to the underlying gingival connective tissue. The present TEM results revealed that hydrogen peroxide in a combination with heat generation caused early alterations in gingival mucosa at the 24 hours (group 2) following the bleaching application compared to group 1. This indicates that the gingival tissue did not remain healthy. Serious ultrastructural changes encountered epithelial cell pattern and morphology, in addition to the extracellular matrix and cytoplasmic alterations. The cytoplasmic organelles including mitochondria, RER, ribosomes revealed some sort of distortion. This distortion extended to include nuclear membrane and chromatin in addition to the mitotic figures. Separation between the cell layers with appearance of clefts was obvious. Moreover, similar degenerative observation was seen in the fibroblasts including reduction in cytoplasmic and nuclear volume with partial loss of the intercellular junctions between the fibroblastic cell processes. The presence of inflammatory cells in between the transversal and longitudinal collagen fibers was prominent feature. Similarly, gingival tissues of dogs respond to a continuous application of 1% H₂O₂ solution over 48 hours. Features of edema, followed by epithelial vacuolisation and finally destruction and sloughing of the cornified layer were reported. A

cellular response similar to that in acute inflammation occurred. In addition, increase in vascular permeability is likely, as there is severe edema, a large number of acute inflammatory cells, hemoconcentration in blood vessels and presence of fibrin strands were seen.⁽²⁰⁾ At a cellular level, Schraufstatter *et al.*⁽²¹⁾ demonstrated an induction of poly-ADP-ribose polymerase activation followed by NAD depletion and a fall in ATP, resulting eventually in cell death

In general, the results of the present study are considered as representative with the most commonly observed clinical effects of treatments with tooth whiteners of oral mucosa in some.^(6,10,12) Some patients have also reported burning palate, throat and gingiva.⁽¹⁸⁾ Several simultaneous developments to cause any observable effects: direct contact of hydrogen peroxide with tissues, the failure of normal human antioxidant defenses, the access of free radicals to target DNA, and the failure of damaged DNA to repair itself.⁽¹⁵⁾ As hydrogen peroxide is capable of producing free radicals (oxygen species with an unpaired electron) which are highly reactive, it can damage proteins, lipids, and nucleic acids.⁽¹⁰⁾

The mechanism by which epithelial cells are irritating could be explained as the initial diffusion of peroxide into and through the epithelial layers reach the connective tissue elements by the oxidizing hydrogen peroxide irritation that is hypothesized to originate from the dehydrating effects of ingredient used to carry the active bleaching ingredient. The irritation is presumed to result from escape of the bleaching material into the gingival margin area where salivary flow is typically low, allowing the material to sit relatively undisturbed. The hydrogen peroxide can cause an acute inflammatory reaction and some of the other components of the bleaching solution can dehydrate tissues. Noticeably, gingival irritation was much more likely to occur when the overlying epithelium was abnormally thin or permeable.^(22, 27, 28) that was detectable in group 2 where the basal lamina was markedly interrupted along its course. Also the anchoring fibers were not detected clearly in samples of this group.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at one week period (group 3), comparing to group 2, and revealed moderate cellular and nuclear alterations. Nuclear mitosis was very dominant. Although, the basement membrane was in favorable situation with well defined anchoring fibrils into the underlying connective tissue, the subepithelial connective tissue elements showed noticeable dilatation of vascular channels. This indicates to the incidence of adverse reactions to include the subepithelial connective tissue elements through a permeable basement membrane.

At two weeks period (group 4), the TEM results revealed that hydrogen peroxide in a combination with

heat generation revealed minimal alterations, comparing to group 3. The basal cell layer of the epithelial layer exhibited nearly regular orientation with irregular cell membrane and enlarged nuclear-cytoplasmic ratio. Interestingly, the concomitant features of mitosis of the previous groups are not present. Mitochondria showed severe hyalinization and loss their internal cristenae. RER appeared with detached ribosomes. The intercellular spaces are still wide and nearly nil desmosomes could be detected clearly between the basal cells. The anchoring fibers could not be seen clearly as in group 3. The superficial part of the cornified layer showed complete separation in some areas, while the deeper layer retained their nuclei. The remaining cell layers of gingival epithelium and lamina propria attained nearly the same ultrastructural features as seen in group 3. Similarly, Up to two weeks, Matis *et al.*⁽²⁵⁾ reported 79% incidence of gingival sensitivity. Contrarily, Nathoo *et al.*⁽²⁴⁾ reported no adverse effects of 2-weeks bleaching with 10% carbamide peroxide. Also, Kowitz *et al.*⁽²³⁾ reported almost no adverse events were reported during this 2-weeks exposure except 1% tooth sensitivity.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at one month-period (group 5), compared to group 4, revealed minimal alterations. The basal cell layer almost had regular orientation. Their cell membrane showed more or less regular and smooth outline. Their nuclei were markedly open faced with regular nuclear membrane and chromatin distribution. The intercellular spaces were still present but lesser than in the group 4. Intercellular cell junctions, mostly desmosomes, were numerous and dominant especially between the basal cells. The spinosum cells retained their polyhedral shape. The intercellular spaces appeared edematous and vacuolated. Their cytoplasm expressed less vacuolization than that of the previous group. The nuclei showed regular nuclear membrane and peripheral nuclear chromatin condensation. The granular cell layer showed regular orientation. However, the extracellular spaces were still evident. The cornified layer appeared regular and their basal part retained some nuclei. No separation could be seen between the cornified layer and the underlying granular cell layer, compared to that seen in samples of groups (2). The fibroblastic cell in the lamina propria appeared in much favorable condition than the previous group, the cytoplasmic contents appeared more preserved. The nucleus appeared elongated, open faced with regular nuclear membrane and peripheral chromatin condensation. The intercellular junctions between their cytoplasmic processes could not be detected clearly in this sample. Similarly, Up to one month: a 3-weeks exposure to 10% carbamide peroxide, 95 of the subjects reported tooth sensitivity and 32% reported minor oral discomfort.⁽⁸⁾ Contrarily, Kozlovsky *et al.*⁽⁹⁾

reported that none of the subjects had oral soft tissue irritation.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at two months-period (group 6), comparing to group 5, revealed almost normal finding, comparing to group 1. Contrarily, Beyond one month, A whitening product with 10% carbamide peroxide was used for 5 weeks on 5 women smokers and 6 women who were not smokers. The authors found with the use of biopsies an increase in the thickness of the epithelium producing an increase in cellular proliferation in the basement and parabasal membranes of the gingival epithelium. The authors pointed out that it is not possible to conclude that 10% carbamide peroxide is carcinogenic in clinical situations, but, it was possible to observe that it alters cellular proliferation and consequently, it could act as a tumor promoter.⁽¹⁶⁾

The gradual decreasing of the irritating effect of bleaching on gingival tissue elements through the experimental periods is in consistence with other studies where the adverse effects were transient.⁽²³⁻²⁵⁾ This suggests that bleaching has only a limited and/or reversible. This can be attributed as: in gingiva, there is sufficient antioxidant defensive mechanisms that protect the tissue from radicals generated from the reaction of hydrogen peroxide, and defense mechanism of the gingiva would significantly reduce available levels of hydrogen peroxide. The reason why hydrogen peroxide is considered as a risk factor to our health is because it is a highly oxidative compound and easily decomposes into hydroxyl radicals. As a free radical with an unpaired electron, the hydroxyl radical readily attacks other molecules in its proximity and produces a new free radical and so on. The resulting damage, referred to as oxidative stress, leads to molecular and cellular dysfunction. The destruction of essential macromolecules by oxygen-based reactants is the basis of some diseases and is also believed to be involved in the processes of aging.⁽²⁶⁾

In conclusion, caution should be exercised with the application of peroxide products used for bleaching due to the possibility of chemical irritation of oral soft tissues with injudicious use. The volumes of material and application times should be controlled carefully. It is recommended to use isolation because it is vital in the chair side process to prevent tissue irritation. Similarly, retractors are important to provide complete protection of the lips and soft tissue while allowing access to the facial side of teeth and gums.

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5.References:

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