Scanning electron microscopic study of visible light curing effects on the oral mucous membrane

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دراسة تفرّسية بالمجهر الإلكتروني لتأثيرات المعالجة بالأشعة الضوئية المرئية على الغشاء المخاطي للفم

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خلاصة: أجريت هذه الدراسة على الفئران لتوضيح التغيرات البنيوية التي حدثت بعد تعريض اللثة لوحدة معالجة الأسنان بالأشعة الضوئية المرئية. فتم تعريض ستة عشر فأراً للأشعة، بينما اعتبر أربعة فئران كمجموعة شاهدة. وأسقطت الأشعة على الضرس الأول الأيمن بالفك السفلي لمدة 40 ثانية. ثم قسمت الحيوانات إلى أربع مجموعات (بكل منها أربعة فئران تجريبية وفأر من المجموعة الشاهدة). وذبحت إحدى هذه المجموعات على الفور، والثانية بعد يومين من التعرض والثالثة بعد أربعة أيام والأخيرة بعد أسبوعين. وأخذت عينات للفحص بالمجهر الإلكتروني والمجهر الضوئي. وتبيّن النتائج أن الأشعة المنبعثة من وحدات معالجة الأسنان بالأشعة الضوئية يمكن أن تؤثر على الغشاء المخاطي للفم وربما تخفض قدراته الوظيفية.

ABSTRACT The present investigation was undertaken on rats to demonstrate the structural changes that took place after the exposure of the gingiva to a dental visible light curing unit. Sixteen rats were irradiated and four were considered as controls. The mandibular right first molar was exposed to radiation for 40 seconds. The animals were classified into four groups (4 experimental and 1 control) and were sacrificed immediately, 2 days, 4 days and 2 weeks after exposure. Specimens were processed for ultrastructural and light microscopic investigations. The results indicate that emission from dental light curing units can affect the oral mucous membrane and may reduce its functional abilities.

Etude au microscope électronique à balayage des effets de la photopolymérisation sur la muqueuse orale

RESUME Cette étude a été réalisée sur des rats pour démontrer les changements structurels qui s'opèrent après exposition des gencives à un appareil de photopolymérisation. Seize rats ont été irradiés et quatre autres ont été considérés comme témoins. La première molaire mandibulaire droite a été exposée aux radiations pendant 40 secondes. Les animaux ont été classés en cinq groupes (quatre expérimentaux et un témoin) et ceux qui ont été irradiés ont été sacrifiés sur-le-champ, 2 jours, 4 jours et 2 semaines après l'exposition. Les spécimens ont été traités pour procéder à des examens ultrastructurals et au microscope optique. Les résultats indiquent que l'émission des appareils de photopolymérisation peut affecter la muqueuse orale et réduire ses capacités fonctionnelles.

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Introduction

Composite resins activated by ultraviolet light and visible light have become an essential part of the armamentarium of the dentist undertaking restorative, preventive and orthodontic dentistry [*1,2*]. Visible light in the blue region of the spectrum has gained popularity as a source over ultraviolet light because of its ability to produce a greater depth of polymerization [*3*], and to avoid possible eye damage [*4,5*].

Ophthalmic research into the appearance of blue light lesions has not ruled out the possibility that short wavelength light (visible light with wavelengths of less than 500 nm) may contribute to premature aging of the retina and to senile macular degeneration [*6*]. Near ultraviolet and blue light may also cause the formation of cataract [*7*]. This effect is photochemical rather than thermal or structural [*8*]. Visible light generators produce heat which may elevate the intrapulpal temperature and/or dehydrate the dentin [*9,10*]. In addition, the condition and quality of the pulpal vascularity may determine the degree of damage caused by thermal trauma [*11*].

Recently, visible curing lights have been used as an aid in oral diagnosis. More specifically, these lights have a potential application for use in transillumination with white light for the detection of caries and calculus and the examination of existing restorations [*12*]. However, the current use of blue light is not without its disadvantages since the oral soft tissues surrounding the tooth cannot be completely excluded from the area of light application.

The present study was carried out experimentally on rats to demonstrate the structural changes that occur after exposure of the gingiva to a dental visible light curing unit.

Materials and methods

Twenty (20) healthy adult male albino rats weighing 220–250 g were used as test animals. The animals were kept on a standard laboratory diet during the study.

The mandibular right first molar was exposed to radiation from a dental light-curing unit for 40 seconds using Litex 600. The fibre optic probe was held 1 cm from the area exposed to the light. Sixteen animals were irradiated and four were considered as controls. The animals were classified into four groups, each comprising four experimental and one control, and they were sacrificed after exposure as follows:

- *Group A*: sacrificed immediately after exposure
- *Group B*: sacrificed 2 days after exposure
- *Group C*: sacrificed 4 days after exposure
- *Group D*: sacrificed 2 weeks after exposure.

The rats were anaesthetized with ether and sacrificed by cervical dislocation. The specimens for histological examination were dissected from the experimental sites and the oral soft tissues surrounding the exposed mandibular first molar were excised. The specimens were divided into halves and immediately fixed in glutaraldehyde–formaldehyde fixative. Half of the specimens were fixed and processed for the scanning electron microscopic investigation: critical-point dried in liquid carbon dioxide, glued to aluminium stubs, sputter-coated with gold in a fine coat (Jeol JFC 1100 E ion sputting device) and examined under the scanning electron microscope (SEM) at 25 kV and 10^{-6} Å beam current (Jeol JSM–5300). The other halves were processed and embedded in paraffin by

conventional methods for the histological investigations using H&E stain and for the histochemical demonstration of Masson's trichrome and modified Mallory stain [*13*] and examined under the light microscope.

Results

Scanning electron microscopic findings

- *Control group.* Normal configuration of the epithelial projections and ridges (Figure 1) was detected, with normal appearance of the surface keratin cells where they appeared polygonal in shape with a pitted surface (Figures 1 and 2).
- *Group A.* Disappearance of the projections due to the effect of the light generated during exposure was noted, which resulted in intracellular oedema in comparison with the controls. Flattening of the undulated areas was apparent where the ridges were smoother and not covered by small blunt projections (Figure 3).
- *Group B.* Oedema resulted in destruction of the intercellular junctions leading to partial or complete loss of cellular adhesion. Areas of separation and sloughing were also noted. Flattening of the undulated areas was still apparent; they were covered with numerous small blunt projections arranged in bands which were separated by areas devoid of projections (Figure 4).
- *Group C.* Partial detachment of the keratin flakes was noted with missing squamae in some areas. Wide intercellular spaces between the superficial epithelial cells were still apparent (Figure 5).
- *Group D.* Delayed healing and regeneration of the keratin layer was seen with few areas of sloughing. The intercellu-

Figure 1 **SEM showing normal configuration of the epithelial projections and ridges (control x 3500)**

Figure 2 **SEM showing normal appearance of the surface keratin cells where they appear polygonal in shape with a pitted surface (control x 3500)**

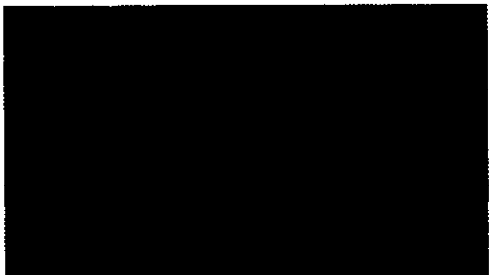

Figure 3 **SEM showing flatter surface area and disappearance of projections. The ridges are smoother and are not covered by small blunt projections (group A x 3500)**

Figure 4 **SEM showing flattening of the undulated areas which are covered by numerous small blunt projections arranged in bands and separated by areas devoid of projections. Note the sloughed areas (arrows) (group B x 3500)**

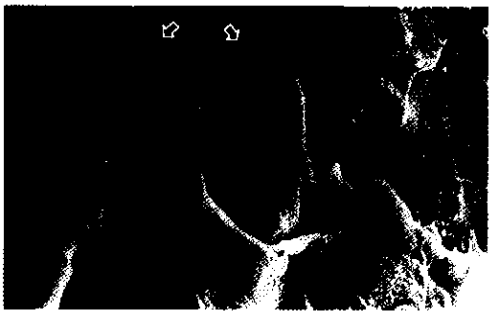

Figure 5 **SEM showing partial detachment of the keratin flakes (arrows) and areas of complete loss of squamae with areas of formation of new ones (group C x 2000)**

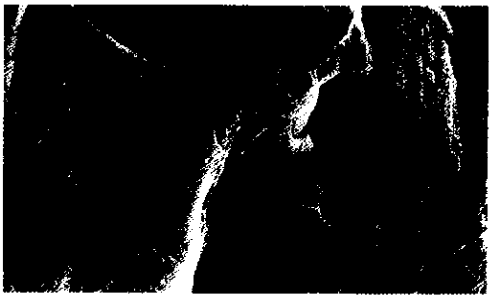

Figure 6 **SEM showing the rhythmic arrangement of the squamae in the surface keratin layer with loss of some superficial squamae (group D x 2000)**

lar spaces had started to be repaired with normal architecture (Figure 6).

Light microscopic findings

- *Control group*. The control group revealed regular configuration of the epithelial cell layers and underlying connective tissue (Figure 7).
- *Group A*. As a result of the immediate effect of light, the keratin layers were separated from each other with spaces in between (Figure 8). In some areas the keratin layer was completely lost (Figure 9).
- *Group B*. The effect of light on the keratin layer was still apparent; the whole thickness of the keratin layer was completely separated from the underlying epithelial layers and had started to shrink and wrinkle with rapid formation of a new layer of keratin where the cells attained their nuclei. The deep layers of the epithelium appeared active showing deeply stained enlarged nuclei indicating increased activity and mitotic figures. The subepithelial connective tissue (CT) showed alterations, including areas of oedema with the presence of inflammatory cells in between the collagen fibres (Figure 10).
- *Group C*. Restoration of the keratin layer had begun and it appeared homogeneous in structure. The basal cell layer appeared disturbed with prominent mitosis (Figure 11). The oedema in the subepithelial CT layer persisted, causing widening of the intercellular spaces in the deep layers of the CT, and dense, coarse collagen fibres and bundles directly beneath the basal cell layer were noted with dilated blood vessels and extravasated red blood cells (Figure 12).
- *Group D*. Increased epithelial thickness was noticed with a marked increase of

clear cells reaching the granular cell layer. The keratin layer appeared homogeneous with increased thickness (Figure 13). Early keratinization was evident near the prickle cell layer which obscured the granular layer (Figure 14). As a result of the increased mitotic figures at the basal cell layer, irregular configuration of the basement membrane with lateral extensions of the epithelial ridges gave rise to the drop-shaped appearance seen (Figures 14 and 15).

Discussion

Recent developments in filling resin technology for tooth repair and caries prevention have led to the widespread use of visible light curing systems [*14*]. Most resins are polymerized by light sources which have a powerful emission of visible light [*9,10*].

Possible eye damage induced by such light has been investigated [*7,8*]. Most studies were initiated after image experiences, hue discrepancies and loss of resolution were reported by users of visible-light-curing units [*15*]. The preliminary results have indicated ocular damage to individuals in close proximity to the emitting tip or reflected field [*4,6,15*].

All previous investigations on visible light curing units used in dentistry have concentrated on the dental filling materials to be cured and the efficiency of the different light curing units, but none has discussed their effect on the surrounding exposed tissues. The first attempt was an experimental pilot study to show the effect of irradiation with dental polymerized lamps on Langerhans cells (LC) in human skin transplanted to nude mice. They reported that radiation with small therapeutic doses from a dental light curing unit with a small fraction of ultraviolet light reduced or depleted the monoclonal T_6 surface markers of LCs in human epithelium [*16*].

In the present study, visible light radiation was applied to the mandibular first molar of rats for a period of 40 seconds, the shortest exposure time used in humans. An increase in exposure time has been recommended up to 180 seconds and more with the aim of decreasing the marginal contraction gap in dentine cavities, which is thought to reduce significantly from 6.1 µm to 3.7 µm [*17*]. Other studies have reported that the reduction in hardness with increased curing depth in teeth suggests that extending exposure times beyond those indicated will improve the depth of cure in clinical situations [*18*].

The temperatures produced by the curing methods differ significantly from one device to another. Bennett et al. reported that temperature increases have caused pulpal stigmata in monkeys [*14*], which is in agreement with other researchers [*9*] who concluded that light-curing units apply enough heat to the tooth surface to potentially damage the pulp. Wavelengths above 500 nm are responsible for the heat production but contribute little to the heat curing of the composite [19].

In the present study, the temperature changes that occurred during light exposure and the heat generated have been shown ultrastructurally to produce complete loss of keratin and flattening of the undulated areas with smooth ridges immediately after exposure. Further effects were noted with time and by two weeks healing and regeneration were starting. The histological findings confirmed the scanning electron microscopic results. These findings are in agreement with a similar study which was carried out using ultraviolet light instead of visible light on rat gingival mucosa, although they did not observe an

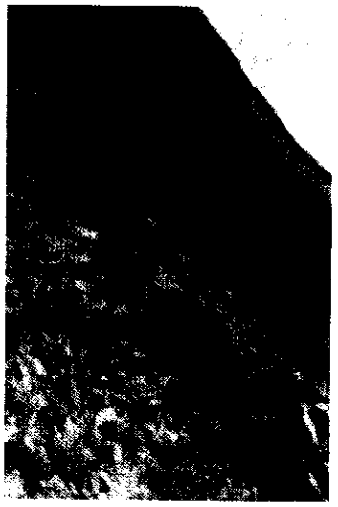

Fig. 7

Fig. 8

Fig. 9

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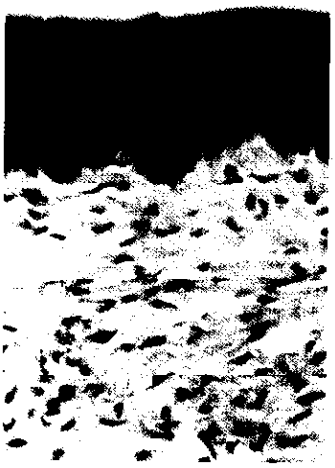

Fig. 11

Figure 7 **Photomicrograph showing normal architecture of the epithelial cell layers and underlying connective tissue (control, H&E x 400)**

Figure 8 **Photomicrograph showing wide intercellular spaces between epithelial cell layers. The keratin layers appear separated from each other with spaces in between them (group A, Mass. trich. x 400)**

Figure 9 **Photomicrograph showing complete loss of the keratin layer in some areas (group A, Mass. trich. x 400)**

Figure 10 **Photomicrograph showing complete detachment and wrinkling of keratin layer with rapid formation of new keratin attaining nuclei just beneath it. There is increased mitosis in the basal and suprabasal layers (group B, Mass trich x 400)**

Figure 11 **Photomicrograph showing disturbance of the basal cell layer with prominent mitotic figures and persisting oedema among the collagen fibres within the lamina propria of the connective tissue (group C, Mass. trich. x 400)**

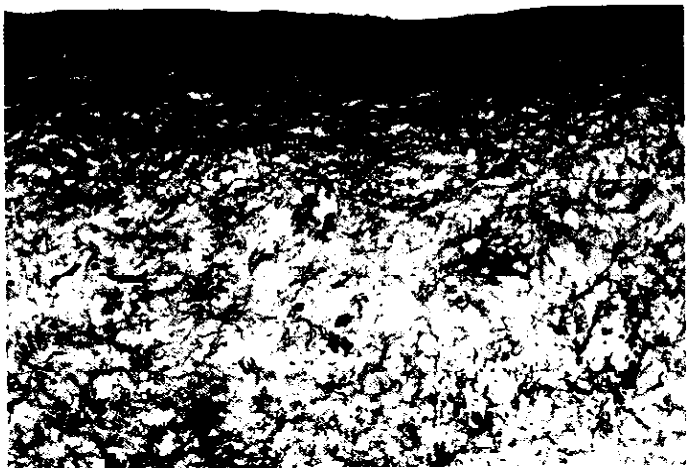

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Figure 12 **Photomicrograph showing thick, coarse, densely packed collagen fibres and bundles directly beneath the basal cell layer with the presence of oedema, dilated blood vessels and extravasated red blood cells in the deeper layers of the connective tissue with increased thickness of keratin (group C, mod. Mallory × 100)**

Figure 13 **Photomicrograph showing increased epithelial thickness with a marked increase of clear cells reaching the granular cell layer. Surface keratin appears homogeneous with increased thickness. The mitotic figures are still a prominent feature of the basal cell layer (group D, H&E × 400)**

Figure 14 **Photomicrograph showing increased mitotic figures within the irregular basal cell layer. Early keratinization is obviously marked near the prickle cell layer obscuring the granular layer (arrows). The collagen fibres are regular and coarse in appearance within the lamina propria (group D, mod. Mallory × 400)**

Figure 15 **Irregular configuration of the basement membrane with increased epithelial ridges and irregular arrangement of the basal cell layer with even thickness of the keratin layer (group D, Mass. trich. × 400)**

apparent pattern of increase in thickness of epithelium and keratin as we did. They concluded that substantial amounts of long-wave radiation are transmitted by the oral mucosa but with no permanent damage [*20*].

The findings in the present study may be attributed to the fact that epithelial cells take about 5 days to a week to reach the surface layer [*21*]. Because of the highly active nature of the oral mucosa, some morphological variations from its normal appearance could possibly occur supporting the above-mentioned study, which found that the epithelial lining in rats was restored to its normal condition after exposure to radiation.

The increased mitotic figures in the basal and suprabasal layers, resulting in the drop-shaped appearance of the epithelial ridges with lateral extensions, may be considered a reflex reaction of the epithelium to compensate for the missing superficial layer of keratin due to light exposure; it could be considered as a defence mechanism of the epithelium to protect the underlying connective tissue.

Conclusion and recommendations

The present study has shown that radiation, even with small therapeutic doses from a visible light curing unit used in clinical practice, affects the gingival tissues adjacent to the exposed tooth. It should be noted that although the results indicated that the effects of the radiation were only temporary, it does not mean that long-term effects might not result. Structural changes may be accentuated with prolonged exposure time to curing units.

The soft oral tissues must be taken into consideration and protected from the area of exposure to light to prevent damage and to guard against hazardous alterations. With new units entering the market, temperature control should be a factor of utmost concern and priority. If the soft tissues cannot be protected from the area of exposure, another type of filling that does not need light curing should be considered.

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