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Shahid H. Ashrafi
University of Illinois at Chicago

Krzysztof Slaski
University of Illinois at Chicago

Kyaw Thu
University of Illinois at Chicago

Eli S. Neiman
University of Illinois at Chicago

Michael P. Cunningham
University of Illinois at Chicago

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SCANNING ELECTRON MICROSCOPY OF CYCLOSPORINE-INDUCED GINGIVAL OVERGROWTH

Shahid H. Ashrafi[1,2,*], Krzysztof Slaski[3], Kyaw Thu[1], Eli S. Neiman[1] and Michael P. Cunningham[3]

[1]Dept. Oral Biology, [2]Dept. Oral Medicine and Diagnostic Science, and [3] Dept. Periodontics, College of Dentistry, University of Illinois at Chicago, Chicago, IL 60612

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Abstract

Overgrown human gingival specimens were examined histologically and by scanning electron microscopy (SEM) to study structural changes caused by cyclosporine. The biopsy specimens were from organ transplant recipients receiving cyclosporine to suppress the rejection of the transplanted organ. The epithelium of the overgrown gingiva was thickened, acanthotic and parakeratotic. Retepegs were anastomosing and extending into connective tissue. The SEM examination of the outer surface of the attached gingival showed loss of cellular attachments and cells were exfoliating. The normal honeycomb structure formed by interconnecting microvilli surrounding the pits was distorted. Outer gingival cell surface showed numerous round, ovoid and dome-like structures instead of parallel, reticular or fingerprint-like microridges.

It was concluded that cyclosporine not only caused hyperplasia but also changed the structure of the outer epithelial cell surface.

Key Words: Gingival overgrowth, cyclosporine, human, scanning electron microscopy, keratinization, microridges.

*Address for correspondence:
Shahid H. Ashrafi
Department of Oral Biology (M/C 690),
UIC College of Dentistry,
801 South Paulina Street,
Chicago, IL 60612
Telephone number: (312) 996-8559
FAX number: (312) 996-1022

Introduction

Cyclosporine is widely used in therapy for patients undergoing organ transplants to prevent rejection of transplanted organs (Calne *et al.*, 1979). It is a cyclic polypeptide produced by the metabolism of two fungi (Wenger, 1982). The mechanism involved in cyclosporine action on immune response is not clear but widespread continued use of cyclosporine produces nephro- and hepatotoxicity, hypertension, thrombosis, stenosis of renal arteries (Bennett and Norman, 1986) and gingival overgrowth (Tyldesley and Roter, 1984; Rostock *et al.*, 1986; Savage *et al.*, 1986; Niimi *et al.*, 1990). Most of the studies related to cyclosporine induced gingival overgrowth (CsG) deal with light microscopic observations and a few with transmission electron microscopy (Bennett and Christian, 1985; Lambertenghi-Deliliers *et al.*, 1986, Belazi *et al.*, 1993; Mariani *et al.*, 1993). Pisanty *et al.* (1990) studied the effects of cyclosporine A on epithelial-stromal function by using scanning electron microscopy (SEM) and showed irregularly extended epithelial projections. They concluded that enlargement of gingival tissues was not due to increased collagen production, but rather implicated the epithelium as the main cause. To our knowledge, there is no detailed report about the effect of cyclosporine on the cell surface structure of the attached gingival overgrowth induced by cyclosporine (Gonzalez-Jaranay *et al.*, 1990). Therefore, the present work was undertaken to study in more detail the epithelial cell surface structure of the attached gingival overgrowth induced by cyclosporine, by using SEM.

Materials and Methods

The cyclosporine-induced overgrown gingival biopsies from 3 patients and 2 normal human gingival biopsies were obtained. Controls consisted of uninflamed biopsies of attached gingiva taken during fixing crowns on teeth. These control biopsies specimens were prepared in a similar way as cyclosporine-induced gingival overgrowth tissues. Biopsies were washed in

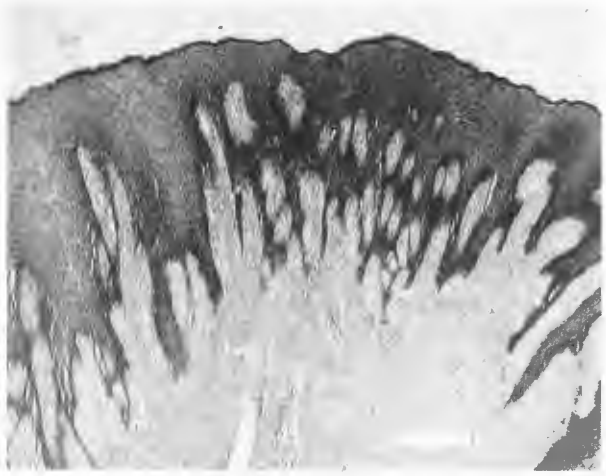


Figure 1. Light micrographs of a hematoxylin and eosine stained section of cyclosporine induced gingival overgrowth showing thickened, parakeratotic, acanthotic epithelium with irregularly elongated retepegs. Bar = 100 μm.

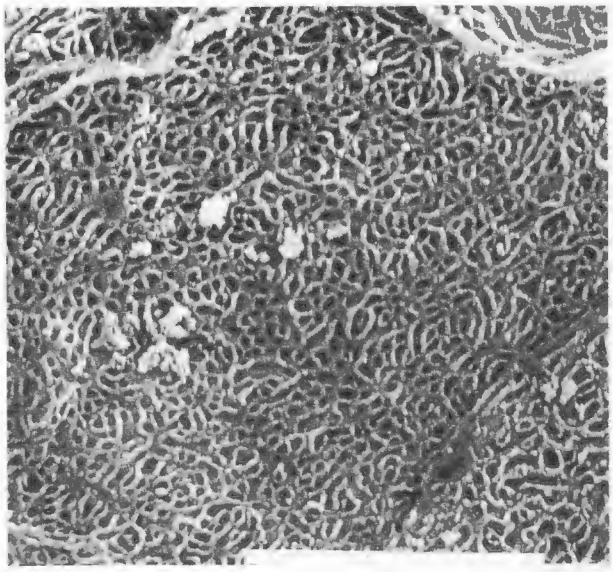
cold phosphate buffered saline for 30 minutes. They were fixed in 10% formalin for light microscopy and in 2.5% glutaraldehyde made in 0.1 M phosphate buffer of pH 7.2, for four hours at 4°C.

Portions of overgrown gingival tissue fixed in formalin were embedded in paraffin, and 4 μm thick sections were cut for light microscopy. Routine staining with hematoxylin and eosine was carried out for histological examination of hyperplastic and hyperkeratotic tissue morphology.

The specimens fixed in glutaraldehyde for SEM were washed three times with 15 minutes interval each time. Then, they were dehydrated in graded series of alcohol and critical point dried, employing liquid carbon dioxide. The dried specimens of the attached gingiva, outer surface facing up, were glued to the SEM-aluminum studs. They were coated with 20 Å thick film of gold-palladium in a Polaron coating unit E5150. The cell surface pattern of the outer gingival epithelium was examined using a JEOL-35C scanning electron microscope (JEOL USA, Peabody, MA). The specimens were examined at 0° tilt angle and a working distance of 15 mm, with a lens current of 90 μA and an accelerating voltage of 20 kV.

Results

The gingival overgrowth induced by cyclosporine begins in the interdental papillae area and extends to the labial region on the vestibular side. The surface was of a nodular type. Histological features of gingival overgrowth showed a thickened, parakeratotic and acanthotic epithelium and long retepegs extending into lamina propria (Fig. 1). Necrotic areas showing collagen destruction, chronic infiltration of inflammatory cells, irregularly arranged connective tissue and some fibroblast were also noticed.



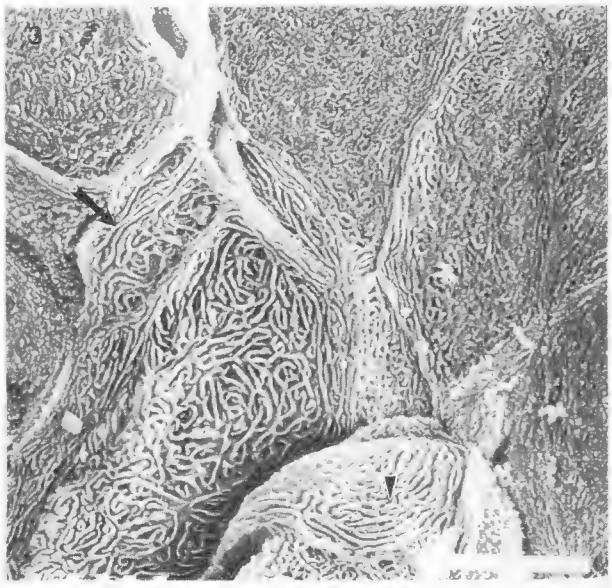


Figure 2. Scanning electron micrograph of keratinized normal human attached gingiva showing a network of interconnected microridges surrounding pits with a honeycomb appearance. Bar = 10 μm.

Figure 3. Scanning electron micrograph of normal human gingiva showing a cell surface with a different arrangement of microridges. Note parallel (arrow) and fingerprint-like pattern (arrowhead) of microridges. Bar = 10 μm.

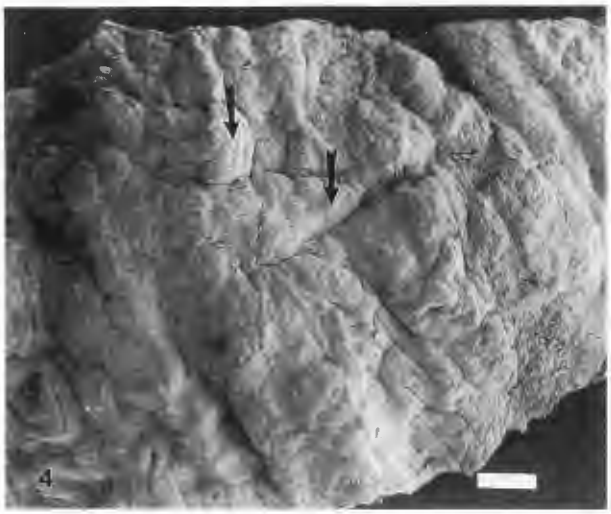


Figure 4. Scanning electron micrograph of the surface of cyclosporine-induced human gingival overgrowth (CsG) showing shiny irregular folds (arrows). Bar = 200 μm.

Figure 5. Higher magnification (of Fig. 4) of CsG showing keratin plugs (KP), a necrotic area (arrows) and desquamating cells. Bar = 100 μm.

Scanning electron microscopy

The SEM examination of the normal attached gingival epithelial surface showed various arrangements of microridges. The prominent feature was a type of honeycomb where microridges were encircling the holes of different sizes (Fig. 2). The surface of other epithelial cells showed a parallel arrangement of microridges and curved rows of microridges forming finger-print type

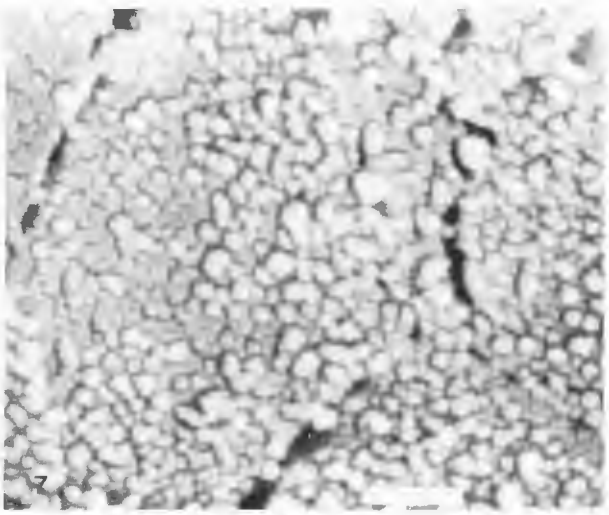


Figure 6. Scanning electron micrograph of CsG showing squames forming keratinized folds. Note changes in the pattern of microridges, parallel (P), fingerprint-like (F) and round (R) microridges. Bar = 10 μm.

Figure 7. Higher magnification (of Fig. 6) of CsG cell surface laced with oval to round projections. Bar = 1 μm.

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arrangement. The cell boarders forming margins of individual squames were distinct and demarcated by macroridges (Fig. 3).

At low magnification, the outer epithelial surface of CsG specimens showed a shiny and irregular surface (Fig. 4). A few overlapping desquamated cells were present (Fig. 5). Some epithelial areas were necrotic. In other areas, the continuous addition of keratin squames forming keratin plugs at the surface, and wavy keratin folds were noticed. At a higher magnification, the cell surface of keratin forming folds showed various

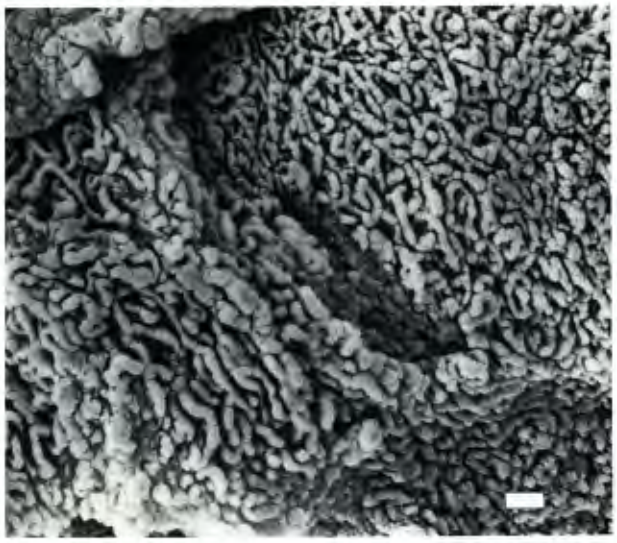

Figure 8. Scanning electron micrograph showing CsG surface with beaded shaped microridges. Bar = 1 μm.

patterns of microridges. The surface of some cells showed parallel microridges, the other squamous surface showed a honeycomb arrangement of microridges changing into a reticular type (Fig. 6). In some areas, cell borders were not clearly defined, and the cell surface was covered with numerous round to oval, dome-shaped structures instead of different patterns of microridges (Fig. 7). The cell surface of some keratin cells showed thickened somewhat parallel microridges with a beaded appearance (Fig. 8). The cell surface from other gingival biopsies exhibited a distorted honeycomb arrangement of microridges. The microridges were of various sizes. Some were small and lacing the whole surface. The intercellular spaces were widened and connecting the cells to neighboring cells by extending cytoplasmic projections (Fig. 9). Some cells showed indistinct cell boarders and microridges of various shape and sizes on their surface (Fig. 10).

Discussion

The light microscopic examination of the attached mucosa of the gingival overgrowth induced by cyclosporine in organ transplant patients showed areas of collagen destruction and invasion of inflammatory cells in the lamina propria. Similar findings have been reported by Bartold (1987). Epithelial hyperplasia, acanthosis and parakeratosis as reported by Wysocki *et al.* (1983), Tyldesky and Roter (1984), Pisanty *et al.* (1988, 1990) and Niimi *et al.* (1990) were also observed by us in the attached gingival epithelium of CsG. An increased number of retepeg processes was observed in phenytoin

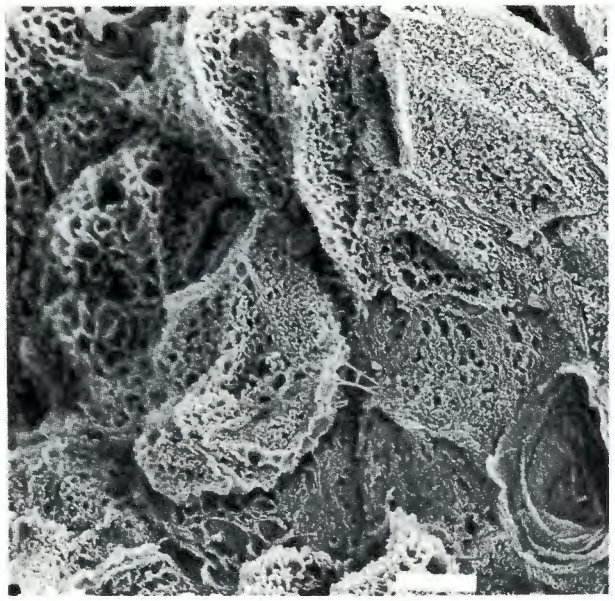

Figure 9. Scanning electron micrograph of CsG showing widened intercellular spaces. The cell surface is covered with different sizes of microridges. Bar = 10 μm.

induced hyperplastic and acanthotic epithelium by Hassell (1981) and Ashrafi *et al.* (1993). However, the increase in the number of retepeg processes with branched endings was more prominent in CsG than in phenytoin induced gingival overgrowth. Ashrafi *et al.* (1993) also suggested that phenytoin not only acted on gingival fibroblasts (Hassell and Hefti, 1991) but increased the growth of epithelium. The present study supports the findings of Pisanty *et al.* (1988, 1990) that epithelium plays a significant role in the gingival overgrowth induced by cyclosporine. The concept of Niimi *et al.* (1990) that CsG or phenytoin induced gingival overgrowth is due to enhanced keratinocyte life span rather than keratinocyte proliferation rate needs investigation under *in vivo* conditions. We believe that hyperplasia is due to a change in the proliferation rate and that parakeratosis is enhancing the life span of keratinocytes, as they are not desquamating at their normal rate.

The SEM examination of normal human attached gingival cell surface showed a honeycomb structure as described by Kaplan *et al.* (1977) or a pitted appearance as noted by Kullaa-Mikkonen (1987) or what was called a reticular pattern called by Moreu *et al.* (1993). The squamous surface of some other normal cells showed a fingerprint-like or somewhat parallel arrangement of microridges (Kulla-Mikkonen, 1987; Moreu *et al.*, 1993).

The SEM examination of the attached epithelial cell surface of CsG showed ulcerated areas. Remnants of necrotic shrunken epithelial cells and several perforations

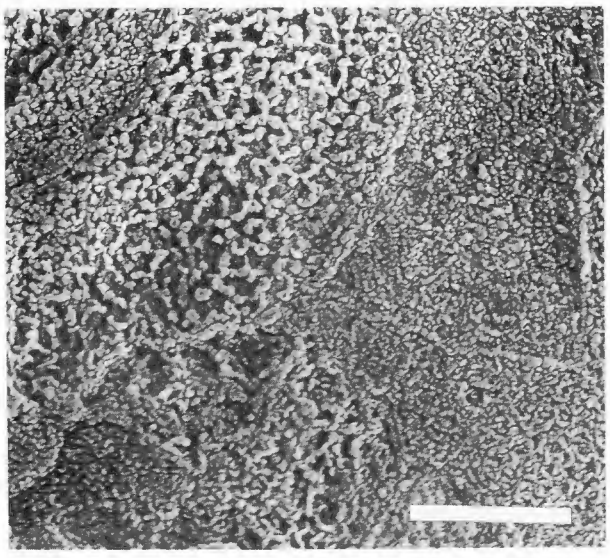

Figure 10. Scanning electron micrograph of CsG showing cell surface with large scanty microridges and small closely crowded microridges. Bar = 10 μm.

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on the epithelial surface were similar to those reported by Shafik *et al.* (1987) in the most apical part of the pocket wall in juvenile periodontitis. Some areas showed accumulation of keratin cells forming keratin plugs. The overall surface had a rippled appearance with a pattern of deep fissures. Ashrafi *et al.* (1992) showed a similar effect on the surface of the snuff-treated keratinized hamster cheek pouch epithelium. The polyhedral epithelial squames were delineated by raised cell boundaries, macroridges, in most areas of the epithelium. The usual honeycomb structure of microridges enclosing pits in the surface of keratinized cells (Kaplan *et al.*, 1977) was changed into a different pattern of microridges, as was seen by others in 7-12 dimethylbenz(α)anthracene-treated hamster cheek pouch (Hassanin and Ashrafi, 1988), snuff-related oral epithelial lesion in humans (Jungell and Malmström, 1985) and in snuff-treated hamster cheek pouch (Ashrafi *et al.*, 1992). Dourov (1984) observed irregular elevation of microridges surrounding pits in phenytoin-treated gingival overgrowth. The delicate and fragile microridges surrounding irregular pits on the epithelial surface were seen in the case of phenytoin induced gingival overgrowth epithelium in mice (Ashrafi *et al.*, 1993). The alterations in the pattern of microridges at the cell surface of the cyclosporine-induced human gingival overgrowth were more pronounced in some areas than other. This may be due to mild to severe changes occurring during cyclosporine-induced gingival overgrowth (Daley *et al.*, 1986). The surface of some cells showed a honeycomb arrangement of microridges changing into parallel, reticular or fingerprint-like patterns. These arrangements of microridges were similar to those described by Kullaa-Mikkonen (1987) and Moreu *et al.* (1993) for human gingival epithelium. Most of the surface area of the cells was covered by stubby, club-formed and spherical projections. These changes in microridge were similar to those found by Reichart and Althoff (1979), in human oral lesions, and by Jungell *et al.* (1987), for early lichen planus in human oral mucosa.

It was concluded that the surface structure of cells was changed in response to cyclosporine. The transitional changes in microridge pattern were occurring in an area of mild to severe gingival overgrowth caused by cyclosporine. SEM enabled us to study the most superficial layer of the epithelium and various changes in the arrangement of microridges. These may be expressed as changes occurring at the surface of oral epithelial cells induced by drugs or carcinogens or disease processes.

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Discussion with Reviewers

A. Campos: Your morphological description, although precise, is not quantitative in nature. Do you see any potential to use these patterns in quantitative analysis to evaluate the degree of gingival overgrowth?
Authors: We are planning to do quantitative analysis of alterations in the pattern of microridges to evaluate the degree of gingival overgrowth.

A. Campos: Surface pattern have been related with the degree of keratinocyte differentiation. Do you think that cyclosporine influences this process?
Authors: We think surface pattern is related with keratinocyte differentiation and cyclosporine influences this process.

Reviewer II: What is the time duration of cyclosporine therapy in your 3 cases? Do you find some relationships

of importance related to alterations?
Authors: The time duration of cyclosporine therapy in 3 cases was 2-3 years. A three year therapy period produced more alterations than a 2 year period.

Reviewer II: Have you observed crystallite depositions on the surface of the gingival epithelium such as observed by Pisanty *et al.* (1990)?
Authors: We did not observed crystallite deposits on the outer surface of gingival epithelium.

Reviewer II: Do you have some information about the gingival mast cells, degranulation or intraepithelial localization?
Authors: We have seen gingival mast cells in CsG epithelium in connective tissue and intraepithelial regions.

Reviewer II: Are there alterations in the pattern of microridges related with senescence of the cell, or do they correspond to the deeper origin of the gingival epithelial cells or are they characteristic for parakeratosis?
Authors: At this stage, it is difficult to offer any explanation to relate alterations in microridges with senescence of the cells or due to deeper origin of the gingival epithelial cells. Some alterations in honeycomb pattern could be correlated with parakeratotic conditions. During parakeratosis microridges surround irregular holes or pits and microridges are not of uniform thickness or elevation (Dourov, 1984; Ashrafi *et al.*, 1993).

M. Fartasch: Do you not think that changes of the epithelial cell surface can be found in every parakeratotic condition? Are the described alterations of the outer epithelial cell surface specific for CsG, or are they regularly found in the parakeratotic/acanthotic changes of different epithelial disease?
Authors: Yes, the changes described in this paper can also be found in other parakeratotic/acanthotic conditions and are not specific for CsG.

M. Fartasch: Are the changes different form other drug induced hyperplasia (like phenytoin-treated gingival overgrowth, etc.)?
Authors: The changes in phenytoin-treated gingival overgrowth were noticed in a honeycomb pattern of microridges (Dourov, 1984; Ashrafi *et al.*, 1993). In CsG, the alterations in microridges were seen not only in honeycomb pattern but also showed numerous round, ovoid and dome-like structures on the cell surface.