

Cell surface patterns in normal human oral gingival epithelium. A quantitative scanning electron microscopy approach

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Summary. We used scanning electron microscopy to study the morphological surface patterns of cells that cover the attached gingiva and intervestibular papilla of the human oral gingival epithelium. Five patterns are described on the basis of the overall appearance of morphological surface markers: microvilli, parallel, fingerprint, reticular and pitted. Statistical analyses detected significant differences in the frequency of each pattern in both regions of the oral gingival epithelium, and showed the reticular and fingerprint types to predominate. We propose that our description of the different morphological surface types may be of use as a standard for subsequent cytological studies and characterizations of morphological alterations in diseased gingiva.

Key words: Pattern, Microuplications, Gingiva, Epithelium, Quantitation

Introduction

The oral gingival epithelium (OGE) is a stratified squamous epithelium covering the surface from the mucogingival junction and the masticatory mucosa of the hard palate to the gingival margin (Avery, 1987). Scanning electron microscopy (SEM) has been a widely-used tool in human oral mucosa to describe the morphology of the stratum corneum and to analyze features in the normal oral cavity (Cleaton-Jones et al., 1978; Kullaa-Mikkonen, 1986, 1987). Many studies have associated specific morphological prototypes and cell surface patterns with different areas of the oral mucosa (Kullaa-Mikkonen, 1986). These patterns have been used as a point of reference in a number of electron microscopic studies of pathological conditions (Banoczy et al., 1980; Jungell et al., 1987; Robertson et al., 1987; Shafik et al., 1987). In normal human OGE, two distinct cell surface patterns have been described in Kullaa-

Mikkonen's SEM study (1986) as characterizing the attached gingiva (the more apical region of the OGE) and the interdental papilla (the vestibular or oral portion of the gingiva between adjacent teeth). The former is characterized by a pitted cell surface, whereas the latter presents parallel or branched microplacae. Because the OGE is a target area of drug-induced hyperplasia (González-Jaranay et al., 1990) increasing attention is being devoted to this tissue. However, as Kullaa-Mikkonen (1987) has pointed out, many gaps still exist in our knowledge of the oral mucosa. For example, little is known of the homogeneity or heterogeneity of cell surface patterns assigned to different regions of the oral cavity. In this connection, the homogeneous SEM cell patterns proposed for different areas of the OGE are rather general in nature, in contrast with classical exfoliative cytological studies based in light microscopic observations, which have identified several different types of cells (Miller et al., 1951; Montgomery, 1951; Trott, 1958; Lange and Lange, 1964).

The aims of the present study were to describe the SEM cell patterns in the attached gingiva (AG) and interdental vestibular papilla (IVP) of normal human OGE, and to quantify their frequencies. As noted by Kullaa-Mikkonen (1987), quantitative approaches have rarely been used to analyze these structures. The objectives of studies published to date have been mainly to examine variations in microplacal density and other morphological surface markers in outer cells; there are no comprehensive studies of the frequencies of such cells in different regions of the oral cavity. A more precise characterization of the SEM surface features of different regions of the human OGE should contribute to our understanding of the epithelial substrate involved in drug-induced hyperplasia and other disorders of the oral mucosa.

Materials and methods

We examined one oral gingival biopsy from each of 12 patients seen at the University of Granada Dental School. All subjects had clinically normal periodontal tissues, but required dental extraction as a part of

treatment for nonperiodontal conditions. Informed consent was obtained from each patient prior to surgery. The criteria for selection of specimens were lack of bone loss, lack of attachment and absence of bleeding on probing.

Before fixation, the specimens were treated with 0.3% collagenase (Sigma, St. Louis, USA) in cacodylate buffer for 30 minutes. The material was fixed in cacodylate-buffered 2.5% glutaraldehyde (pH= 7.4), and postfixed in 1% osmium tetroxide. After fixation, the samples were dried in an ascending series of acetones, critical point dried, gold sputter-coated and examined with a Philips 505 Scanning electron microscope.

To quantify the frequencies of different cell surface patterns, 50 cells from each specimen were counted: 25 from the AG and 25 from the IVP. The differences between the frequencies of surface patterns in both zones were statistically analyzed with one-way analysis of variance (ANOVA) and Student's test, with $p \leq 0.05$ considered to denote significance.

Results

Five distinct microscopic patterns in the surface of the most external cells of the gingival epithelium were identifiable on the basis of our SEM findings (Fig. 1). The type I pattern was characterized by microvilli as a morphological surface marker of the keratinocytes. The finger-like projections had a maximum height of 1 μm , and were variably distributed on different cells; some

cells showed scarce microvilli, whereas in other cells they covered most of the surface. The type 2 pattern consisted of straight, parallel rows of microplacae,

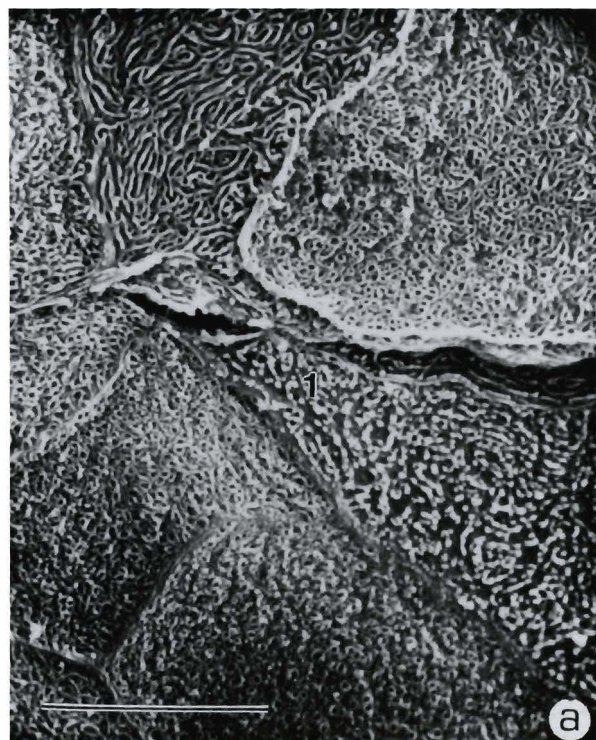
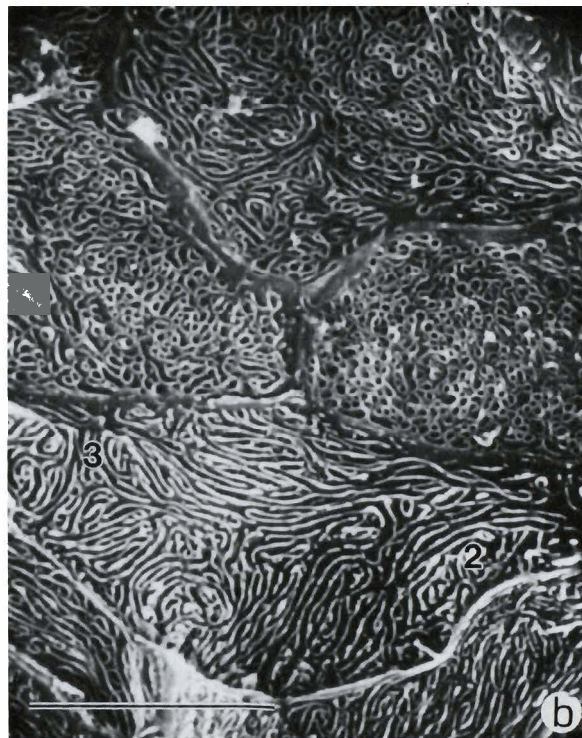


Fig. 1. Morphological surface pattern in normal human oral gingival epithelium. A. Type 1, microvilli; B. Type 2, parallel and Type 3, fingerprint-like; C. Type 4, reticular and Type 5, sponge-like. Scale bar=10 μm

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Table 1. Mean values with standard error for the frequencies of five cell surface patterns in two zones of human adult oral gingival epithelium. One-way ANOVA was used to compare the frequencies.

Oral gingival epithelium	SEM CELL PATTERNS					p
	1	2	3	4	5	
Attached gingiva	2.7±1.2	1.9±0.8	7.8±2.1	10.5±2.3	1.9±1.2	p<0.001
Interdental vestibular papilla	2.1±0.8	1.0±0.4	6.4±1.6	13.5±2.1	1.9±1.6	p<0.001

measuring, on average, 0.1 µm thick by 0.3 µm high. On most type 2 keratinocytes, the microplacae formed a series of parallel furrows similar in appearance to a ploughed field. Keratinocytes showing the type 3 pattern were characterized by concentric curved rows of microplacae similar in size to type 2 surface markers, but arranged on the cell surface in fingerprint-like patterns. In the type 4 pattern, the microplacae were distributed as branching, confluent crests of reticular appearance. The morphological surface marker of keratinocytes showing the type 5 pattern was characterized by clearly delimited pits of not more than 1.0 µm in diameter, framed by a faint network of flattened, slightly raised crests, giving an overall impression of a weakly pitted sponge-like surface.

In our material, most cells displayed one of the above-mentioned patterns. Some cells showed a combination of two consecutive patterns (eg, 1/2, 2/3, 3/4 or 4/5); other combinations were rarely observed. In the quantitative study, cells which displayed two patterns were selected only if one of the patterns covered at least two-thirds of the cell surface. Mean values plus standard error for the frequencies of the five cell patterns in the two zones of the OGE (AG and IVP) are given in Table 1. ANOVA revealed significant differences in both zones in the frequencies of the different patterns (F test). The comparisons between types that showed significant differences in frequency, according to Student's test, are summarized in Table 2.

Discussion

The correct interpretation of SEM findings in the study of buccal samples requires a number of factors, the most important of which are the correct collection and handling of the specimens, meticulous cleaning of the surface to be examined to remove mucus, blood, or tissue fluid, and appropriate fixation, dehydration and drying to minimize curling and shrinking.

We used the methodological guidelines recommended by various authors for oral mucosa. As has been suggested for other epithelia (Hudspeth, 1983; Cañizares et al., 1985) we substituted saline solution (Kullaa-Mikkonen, 1987) for diluted collagenase and fixative buffer solution as the agent used to clean the surfaces to be observed. Cacodylate was chosen instead of phosphate buffer to avoid salt precipitation during

Table 2 Significant differences in frequencies of cell surface patterns, calculated with Student's test.

Oral gingival epithelium	SEM CELL PATTERNS				
	1/3	1/4	2/3	2/4	4/5
Attached gingiva	NS	p<0.05	p<0.02	p<0.01	p<0.02
Interdental vestibular papilla	p<0.05	p<0.001	p<0.01	p<0.001	p<0.01

fixation (Carrasi et al., 1988). Acetone was used as the dehydrating agent to avoid dense precipitations in tissues with the use of alcohol (Brunk et al., 1981; Crespo et al., 1987).

The five SEM patterns we describe in both AG and IVP are based on the types proposed by Takagi (1976) and Kullaa-Mikkonen (1986, 1987) for all oral mucosa. Our patterns facilitate the identification of these tissues when examined in SEM images, thanks to their emphasis on the overall morphology of the microplacae on the cellular surface (parallel, fingerprint, reticular, etc.) rather than the presence of specific types of microplacae, usually described by other authors (Nair and Schröder, 1981; Dourov, 1984).

In contrast with previous descriptions of the two regions in the OGE (eg, a pitted surface for AG, and parallel or branching microplacae in IVP), we observed that all morphological patterns could be found in both regions. The frequencies of the different types, however, differed significantly, a finding that appears to confirm the superficial heterogeneity of the OGE.

The most frequent cellular patterns covering both surfaces were the reticular and fingerprint types. The frequencies of these patterns differed significantly from those of most of the other cell types present, both in AG and IVP.

Many workers have wondered what the function of the more frequent SEM morphological markers might be in the OGE. Most have surmised that in the oral mucosa, these patterns are involved in two essential functions: 1) cell-cell adhesion, a phenomenon related to differentiation; and 2) the flow of secretions across the gingiva.

With regard to cell-cell adhesion, as the keratinocyte ascends through the stratified epithelium, it loses its desmosomes, the structures that facilitate cohesion in deeper layers. The type 2, 3 and 4 patterns observed in SEM would appear to represent different mechanisms of intercellular association; the precise mechanical-topological and physicochemical features of these systems will require detailed study. Hodgkins et al. (1978) used adhesive tape to strip away cell layers in the OGE, and observed the cell surfaces in different epithelial strata. Less differentiated cells of the middle and deep layers, associated by desmosomes, were covered exclusively with microvilli. The upper surface of the superficial cells was covered with ridges, whereas

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the undersurface contained only microvilli. Desmosomes became progressively scarcer in these more superficial, well-differentiated cells. Cells displaying a pitted surface have been considered the most superficial, and hence, the most well-differentiated (Cleaton-Jones, 1975; Dourov, 1984).

A second function of these markers is their role in the flow of secretion across the cell surface, in the spaces between the microplicae. This functional activity is thought to occur on epithelial surfaces with secretory cells or with glands close to this type of surface. Although this activity was demonstrated in an elegant study (Sperry and Wassersug, 1976), it appears not to be applicable to the OGE, as this tissue lacks secretory cells and glands in the underlying connective tissue (Avery, 1987).

The presence of all cell surface patterns in both AG and IVP implies that these epithelia are probably covered by cells of different degrees of differentiation. Our observations should therefore find wider application at two levels: 1) The description of different cell types found in conventional light microscopic studies of exfoliative cytology; and 2) pathological studies of the gingiva. We suggest that our description of the presence of the different morphological surface types in both regions of the OGE may be of use as a standard for subsequent cytological studies and characterizations of morphological alterations in diseased gingiva.

If SEM images facilitate differential diagnosis of different disease processes in the oral cavity (Matravers and Tyldesley, 1978a,b; Banoczy et al., 1980; Dourov, 1984; Jungell et al., 1987), the straightforward identification of the patterns described in this study should help simplify the diagnosis of gingival disease in both regions of the OGE, and make differential diagnosis more reliable.

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