Histol Histopath (1994) 9: 149-153

Histology and Histopathology

Regional differences in cell surface patterns in normal human sulcular epithelium

M.C. Sánchez-Quevedo[1], G. Moreu[2], A. Campos[1], J.M. García[1] and M. González-Jaranay[2]

[1]Department of Histology and Cell Biology and [2]Department of Periodontics,
Faculty of Medicine and Dentistry, University of Granada, Granada, Spain

Summary. Studies with scanning electron microscopy in the normal human sulcular epithelium are scarce, and no precise information exists about cell surface patterns along the epithelium, the frequencies of these patterns, or possible regional differences within the mouth. In five periodontal biopsy specimens each from the anterior and posterior region of the mouth, we observed three cell patterns on the basis of the overall appearance of morphological surface markers in the coronal and apical zones of sulcular epithelium: microvilli; microplicae; and pits. The percentage of keratinocytes showing the microvillous pattern in the surface of apical sulcular epithelium of the posterior region of the mouth was significantly higher than in the anterior region. We posit that the presence, in the bottom of the normal sulcular epithelium in the posterior region of the mouth, of mainly microvillous keratinocytes (the most undifferentiated and least desquamative type of keratinocyte, and thus the most vulnerable to bacterial colonization) can be associated with observations of longitudinal clinical studies of periodontal disease, which suggest that more severe clinical findings are found in the region of the molars.

Key words: Scanning electron microscopy, Normal sulcular epithelium, Regional differences

Introduction

The sulcular epithelium which surrounds the cervical area of all teeth is of particular importance as a barrier in which interaction takes place between the contents of the oral cavity, especially in the environment of the dentogingival space, and the periodontal tissues, which support the teeth (Garnick and Ringle, 1988; Shafik et al., 1988). The stratified epithelium, together with the enamel, cemental or dentinal tooth surface, delimits a space, called the sulcus, in which bacterial plaque can form and in which inflammatory cells can be found (Saglie, 1977, 1988). The structural characteristics of this epithelium have been the object of intensive study aimed at elucidating the pathogenesis of periodontal disease, especially bacterial invasion in this area (Saglie, 1988; Shafik et al., 1988).

In recent years, scanning electron microscopy (SEM) has become a powerful and widely used tool to investigate the cell surface patterns in all regions of the oral mucosa under both normal and pathological circumstances (Cleaton-Jones et al., 1978; Dourov, 1984; Kullaa-Mikkonen, 1986, 1987; Jungell et al., 1987). The main advantages of SEM are its larger depth of focus and better resolving power than available light microscopy. Moreover, in comparison with transmission electron microscopy, it allows observations of large surface areas, an important advantage in the oral cavity. SEM has also been used to study the gingival pocket, a deeper extension of the normal sulcus which arises in periodontal disease, and which especially involves the sulcular epithelium (Shafik et al., 1988).

Earlier studies of periodontal pockets in humans were based either on a limited number of cases (Carranza et al., 1983), on certain aspects of the pocket wall, e.g. bacterial invasion, or on some morphological features of epithelial surface (Kaplan et al., 1977; Saglie et al., 1982, 1985; Cobb et al., 1988).

Studies with SEM in the human sulcular epithelium under normal conditions are therefore scarce, and no precise information exists about the cell surface patterns along the epithelium, the frequencies of these patterns, or possible regional differences within the mouth. Because the sulcular epithelium forms a target area in periodontal disease, and because clinical differences between the anterior and posterior areas of the oral cavity have been reported in this process (González-Jaranay, 1987; Albandar et al., 1991), the aims of the present study were to describe the SEM cell patterns in the coronal and apical zones of the sulcular epithelium of the normal human periodontal area, and to quantify their frequencies in the anterior and posterior regions

Offprint requests to: Prof. Antonio Campos, Departamento de Histología y Biología Celular, Facultad de Medicina, Universidad de Granada, Avda. de Madrid nº 9, E-18071 Granada, Spain

of the mouth. Better knowledge of the SEM surface features in the two regions should contribute to our understanding of the epithelial substrate involved in the pathology and treatment of periodontal disease.

Materials and methods

A biopsy specimen of the marginal periodontal area was obtained from 10 patients ranging in age from 23 to 52 years. Five specimens each were taken from the anterior and posterior periodontium. The anterior region was defined as that surrounding the incisors and canines, as far as the interdental papilla between the canines and first premolars. The posterior region comprised the periodontium surrounding the premolars and molars. All subjects had clinically normal periodontal tissues, and required extraction as part of treatment for non-periodontal conditions. Informed consent was obtained from each patient prior to surgery. The patients selected for biopsy satisfied the following clinical criteria: lack of bone loss, lack of attachment loss and absence of bleeding on probing. The depth of the sulcus on probing was never more than 2 mm. Because the extension of the coronal sulcular epithelium (CSE) into the depth of the sulcus varies depending on the depth of each sulcus, for sampling purposes we considered CSE to be epithelium from the coronal half of each sulcus, and apical sulcular epithelium (ASE) as that from the apical half of each sulcus (Fig. 1).

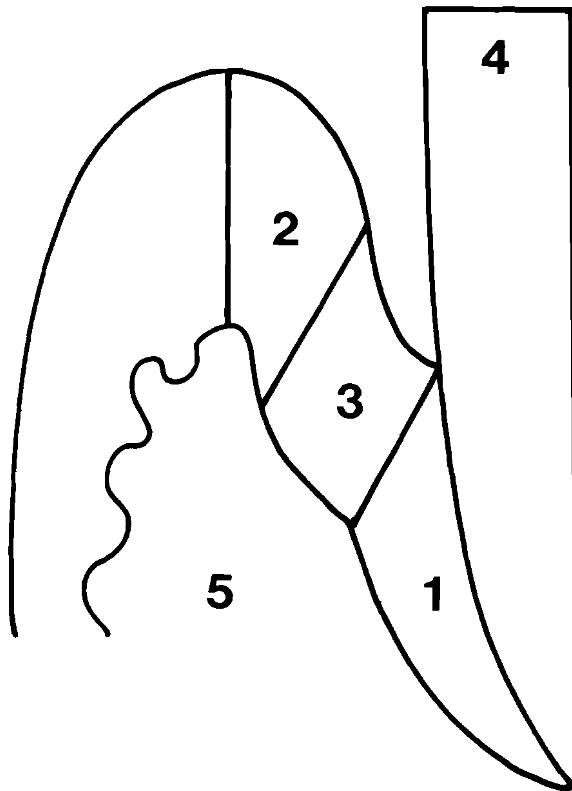

Fig. 1. Schematic drawing that illustrates the junctional epithelium (1) and the two areas of sulcular epithelium sampled in this study. Epithelium from the coronal half of the sulcus was considered coronal sulcus epithelium (2) and the epithelium from the apical half was considered apical sulcular epithelium (3). Tooth (4). Connective tissue (5).

Before fixation, the specimens were treated with 0.3% collagenase (Sigma) in cacodylate buffer for 30 min. The material was fixed in cacodylate-buffered 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. After fixation, the samples were dehydrated in increasing concentrations of acetone, critical point dried, gold sputter-coated and examined in a Philips 505 scanning electron microscope.

To quantify the frequencies of cell surface patterns, 25 cells in each zone of epithelium were counted, giving a total of 50 cells from each specimen. The differences between the frequencies of surface patterns in the two regions were statistically analyzed with nested analysis of variance and paired Newman-Keuls comparisons.

Results

Our SEM observations showed three basic cell patterns in the outermost surface of the keratinocytes in both the coronal and apical zones of the epithelium. Some cells were characterized by microvilli on their surface. This morphological marker was variably distributed on different cells, usually covering most of the surface. The highest microvilli measured 1μm (Fig. 2). A second pattern was characterized by microplicae on the cell surface. This morphological marker consisted of straight, curved or branching rows of microplicae giving rise to different features such a parallel furrows, fingerprint-like or reticular images (Fig. 3). A third pattern was characterized by clearly delimited pits of no more than 1.0 μm in diameter, framed by a faint network of flattened, slightly raised crests, giving an overall image of a weakly pitted sponge-like surface (Fig. 4). In our material some cells displayed a combination of two patterns. However, in the quantative study, cells which showed two patterns were only selected if one of the patterns covered at least two-thirds of the cell surface.

Mean values with standard error for the percentage frequencies of the three cell surface patterns are given in

Table 1. Percentge ($\bar{x}$±SEM) frequencies of the three cell surface patterns in apical and coronal sulcular epithelium from the anterior and posterior region of the oral cavity.

REGION	SULCULAR EPITHELIUM	SEM CELL PATTERN		
		Microvilli	Microplicae	Pits
ANTERIOR	Coronal	42.0±14.2	50.0±14.7	8.0±2.7
	Apical	17.3±8.4*	65.3±11.2	17.3±9.7
POSTERIOR	Coronal	19.0±7.1	74.0±6.0	6.6±4.3
	Apical	62.0±13.8*	32.0±10.9	6.0±3.8

*: $p<0.05$ when compared with nested ANOVA and Newman-Keuls test.

Table 1 for the coronal and apical zones of the epithelium and the anterior and posterior regions of the periodontal area. Our statistical study revealed a significant difference in the frequency of cells with the microvillous surface pattern between the ASE of the anterior and the posterior region of the oral cavity ($p<0.05$).

Discussion

A number of factors influence the interpretation of SEM findings in oral samples, e.g. 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 artifacts (Moreu et al., 1993). In this study, samples were prepared according to the guidelines recommended by Kullaa-Mikkonen (1987) for oral mucosa and other epithelia (Hudspeth and Jacobs, 1979), and our own experience with gingival samples (González-Jaranay et al., 1990; Moreu et al., 1993). We used diluted collagenase and buffered fixative solution instead of saline solution to clean the surfaces to be observed. Phosphate buffer was replaced with cacodylate to avoid salt precipitation during fixation (Carrasi et al., 1988). Acetone was used as the dehydrating agent to prevent dense precipitations in tissues treated with alcohol (Brunk et al., 1981).

The three patterns we describe in the two zones of the epithelium are based on the types proposed by Dourov (1984) and Kullaa-Mikkonen (1986, 1987) for all oral mucosa. These patterns, as Shouthgate et al. (1987) have recently demonstrated in a study of oral epithelium in culture, are related to the degree of differentiation of the keratinocytes. Keratinocytes with pits or a honeycomb appearance and keratinocytes with parallel or branched microplicae are now thought to be clearly related, as different authors have shown, with keratinized (with stratum corneum) and non-keratinized epithelium (without stratum corneum) (Kullaa-Mikkonen, 1986).

Moreover, although in early nonquantitative reports, cells with microvilli were described on the surface of keratinized epithelium (Cleaton-Jones, 1975), more recent observations have demonstrated that these microvilli-bearing cells are the least differentiated of the keratinocyte lineage. This was shown by Hodgkin et al. (1978), who studied the SEM appearance of different strata of human gingival epithelial by using adhesive tape to strip away cell layers, and by Southgate et al. (1987), who studied primary cultures of human oral epithelia. According to Hodgkin et al. (1978), cells of the middle and deep layers - the least differentiated cells of the keratinocyte lineage - were covered esclusively by microvilli. In Southgate's material the microplicae

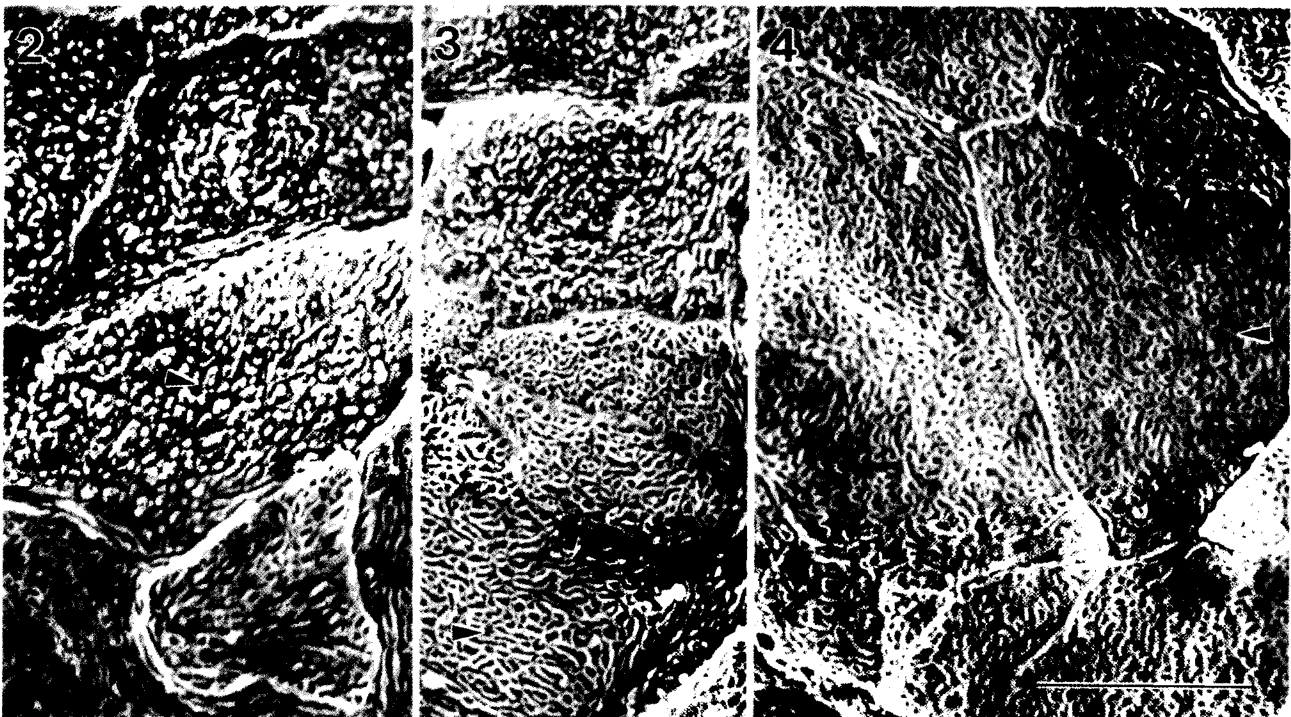

Fig. 2. Normal human sulcular epithelium showing a cell surface pattern characterized by microvilli (arrowhead). Scale bar = 10 µm

Fig. 3. Normal human sulcular epithelium showing a cell surface pattern characterized by microplicae (arrowhead). Scale bar = 10 µm

Fig. 4. Normal human sulcular epithelium showing a cell surface pattern characterized by pits (arrowhead). Scale bar = 10 µm

closely replaced packed microvilli as the cells matured.

In contrast with previous studies in which corrugations and microplications were described for the human sulcular and pocket wall surface (Shafik et al., 1988), our results obtained from epithelia in sulci of a maximum depth of 2 mm, i.e. within the range of clinically normal gingival sulci (Smith, 1982; Schroeder, 1986; Garnick and Ringle, 1988; Melfi, 1988; Carranza, 1990) showed three surface patterns, suggesting that the outer surface of these epithelia in both ASE and CSE is covered by cells of different degrees of differentiation. With regard to the frequencies of the types of keratinocytes, significant differences were found in our study only in the percentage of keratinocytes with microvilli in the ASE between the anterior and posterior region of the periodontal area, microvillous keratinocytes being more numerous in the latter. The heterogeneity in sulcular epithelia in both ASE and CSE is supported by quantitative data from normal human gingival epithelia (Moreu et al., 1993) and buccal mucosa after radication (Robertson et al., 1987). In the latter case, the increase in keratinocytes bearing microvilli on the epithelial surface resulted from the effects of irradiation on the earlier differentiation of cells of keratinocytic lineage.

Therefore, the epithelium in the deeper half of the normal sulcus is composed predominantly of keratinocytes with microvilli, which offer less resistance to microbial invasion and could be considered an important factor leading to more severe clinical findings in the posterior region of the mouth. In this sense, longitudinal clinical studies on the prevention, progression and treatment of periodontal disease can be associated with the data described above. Molars, rather than premolars or incisors, are most frequently lost (Axelsson et al., 1991); moreover, periodontal progression occurs mainly in the first molar (Albandar et al., 1991). These observations appear to reflect the depth of the periodontal pocket and the subsequent loss of tooth attachment. These two circumstances are compatible with the observation that the number and distribution of bacteria are linked with the degree of keratinocyte differentiation (Aufdemorte and Cameron, 1981), changes in the characteristics of exposed microvilli (Robertson et al., 1987) and especially with the rate of epithelial cell turnover (Cobb et al., 1988).

As Cobb and Killoy (1990) have suggested, desquamation of epithelial cells is an important adjunct to host defenses. If desquamation occurs only in normal stratified epithelia where cells are highly differentiated, displaying pitted or microridged surfaces, less well differentiated cells bearing microvilli may well facilitate long-term adhesion processes and thus favour microbial colonization and bacterial invasion. Only further studies will show whether our findings of a higher percentage of keratinocytes with microvilli in the ASE of the posterior region of the periodontium can be related with clinical data in the literature that show a greater incidence of periodontal disease in this region.

Acknowledgements. We thank Mrs. Maria Angeles Robles for her competent technical assistance, and Ms. Karen Shashok for assisting in the translation of the original text into English.

References

Albandar J.M., Baghdady V.S. and Ghose L.J. (1991). Periodontal disease in teenagers with no preventive dental care provisions. J. Clin. Periodont. 18, 300-304.

Aufdemorte T.B. and Cameron I.L. (1981). The relation of keratinization to bacterial colonization on the Baboon tongue as demonstrated by scanning electron microscopy. J. Dent. Res. 60, 1008-1013.

Axelsson P., Lindhe J. and Nyström B. (1991). On the prevention of caries and periodontal disease. J. Clin. Periodont. 18, 182-189.

Brunk U., Collins P. and Arro E. (1981). The fixation, dehydration, drying and coating of cultured cells for SEM. J. Microsc. 123, 121-131.

Carranza Jr. F.A. (1990). Glickman's clinical periodontology. 7th edition. W.B. Saunders Company. Philadelphia. p 14.

Carranza Jr. F.A., Saglie R., Newman M.G. and Valentin P.L. (1983). Scanning and transmission electron microscopic study of tissue-invading microorganisms in localized juvenile periodontitis. J. Periodontol. 54, 589-617.

Carrasi A., Abati S. and Santarelli G. (1988). The role of scanning electron microscopy in periodontal research. Scanning Microsc. 2, 1123-1138.

Cleaton-Jones P. (1975). Surface characteristics of cells from different layers of keratinized and non-keratinized oral epithelia. J. Peridont. Res. 10, 79-87.

Cleaton-Jones P., Buskin S.A. and Volchansky A. (1978). Surface ultrastructure of human gingive. J. Peridont. Res. 13, 367-371.

Cobb Ch.M. and Killoy W. (1990). Microbial colonization in human periodontal disease: an illustrated tutorial on selected ultrastructural and ecologic considerations. Scanning Microsc. 4, 675-691.

Cobb Ch.M., Rodgers R.L. and Killoy W. (1988). Ultrastructural examination of human periodontal pockets following the use of an oral irrigation device in vivo. J. Periodontol. 59, 155-163.

Dourov N. (1984). Scanning electron microscopy contribution in oral pathology. Scan. Electron. Microsc. 1, 243-248.

Garnick J.J. and Ringle R.D. (1988). The dento-gingival junctions as seen with light microscopy and scanning electron microscopy. Scanning Microsc. 2, 1113-1122.

González-Jaranay M. (1987). Tratamiento no quirúrgico de la enfermedad periodontal. Avances en Odontoestomatología. 3, 193-201.

González-Jaranay M., Fernández-Segura E., Moreu G. and Campos A. (1990). Gingival epithelium overgrowth in renal transplant. Electron Microsc. 3, 336-337.

Hodgkings J.F.W., Watkins R. and Walter D.M. (1978). Correlated scanning and transmission electron microscopy of cells surfaces at various levels in human gingival epithelium. Arch. Oral Biol. 23, 355-360.

Hudspeth A.J. and Jacobs R. (1979). Stereocilia mediate transduction in vertebrate hair cells. Proc. Natl. Acad. Sci. USA. 76, 1506-1509.

Jungell P., Malmstrom M., Wartiovaara J. and Konttien Y. (1987). Ultrastructure of oral leukoplakia and lichen planus. II. A correlated scanning and transmission electron microscopic study of epithelial surface cells. J. Oral Pathol. 16, 287-293.

Kaplan G.B., Ruben M.P. and Pameijer C.H. (1977). Scanning electron microscopy of the epithelium of the periodontal pocket. Part II. J. Periodontol. 48, 634-638.

Kullaa-Mikkonen A. (1986). Scanning electron microscopy study of surface of human oral mucosa. Scand. J. Dent. Res. 94, 50-56.

Kullaa-Mikkonen A. (1987). Scanning electron microscopy in oral mucosal research: a review. Scanning. Microsc. 1, 1145-1155.

Melfi R.C. (1988). Permar's oral embryology and microscopic anatomy. 8th edition. Lea & Febiger. Philadelphia.

Moreu G., Sánchez-Quevedo M.C., López-Escámez J.A., González-Jaranay M. and Campos A. (1993). Cell surface patterns in normal human oral gingival epithelium. A quantitative scanning electron microscopy approach. Histol. Histopath. 8, 47-50.

Robertson A.G., Wilson P., Wilson D.J., Carr K.E. and Hunter I. (1987). Microplication patterns on human buccal epithelia following radiotherapy: a scanning electron microscopic analysis. J. Submicrosc. Cytol. 19, 515-521.

Saglie R. (1977). A scanning electron microscopic study of the relationship between the most apical localized subgingival plaque and the epithelial attachment. J. Periodontol. 48, 105-115.

Saglie R. (1988). Scanning electron microscope and intragingival microorganisms in periodontal diseases. Scanning Microsc. 2, 1535-1540.

Saglie R., Carranza Jr.F.A., Newman M.G. and Pattison G.A. (1982). Scanning electron microscopy of the gingival wall of deep periodontal pockets in humans. J. Periodont. Res. 17, 284-293.

Saglie F.R., Carranza Jr.F.A. and Newman M.G. (1985). The presence of bacteria within the oral epithelium in periodontal disease. I. A scanning and transmission electron microscopic study. J. Periodontol. 56, 618-624.

Schroeder H.E. (1986). The periodontium. Springer-Verlag. Berlin.

Shafik S.S., Zaki A.E., Ashrafi S.H., Nour Z.M. and ElNesr N.M. (1988). Comparison of scanning and transmission electron microscopy of the epithelial pocket wall in juvenile and adult periodontitis. J. Periodontol. 59, 535-543.

Smith R.G. (1982). A longitudinal study into the depth of the clinical gingival sulcus of human canine teeth during and after eruption. J. Periodont. Res. 17, 427-433.

Southgate J., Williams H.K., Trejdosiewicz L.K. and Hodges G.M. (1987). Primary culture of human oral epithelial cells. Growth requirements and expression of differentiated characteristics. Lab. Invest. 56, 211-223.

Accepted October 25, 1993