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SCANNING ELECTRON MICROSCOPY OF ORAL MUCOSA IN VIVO AND IN VITRO: A REVIEW

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Abstract

The oral mucosa is classified by function into lining, masticatory and specialized oral mucosa, with regional structural adaptation. In this review, the surface structures of the human oral mucosa have been studied in the scanning electron microscope (SEM). Regional variations in regard to keratinization, cell arrangements and microplications with related specific structures observed in SEM are described and correlated with the appearance of similar areas observed in the light microscope. Furthermore, human oral tissue and cell cultures have also been studied. These systems offer usable and complementary models for performing similar studies in vitro under controlled experimental conditions. We now show that explant cultures of human oral mucosa can propagate both normal epithelial cells and fibroblasts. The surface morphology of both cell types has been investigated in SEM.

KEY WORDS: Cell culture, epithelial cells, fibroblasts, keratinization, microplicae, oral mucosa, oral pathology, scanning electron microscope, taste buds.

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Introduction

The surface patterns of normal human oral mucosa have been studied by scanning electron microscopy (SEM) (4, 23, 43-45, 51,68). Similar studies of other mammalian species have been reported (2, 25, 27, 41, 53, 55-57).

The SEM technique has also been used to characterize surface changes of the oral mucosa such as denture stomatitis (70,90), lichen planus (52, 68), oral leukoplakia (11,71) and oral carcinoma (23,52,69), for a recent review, see Dourov (28).

In biomedical research, tissue and cell culture techniques are now widely used. They provide good models for in vitro study of cell behaviour under controlled experimental conditions. Tissue cultures of the oral mucosa have been used mainly to study epithelial cells and fibroblasts and their capacity to undergo differentiation (73, 80, 81, 89). There are very few studies of SEM techniques applied to cultures of oral epithelial cells and fibroblasts (14, 58, 67). Therefore, some findings from the use of SEM technique to characterize cells originating from oral mucosa are included in this review.

Morphology of oral mucosa

The epithelium of the normal oral mucosa (fig. 1) consists of several layers of closely packed cells. The covering epithelium shows wide regional variation in thickness and in type of keratinization (24,87). Orthokeratinizing, parakeratinizing and non-keratinizing mucosae occur intraorally. The epithelium is supported by a connective tissue, the lamina propria, containing ground substance, fibers and cells. The lamina propria and the form of epithelial-connective tissue junction reflect the functional demands of the different regions of the oral cavity. There are also differences in the nature of the submucosa, when present, and the attachment of the mucosa to the underlying structures.
The human oral mucosa is commonly classified into lining, masticatory and specialized mucosa (83, 88). This classification is widely used but has been questioned (74) because of regional differ-

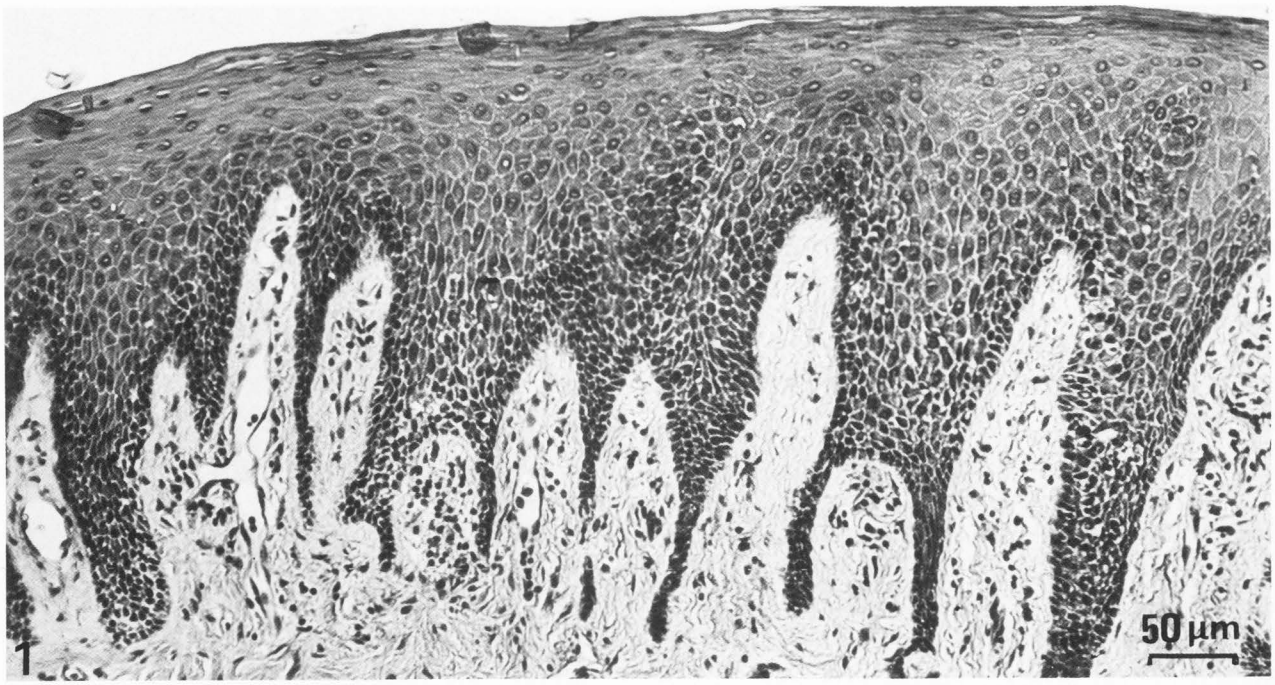


Figure 1. A histological section through the human hard palate. The epithelium is supported by the lamina propria. Hematoxylin and eosin.

ences within each group. The classification used in this study is summarized in Table 1.

Lining mucosa

The lining mucosa covers the free gingiva, the inside of the lips, the soft palate, the ventral surface of the tongue, the floor of the mouth and the labial and buccal mucosa. The epithelium of lining mucosa is thicker than that of the other types of oral mucosa and is mostly non-keratinized. The surface is thus flexible and able to withstand stretching. Parakeratinization may occur in some areas such as the lips, the free gingiva and the soft palate.

In the scanning electron microscope, at low magnification, the lining mucosa (fig. 2) usually appears as a very uneven, corrugated surface layer (2, 27, 65). Cell boundaries are generally indistinct and the underlying cell or nuclear contours are not distinguishable, but there are great variations. Zoghby and Moussa (91), however, have shown that the human buccal mucosa has a mosaic-like arrangement of polygonal cells, with fairly sharp cell borders and loop-like ridges along the junction. At higher magnification the epithelial cells of the non-keratinized surface have winding ridge-like surface folds (fig. 3). These structures have also been described as cytoplasmic folds (59), microvillar ridges (36), microridges (82), microrugae (11) or microplicae (25,27). Nair and Schroeder (64) described eight variations of these ridge-like surface folds or microplications; bifurcating, bridge-like, ring-like, simple ending, U-turn ending, looped ending, hooked ending and microvilli. The density of microplications per 100 μm^2 cell surface area varied considerably, from 120 to 550 µm (64). The most frequent pattern, the sinuous interlocking

TABLE 1.

DIFFERENT TYPES OF HUMAN ORAL MUCOSA

Types of oral mucosa	Topographical distribution	Degree of keratinization
Lining mucosa	Free gingiva	Para- or nonkeratinized
	Lips	Para- or nonkeratinized
	Soft palate	Para- or nonkeratinized
	Ventral surface of tongue	Nonkeratinized
	Floor of mouth	Nonkeratinized
	Labial and buccal mucosa	Nonkeratinized
Masticatory mucosa	Hard palate	Ortho- or parakeratinized
	Attached gingiva	Parakeratinized
Specialized mucosa	Dorsal surface of tongue	Ortho- or parakeratinized

pattern, presented microplications running in randomly winding paths, branching, taking U-turns or terminating in a variety of other features. Sinuous interlocking was observed in 72.5% of the total area of the cheeks and in 71.9% of the lips. Nair and Schroeder (64) suggested four possible correlations with the different patterns of microplications as adhesion, protection, channel formation for liquid transport and reserve for stretching.

Masticatory mucosa

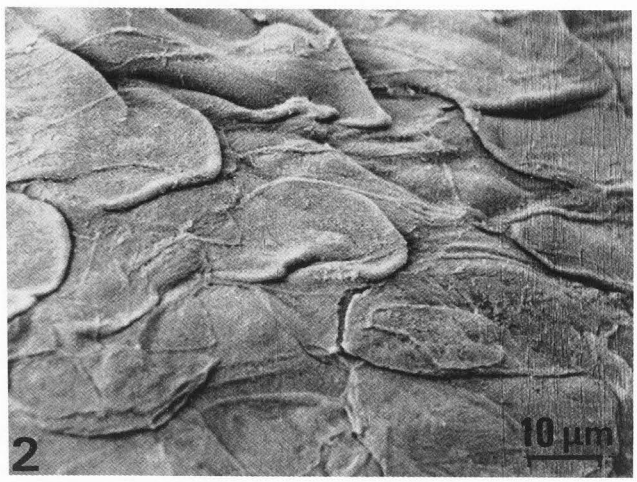
The masticatory mucosa covers areas of the oral cavity exposed to compression, shear force and to abrasion during mastication of food, for example the hard palate and the attached gingiva. The upper surface of the tongue has the same functional role as the hard palate and the attached gingiva, but because of its specialized structure it is considered separately. The epithelium of masticatory mucosa is moderately thick compared to the lining mucosa. It is frequently orthokeratinized, but parakeratinized areas of the gingiva and occasionally of the hard palate also occur normally.

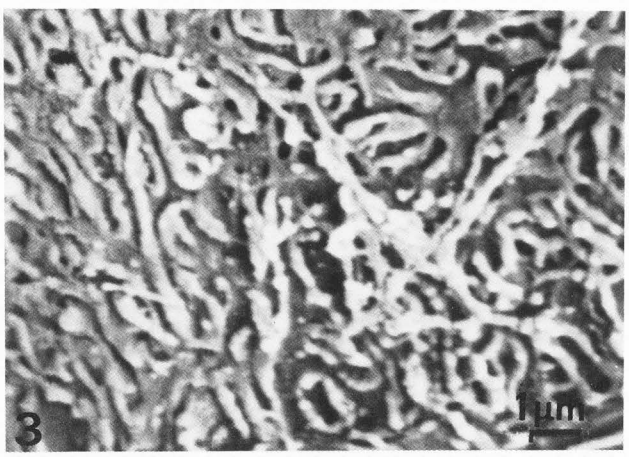
Under low magnification, the surface area (fig. 4) of densely or completely keratinized epithelium appears flat and the "cobble-stone" arrangement of the epithelial cells is clearly visible as described elsewhere (2,25,27,55,57,65). Cell outlines are distinct and appear in a mosaic-like pattern of polygonal squamous cells of varying size, indicating overlapping of individual cells (fig. 5). At higher magnification, the surface cells have been commonly described as pitted or spongy in appearance (2,25-27,55,65). Numerous minor salivary glands in the palatal mucosa (fig. 6) maintain its characteristically moist surface.

Immediately before fixation, the mucous membrane of the biopsy is usually thoroughly washed with a jet of saline (18). The oral mucosa as well as the teeth are *in vivo* rapidly covered with a protective film containing salivary macromolecules and different bacteria which are partly removed by thoroughly rinsing (fig. 7). The protective role of the oral mucosa should be considered not only in terms of resistance to mechanical insult but also as a biological barrier to micro-organisms and toxic compounds.

Specialized mucosa

The mucosa covering the upper surface of the tongue is unlike that anywhere else in the oral cavity in that it has different kinds of lingual





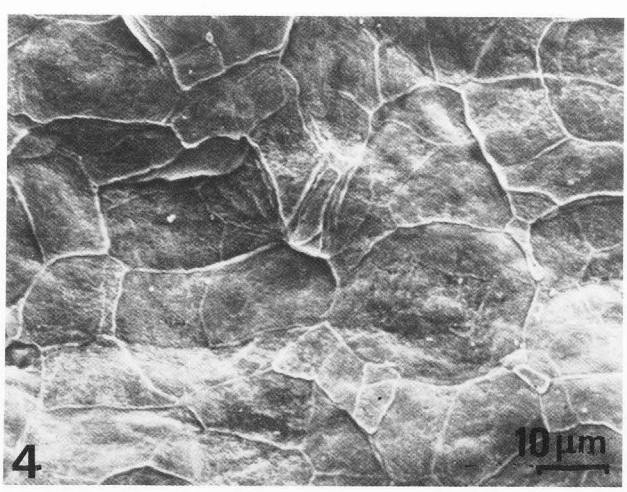
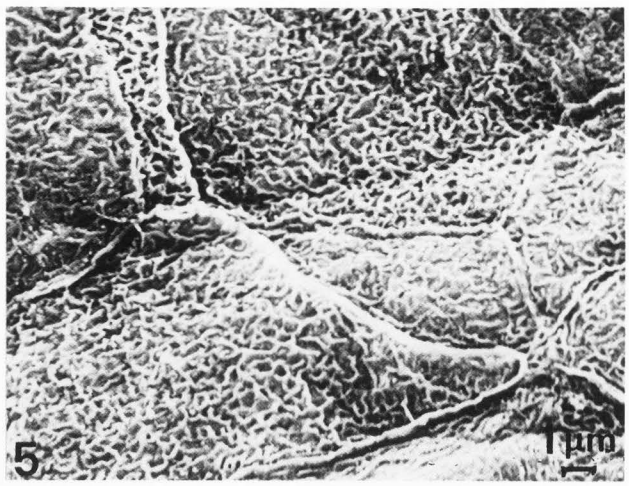
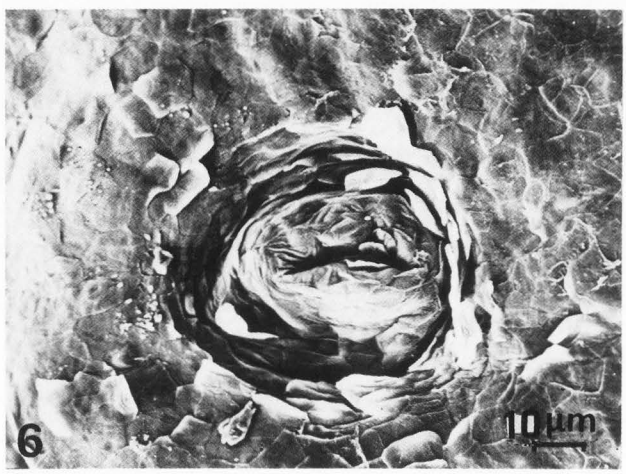


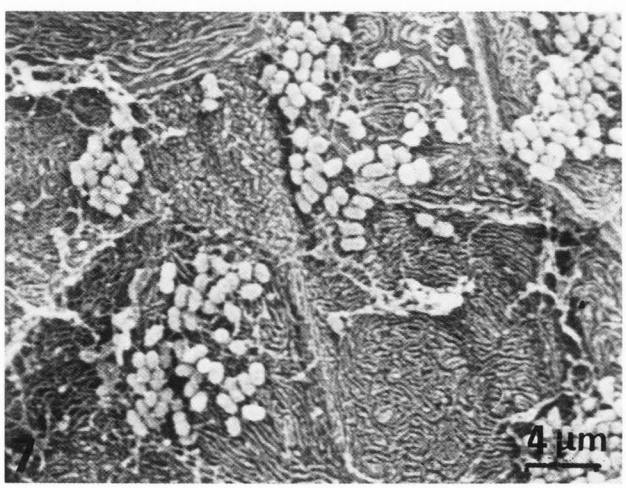
Figure 2. Oral surface of human buccal mucosa showing an uneven corrugated surface.

Figure 3. Sinuous interlocking pattern of microplications of cells of nonkeratinized human buccal mucosa.

Figure 4. Oral surface of human hard palate showing a mosaic-like pattern of squamous cells.







papillae (6,15,16,22,42,45,46). Four different kinds of lingual papillae are found on the upper surface of the tongue; namely the circumvallate, foliate, fungiform and filiform papillae. The circumvallate, foliate and fungiform papillae bear taste buds and have a sensory function. The filiform papillae have only a mechanical function. Scanning electron micrographs of different papillae in the rabbit, rat and dog have been presented by Beidler (12,13) and Bradley (19).

The SEM technique has been used only to a very limited extent for the study of the human tongue. Filiform and foliate papillae have been examined, at different ages, by Kullaa-Mikkonen and Sorvari (45), Skach and Svejda (79) and Svejda and Janota (85). The epithelium of human fetal tongue, adult tongue and a brief description of the bacteria on adult tongue have been reported by Boshell et al. (16).

The anterior and posterior parts of the tongue have different embryologic origins (for a recent review, see 88). The embryology of the tongue and taste buds has been studied by several authors (for reviews, 20,21). Scanning electron micrographs have also been used to illustrate topographical changes during the development of the circumvallate, fungiform and filiform papillae of the rat (61) and during atrophy of the lingual mucosa of the cat after nerve transection (62).

Between the anterior and posterior parts of the tongue, close to the foramen caecum, the circumvallate papillae, in man usually 8-12, are organized in a V-shape. The circumvallate papillae are embedded in the surface of the mucous membrane and each papilla is surrounded by a deep circular furrow (fig. 8). These papillae have a connective tissue core covered on the superior surface with keratinized epithelium. The epithelium on the lateral walls of the circumvallate papillae is non-keratinized and is usually the site of many taste buds (fig. 9).

Figure 5. Keratinized oral cells from human hard palate at a higher magnification than in fig. 4.

Figure 6. The opening of a minor salivary gland of the human hard palate.

Figure 7. Human hard palate showing bacteria (cocci) and granular materials. The cocci are randomly distributed in small clumps.

Figure 8. Oral surface of human circumvallate papillae. Note the desquamation of epithelial cells.

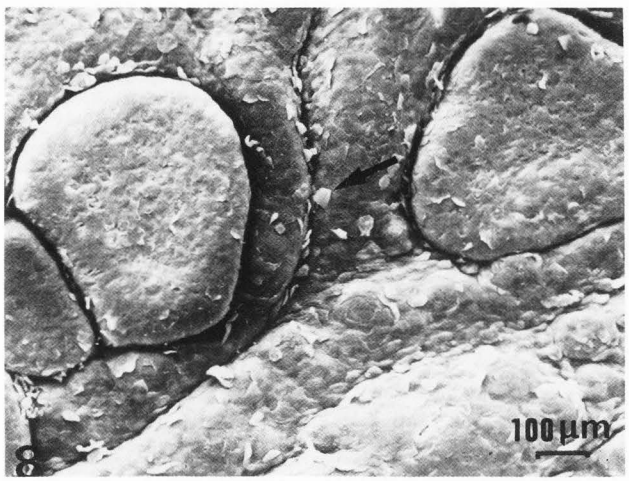
Figure 9. Light micrograph of a longitudinal section of a human circumvallate papilla with numerous taste buds on the wall. Haematoxylin & eosin.

Figure 10. A human fungiform papilla. The location of rounded nuclei is indicated by the presence of smooth elevations (arrow).

Figure 11. Light micrograph of a longitudinal section of a human fungiform papilla showing a taste bud on the upper surface (arrow).

Figure 12. Numerous filiform papillae from the anterior part of a human tongue.

Figure 13. Surface area of a human fungiform papilla showing openings of two taste buds (arrows).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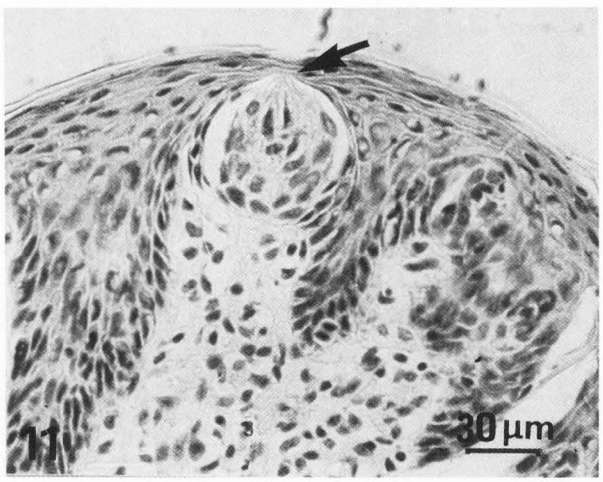
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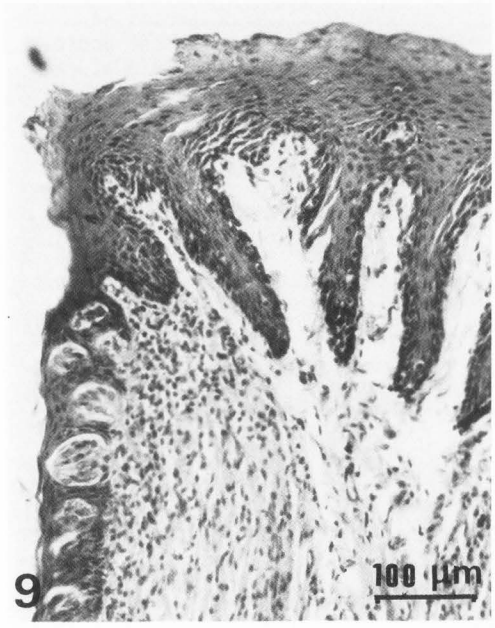
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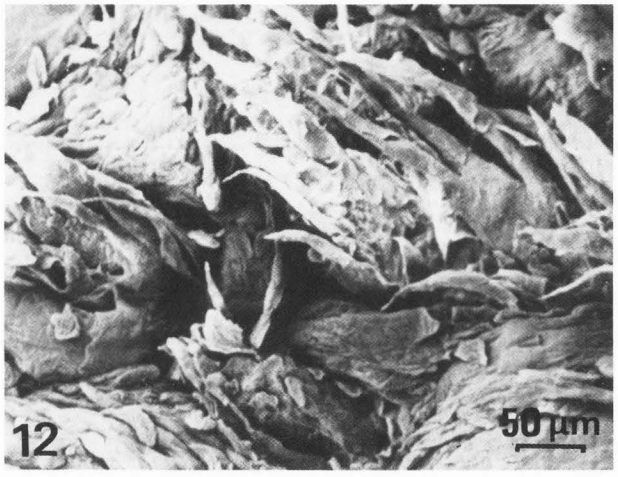
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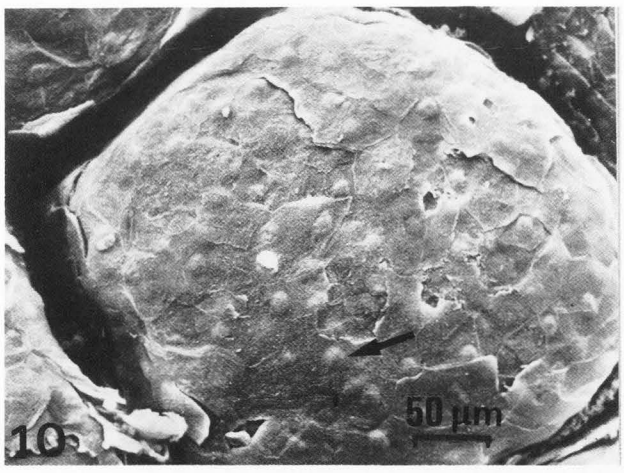
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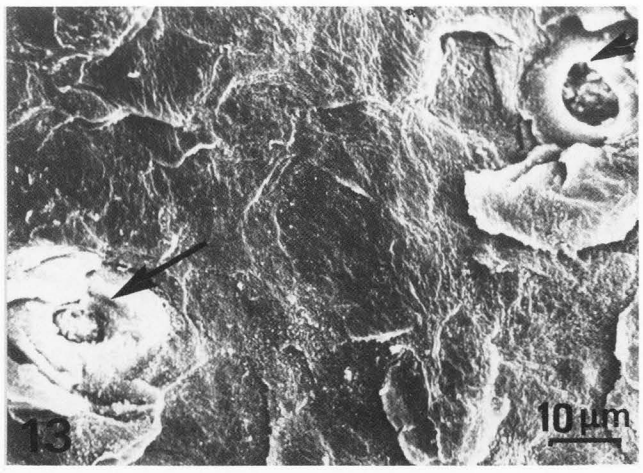

13
10 µm

The foliate papillae appear in parallel folds on the lateral parts of the tongue, close to the circumvallate papillae. In man, few taste buds are found in the epithelium of the lateral walls of the folds. In the rabbit, however, the foliate papillae and their taste buds are well developed and used in experimental research (39).

The fungiform papillae (fig. 10) are club-shaped and scattered over the upper surface of the tongue, with most at the tip and on the lateral margins. In man it is estimated that there are about 150-400 fungiform papillae per tongue (1). Taste buds (fig. 11), when present, are found in the non-keratinized epithelium on the superior surface of these papillae. By using light microscopy and scanning and transmission electron microscopy the location, number and frequency of taste buds on fungiform papillae in man and monkey have been described in detail by Arvidson (4-6), Arvidson and Friberg (10) and Arvidson et al. (8,9).

Filiform papillae (fig. 12) cover the anterior part of the tongue and consist of pointed, cone-shaped papillae containing a core of connective tissue covered with keratinized epithelium. In the cat, marked regional variations in size, shape and organization of the filiform papillae were shown by Boshell et al. (17) in microscopic studies.

Taste pore

The taste bud communicates with the oral cavity through a taste pore. The taste pore probably plays an important role in taste transmission, permitting access to taste stimuli to the taste bud. SEM studies can contribute to a more detailed understanding of the mechanism of taste. SEM studies on taste pores of several species have been reported. Graziadei (30,31) examined, for example, fungiform papillae in the rat and the frog, as well as taste buds in the lips of fish. SEM studies on the taste organ of the frog have also been reported by Shimamura and Tokunaga (75) and Graziadei and DeHan (32). Shimamura et al (76) were the first to investigate in greater detail the pore of mammalian taste buds. They studied circumvallate and foliate papillae in the rabbit and described the occurrence of two types of cellular projections or taste hairs.

Arenberg et al. (3) studied the outer taste pore of human fungiform papillae. Arvidson (4) showed that in both human and simian fungiform papillae, the taste pores opened as rounded craters, slightly elevated above the surface of the papilla. The wall of the crater was formed by three to four squamous epithelial cells lying side by side. The diameter of the opening varied between different taste buds within a range of 1-12 µm and most of the taste pores had a diameter of about 5-7 µm (fig.13). These results were later confirmed by Kullaa-Mikkonen and Sorvari (45). The corresponding figures for rat and rabbit were 1-2 µm and up to 4 µm, respectively (30,31,76). In large-bore pores of human and simian fungiform papillae there were finger-like protrusions or microvilli, which were irregularly arranged and did not extend to the free margin of the pore (4).

Regional variations of the normal oral mucosa

In the light microscope the different regions of the oral mucosa as well as keratinized and non-keratinized oral mucosa, can easily be identified (83,88). The upper surface of the tongue with its various types of papillae can also be easily identified in SEM (45). Identification of the other types of oral mucosa in the SEM seems to depend on the degree of keratinization and nature of mechanical retention between the cells (26,27) and may also be affected by various pathological changes (23,28, 51).

Stereological technique has been used by Nair and Schroeder (64) on normal oral lining mucosa, viz., the buccal and labial mucosa of Macaca fascicularis to determine the variation and density of the microplication patterns as described earlier. Matravers et al. (53) used computer analysis to distinguish different areas of the porcine oral mucosa, viz.,the hard palate, buccal mucosa, alveolar mucosa, attached gingiva, central surface of the tongue and lower lip. The following SEM characteristics of groups of spatially related cells observed in the photographs at 2000 x were recorded; individual cell shape described as either polygonal or irregular, contact relationships of adjacent cells recorded as ridged, overlapped, smoothly abutting or grooved, contours of underlying cell-contact relationships and nuclear contour (53). Furthermore, these authors classified at 5000 x the superficial morphology of individual cells. The following features were recorded; microvilli or short finger-like projections, pits surrounded by prominent ridges, pits without prominent surrounding ridges, rounded ridges without pits, continuous parallel ridges, short discontinuous ridges, whorled spaghetti-like arrangement of ridges and amorphous pattern (53).

The conclusions from these studies(53,64) are that although keratinized and non-keratinized mucosa could be consistently distinguished, the analysis offered no advantages as a means of individual tissue identification over conventional histological examination. Recently, computerized image analysis systems have been developed for use in SEM research (44). Such systems should prove applicable in quantitative analysis of morphometric parameters.

Figure 14. Phase-contrast observation of human buccal fibroblasts.

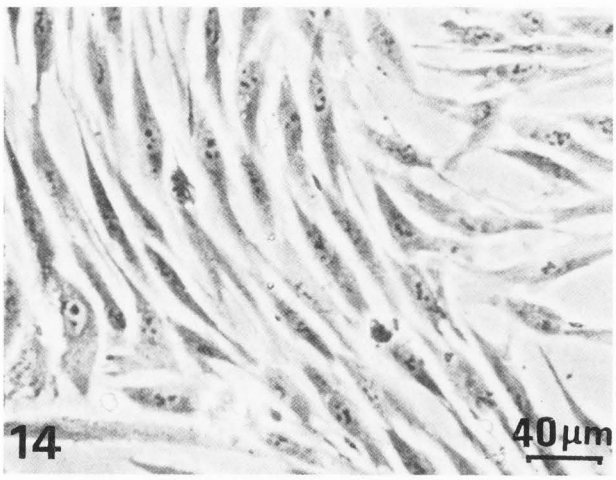
Figure 15. Phase-contrast observation of human buccal epithelial cells.

Figure 16. SEM observation of human epithelial cells grown on a plastic dish.

Figure 17. SEM observation of an epithelial cell showing numerous medium sized microvilli.

Figure 18. TEM observation of epithelial cells cultured for 2 weeks. Note numerous tonofilaments (t), and interdigitations (i). One desmosome (d) between two epithelial cells is visible.

Figure 19. SEM observation of a part of a fibroblast.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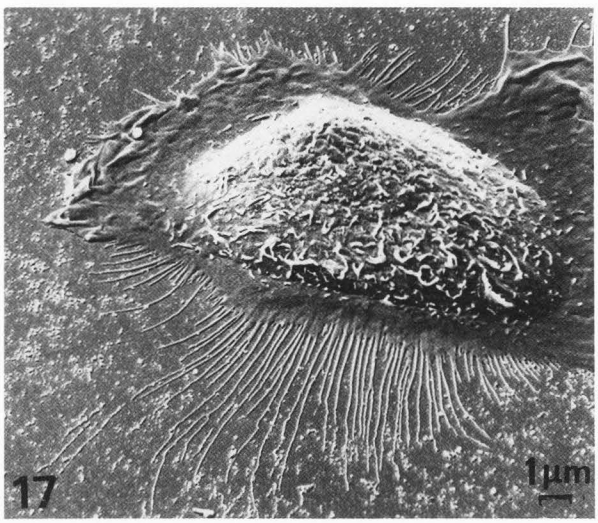
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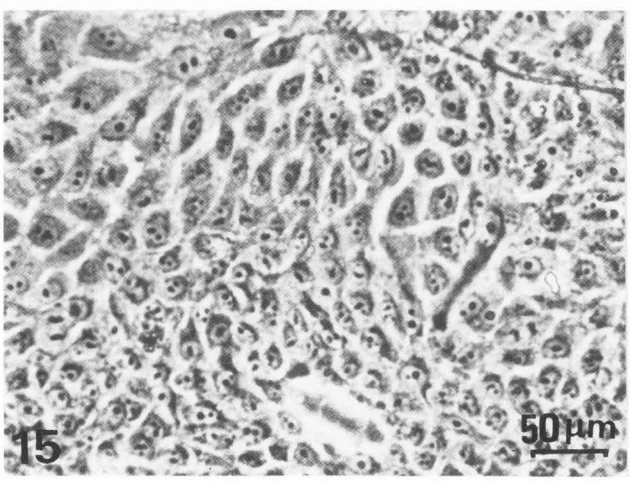
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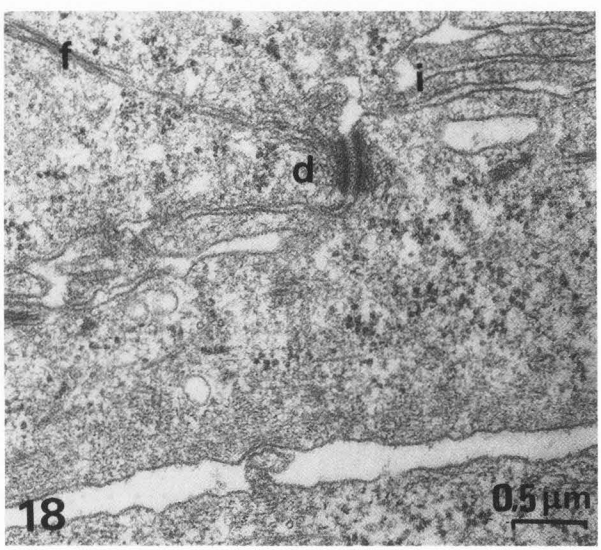
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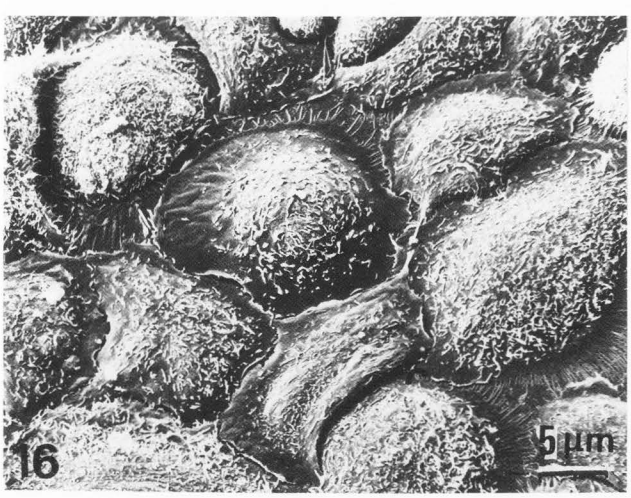
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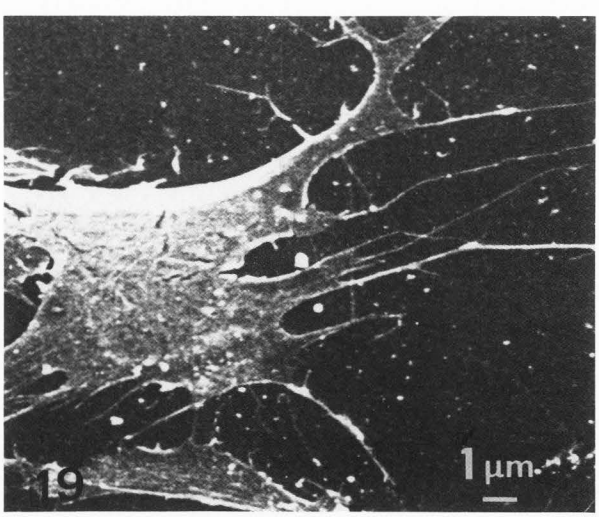
19
1µm

In vitro studies of oral mucosa

The cell and tissue culture technique was introduced by Harrison in 1907 (37). Such experimental systems have since then been applied in many fields of medical and biological research (35). Among these are studies on the effects of carcinogens on cells (34), nutritional requirements of cells (54,63), growth factors (47,49), cell division and cell differentiation (50).

Mainly two types of cells can be obtained in vitro from oral mucosa, fibroblasts and epithelial cells. Oral mucosa is, however, of particular interest for studies of keratinization as it encompasses a spectrum of epithelial surfaces ranging from the non-keratinizing areas of the lining mucosa to the frankly orthokeratotic parts as the hard palate. Tissue culture of normal oral mucosa of human and other mammalian origin has therefore been carried out by many investigators. Meyer et al. (60) compared the mitotic activity in cultivated mucosa from four different regions of the mouse oral mucosa (the cheek, the floor of the mouth, and the lateral and central parts of the hard palate). Porter (66) cultured the masticatory mucosa from rat fetus to determine both normal growth and maturation patterns of mucosa in vitro and tissue repair. Silverman and Vaeth (78) cultured normal gingiva to investigate some of the problems encountered in comparing explanting and cultivating oral cells and the growth behaviour of malignant tumors. Silverman (77) also cultured normal oral mucosa from the tongue to study the ultrastructure of epithelial-like and fibroblast-like cells and compared them with explant sources and tissue in culture. Smulow and Glickman (81) cultured adult human attached gingiva and established a permanent epithelial cell line from clinically normal adult gingiva. Flaxman et al. (29) grew buccal mucosa in vitro and stated that epithelial cells were able to mature in an organized way. The epithelial outgrowth from adult buccal mucosa, in the absence of underlying connective tissue, formed multilayers with a consistent pattern of organization. Jepsen (40) studied the oral mucosa of the rodent.

Rheinwald and Green (72) reported a method for long-term cultivation of epidermal keratinocytes using feeder layers of mouse 3T3 fibroblasts. This method has also been applied to the cultivation of human gingival and buccal epithelial cells (33,86,87). Lechner et al. (49) reported improved conditions for clonal growth of normal bronchial epithelial cells with neither serum nor feeder cells.

There are principally two main ways of harvesting cells from oral mucosa for cell culture studies. One technique involves the use of digestive enzymes for the release of cells from the tissue (38). In the other method, the cells are allowed to grow out from a biopsy. The latter method is usually referred to as the explant technique (7).

Cell culture methodology

Our laboratory has been involved in the development of explant techniques to grow epithelial cells and fibroblasts originating from normal oral mucosa from adult donors. Human buccal mucosa was obtained at autopsy or surgery. Under sterile conditions the tissues were cut into explants of 0.5 cm^2 which were placed in the center of 60 mm tissue culture dishes and incubated in a growth medium. The growth medium for obtaining fibroblasts was CMRL 1066 medium supplemented with 10% fetal calf serum and factors (84). The medium used for epithelial cells was a slightly modified 1:1 mixture of LHC and EGM medium (48,54) containing only 0.6% fetal calf serum.

With both types of media outgrowths of fibroblasts and epithelial cells were initially obtained as could be easily distinguished by their typical morphology under phase contrast microscopy. After another 2-3 weeks of culture (figs. 14,15) the cells were processed for scanning and transmission electron microscopy.

Cultured material for SEM was usually produced by a method which facilitates handling during SEM preparation. On the plastic tissue culture dish cells were punched out of suitable diameter for passage through fixation vessels and specimen holder for critical point drying and microscopy. For SEM the cells were initially rinsed several times with Hepes buffered saline solution for 10 minutes and fixed 2 h in 2% glutaraldehyde buffered to pH 7.4 with 0.1 M cacodylate. The cells were then postfixed in 1% osmium tetroxide for 1 h and dehydrated with ascending grades of ethanol. The cells were critical point dried, mounted on metal stubs and coated with gold-palladium. The scanning microscope used was a Philips SEM 501.

For TEM, half of the culture media was poured off and compensated with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 30 minutes at room temperature. The mixture was poured out and the cells were fixed for another 2 h in 2% glutaraldehyde in the same buffer at 4°C. The cells were postfixed for 2h in 1% osmium tetroxide, rinsed in buffer, dehydrated and embedded in LEX 112 (Ladd Research Industries Inc., Burlington, VT). After examining 1 µm survey sections stained with toluidine blue, ultrathin sections of selected areas were cut with an LKB IV ultramicrotome. The ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 401.

The epithelial outgrowths were comprised of flattened polygonal cells (fig.16) containing abundant tonofilaments and typical desmosomes (fig.17), characteristics of cells exhibiting the ability to undergo keratinization. The cells showed medium sized microvilli (fig.18). In contrast, the fibroblasts had a spindle-shape containing few microvilli and were not joined by desmosomal junctions (fig.19).

Ongoing studies indicate that the cellular fine structure of epithelium and connective tissue cells can easily be seen when SEM is used after in vitro maintenance of explants.

Conclusions

SEM can be used to study effects on the three-dimensional morphology of the oral mucosa related to several physiological functions including: sensation, secretion and protection. Furthermore, tissue progression through different developmental stages can also be studied. With regard to differentiation, it is mostly possible to distinguish keratinized from non-keratinized mucosa.

Furthermore, cultured oral epithelial cells and fibroblasts can also be studied with SEM to examine specific cellular fine structures. Thus, SEM will be useful for following morphological changes during proliferation, maturation and interaction between different cell types in culture. Finally, the possible influence of various foreign compounds on the ultrastructure of human oral mucosa could also be studied both on tissue and cellular levels.

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Discussion with Reviewers

SH Ashrafi: What type of bacteria did you see in Fig. 7?

Authors: In this study the types of bacteria present on the hard palate was not characterized. It is commonly known, however, that streptococci are prevalent in dental plaque formation and the adhesion to the mucosa.

A. Kullaa-Mikkonen: Do you find differences between keratinized and non-keratinized epithelial cells in cultured material?

Authors: In culture, both proliferating, non-differentiated and highly differentiated (squamous differentiation) buccal/gingival epithelial cells express keratins. Our ongoing studies indicate that different types of keratin are formed during various stages of differentiation. These findings are preliminary and should be considered as unpublished information. In Reference 24, information about keratin expression during differentiation of different human oral epithelia can be found.

JP Waterhouse: The authors state accurately and succinctly in reference to normal mucosa - "identification of the other (than lingual) types of oral mucosa in the SEM seems to depend on the degree of keratinization and nature of mechanical retention between the cells". In their review, they cite quite numerous accounts of the findings in oral mucosa as affected by various pathologic states. To what extent can generalizations be written at this time to summarizing the SEM changes in oral mucosa in pathologic states, if these are characterized by the pathologic process that led to them? Three important pathologic processes that could be chosen are acute inflammation, degenerative change (due to toxic substance), or malignant neoplasia leading to, e.g. squamous carcinoma. Would such general descriptions of the SEM findings in mucosal lesions which result from the effects of these named pathologic processes correspond well to those which would be anticipated if they caused mainly differences in: "degree of keratinization and nature of mechanical retention between the cells?"

Authors: To determine pathological states of oral mucosa, the use of SEM methodology is a valuable complement in analysis of tissue sections on both light and ultrastructural levels. More information concerning use of SEM for analysis of pathological changes has been reviewed in Reference 28.