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Scanning Electron Microscopy of Different Types of Oral Leukoplakia: Comparison With Normal and Malignant Oral Mucosa

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The present study analysed surface architecture of normal, premalignant and malignant oral mucosa using scanning electron microscopy to evaluate its role in early diagnosis of potentially malignant oral lesions. The surface ultrastructure of the buccal mucosa in tobacco chewers showed variations from that of non-chewers. Homogenous leukoplakia demonstrated well-defined intercellular junctions and the microrugal surface pattern as seen in normal mucosa. In verrucous leukoplakia, the surface layer consisted of characteristically-shrunken desquamated hyperkeratotic cells. Erosive leukoplakia had a discontinuous superficial layer along with complete loss of intercellular ridges. Speckled leukoplakia also showed marked abnormalities such as thickened irregular protrusions and evidence of a villus-like pattern. These villus-like structures were comparatively prominent in leukoplakia showing dysplasia. Oral carcinoma showed marked altered surface ultrastructure and had a pattern similar to dysplastic lesions. The irregular swollen elongated protrusions with villous-like structures that were observed in carcinoma and dysplastic lesions can, therefore, be considered as surface markers for potentially malignant leukoplakia.
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INTRODUCTION

CANCER of the oral cavity is an important contributor to the overall cancer burden, especially in developing countries. The high incidence of oral cancer in India has long been linked with the habit of betel quid chewing, incorporating tobacco [1, 2]. Longitudinal studies have revealed that these tumours do not always arise directly from the oral epithelium. The epithelium probably goes through stages of initiation and promotion leading to well-defined precursor lesions, of which oral leukoplakia is the most common [3]. It has been reported that more than 75% of oral cancers develop from pre-existing leukoplakia [4]. Clinically, these leukoplakia can be classified into homogenous leukoplakia (simplex) and non-homogenous leukoplakia, the latter including verrucous, speckled or nodular and erosive or erythroleukoplakia [5]. Various follow-up studies have reported that speckled and erosive leukoplakia have a greater tendency to undergo malignant transformation [4, 6, 7]. Histologically, leukoplakia can be subdivided into dysplastic and non-dysplatic types and it has been shown that dysplastic lesions have higher malignant potential than non-dysplastic types [7]. Various techniques have been reported for the early detection of malignant potential in oral leukoplakia [8]. We have described previously the role of transmission electron microscopy in predicting the malignant potential of oral leukoplakia [9].

Scanning electron microscopy (SEM) has been performed by many investigators to study the surface morphology of various tissues [10–13]. Several studies have described the cell surface characteristics of normal and malignant oral mucosa [13–15], but only a few comparative investigations on different clinical and histological types of oral leukoplakias have been reported [16–18]. In the present study we have evaluated the surface ultrastructure of normal mucosa, both in subjects with and without the habit of tobacco chewing, various clinical and histological types of oral leukoplakia and carcinoma. We have also compared the surface ultrastructural changes of these lesions to identify the surface markers of malignant transformation in oral leukoplakia.

MATERIALS AND METHODS

Patients with lesions in the buccal mucosa were included in the present study. SEM was performed on 7 normal control, 14 leukoplakia and 3 carcinoma patients. Normal controls

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Table 1. Various types of oral mucosal epithelium analysed

Clinico-pathological diagnosis	No. of cases
Normal buccal mucosa	
Without chewing habit	3
With chewing habit	4
Non-dysplastic leukoplakia	
Homogenous (simplex)	3
Verrucous	3
Erosive (erythroleukoplakia)	2
Speckled (nodular)	3
Dysplastic leukoplakia	
Erosive	1
Speckled	2
Squamous cell carcinoma	3

were grouped on the basis of their betel-tobacco chewing habit and leukoplakia lesions were classified by both clinical and histological characteristics [5, 7]. The numbers of lesions in each group are shown in Table 1. An incision biopsy was taken from the lesion and cut into two portions. One portion was used for SEM and the other was fixed in buffered formalin for routine histopathological examination. In the cases of normal controls, a small bit of mucosa was taken from patients undergoing oral and maxillofacial surgery and processed for SEM. Tissue portions for electron microscopy were washed in normal saline to remove blood and fixed in 2.5% phosphate-buffered glutraldehyde (pH 7.4) for 2 h. The tissues were cut into 3-mm^2 pieces and dehydrated in a critical point dryer. Specimens were mounted on brass studs and coated with a thin film of gold using an ion sputtering device. Observations were carried out in a JSM-35 SEM at 12–15 KV accelerating voltage.

RESULTS

SEM of normal buccal mucosa showed dense and closely-packed polygonal cells with a mosaic-type arrangement. The cell borders were fairly demarcated and the intercellular junctions formed loop-like elevated ridges, termed intercellular ledges [10] (Fig. 1). At higher magnifications, cells also exhibited ring-shaped arborising projections, the microridges or microrugae [11] of which were arranged in an orderly fashion on the cell surface. Very few small round and often pedunculated structures were seen to arise from the delicate reticular surface (Fig. 2).

The buccal mucosa of betel chewers revealed deposits of betel constituents on the epithelial surfaces. The free surface was characterised by regular cell borders and with signs of cell desquamation. Cell surfaces were more or less smooth. On higher magnification, the microridges were not distinguishable, as seen in the non-chewer's mucosa but appeared as minute parallel elevations (Fig. 3).

In homogenous leukoplakia, polygonal cells with reticular surface pattern of microrugae similar to normal buccal mucosa were observed. In some areas, occasional dissociation of epithelial cells was noticed with evidence of overlapping of cell junctions (Fig. 4).

A complete loss of polygonal cell outline and mosaic pattern as seen in verrucous leukoplakia. The cells showed marked dissociation and a number of cells were hanging from the surface. These keratinised desquamated cells were shrunken, amorphous and often navicular in appearance (Fig. 5).

The intimate association of polygonal cells as completely disturbed in erosive leukoplakia. The elevated ridges of the intercellular junctions seen in their normal counterparts had

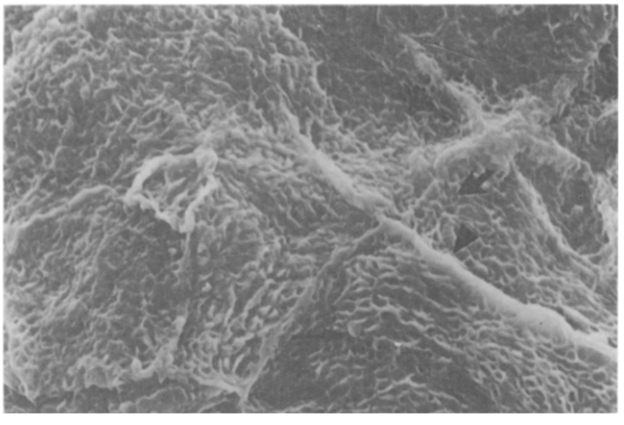

Fig. 2. Normal buccal mucosa showing ring shaped arborising microrugae (arrow), intercellular ledges (arrow head) and round pedunculated structures (small arrow) (original magnification 3000 ×).

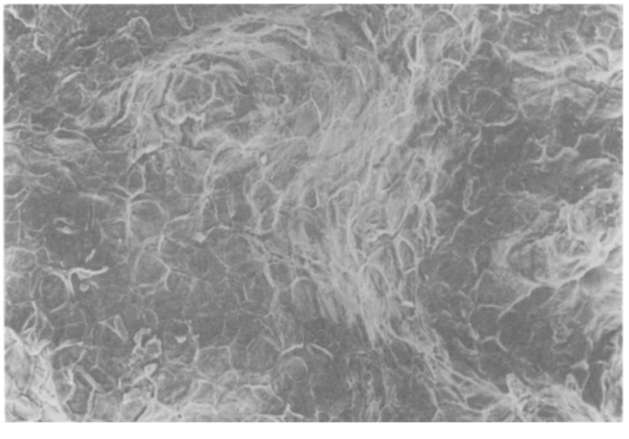

Fig. 1. Normal buccal mucosa showing dense and closely-packed polygonal cells with mosaic pattern and fairly demarcated cell borders (original magnification 300 ×).

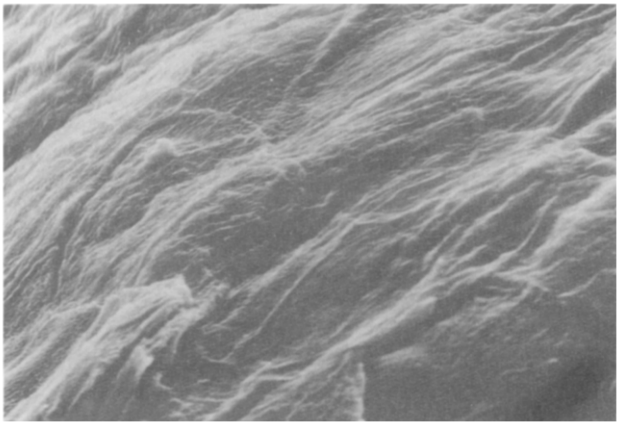

Fig. 3. Buccal mucosa of a betel chewer showing smooth cell surfaces with parallel elevations (original magnification 1600 ×).

completely disappeared. Cells were overlapping, resulting in the formation of scattered empty spaces (Fig. 6). These areas were often occupied by desquamated cells. At higher magnification, solitary cells displayed the loss of mosaic pattern and microridges were irregular. Occasional surface invaginations and protrusions with varied sizes and shapes were noticed on the cells (Fig. 6).

In speckled leukoplakia, the epithelial surface was composed of thickened, elongated, often zig-zag-shaped, swollen, irregular protrusions. A complete loss of polygonal cell outline, regular mosaic pattern and intercellular junctions was seen (Fig. 7). At higher magnification, the cell surface showed small club-tipped projections and occasional invaginations.

Dysplastic leukoplakia exhibited more or less similar surface features irrespective of its clinical type. The common characteristic feature was an irregular surface pattern with loss of distinct borders and cell contact. At higher magnification the surface showed discontinued parallel microridges, a pitted surface and club-tipped microvilli. In one sample of dysplastic speckled leukoplakia, the surface contained an elongated, filament-like structure piercing into the tissue, which probably could be a fungal hyphae (Fig. 8).

The surface morphology of oral carcinoma under lower magnification revealed a cauliflower-like appearance (Fig. 9). Cells were round, irregular in size and arranged in dense clusters. Cells were usually found to be piled one over the other. The surface was composed of many spherical stubby and cylindrical villous-like structures of variable size and length (Fig. 10).

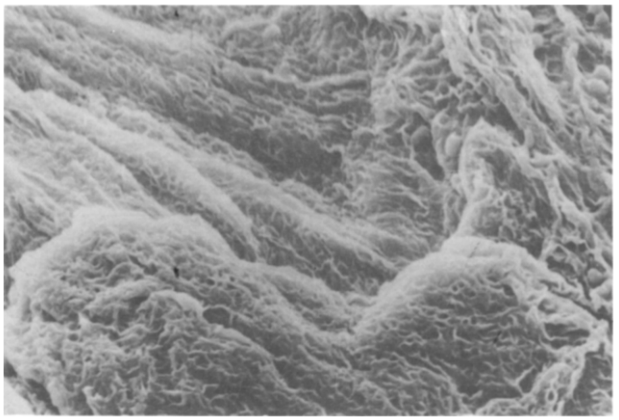

Fig. 4. Homogenous leukoplakia showing cells with reticular pattern of microrugae and overlapping of cell junctions (original magnification 3000 ×).

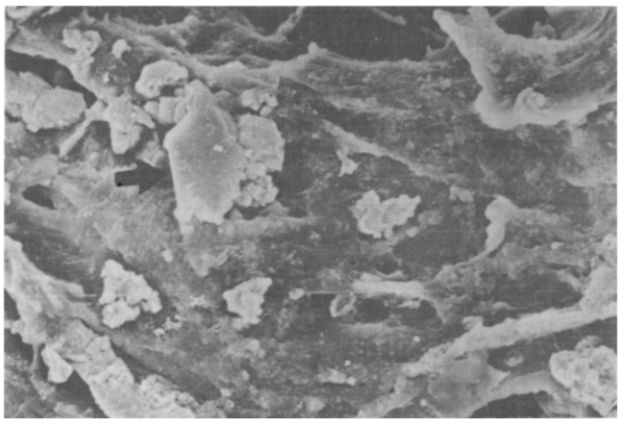

Fig. 5. Verrucous leukoplakia showing marked dissociation, desquamated, keratinized and navicular cells hanging from the surface (arrow) (original magnification 1600 ×).

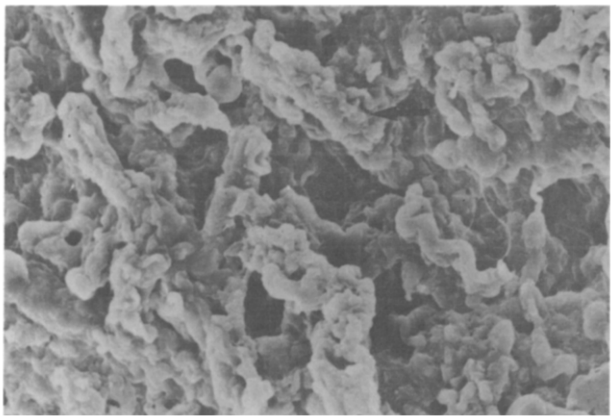

Fig. 7. Speckled leukoplakia showing thickened, elongated and swollen irregular protrusions (arrow) with complete loss of mosaic pattern and intercellular junctions (original magnification 1600 ×).

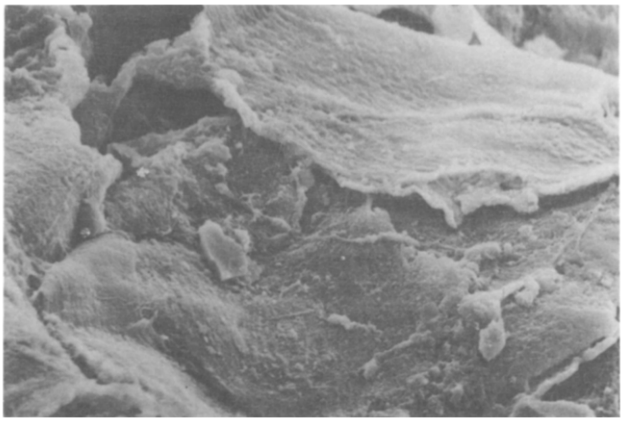

Fig. 6. Erosive leukoplakia showing disruption of mosaic pattern, presence of crevices and cellular overlapping (original magnification 1600 ×).

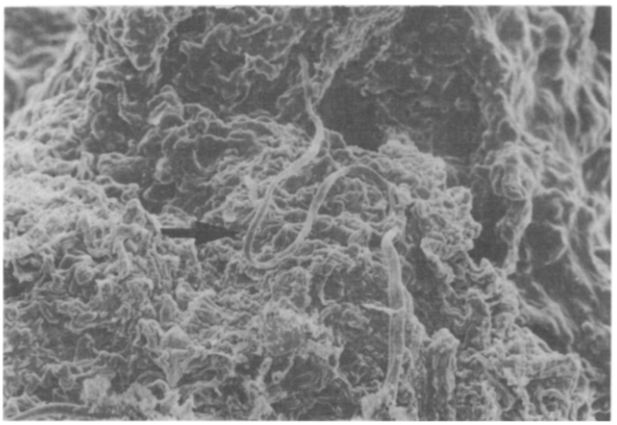

Fig. 8. Speckled leukoplakia with dysplasia showing an irregular surface with an elongated fungal filament invading the epithelium (original magnification 300 ×).

DISCUSSION

The oral epithelium exhibits regional variations in the degree of keratinisation [19]. Ultrastructural appearance of keratinised and non-keratinised oral epithelial surfaces have been reported [14, 20]. Matravers and Tyldesley [14] have shown that the presence of microridges arranged in parallel rows or in an irregular honeycomb pattern appears to be a characteristic of an epithelium undergoing keratinisation. Maclord [21] observed regular surface microplications on exfoliated healthy oral squamous cells. The term "microplications" was used by Cleaton-Jones and Fleisch [22] to describe folds of the cell membrane running parallel to each other. This microplication pattern forms part of the cellular interdigitation mechanism in stratified squamous epithelium. Similar microridges have also been observed in squamous cervical epithelium undergoing keratinisation [20]. The difference may well be that a keratinised epithelium with its protective role would need such a mechanism, where it may be less necessary in a non-keratinised epithelium [14]. In our study, the non-keratinised buccal mucosa cells appeared to be characterised by fine circumvallated or ring-shaped microridges. The smooth appearance of the epithelial surface with reduced parallel microridges in betel-tobacco chewers may reflect evidence of epithelial keratinisation. Similar observations were also reported in the oral mucosa of betel chewers by Reichart *et al.* [23].

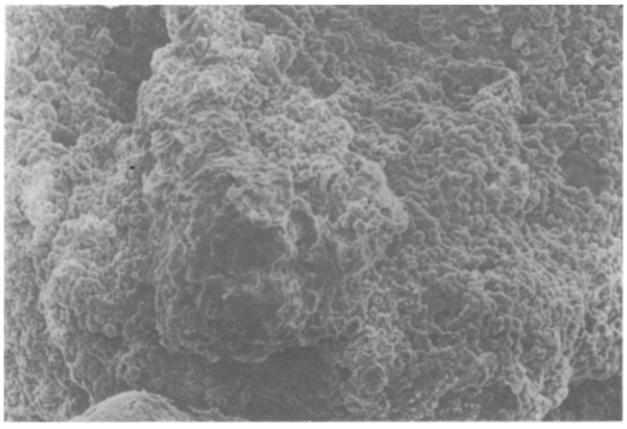

Fig. 9. Squamous cell carcinoma showing a characteristic cauliflower-like appearance (original magnification 300 x).

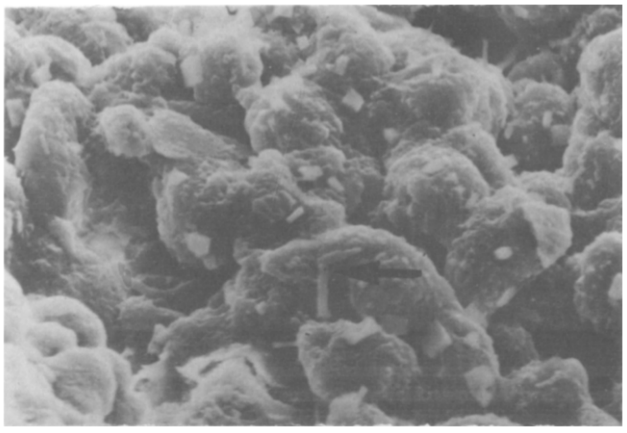

Fig. 10. Squamous carcinoma cells showing many spherical stubby and cylindrical villous-like structures (arrow) (original magnification 3000 x).

Bahr and Bahr [24] reported that the sharp cell borders in normal superficial cells are due to the very thin cytoplasm at their perimeters. This was reinforced by a bulge in which the microridges assume a dove-tail function for cell-to-cell adhesion. The sharp delineation of superficial cells caused by the peripheral bulge may be a sign of cell maturity. Conversely, the absence of this feature in dysplastic lesions suggests cell immaturity. In carcinoma, the surface exhibited a totally irregular pattern, indicating the complete loss of co-ordination in cell maturation and differentiation. It may also be the result of total loss in contact cell inhibition, a characteristic feature of malignancy. Our recent reports on transmission elecron microscopic studies on various histological types of normal, premalignant and malignant oral mucosa revealed that loss of desmosomes and marked spongiosis are the common ultrastructural features in different types of carcinoma [9, 25]. These features were also prominent in potentially malignant leukoplakia. This loss of intercellular contact may lead to aberrant differentiation or maturation of the cells that may be clearly visualised in the epithelial surface of dysplastic and malignant lesions.

Comparing the surface pattern of various non-homogenous types of leukoplakia with carcinoma, it was seen that speckled leukoplakia had many features in common with carcinoma. Follow-up studies have shown that among the various types of leukoplakia, the speckled type had the highest transformation rate [4, 6]. Another supporting finding about the behaviour of speckled leukoplakia from this study is that dysplastic leukoplakia (irrespective of its clinical features), had a similar surface ultrastructure as that seen in non-dysplatic speckled leukoplakia. A comparative study in different types of oral leukoplakias by Banoczy *et al.* [16] observed that erosive leukoplakia had more surface alterations than seen in verrucous or homogenous leukoplakia. Thus, the irregular swollen elongated protrusions seen in these lesions may have some diagnostic and/or prognostic importance and can also be used as a surface marker. However, to establish its validity, these features should be examined in large numbers of patients with regular follow-up.

1. Sankaranarayanan R, Duffy SW, Day NE, Nair MK, Padmakumary G. A case–control investigation of cancer of the oral tongue and the floor of the mouth in Southern India. *Int J Cancer* 1989, **44**, 617–621.
2. Sankaranarayanan R, Duffy SW, Padmakumary G, Day NE, Nair MK. Risks factors for cancer of the buccal and labial mucosa in Kerala, Southern India. *J Epidemiol Comm Health* 1990, **44**, 286–292.
3. Gupta PC, Mehta FS, Daftary DK, *et al.* Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up of Indian villagers. *Dent Oral Epidemiol* 1980, **8**, 287–333.
4. Sankaranarayanan R. Oral cancer in India: an epidemiologic and clinical review. *Oral Surg Oral Med Pathol* 1990, **69**, 325–330.
5. Axell T, Holmstrup P. Kramer IRH, Pindborg JJ, Shear M. International seminar on oral leukoplakia and associated lesions related to tobacco habits. *Comm Dent Oral Epidemiol* 1984, **12**, 145–154.
6. Banoczy J. Clinical and histopathological aspects of premalignant lesions. In Vanderwall I, Snow GB, eds. *Oral Oncology*. Boston, Martinus Nighoff Publishing, 1984, 3–31.
7. WHO Collaborating Center for Oral Precancerous Lesions. Definitions of leukoplakic and related lesions. An aid to studies on oral precancer. *Oral Surg* 1978, **46**, 518–539.

8. Burkhardt A. Advanced methods in the evaluation of premalignant lesions and carcinomas of the oral mucosa. *J Oral Pathol* 1985, **14**, 751–778.
9. Kannan S, Balaram P, Pillai MR, *et al.* Ultrastructural variations and assessment of malignant transformation risk in oral leukoplakia. *Pathol Res Pract* 1993, **189**, 1169–1180.
10. Whittaker DK, Adams D. The surface layer of human foetal skin and oral mucosa: a study by scanning and transmission electron microscopy. *J Anat* 1971, **108**, 453–464.
11. Kaplan GB, Rubès MP, Pameijer CH. SEM of the epithelium of the periodontal pocket. *J Periodontol* 1977, **48**, 634–638.
12. Rubio A, Krantz I. The exfoliating cervical epithelial surface in dysplasia, carcinoma *in situ* and invasive squamous carcinoma I. Scanning electron microscopic study. *Acta Cytologica* 1976, **20**, 144–150.
13. Kullaa-Mikkonen A. SEM in oral mucosal research. *Scanning Microscopy* 1987, **1**, 1145–1155.
14. Matravers J, Tyldesley WR. Scanning electron microscopy of oral epithelial cells. Part I. Normal and malignant tissue. *Br J Oral Surg* 1977–1978, **15**, 193–202.
15. Chomette G, Auriol M. Morphology of precancerous and cancerous cells of the oral mucosa. Changes in the epithelial surfaces (scanning electron microscopy). *Rev Stomatol Chir Maxillofac* 1990, **91**, 259–261.
16. Banoczy J, Lapis K, Albrecht M. Scanning electron microscopic study of oral leukoplakia. *J Oral Pathol* 1980, **9**, 145–154.
17. Reichart PA, Althoff J. Oral leukoplakia; a scanning electron microscopic study of epithelial surface patterns. *Int J Oral Surg* 1983, **12**, 159–164.
18. Matravers J, Tyldesley WR. Scanning electron microscopy of oral epithelial cells. Part II. Potentially malignant lesions. *Br J Oral Surg* 1977–1978, **15**, 203–214.
19. Morgan PR, Leigh IM, Purkis PE, Gardner ID, Muijen GNP, Lane EB. Site variation in keratin expression in human oral epithelia. An immunocytochemical study of individual keratins. *Epithelia* 1987, **1**, 31–43.
20. Zoghby SE, Moussa M. Regional variations of the normal oral mucosa: a study by scanning electron microscopy. *Egypt Dent J* 1986, **32**, 39–56.
21. Macleod RI. Applications of cytology of the diagnosis and prognosis of oral squamous cell carcinoma. *Diss Abstr Int (B)* 1990, **51**, 1202.
22. Cleaton-Jones P, Fleisch L. A comparative study of the surface of keratinized and non-keratinized oral epithelia. *J Periodontal Res* 1973, **8**, 366–370.
23. Reichart P, Boning W, Srisuwan S, Theetranont, Mohr U. Ultrastructural findings in the oral mucosa of betel chewers. *J Oral Pathol* 1984, **13**, 166–177.
24. Bahr GF, Bahr NI. Study of the cell. In Wied GL, Keebler CM, Koss LG, Reagan JW, eds. *Compendium on Diagnostic Cytology, Tutorials of Cytology*. Chicago, Illinois, 1988, 5–17.
25. Kannan S, Kartha CC, Chandran GJ, *et al.* Ultrastructure of oral squamous cell carcinoma: a comparitive analysis of different histologic types. *Oral Oncol, Eur J Cancer* 1994, **308**, 32–42.

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