



Scanning Electron Microscopic Study of Surface Epithelial Cells in Erosive and Nonerosive Oral Lichen Planus

Manjiri Thakur, Vinay Hazare

ABSTRACT

Aim: Aim of the study is to study the surface epithelial cells of erosive, nonerosive lichen planus by scanning electron microscope (SEM) and to correlate it with that of normal mucosa and oral leukoplakia.

Materials and methods: Twenty biopsies of lichen planus and five biopsies of normal mucosa were taken. Half piece of each biopsy was processed for H&E stain and another half for SEM study.

Result: Surface epithelial cells were showing different features.

Conclusion: Definite different features were seen in lichen planus-leukoplakia group than in normal oral mucosa. These changes are due to abnormality in cell maturation and differentiation.

Clinical significance: SEM study is helpful in early detection of dysplasia. It can lead to early treatment of the lesion and thus prevention of squamous cell carcinoma.

Keywords: Scanning electron microscopy, Erosive lichen planus, Nonerosive lichen planus, Oral leukoplakia, Normal oral mucosa.

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INTRODUCTION

Oral squamous cell carcinoma is the most common cancer in men and third most common in women in India. Many of these cases are preceded by premalignant lesions and conditions. So diagnosis and treatment of these lesions is of utmost importance. Investigations which define different stages in development of neoplasm, in relation to morphological parameters may provide a method more effective than routine histology alone. One parameter is that, study of surface structures of premalignant and malignant oral lesion by scanning electron microscope.

Scanning electron microscope (SEM) may be helpful in early diagnosis of dysplasia. Review of literature reveals relative paucity of SEM studies of oral lichen planus.

SEM has been used to describe surface pattern of surface epithelial cells in pathological and normal mucosa. SEM was first applied in field of dentistry by Boyde and Stewart. Oral lichen planus (OLP) was studied by SEM by Matravers and Tyldesley, Reichart and Althoff, Jungell et al, and Thongprasom et al.

MATERIALS AND METHODS

Fifteen patients of oral lichen planus were selected for the study. Of these, 11 were of erosive type and four were of nonerosive type of lichen planus. In five patients of erosive OLP, biopsy was taken from erosive as well as non-erosive site of OLP. So total number of biopsies were 20. Five cases of normal mucosa were studied as control.

Under LA punch, biopsy was taken from selected site. It was divided into two pieces; one was processed for H&E and PAS stain and other was processed for SEM studies.

Methodology for SEM Study

Biopsy was washed in buffer saline and then kept in 2.5% glutaraldehyde for minimum 24 hours. After fixing it was washed 6 to 8 times in 0.1M sodium phosphate buffer to remove all traces of aldehyde. Then dehydration was done with various concentrations of ethyl alcohol. Finally, two changes of absolute alcohol 30 minutes each was used. Specimen was then air dried. Correct orientation was done on aluminum stub and it was glued with silver paste. Then it was transferred to gold sputter coating unit for gold coating. Finally, it was transferred to electron column and was examined under a Cambridge Stereoscan 250 MK 3 SEM, operating at 9.8 to 10 kV at working distance of 8 to 10 mm and tilt angle of 25 degrees. Complete surfaces were

scanned, areas of interest were identified and photomicrographs were taken.

OBSERVATION

Specimens were studied by H&E stain to confirm the diagnosis of lichen planus.

Scanning Electron Microscopic Observations

Clinically Normal Buccal Mucosa

SEM showed surface of buccal mucosa, consisted of polygonal cells in mosaic like arrangement. Cell borders were well-demarcated and made-up of thick ledges. At higher magnification, cells were showing protrusions called as microridges or microrugae. These microridges were running parallel either continuous or discontinuous. They were intercommunicating. Honeycomb pattern was seen in one case. Pitted surface was seen in two cases (Table 1).

S. No.	Features	Total cases	% of cases
1.	Smooth surface	-	-
2.	Granular surface	-	-
3.	Parallel microridges	5	100
4.	Honeycomb patterns	1	20
5.	Pitted surface	2	40
6.	Finger like microvilli	-	-
7.	Club tip microvilli	-	-
8.	Spherical structure	-	-

Nonerosive Oral Lichen Planus

In low magnification, overall picture was of mosaic like arrangement of flattened polygonal cells. But in many areas, overlapping cells were observed. Overlapping indicates loss of cell contact and increase desquamation. It indicates increased keratinization on surface.

At higher magnification, cell surfaces were seen consisted of raised microridges. Individual microridges were loosely arranged. Cell borders were seen as elevated ridges.

Certain cellular and intercellular disturbances were observed like wide variation in cell size, changing shape of cell, irregular surface pattern, i.e. regional variation in surface pattern, irregular group pattern, i.e. different surface pattern of cells in a group, lack of distinct cell border, loss of cell contact, dense cell group.

Erosive Oral Lichen Planus

In low magnification, cells appeared shrunken. In many fields, cells were overlapped giving fish scaled appearance. Cell borders were rolled and thickened. Areas of erosion without keratinized cells were seen.

At higher magnification, cellular features appeared more irregular. Cell surfaces were covered with more irregularly and loosely arranged microridges (Table 2).

From all the above observations, it becomes clear that changes were more irregular in erosive lesions than in non-erosive lesions.

DISCUSSION

Lichen planus is a relatively common, mucocutaneous disorder of yet unknown etiology. Lichen planus was first described by Erasmus Wilson in 1869. Audry in 1894 described oral lesion without skin lesions. Clinically six forms of oral lichen planus are described viz reticular, papular, plaque like, atrophic, erosive and bullous.^{1,2} Various etiological factors include autoimmune, immunodeficiency, cell mediated immune response, drugs, chemicals, trauma, psychosomatic, hereditary, infection, neurogenic, etc.¹⁻⁵ Various diagnostic aids include histopathology, immuno-histochemistry, direct and indirect immunofluorescence, serum immunoglobulin level and study by transmission electron microscope.⁷⁻⁹ Lichen planus is considered as a precancerous condition.^{1,6}

Lichen planus is predominantly a disease of middle age. Peak incidence is reported in 3rd to 5th decade with age range of about 30 to 70 years. In the present study, the age range was 30 to 70 years with peak incidence in 4th, 5th and 6th decade.¹

In the present study, slightly greater incidence was seen in males (60%) as compared to females (40%). It is comparable to that given by Shklar G and McCarthy PL³ Shafer et al¹ and Moschella et al² have reported equal sex distribution. Female predominance has been reported by many workers.

According to Shklar, 65% patients have discomfort in form of pain and burning sensation.^{3,4} In the present study, the same complaint was observed in 86.6% of cases.

Histological features of OLP are as follows:^{1,2,4}

- Hyperparakeratosis
- Hypergranulosis
- Acanthosis
- Liquefaction degeneration in basal cell layer
- Thickening of basement membrane
- Presence of civatte bodies
- Mild epithelial dysplasia
- Juxtaepithelial chronic inflammatory infiltrate.

These findings are similar to that of Matravers J, Tyldesely WR,^{10,11} Jungell P,¹² Reichart PA, Althoff J.^{13,15}

Scanning Electron Microscopic Assessment

Normal buccal mucosa shows polygonal cells with often parallel microridges and occasionally honeycomb

Table 2: SEM observations of oral lichen planus

Features	Total cases		% of cases	
	E	NE	E%	NE%
1. Changes in a cell as a whole				
a. Wide variation in cell size	6	2	54.54	22.22
b. Aberrance in form of cell	7	4	63.63	44.44
c. Degenerative changes	-	-	-	-
d. Irregularity of surface pattern	9	2	81.81	22.22
2. Changes in intercellular relationship	-	-	-	-
a. Irregularity of group pattern	7	3	63.63	33.33
b. Lack of distinct cell border	5	4	45.45	44.45
c. Dense cell group	7	4	63.63	44.44
d. Apparent cell cannibalism	-	-	-	-
e. Loss of cell contact	10	8	90.90	88.88
3. Variation in cell surface pattern				
a. Smooth surface	-	-	-	-
b. Granular surface	-	-	-	-
c. Parallel microridges	10	9	90.90	100
d. Honeycomb pattern	3	4	27.27	44.44
e. Pitted surface	5	5	45.45	55.55
f. Finger like microvilli	3	-	27.27	-
g. Club tip microvilli	4	2	36.36	22.22
h. Spherical structures	2	2	18.18	22.22
i. Cylindrical structures	-	-	-	-
j. Surface evagination	-	-	-	-
k. Slender strands	-	-	-	-
l. Ruffles	-	-	-	-
m. Fillipodia	-	-	-	-

micro-ridges. Cells are closely packed in mosaic like arrangement, cell borders are well-demarcated and made-up of thicker ledges (Figs 1 and 2). Normal keratinized mucosa, i.e. of hard palate, was studied by Matravers and Tyldesley.¹⁰ According to them, cells of hard palate are more elongated and their surfaces show either pitted appearance or short stubby microvilli.

In lichen planus, individual microridges were thickened, loosely and irregularly arranged. Cell borders were seen as elevated ridges. It may be due to decrease in number of desmosomes and widening of intercellular spaces (Figs 3 to 5).

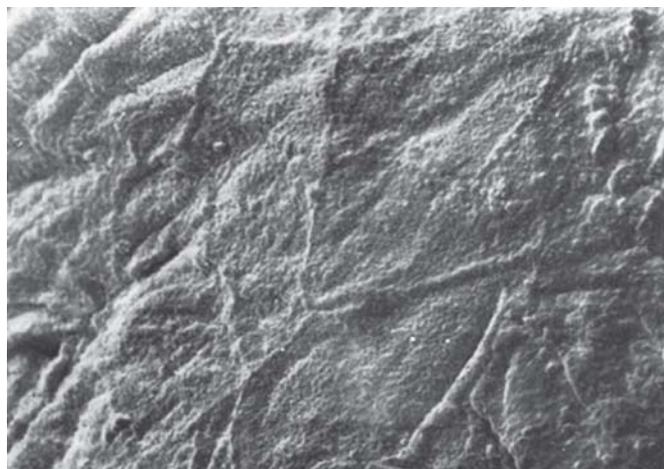


Fig. 1: Normal buccal mucosa. Polygonal epithelial cells are arranged in a mosaic like pattern. Cell junctions can be seen as elevated ridges ($\times 1500$)

In erosive lichen planus, more commonly found features were irregular surface pattern, irregular group pattern, dense cell growth, variation in cell shape, loss of cell contact, wide variation in cell size, indistinct cell borders. Less commonly seen features were, honeycomb pattern, club tip microvilli, finger tip microvilli and spherical structures (Figs 6 to 8).

These findings are similar to that of Jungell et al,¹¹ Reichart and Althoff,¹² Matravers and Tyldesley.¹³

Scanning electron microscopic features of lichen planus were compared with SEM features of oral leukoplakia, studied by Dr Ahuja in the Department of Oral Pathology, GDCH, Nagpur, Maharashtra, India.¹⁴ He studied 40 cases

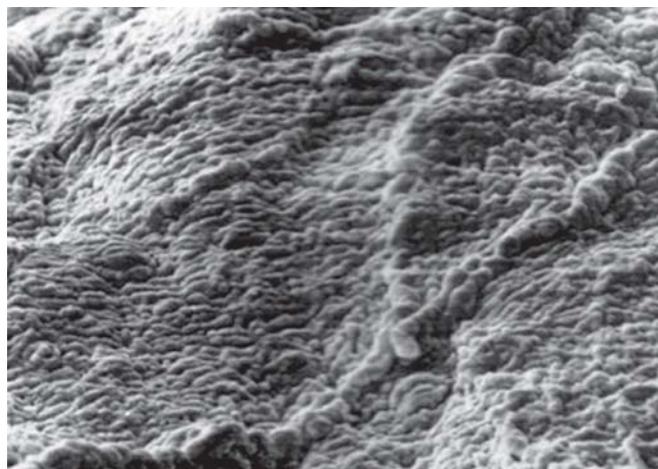


Fig. 2: Normal buccal mucosa. Higher magnification shows mosaic like arrangement of cells. Microridges are densely arranged. Cell borders are seen as elevated ridges ($\times 3300$)



Fig. 3: Nonerosive lichen planus. A cell showing total loss of cell junction which is seen as clear space between the cells. Microridges show parallel arrangement ($\times 3000$)



Fig. 6: Erosive lichen planus. A cell showing thickened cell border, irregular cell border and two separate cell borders, showing early loss of cell contact ($\times 2000$)

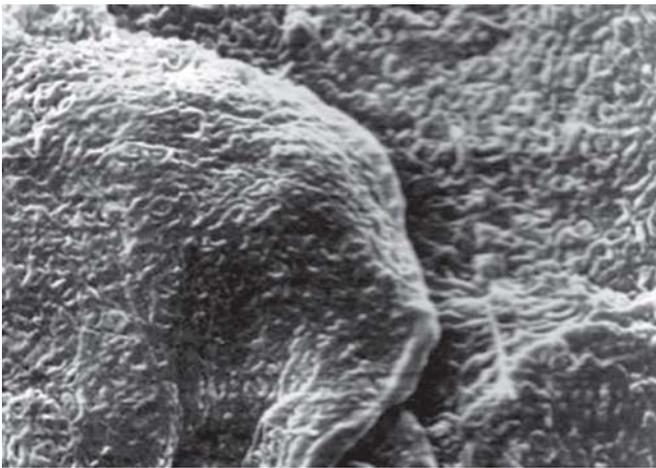


Fig. 4: Nonerosive lichen planus. Higher magnification shows overlapping cell border which indicates loss of cell contact. Microrugae pattern is similar to normal ($\times 5200$)



Fig. 7: Erosive lichen planus. Showing variation in cell size and shape with loss of cell contacts, which indicate early desquamation ($\times 1030$)

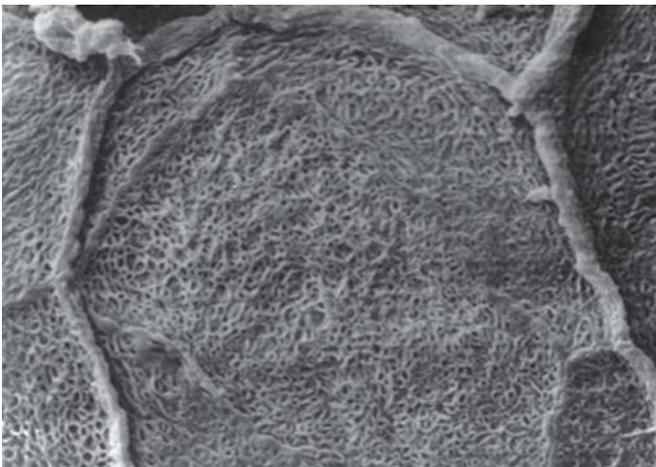


Fig. 5: Nonerosive lichen planus. Cell borders are excessively thickened. Cell surface shows honeycomb arrangement of microridges ($\times 3000$)

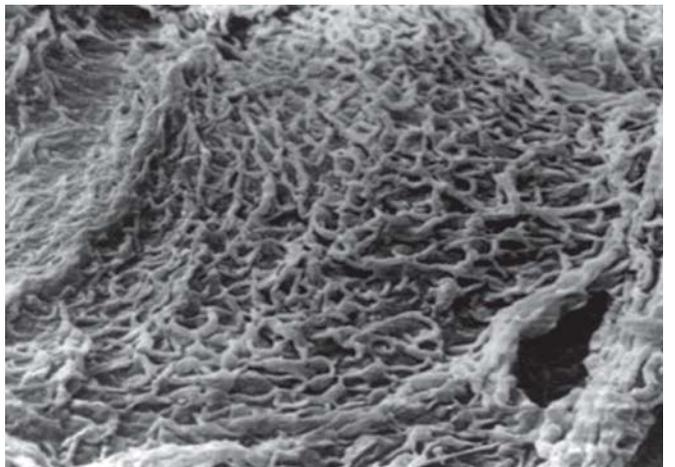


Fig. 8: Erosive lichen planus. Cell shows thickened and loosely arranged microridges, giving pitted appearance. Loss of cell contact is also seen. This appearance is not seen commonly in normal mucosa and nonerosive lichen planus ($\times 6000$)

Table 3: SEM observations of oral leukoplakia

Feature	No. of cases	% cases
1. Abnormal size variation	6	42.9
2. Aberrant forms	2	14.3
3. Degenerative changes	-	-
4. Surface pattern irregularity	8	57.18
5. Absence of distinct cell borders	4	28.6
6. Dense grouping of cells	4	28.6
7. Loss of intercellular junction	10	71.4

Courtesy: Dissertation of Dr Ahuja

Table 4: SEM observation of oral leukoplakia

Feature	HL	EL
1. Granular surface	-	100%
2. Parallel microridges	16.7%	100%
3. Honeycomb pattern	83.3%	-
4. Pitted surface	50%	-
5. Finger like microvilli	50%	50%
6. Club tip microvilli	-	100%
7. Other surface structures	-	-

HL: Homogeneous leukoplakia; EL: Erosive leukoplakia
(Courtesy: Dissertation of Dr Ahuja)

with 12 cases of homogeneous leukoplakia and two cases of erosive leukoplakia. Observations, are given in Tables 3 and 4.

These observations were comparable with that of lichen planus. From overall observations it is suggested that the features of leukoplakia lies between features of nonerosive and erosive lichen planus, changes being more irregular in erosive type of lichen planus. Because features like abnormal size and shape variation, irregularity of surface pattern, dense cell growth, indistinct cell borders, loss of intercellular junction, are pronounced in erosive type of lichen planus than that of leukoplakia.

It is comparable with study of Jungell et al¹¹ who stated that in erosive type of lichen planus, changes are more irregular than normal mucosa and leukoplakia. It is in agreement with Matravers and Tyldesley that oral leukoplakia and lichen planus lie between normal and malignant growth, thus may be considered as premalignant lesion and condition respectively.¹³

These observations also suggest that, surface characters of cell depend on the lesion and not on the site of the lesion. The fact is that, cell maturation and differentiation is linked to specific surface morphology.

CLINICAL SIGNIFICANCE

SEM can be used as a valuable aid for determining premalignant lesions and conditions. Early detection of epithelial dysplasia is possible through study by through SEM. It can result in early treatment of the lesion and can prevent occurrence of squamous cell carcinoma.

SUMMARY

It can be concluded that, there is a definite differentiation between normal mucosa and lichen planus—leukoplakia group. Although characters of lichen planus and leukoplakia overlap each other, they can be differentiated from each other. The characters in nonerosive lesions are nearer to normal group than erosive lesions. Histopathologically also, it is seen that epithelial changes are more severe in erosive lesions than that of nonerosive lesions. Thus, SEM and light microscopic features can be correlated. These changes are due to abnormality in cell maturation and differentiation.

Thus, SEM can act as a sophisticated and modern adjunct to the histopathology in cases of premalignant lesions.

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ABOUT THE AUTHORS

Manjiri Thakur (Corresponding Author)

Professor, Department of Oral Pathology and Microbiology
SDKS Dental College and Hospital, Nagpur, Maharashtra, India
e-mail: thakur.manjirimuk@gmail.com

Vinay Hazare

Dean, Department of Oral Pathology and Microbiology, Government
Dental College and Hospital, Nagpur, Maharashtra, India