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Surface structures of aural polyps: a scanning electron microscopic study

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Abstract

Surfaces of aural polyps collected from 30 patients were examined by scanning electron microscopy. In the polyps not associated with cholesteatoma, the epithelial lining showed individually variable metaplasia towards cuboidal 'cobblestone'-type and squamous epithelium covered with microvilli of various shapes and sizes. Squamous epithelium was present on the surface of all polyps with underlying cholesteatoma, with superficial cells possessing elongated microvilli, microplicae of different sizes, grooves and pits. Such surface structures reflect different stages of the keratinization process that seems to be characteristic for the epithelial lining of polyps with underlying cholesteatoma. Incomplete epithelium accompanied by granulation tissue was found in several polyps; in two cholesteatoma-associated polyps plate-like cholesterol crystals were observed.

Key words: Polyps; Ear, External; Cholesteaoma, Middle Ear; Microscopy, Electron, Scanning

Introduction

There have been few studies of the histology of the aural polyp and its relationship with the underlying middle-ear pathology. Milroy *et al.*[1] found histological features of the polyp (keratin flakes, raw granulation tissue) to be indicative of the underlying cholesteatoma, while Hussain[2] failed to demonstrate any predictive value of polyp histology.

The epithelium lining the pathologically unaltered middle ear is an extension of the airway epithelium, being composed of ciliated cells, nonciliated secretory cells (including goblet cells), intermediate cells and basal (undifferentiated) cells. The distribution of the cells and the character of the epithelium are not, however, uniform: columnar/cuboidal epithelium with relatively numerous ciliated and goblet cells predominates in the anterior part of the middle ear, while its posterior part, including the mastoid area, is lined by more flattened, low cuboidal/squamous epithelium with very few ciliated and goblet cells.[3,4]

All authors investigating the histological structure of aural polyps emphasized the variability of the epithelium covering the polyp – from simple cuboidal to stratified squamous. However, the epithelial covering of the aural polyps has not been investigated by scanning electron microscopy, which can demonstrate fine structural details of the surface, and can supplement routine histological findings by revealing additional structural features with potentially diagnostic or predictive significance.

Hence, the aim of the present study was to examine the surface of aural polyps under the scanning electron microscope.

Materials and methods

The material consisted of 30 aural polyps removed surgically from patients (20 men, 10 women, aged 10–80 years, mean 38 years) at the Department of Otorhinolaryngology, Jagiellonian University School of Medicine, during the period 1991–1995. The size of the polyps ranged from 1 mm to 20 mm in diameter. Underlying cholesteatoma was diagnosed in 14 cases.

After surgery, the polyps were gently washed in saline and were halved for light and scanning electron microscopic examination, respectively.

For routine light microscopic histopathology, the polyp halves were fixed in 10 per cent buffered formalin and embedded in paraffin. Six μm sections were cut and stained with haematoxylin and eosin.

For scanning electron microscopy, the polyp halves were processed according to protocol 3 of Toskala,[5] guaranteeing the lowest incidence of artefacts: they were fixed in one per cent paraformaldehyde/1.23 per cent glutaraldehyde in 0.2 M cacodylate buffer, pH 7.3, and 24 hours at 4°C, thoroughly washed several times in the same buffer and postfixed in two per cent osmium textroxide for three hours. After dehydration in a graded series of ethanol followed by absolute acetone, the samples

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TABLE I
TYPES OF EPITHELIAL LINING IN AURAL POLYPS (NO. CASES)

	n	Cb CC	Cb CC SN	Cb	Cb SN	SN	SN SKl	SKl SKh	SKh SKl	SKh
Location										
anterior meso-tympanum	17	1		2		2	2	1	2	7
posterior meso-tympanum	10		2	2	1	3			1	1
mastoid area	3						1			2
Pathology										
cholesteatoma	14							1	3	10
no cholesteatoma	16	1	2	4	1	5	3			

Epithelial types: CC = ciliated columnar/cuboidal; Cb = cobblestone-like; SN = squamous non-keratinized; SKl = squamous keratinized with low degree of keratinization, SKh = squamous keratinized with high degree of keratinization. The dominant epithelium type is listed as first.

were critical-point dried in CO_2, coated with carbon and gold and examined in a Jeol JSM 35 CF scanning electron microscope at 25 kV.

Results

Scanning electron microscopy revealed a wide spectrum of epithelial linings, sometimes different epithelium types were present on the surface of a single polyp. The character of the epithelium covering the polyp showed no apparent correspondence to the site of polyp growth. A summary of data concerning the relationship of the epithelium type to polyp location and to the presence or absence of cholesteatoma is presented in Table I.

Unaltered simple columnar/cuboidal epithelium with ciliated cells and goblet cells (Figure 1) was relatively rare and usually covered only a part of the polyp surface. Cuboidal epithelium with a characteristic 'cobblestone' appearance due to widened intercellular spaces as well as different shapes and sizes of the apical cell surfaces (Figure 2) could be observed more frequently, in approximately a quarter of the polyps. Such epithelium contained only occasional goblet cells and was either completely devoid of ciliated cells, or showed the presence of single, shortened and malformed cilia (Figure 3). The free surface of the cells were covered with microvilli of variable size, shape and distribution, sometimes clumped of fused with their apical parts (Figure 2), or possessing globular, 'inflated' tips (Figure 3).

A transition from 'cobblestone' type cells to flattened, squamous cells with polygonal apical surfaces of a markedly larger area (Figure 4) was observed in a few polyps. In such a transition zone, there were no goblet cells and the squamous cells showed the presence of numerous, densely packed, short microvilli.

All polyps showing the above types of epithelial lining had no underlying cholesteatoma.

The majority of polyps examined, including all polyps with underlying cholesteatoma, were covered with squamous epithelium (Table I). The free surfaces of the cells showed a variety of structural differentiation, which could be related to each other, suggesting a sequence of epithelial transformation.

FIG. 1-12
Scanning electron micrographs of the surfaces of aural polyps without (Figs. 1–4, 11) and with underlying cholesteatomas (Figs. 5–10, 12).



FIG. 1
Apparently normal columnar surface epithelium with cilia and microvilli and occasional goblet cells secreting mucus (arrowhead). Bar = 10 μm

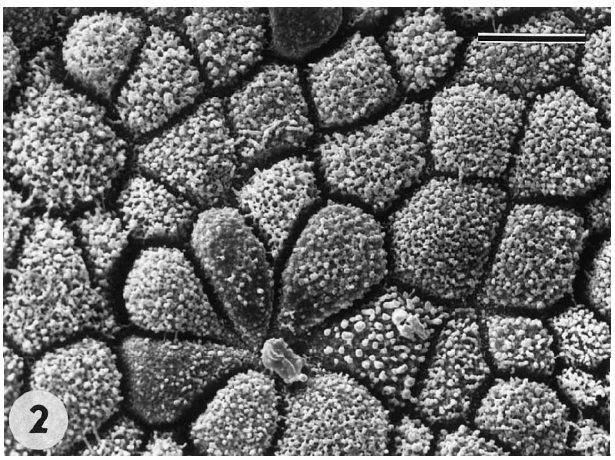


FIG. 2
'Cobblestone' type epithelial cells with microvilli of variable size, shape and density. Bar = 10 μm.

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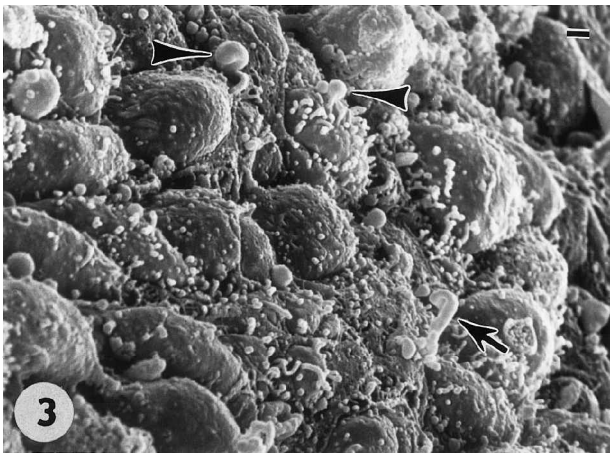


FIG. 3

'Cobblestone' type epithelium with irregular, often bulbous microvilli (arrowheads) and a malformed cilium (arrow). Bar = 1 μm.

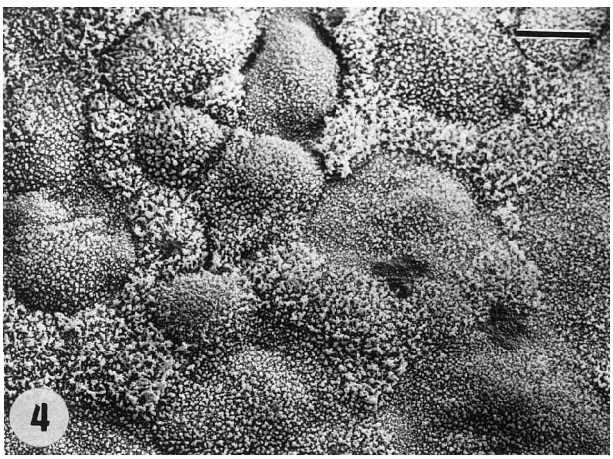


FIG. 4

A transition between 'cobblestone' type epithelium and squamous epithelium characterized by large, flat apical cell surfaces. Bar = 10 μm.

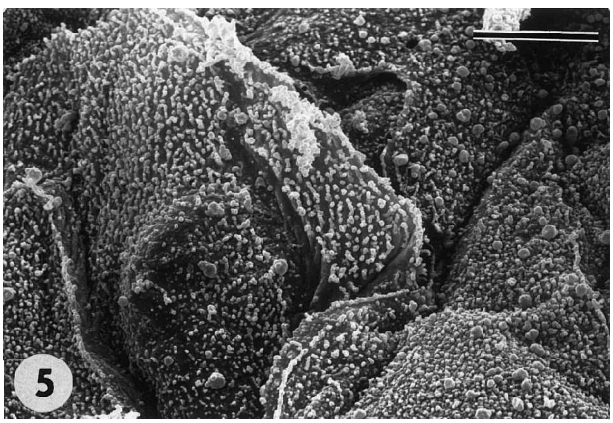


FIG. 5

A squamous cell with a row of longer microvilli along its borders, surrounded by cells with 'inflated', bulbous microvilli. Bar = 10 μm.

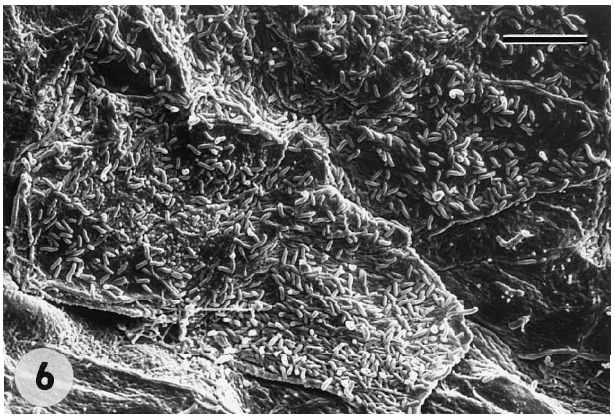


FIG. 6

Squamous cells with long, curled microvilli. Bar = 10 μm.

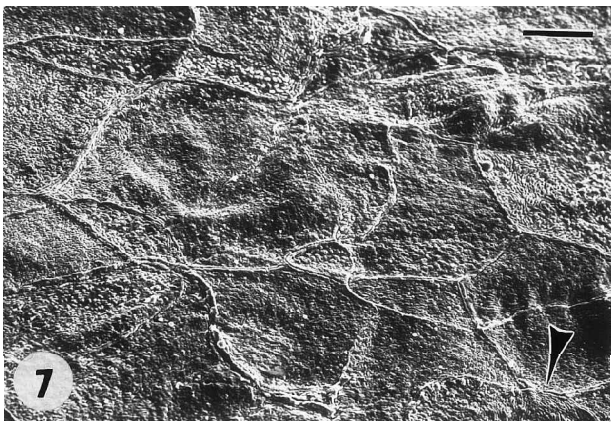


FIG. 7

Squamous cells – some with small microvilli, some with delicate, dense microplicae, occasionally showing parallel arrangement (arrowhead). Note taller borderline microplicae. Bar = 10 μm.

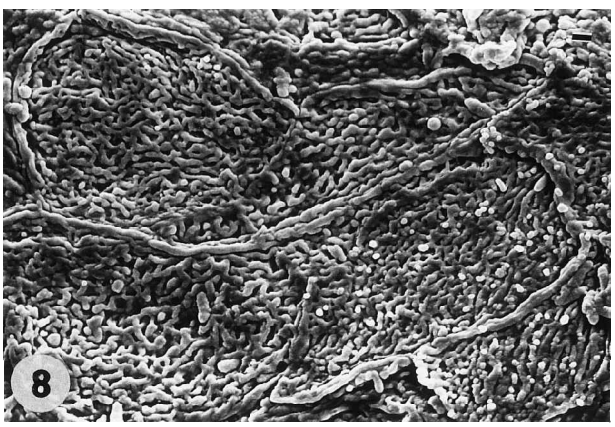


FIG. 8

Squamous cells with prominent networks of microplicae on the surface and marked borderline microplicae. Bar = 1 μm.

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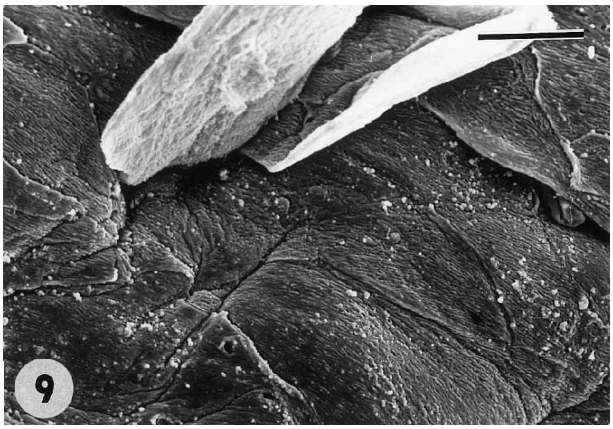


FIG. 9

Squamous cells with flattened, often parallel microplicae. Some cells are in the course of desquamation. Bar = 10 μm.

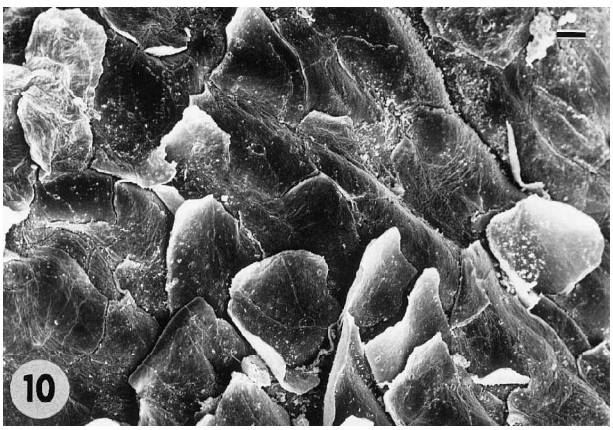


FIG. 10

Squamous, partially desquamating cells showing relatively smooth surface with irregular shallow grooves and pits. Bar = 10 μm.

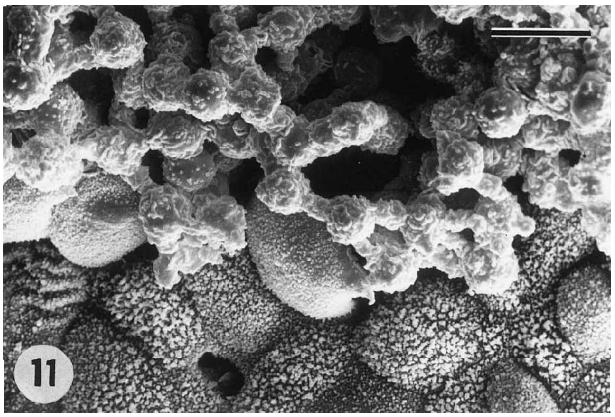


FIG. 11

Polyp surface partially covered by 'cobblestone' type epithelium, and partially by granulation tissue with numerous inflammatory cells. Bar = 10 μm.

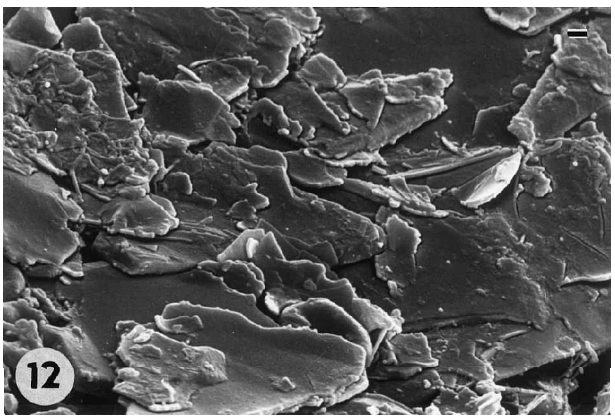


FIG. 12

Surface fragment of a polyp with numerous smooth, crystal-like plates. Bar = 1 μm.

In polyps partly covered by 'cobblestone' cells, the adjacent areas occupied by squamous epithelium were characterized by the diverse size of the polygonal cell surfaces and by the relatively uniform distribution of short microvilli (Figure 4). Only occasional, single cells had markedly longer microvilli. In polyps covered exclusively by squamous epithelium, the surface of superficial cells showed, depending on the polyp or polyp area, several characteristic structural patterns:

(1) short, but widened, 'bulbous' microvilli with different diameters (Figure 5);
(2) moderately long microvilli on the surface of the cells and a single or double row of taller microvilli demarcating the cell borders (Figure 5);
(3) very long, curled microvilli on the surface and single microplicae demarcating the cell borders (Figure 6);
(4) a dense array of delicate microplicae, sometimes showing parallel arrangement, accompanied with taller borderline microplicae (Figure 7);
(5) a complex array of prominent microplicae with distinct borderline microplicae (Figure 8);
(6) smoothened surface, with flatter microplicae, often with parallel alignment (Figure 9);
(7) relatively smooth surface with irregular, shallow grooves and pits (Figure 10).

In the latter two cases, partial detachment of the superficial cells was frequently observed.

The first four patterns were observed on the surfaces of some cells covering polyps belonging to both groups (with and without underlying cholesteatoma), while the other three types of structures occurred exclusively in the latter group.

In four polyps (two from each group), the epithelial lining was incomplete and some areas were covered by densely packed, spherical cells showing microvilli and cell processes of variable size and density (Figure 11). Occasional erythrocytes were also observed in such regions. In two polyps, both with underlying cholesteatoma and with incomplete epithelial covering, the areas without epithelium showed the presence of ideally smooth plates of various size, characterized by straight, polyhedral edges. They often looked 'broken' into smaller fragments (Figure 12).

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Discussion

The type of epithelium covering the aural polyps mostly did not correspond to that observed in the normal middle-ear mucosa at sites from which the polyp developed, the only exception being three polyps growing from the mastoid area, all covered by squamous cells. The epithelial lining of the vast majority of the polyps showed alterations suggesting various stages of metaplasia.

According to the surface structures of the epithelial cells observed by the scanning electron microscope, the metaplastic process begins with subtle changes including malformation and disappearance of cilia, deformations of the microvilli and a decrease in the number of goblet cells. Such changes – in part visible only in the scanning electron microscope – were postulated to reflect the early response of the epithelium to the inflammatory process.[6]

The resulting 'cobblestone'-type epithelium was also described on the surface of nasal polyps by Mygind *et al.*,[7] who considered several surface features as typical for the polyps: cobblestone-like epithelial cells, widened intercellular spaces, and moderate variation of the surface area of individual epithelial cells. Since we have observed all those morphological alterations on the surface of the aural polyps, it seems justified to conclude that both nasal and aural polyps have much in common and that they may share a common mechanism of formation.[8,9] Similar changes were also reported on the surface of normal middle-ear and eustachian tube mucosa in the vicinity of cholesteatoma.[3,6]

The appearance of epithelial cells with vary variable surface areas can be interpreted as further metaplasia towards the squamous epithelium – the largest cells are presumably squamous and in some polyps they predominate in the microscopic picture. The pattern observed in this study corresponds well to the sequence of the metaplastic changes in the epithelium of nasal mucosa proposed by Boysen:[10] pseudostratified (with ciliated and goblet cells)→ stratified cuboidal ('cobblestone')→stratified cuboidal/squamous (cells with variable surface area)-→stratified squamous.

The surface of squamous epithelial cells covering the polyps showed a wide spectrum of structures: microvilli of various sizes and shapes, microplicae of different height and arrangement, grooves and pits. Previous scanning electron microscopic studies of stratified squamous epithelia have demonstrated that such structures can be observed in both normal and metaplastic/dysplastic epithelia in different locations: the oral cavity,[11] nasal cavity,[10,12] as well as in cultural keratinocytes obtained from skin[13] and tympanic membrane.[14] It seems that such different surface configurations may represent the advancement of the keratinization process: the nonkeratinized cells have short, uniform microvilli; the possible sequence of the keratinization-associated changes includes: (1) elongation and/or deformation of the microvilli; (2) their fusion into microplicae, and (3) increase in the size and pattern complexity of the microplicae. In fully keratinized cells, their surface becomes smooth again, showing only shallow grooves and pits.

In our material, squamous cells with surface structures indicative of different stages of keratinization were observed predominantly in cases with underlying cholesteatoma. In the other polyps, even if the squamous epithelium was present, it had only regular microvilli, typical of nonkeratinized cells. Only in three polyps not associated with cholesteatoma, were occasional cells with microplicae indicating the initial phase of keratinization found. Moreover, the vast majority of the polyps associated with cholesteatoma showed a high degree of epithelial keratinization. This observation supports the view that in 'purely inflammatory' aural polyps, epithelial metaplasia mostly leads no further than to nonkeratinized squamous epithelium, while the presence of underlying cholesteatoma is manifested by keratinized epithelium, probably reflecting the origin of the lesion.

The surface of epidermoid cholesteatoma was investigated in the scanning electron microscope by Youngs and Rowles[15] who described regular stacks of hexagonal corneocytes, also characteristic for the meatal skin, that is the source of cells migrating from the retraction pocket in the process of cholesteatoma formation. We have never observed such regular arrangement of squamous cells on the surface of polyps with underlying cholesteatoma. This suggests, that in the course of cholesteatoma development, the cells of ingrowing squamous epithelium may be rearranged, thus losing their original three-dimensional distribution.

In the present study, incomplete epithelial lining with granulation tissue exposed on a part of polyp surface was observed, albeit rarely, in polyps both with, and without, underlying cholesteatoma, confirming the previously reported histopathological findings.[1,8] However, in two cases with underlying cholesteatoma, such areas contained very smooth, regular, crystal-resembling plates – most probably cholesterol deposits, since their size and scanning microscopic appearance was strikingly similar to that of artificially-grown cholesterol crystals.[16]

Conclusion

Scanning electron microscopy is not included in routine histopathological diagnosis. In the case of aural polyps, surface structures of the lining epithelium may reflect its type: squamous cells at different stages of the keratinization process seem to be characteristic for the epithelial lining of polyps with underlying cholesteatoma. Hence, scanning electron microscope examination can be helpful in assessing the risk of cholesteatoma – subtle manifestations of keratinization may elude observation in the light microscope, but are easily identifiable under the scanning electron microscope.

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References

1 Milroy CM, Slack RWT, Maw AR, Bradfield JWB. Aural polyps as predictors of underlying cholesteatoma. *J Clin Pathol* 1989;**42**:460–5

2 Hussain SSM. Histology of aural polyp as a predictor of middle ear disease. *J Laryngol Otol* 1991;**105**:268–9

3 Chao W-Y, Shen C-L. Ultrastructure of the middle ear mucosa in patients with chronic otitis media with cholesteatoma. *Eur Arch Otorhinolaryngol* 1996;**253**:56–61

4 Hentzer E. Ultrastructure of the middle ear mucosa. *Acta Otolaryngol* (Stockh) 1984;(suppl 414):19–27

5 Toskala E. The influence of specimen preparation on artefacts in scanning electron microscopy of respiratory cilia. *Biotechnol Histochem* 1995;**70**:46–51

6 Chao W-Y, Chang S-J. Ultrastructure of eustachian tube mucosa in chronic otitis media with cholesteatoma. *Am J Otolaryngol* 1996;**17**:161–6

7 Mygind N, Bretlau P, Sorensen H. Scanning electron microscopic studies of nasal polyps. *Acta Otolaryngol* (Stockh)1974;**78**:436–43

8 Caye-Thomasen P, Hermansson A, Tos M, Prellner K. Polyp pathogenesis – a histopathological study in experimental otitis media. *Acta Otolaryngol* (Stockh)1995;**115**:76–82

9 Larsen PL, Tos M, Kuijpers W, van der Beek JMH. The early stages of polyp formation. *Laryngoscope* 1992;**102**:670–7

10 Boysen M. The surface structure of the human nasal mucosa. I. Ciliated and metaplastic epithelium in normal individuals. A correlated study by scanning/transmission electron and light microscopy. *Virch Arch B Cell Pathol* 1982;**40**:279–94

11 Kullaa-Mikkonen A. Scanning electron microscopy in oral mucosa research. *Scanning Microsc* 1983;**1**:1145–55

12 Boysen M, Reith A. Surface structure in normal, metaplastic and dysplastic nasal mucosa of nickel workers. A SEM and post-SEM histopathological study. *Scanning Electr Microsc* 1980;**3**:35–41

13 Nielsen EH, Elberg JJ, Ronne M, Jahn H, Bierring F. Surface ultrastructure of cultures of affected psoriatic keratinocytes, unaffected psoriatic keratinocytes and normal keratinocytes. *APMIS* 1987;**96**:303–8

14 Olsen EG, Stenfors L-E. The behaviour of tympanic membrane perforations in tissue culture: a scanning electron microscopic study. *J Laryngol Otol* 1993;**107**:1106–9

15 Youngs R, Rowles P. The spatial organisation of keratinocytes in acquired middle ear cholesteatoma resembles that of external auditory canal skin and pars flaccida. *Acta Otolaryngol* (Stockh) 1990;**110**:115–9

16 Busch N, Lammert F, Marschall HU, Matern S. A new subgroup of lectin-bound biliary proteins binds to cholesterol crystals, modifies crystal morphology, and inhibits cholesterol crystallization. *J Clin Invest* 1995;**96**:3009–15

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