## SEM study of incus surface erosion due to cholesteatoma action

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Cholesteatoma is a noncancerous cystic lesion derived from an abnormal growth of keratinizing squamous epithelium in the temporal bone (1). It causes significant problems due to its erosive and expansile properties, resulting in the destruction of the ossicles. Over 5 million people worldwide are affected of cholesteatomas and gradually loss hearing. (2). In order to provide a prognostic tool useful during surgical procedures, we are performing a SEM morphological analysis of incus surface erosion due to cholesteatoma action; then we will investigate (on the same samples) the relationship among data from SEM analysis and genetical, proteomical, biochemical and histological data. Up to now we have observed 10 incus from patients with cholesteatoma. Samples were fixed immediately upon recovery in 2.5% glutaraldehyde in PBS at 4°C for 48 h, than they were prepared for scanning electron microscopy. Samples were gently sonicated before sputter coating, to remove excess of keratinizing squamous epithelium, that would have prevented erosion observation. The total surface area of the observed side was measured  $(3,54\pm0.21 \text{ mm2})$ . The mean distance from the surgical removal point to the bone far end was measured in order to define a ROI (2,37±0,31 mm2). Five consecutive fields at 100X magnification aligned in 3 raws, the first one proximal and the last one distal to surgical removal point were analized. A total of 60 field for each raw were observed. Degree of erosion was classified as: No erosion=0, light =1, mild=2 high=3. Presence of biofilm was also recorded. Our early data suggest that although a gradient proximal to distal exists, looking to the distribution of eroded areas, grade 3 erosion is not limited only to the area proximal to cholesteatoma (first raw) but is also present in raw 2 and sometimes scattered since raw 3. Grade 3 erosion was observed around nutrient foramina of the bone (65%). Biofilm of bacteria was observed in 50% of analyzed fields, this is consistent with results reported in literature. Our data suggest that relapse of cholesteatoma is due to erosive activity of cells far from surgical removal point.

## References

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## Keywords

Cholesteatoma; Scanning electron microscopy; Incus bone.