

# Cholesteatoma removal efficiency evaluated by Variable Pressure Scanning Electron Microscopy

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## Background incl. aims

Cholesteatomas are well-defined non-cancerous cystic lesion that results from the aberrant development of the keratinizing squamous epithelium within the middle ear. They can erode into the CNS and cause severe complications thus surgical removal is needed and is usually performed by manual dissection 1 however, with the possibility of recurrence. To improve the effectiveness of this technique the combined use of Mesna 5% (sodium 2-mercaptoethanesulfonate) has been introduced, this method is called Chemically Assisted Dissection (CADISS®). Our work aimed to evaluate the effectiveness of this combined surgical procedure during middle ear cholesteatoma removal, in terms of the absence of residual pathological tissue.

## Methods

6 incus bones involved in cholesteatoma and removed during surgery were randomly divided into 2 groups: a) 3 subjected to CADISS-assisted dissection; b) 3 subjected to manual dissection. Samples were fixed in glutaraldehyde 2.5% in PBS (0.1M, pH 7.4) immediately upon recovery for at least 24 hours. Samples were then washed in PBS and underwent OsO<sub>4</sub> post-fixation for 1 h. After washing samples were impregnated with tannic acid 1% for 30 min, then washed, dried on absorbent paper, and directly observed at Hitachi SU 3500 at 30 Pa and 10 kV operating conditions. Images were analyzed by the software Hitachi Map 3D advanced 8.2 (Digital Surf, France) to provide quantitative measurements of cholesteatoma tissue debris on the incus bone surface 2.

## Results

Our results show that CADISS-assisted dissection provides a better outcome in terms of clean surface area concerning manual dissection. Data from software-aided BSE image analysis revealed that the clean area/ total surface area ratio is higher in the CADISS method samples ( $19.7 \pm 3.61$ ) than in the manual dissection group referred to as the control group ( $4.57 \pm 1.66$ ), the difference is statistically significant as revealed by t-Test results ( $t = 20.91$   $P < 0.001$ ). The ability of Variable pressure SEM is that observation of samples in their native hydrated state is possible. The absence of dehydration steps in sample preparation allowed the observation of the samples as they came from the operatory room, without any artifact due to the preparation procedure. It is mandatory to use a procedure that does not modify sample surfaces if a comparison between two different surface cleaning procedures has to be performed.

## Conclusion

The use of VP-SEM allowed sample observation without dehydration procedures, decreasing the risk of losing the pathological tissue while ossicle processing and allowing the comparison of the surgical technique's effectiveness. Our study also shows how still is important a morphological approach in establishing new surgical technique validity and how the application of innovative image analysis software can transform Scanning electron microscopy from a qualitative imaging modality into a quantitative technique.

Keywords

Variable pressure scanning electron

microscopy **Reference:**

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