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Looking Down the Nose Through Large Block-Face (2D) and Serial Section Array (3D) Scanning Electron Microscopy

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The airway epithelium of the nasal and pulmonary compartments shares fundamental characteristics in function, morphology and cellular makeup as part of a unified respiratory airway. In both compartments the epithelium consists of a complex pseudostratified configuration of cells attached to a basement membrane, and is comprised of several primary cell types that play roles in mucociliary clearance, mucus production and lubrication, and epithelial maintenance. Unlike the lung, where mechanisms of development and self-renewal are well described [1-4], comprehensive ultrastructural characterization has not been performed for the nasal cavity, sinuses and enveloping epithelium [5-8]. Understanding of this epithelial barrier interface would greatly assist our understanding of maintenance, repair and regeneration in the nasal cavity. The new field of ultrastructural analysis through SEM imaging of thin sections, previously associated with Transmission EM, is here further developed in a temporal study of the ultrastructure of complete and uninterrupted nasal areas, which were previously impossible to analyze with the small sample size associated with TEM techniques. In comparison to Serial Block-Face SEM[9], collection of a 'library' of sections for Serial Section Array -SEM allows 3D computation of various regions in the same section, and revisiting of sections for further analysis.

Wild type C57BL/6 mice were used to characterize the cells lining the murine nasal cavity with advanced techniques of (1) Large Block-Face (LBF)-SEM and (2) Serial Section Array (SSA)-SEM [10]. Fixed whole skull specimens from embryonic (stage E13 and E19), adult (P180) and advanced aged (P720) mice were processed for Epon resin embedding, with additional *en bloc* staining methods to increase conductivity and contrast in thin sections [11]. Arrays of serial thin sections (100-200nm) were produced with ultramicrotomy on glass slides, and treated for scanning with FESEM as described recently [10]. FESEM analysis involved two novel aspects: (1) stitching of individual serial images captured at 1,000-2,000x magnification of a single large section of the entire nasal epithelial lining, at 4 ages (E13, E18, P180, P720), to investigate changes in cellular populations and epithelial properties during development (2) 3D reconstruction of arrays of serial sections of the main areas of interest in the nasal cavity at various ages to clarify the interrelationship of cells in the nasal epithelium. Parallel samples were prepared for conventional FESEM using standard techniques. Imaging was performed with a Zeiss Sigma FESEM using BSD at 5-6kV and inLens SE detection at 2kV.

FESEM analysis confirmed the existence of ultrastructurally discrete cell types previously described [12], as well as previously undescribed cell types in specific subsites of the murine nasal cavity [Figs 1&2]. Not only was there a distinct, and previously unappreciated association of ultrastructural features with age and location, but our findings also emphasize the structural disparities between epithelial areas exposed to high vs. low air flow. The relative abundance of goblet cells associated with mucus secretion, and presence of regionally specific ciliated cells, were computed through 3D reconstruction at various developmental stages. Previously poorly defined cells in the region of the nasal floor (crypt) can now be appreciated and suggest a stem cell function [13].

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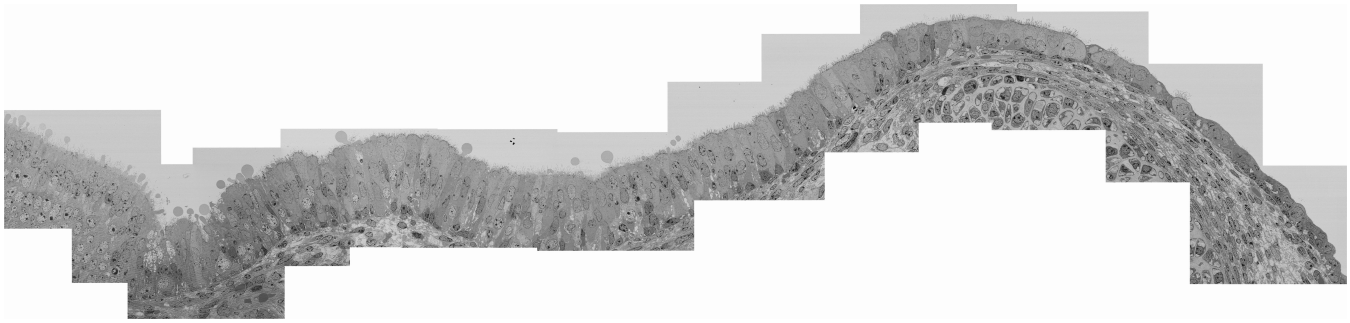

Figure 1. Large Block-Face SEM of embryonic stage E19 (partial mosaic of epithelial lining, 13 images at 1,000x magnification)

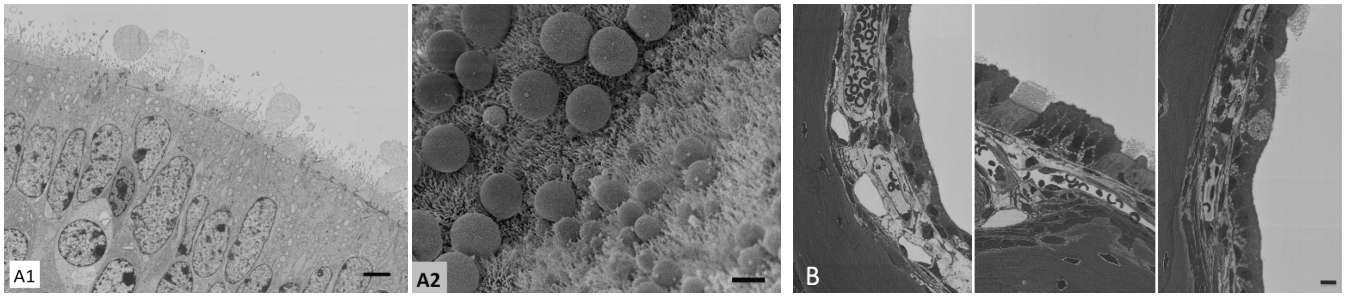

Figure 2. (A) Ultrastructural correlation of LBF-SEM image (A1:BSE detection) with Conventional FESEM (A2: InLens SE detection) characterizes cell types in nasal cavity of mouse embryo E19, indicating high metabolic activity of goblet cells preparing to secrete mucus, and dense ciliated covering in areas that will shortly be exposed to air (B) Unidentified cells that line the nasal cavity, not in direct contact with the main airway, appear to be novel cell types not previously characterized.
Scalebar = 4μm

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