Introduction
Pediatric chronic rhinosinusitis (CRS), a ubiquitous upper airway disease representing one of the most prevalent chronic diseases in children, is characterized by inflammation, mucus hypersecretion, and obstruction of the sinonasal outflow tracts for more than 3 months duration. It is one of the most common chronic illnesses in children with an estimated cost of $1.8 billion/year. Bacteria, viruses, and environmental agents, are thought to play a critical role in initiating and sustaining the altered immune response that results in mucus hypersecretion. However the molecular pathways that lead to CRS remain elusive.

Further complicating the understanding of this disease is the fact that CRS presents with two different phenotypes defined by the presence or absence nasal polyps (NP), which are outgrowths of hyperplastic sinus mucosa that often extend into the nasal cavity. Histologically, pediatric CRS with NP (CRSwoNP) is characterized by edematous tissue, a paucity of submucosal glands and stromal fibrosis with mixed infiltrates of mononuclear cells and eosinophils. Pediatric CRS without NP (CRSsNP) characteristically shows an infiltration of mixed mononuclear cells and neutrophils, with an increase in submucosal glands and stromal fibrosis. Epithelial goblet cell hyperplasia may also be present.

The differences in nasal polyp CRS and CRSwoNP is distinct despite differences in management of children with CRSwoNP. Basal membrane extracellular matrix (ECM) provides cues for cell proliferation and differentiation and ultimately directs morphology. The two most commonly used ECMs for in vitro 3-dimensional (3D) cell cultures are Matrigel and Collagen I. Matrigel is an extract isolated from Engelbreth-Holm-Swarm murine tumors and composed of laminin (69%), collagen IV (30%) and entactin (7%). Collagen I exists in vivo as fibers and is a major component of connective tissue. We have shown that human nasal epithelial (HNE) cells, when grown on Matrigel or Coll I, differentiate to acini or tubules respectively which reflect specific glandular structures of 3D models (Figure 1), implicating the presence of pluripotent cells in human nasal mucosa that can be directed to differentiate into glandular substructures depending on the ECM used. We postulated that these similar pluripotent cells in pediatric human sinus epithelial (HSE) cells. To evaluate this, we proposed to culture HSE cells using the Matrigel and Coll I 3D in vitro models and determine if the sinus mucosa from children with CRSsNP and CRSwoNP could be directed to replicate the submucosal glandular hyperplasia seen in pediatric CRS. Moreover, due to the clinical differences in pediatric CRSsNP and CRSwoNP, we postulated differences in the ability of sinus mucosal progenitor cells to differentiate in these two CRS phenotypes.

Methods and Materials

Human sinus epithelial cells (HSE) were collected from the mucosa of three cohorts of children: CRSsNP (n=3), CRSwoNP (n=3), and disease control (patients n=3).

In Vitro 3D Models:
3D Matrigel Culture: Plates or chamber slides were coated with Matrigel and placed in 37°C incubator to solidify. A suspension of HSE cells in Bronchial Epithelial Growth Medium (BEGM, Lonza) was mixed 1:1 with a separate stock medium containing 4% Matrigel and 20 ng/ml EGF. The single cell suspension was plated on top of the solidified Matrigel, corresponding to a final overlay solution of 3.2 x 10^5 cells/cm^2 in medium containing 2% Matrigel and 5 ng/ml EGF at day 0. Cell cultures were maintained in a 5% CO2 humified incubator at 37°C and fed with fresh medium containing 2% Matrigel and 10 ng/ml EGF every 2-4 days as needed.

3D Culture within Collagen Gels: Collagen I gels were prepared using a published protocol. Eight volumes of rat tail collagen type I (BD Bioscience) stock solution were mixed with 1 volume of 10x PBS and 1 volume of sodium bicarbonate and kept on ice. HSEBCs were suspended in the cold gel mixture; aliquots were dispensed into plastic culture dishes and allowed to gel for 10 minutes at 37°C before adding BEGM culture medium with 15% EGF.

Antibodies and Immunofluorescence:
Immunofluorescence was used to test for the presence of MUC5B, a secretory mucin predominant in CRSwoNP glands and glandular acinar marker for mucosal cells in submucosal glands.

Results
Single HSE cells from CRSwoNP cultured on Matrigel proliferated into large cellular spheres up to day 15. The acini’s fold change in size was measured on days eight and 15. The acini were significantly 3-fold larger compared to those from CRSsNP controls and compared. Acini from the sinus mucosa of CRSwoNP patients were larger than acini from HSE cells of CRSsNP children (Figure 2A, B). HSE cells from CRSsNP and HSE cells from CRSwoNP cultured on Coll I differentiated into tubule structures on day seven. The tubular length fold change in size and length was measured and tubules were larger in size and longer in length in HSE cells from CRSwoNP compared to CRSsNP HSE cells (Figure 3A, B). Glandular markers were detected in HSE tubules from CRSsNP and CRSwoNP on day 10. Expression of the glandular marker MUC5B was significantly increased in the acinar structures from CRSsNP patients (Figure 4). Expression of this glandular acinar marker for mucous cells in submucosal glands demonstrates the ability of pluripotent cells to differentiate into glandular cells.

Discussion

HSE from CRSsNP, compared to HSE from CRSwoNP, appear to have an enhanced pluripotent capacity for in vitro glandular development of acini and of tubules. The morphological differences may reflect inherent properties in the HSE of these two CRS phenotypes.

Conclusions

CRSsNP and CRSwoNP behave differently in the clinical setting and pose challenges for the clinician in their respective management. Understanding the differences in the pathophysiology is crucial to alleviate CRS disease impact and burden. The presence of nasal polyps often occurs in children with cystic fibrosis and warrants further investigation due to the increased prevalence of CRSsNP in that cohort. By using pluripotent HSE cells to create 3D models that mimic the CRSsNP and CRSwoNP phenotypes, we may be able to identify mediators that cue for cellular proliferation in the basement membrane and hence find potential areas for targeted therapies in clinical intervention and future experimental medicion.

Future Directions
CRSsNP and CRSwoNP behave differently in the clinical setting and pose challenges for the clinician in their respective management. Understanding the differences in the pathophysiology is crucial to alleviate CRS disease impact and burden. The presence of nasal polyps often occurs in children with cystic fibrosis and warrants further investigation due to the increased prevalence of CRSsNP in that cohort. By using pluripotent HSE cells to create 3D models that mimic the CRSsNP and CRSwoNP phenotypes, we may be able to identify mediators that cue for cellular proliferation in the basement membrane and hence find potential areas for targeted therapies in clinical intervention and future experimental medicion.

References