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Submucosal gland mucus strand velocity is decreased in chronic rhinosinusitis

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Abstract

Background: Chronic rhinosinusitis (CRS) may be initiated by innately impaired host defense mechanisms that predispose the upper airways to infection. Recent evidence suggests tethering of submucosal gland mucus strands represents an inciting event within CF airways, occurring prior to onset of chronic infection. Submucosal gland hypertrophy and defective mucociliary clearance (MCC) are present in actively inflamed sinuses, but mucus strand velocity may also be affected as a secondary event, further contributing to chronic disease. The objective of this study is to assess whether mucus strand velocity is decreased in patients with CRS.

Methods: Mucosal explants from patients with and without CRS were submerged in Ringer's solution mixed with fluorescent nanospheres. Methacholine was then added, and videos demonstrating strand growth and detachment were generated from a time-lapse of z-stack images using a multiphoton confocal microscope. Dynamic mucus strands were identified and individual velocities quantified with the MTrackJ plugin of ImageJ.

Results: Fifteen patients met criteria for *ex vivo* analysis of mucus strand velocities (CRS, n = 9 vs. Controls, n = 6). Mucus strands were recorded (pixels/second) streaming from the submucosal gland openings. Average mucus strand velocities were significantly decreased in patients with CRS (1.53±0.67 vs controls, 4.86±1.68; p<0.001).

Conclusions: This study is the first to report evidence of abnormal mucus strand velocity from submucosal glands in diseased sinonasal mucosa. Future pharmacologic studies targeting this critical component of MCC are warranted.

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Level of Evidence: NA.

Keywords

mucociliary clearance; mucociliary transport; sinusitis; chronic sinusitis; chronic rhinosinusitis; mucus tethering; mucus strand; chronic rhinosinusitis

INTRODUCTION

In humans, the upper and lower airways are constantly exposed to environmental insults in the form of inhaled ambient pathogens, allergens, and other particulate matter.^{1,2} Mucociliary clearance (MCC) is the primary innate defense mechanism by which inhaled particulate matter is eliminated from the airways.^{1,3,4} Proper functioning of MCC relies on two, yet equally important constituents: mucus production and mucus transport⁵, both of which are contingent upon normal ciliary beating, adequate hydration of airway surface liquid (ASL), periciliary liquid (PCL), and mucus viscosity.⁶ Abnormalities in any one of these critical components may result in dysfunctional MCC, irrespective of the underlying etiology. Impaired MCC is a fundamental finding that is characteristic of many airway diseases, including chronic obstructive pulmonary disease and chronic rhinosinusitis.^{7–10} In patients with chronic rhinosinusitis (CRS), for example, dysfunctional MCC predisposes the upper airways to a perpetuating cycle of obstruction and infection, with a consequent decrease in overall quality of life (QOL).^{7,11,12} This translates into more dire consequences in patients with cystic fibrosis (CF), the majority of who develop CRS, as there is evidence to suggest that CRS may contribute to bacterial seeding of the lower airways and facilitate the onset of lung disease.^{13–17}

There is a relative paucity of methods currently used for quantitatively assessing MCC, particularly in the upper airways. As a result, our ability to fully comprehend early pathogenesis of CRS has been severely limited. However, there have been a number of studies and a variety of methods for assessing MCC when evaluating lower airway pathology, especially CF airways. Recently, one study reported impaired mucus strand detachment as an inherently defective mechanism responsible for aberrant MCC in CF piglet tracheas.¹⁸ In this study, CF mucus strands often failed to detach from submucosal glands or became tethered to nearby anchored strands rather than exhibiting normal elasticity and release from the glands as was seen in non-CF piglet tracheas.¹⁸ While this was demonstrated when there was a genetic absence of functional CFTR, acquired CFTR deficiency in non-CF patients likely contributes to continued pathogenicity in diseases of the airway, including chronic obstructive pulmonary disease and CRS.^{6,8,9,19} Thick abundant mucus is a hallmark of inflamed and/or infected sinuses. How mucus is secreted under these circumstances from submucosal glands may impact the clinical manifestations of the disease.

The objective of the present study is to evaluate differences in submucosal gland mucus strand velocities in patients with CRS as compared to those with non-diseased, healthy tissue.

MATERIALS AND METHODS

Human subjects/Tissue samples

Institutional Review Board approval was obtained from the University of Alabama at Birmingham prior to commencement of the study. Human sinonasal mucosa was obtained from patients undergoing endoscopic sinus surgery for CRS or non-inflammatory conditions such as cerebrospinal fluid leak repair. No decongestants or injections were performed prior to tissue harvest. All tissue was harvested from the area of the ostiomeatal complex so that only uncinate process or lateral surface of the middle turbinate was utilized for the study. Patients submitted to a blood draw to test for major Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutations (Cystic Fibrosis Screen, 10458X, Quest Diagnostics, Chantilly, VA). Subjects with positive CFTR mutations were excluded from subsequent analysis. Tissue samples were immediately placed in a Petri dish atop a piece of gauze moistened with $\text{HCO}_3^-/\text{CO}_2$ buffered Krebs-Ringer solution. Tissue specimens were dissected to 1–2 cm in length and pinned flat to a paraffin wax embedded Petri dish using glass-head pins. Samples were then submerged in a sonicated, dilute solution of 11-mL buffered Krebs-Ringer saline admixed with 0.75 μL of 40-nm yellow-green fluorescent nanospheres (ThermoFisher, FluoSpheres, Molecular Probes).¹⁸ To further stimulate mucus secretion from submucosal glands, 1-mL of 1.28×10^{-5} mol/L methacholine was added immediately prior to imaging as previously described.¹⁸

Assessment of ex vivo mucociliary transport (Figure 1)

Experiments were performed using a temperature-controlled stage set at 37°C. Mucus strands labeled with fluorescent nanospheres were recorded using an upright Nikon A1R resonant scanning multiphoton confocal microscope affixed with a 25x water immersion objective lens. Videos demonstrating strand elongation and movement were acquired over a 10-minute real-time period. Dynamic mucus strands were reconstructed at maximum intensity projection using a time-lapse of z-stack confocal images compressed at a speed of 50 or 100 milliseconds.

Videos were subsequently analyzed using the MTrackJ plug-in of ImageJ software (National Institutes of Health, NIH) to manually track moving objects and measure their trajectory paths over a period of time. Videos were first transformed to grayscale and, because the number of frames per second (FPS) in each video may vary depending upon sample thickness and subsequent size of each z-stack, they were further adjusted to reflect the different FPS for each sample when necessary. Individual, mobile mucus strands were then identified and tracked for a minimum of four frames using MTrackJ. Analysis of videos was performed with the reviewer (KT) blinded to the condition of the patient. Mucus strand velocity was measured in pixels/second.

Statistical analysis

Statistical analysis was performed using two-tailed, unpaired t-tests. All values are reported as mean $\pm$ standard deviation. A p-value of <0.05 was considered statistically significant.

RESULTS

Study population

A total of 23 patients were enrolled in the study, 15 of whose videos met criteria for analysis (CRS, n = 9 vs Controls, n = 6). One patient was excluded due to presence of a CFTR mutation (F508del), while tissue from 7 other subjects did not have viable mucosa with active mucus strands detected by CLSM. The CRS cohort included 3 males and 6 females with an average age of 56.7 (range, 26 – 57), while the control group had 3 males and 3 females with an average age of 43.2 years (range, 25–80).

Mucus strand velocities

Several mucus strands were usually visualized in each high-powered field (25x objective) per tissue sample. To stay consistent, the fastest moving strand was analyzed in each sample. Average mucus strand velocities (pixels/second) were calculated (Figure 2). Average mucus strand velocity was decreased in patients with CRS (1.53 ± 0.67) (Figure 3, Supplementary Video 1) as compared to those with non-diseased sinonasal mucosa (4.86 ± 1.68) (Supplementary Video 2). Although mucus strand tethering and strand breaks were observed, we were unable to quantify this with meaningful methods.

DISCUSSION

CRS is a disease of slowed MCC and thick, viscous mucus that affects 29.4 million, or 12.1%, of adults in the United States.^{20,21} In the present study, mucus strand velocity was significantly decreased in CRS subjects when compared to controls. Mounting evidence suggests that chronic airway diseases are perpetuated by the development of acquired CFTR dysfunction resulting in abnormalities of MCC similar to patients with genetic CF. A number of environmental insults can also induce acquired CFTR dysfunction, including hypoxia, tobacco smoke exposure, viruses, and bacterial exoproducts. Thus, similar mucus abnormalities, such as mucus tethering abnormalities, could be present in non-CF airway diseases as well.^{6,8–10,22–25} The decreased mucus strand velocities exhibited in the current cohort of CRS patients may be the result of increased mucus strand tethering and/or decreased submucosal gland detachment as suggested by Hoegger et al.¹⁸ For the current study, we were unable to replicate the broad field evaluation of mucus tethering abnormalities, as multiphoton confocal imaging is not compatible with the lower powered objectives necessary for capturing a large field of view. While we were unable to quantify tethering abnormalities, mucus strand velocities could be measured and allowed a quantifiable aspect to the movement of glandular mucus in sinus mucosa. The reduced velocity of mucus strands in CRS suggests that submucosal gland mucus may have abnormal (high) viscosity slowing egress from the gland or be a byproduct of delayed MCC – both of which are consistent with acquired CFTR dysfunction. To the best of our knowledge, this study represents the first evidence that abnormal mucus strand dynamics are present in human CRS.

Despite adverse effects on quality of life, treatment of CRS remains difficult with highly variable outcomes. Traditional medical management relies on antibiotics and anti-

inflammatories, such as steroids. With antibiotic resistance on the rise and the deleterious side effects of steroids, new targets for treatment are needed. Data in the current study indicates decreased mucus strand velocity and abnormalities of mucus expulsion from submucosal glands may serve as potential targets for early therapeutic interventions. As the abnormalities may be physical manifestations of acquired CFTR dysfunction, Cl⁻ secretagogues (CFTR potentiators, i.e. ivacaftor) developed for CF disease have a high feasibility for translation to therapy for non-CF CRS. Furthermore, the currently described method of *ex vivo* mucus strand tracking using fluorescent nanospheres and Internet freeware demonstrates a relatively simple method for quickly assessing the therapeutic efficacy of future drug therapies.

Limitations of the present study include the small field of view associated with use of a 25x objective lens and subsequent difficulty in imaging more than one area of the tissue samples before they stopped producing strands. Imaging of whole tissue samples and quantitative assessments of stationary mucus strands and impaired mucus detachment were impossible under these conditions. The relatively small numbers in our cohorts are another limitation of the study, which precludes correlating mucus strand velocity with subjective and objective outcome measures of CRS. Cohorts were also not age or gender matched which could influence capability to expel mucus from the glands. Nevertheless, our findings provide evidence that mucus-stranding abnormalities are present in humans with CRS, and this technique appears to be a valid endpoint in human samples and will translate well to evaluation of mucus abnormalities in preclinical animal models.

CONCLUSION

This study is the first to report evidence of abnormal mucus strand velocity from submucosal glands in diseased sinonasal mucosa. Whole tissue samples for quantitative evidence of increased mucus tethering and reduced mucus strand detachment are planned. Future pharmacologic studies should target this critical component of MCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic illustration demonstrating methods for visualization of dynamic mucus strands using a multiphoton confocal microscope.

Figure 2.
Bar graph showing average mucus strand velocity (pixels/second) of control vs CRS cohort.
Error bars represent

Figure 3. Snapshot of multiple mucus strands highlighted by fluorescent nanospheres in a patient with CRS.