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The effects of epinephrine on ciliary beat frequency in human sinonasal mucosa

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ABSTRACT

Mucociliary clearance is an important physiological mechanism for clearing the upper airways. Previously, it has been shown that different disease processes and drugs affect ciliary beat frequency (CBF). Namely, epinephrine has been shown to accelerate CBF in various animal models. Additionally, phase contrast microscopy (PCM) and spectrally encoded interferometric microscopy (SEIM) have been used to image dynamic tissue of the upper airway. Herein, we explore the effects of epinephrine on human sinonasal mucosa through PCM and SEIM. Sinonasal mucosa was harvested from patients undergoing endoscopic sinus surgery (ESS). Tissue was imaged using PCM and SEIM, maintaining physiological temperature through the use of warmed HBSS and a heating plate. Videos were taken before addition of any drugs as baseline. Epinephrine was diluted to 1 mg/mL (1:1000) and 1mL of solution was introduced to the sinonasal mucosa. PCM and SEIM was performed after to determine effects of epinephrine on CBF. Data analysis was performed using MATLAB (Mathworks, Natick, Massachusetts). Human sinonasal mucosa, taken from various anatomic locations, showed CBF values on PCM and SEIM consistent with what has been shown in previous literature. Upon addition of epinephrine to sinonasal mucosa, a marked increase in CBF was observed in both PCM and SEIM. In conclusion, the addition of epinephrine to sinonasal mucosa increased ciliary beat frequency. This validates the use of SEIM for determining CBF in sinonasal tissues. Further studies include adding to our sample size to determine a more accurate magnitude of increase of CBF.

Keywords: Ciliary beat frequency, sinonasal mucosa, optical coherence tomography

1. INTRODUCTION

Respiratory epithelium is covered by mucus that is constantly cleared by surface cilia. This specialized organelle beats in a rhythmic pattern contributing to mucociliary clearance. Compromise of this system has been previously implicated in the pathogenesis of bronchial asthma, chronic lung disease, and COPD.[1–3] Management and prevention of these diseases thus requires further understanding of mucociliary clearance.

The dynamic nature of respiratory tissue makes imaging of the upper airway a challenge. Prior studies have examined the use of phase contrast microscopy (PCM) for *ex vivo* analysis of sinonasal cilia. In addition, Park *et al.* has reported on a novel approach to high resolution imaging using a phase-resolved Doppler spectrally encoded interferometric microscope (SEIM).[4–7] This study will further examine the feasibility of imaging *ex vivo* upper airway tissue.

Herein, we explore the efficiency of these imaging modalities and their ability to characterize changes in upper airway ciliary beat frequency (CBF) in response to topical epinephrine. Epinephrine has previously been shown to increase CBF. *In vitro* rat studies have previously described a dose dependent increase in CBF following the administration of L-adrenalin.[8] Seybold et al have similarly reported epinephrine induced increases in CBF in the sheep model.[9] Additionally, cultured epithelial cells taken from human nasal swabs showed a hyperacute increase in CBF following epinephrine administration lasting 30 minutes. Afterwards, epinephrine showed ciliodepressent effects at the end of the 90 minute observation window.[10] It is possible these conflicting reports are consequences of the differing imaging modalities or the tissue models. Further investigation is necessary to clarify these differences prior to clinical translation.

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2. METHODS

2.1 Sample Acquisition

This study was approved by the University of California, Irvine Institutional Review Board. Tissue was collected from patients undergoing functional endoscopic sinus surgery (FESS) and placed in Hank's Balanced Salt Solution (Fisher Scientific International, Inc., Hampton, NH; HBSS), warmed to physiological temperature, during transport. Tissue was transported in an insulated thermos, which was previously shown to maintain physiological temperature of specimen. Tissue was then placed on a glass bottomed petri dish with a layer of HBSS to prevent desiccation. These petri dishes were then placed on heated stages (37°C; Microscope Temperature Control Stage Slide Warmer, Amscope, Irvine, CA) for PCM and SEIM imaging. Exceptionally large pieces of tissue were cut in half using a razor blade; however, it is important to note that most specimens were miniscule in nature.

2.2 Imaging

Ciliary imaging was performed with an SEIM system as previously described in Park *et al.* SEIM imaging was also verified using phase-contrast microscopy at 60 fps using a smartphone (iPhone 12 Pro, Apple, Cupertino, USA) and a smartphone adaptor.[7]

2.3 Drug Administration

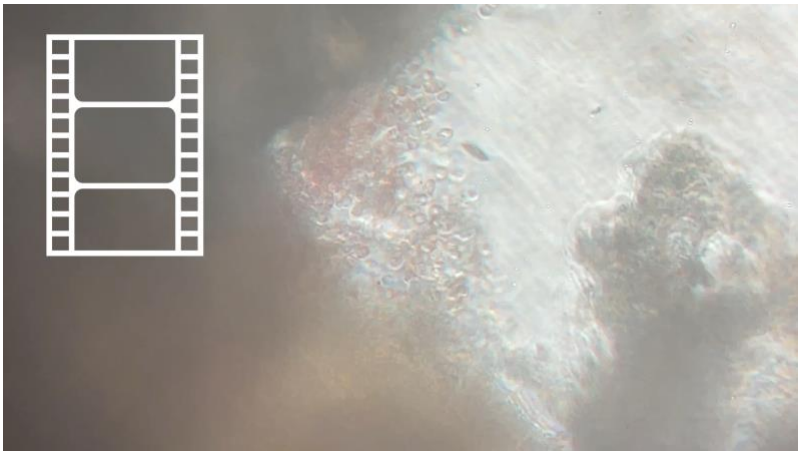
Epinephrine (Sigma Aldrich, St. Louis, USA) was diluted in deionized water yielding a 1mg/mL solution. On imaging, epinephrine was introduced to samples after baseline measurements were recorded. Imaging was continued for one minute after introduction of epinephrine and CBF was calculated pre- and post-epinephrine. Imaging was performed on the same region of interest (ROI) pre- and post-epinephrine introduction.

2.4 Data Analysis

The acquired PCM and SEIM videos were analyzed using MATLAB. The ROI was manually selected before each process, focusing on the areas with cilia movement. Within the selected ROI, a threshold is chosen appropriately to create a mask to minimize the background noise. Once the mask is applied to all the images, fast Fourier transform is used to calculate the CBF. The frequency spectrum is plotted to show the fundamental frequency.

3. RESULTS

PCM of sample one revealed pre-epinephrine CBF was 2.9 Hz and 4.5 Hz post-epinephrine introduction (Video 1). PCM indicated a 228% increase in CBF. On a second sample, SEIM showed a pre-epinephrine CBF of 5.4 Hz and a CBF of 7.0 Hz post-epinephrine introduction (Fig. 1). SEIM exhibited a 128% increase in CBF.



Video 1. PCM video: Addition of epinephrine to sinonasal mucosa in real time. http://dx.doi.org/10.1117/12.2670003.1

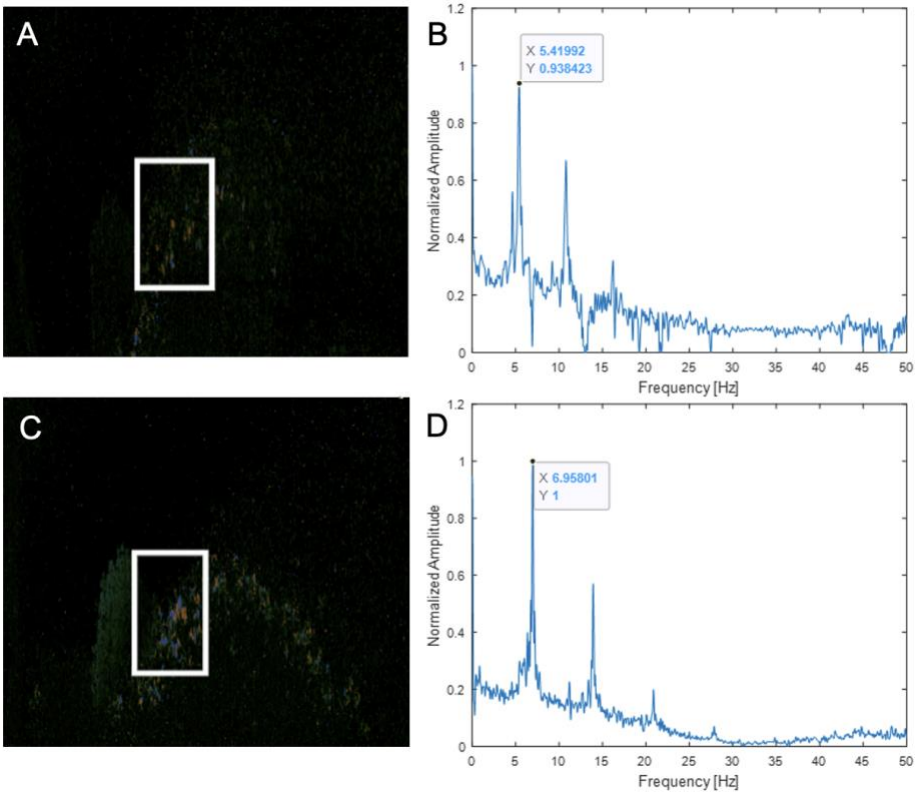


Figure 1. Region of interest selected on SEIM videos to determine CBF (A) pre-epinephrine and (B) post-epinephrine. The first peak (fundamental frequency) represents the calculated CBF (A) pre-epinephrine and (B) post-epinephrine.

4. DISCUSSION

Mucociliary clearance is an important physiological function of the immune system. Many disease processes, such as rhinosinusitis, cystic fibrosis, and allergic rhinitis, have been shown to disrupt ciliary motility. Furthermore, studies have also validated the use of CBF as a surrogate for ciliary health. Previously, we have shown SEIM as a suitable imaging modality for CBF measurements. Herein, we seek to further validate the use of SEIM by introducing epinephrine during SEIM imaging. Epinephrine, an adrenergic agonist, has been shown to increase ciliary activity and thus increase CBF. Traditional methods of calculating this increase in CBF has been through PCM with various pharmaceutical drugs showing varying effects on CBF. It was shown that L-adrenaline (epinephrine) showed marked increase (50-60%) in CBF starting at 0.01 mg/mL and peaking at 1 mg/mL.

The ability to measure CBF and discern magnitude of changes to CBF has various biomedical applications. As previously discussed, reduction of CBF from chronic disease states has been thoroughly discussed in the literature. However, most of these relied on the use of *ex vivo* imaging of tissues. The use of SEIM can allow for avenues in adapting this technology for *in vivo* application in an outpatient setting. As studies have previously shown, disrupted cilia through nasal brushings decreased CBF in the region of sampling.[11] As rhinosinusitis and allergic rhinitis are routinely treated with FESS, the ability to determine a patient's disease state through non-invasive methods may improve patient outcomes. The ability to perform *in vivo* CBF measurements in a non-invasive method may better help the clinicians in deciding whether to operate.

While this further validates the use of SEIM on *ex vivo* human nasal mucosa to determine CBF, several limitations must be considered. The current sample size, n=2, is small. The total number of samples must increase to verify the validity of epinephrine effects on CBF and have a reliable change of magnitude. Another limitation includes the inability to perform SEIM and PCM simultaneously on the same sample. A possible way to combat this problem is to possibly halve the sample; however, the CBF of each specimen half may not be equivalent. Future studies include transitioning from *ex vivo* tissue to an *in vivo* animal model.

In conclusion, the addition of epinephrine to sinonasal mucosa increased ciliary beat frequency. This further validates the use of SEIM in detecting changes in CBF in diseased states.

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