ASSESSING TRACHEAL HEALTH USING OPTICAL METABOLIC IMAGING AND OPTICAL COHERENCE TOMOGRAPHY

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Key words: autofluorescence, metabolism, cilia, optical coherence tomographyThe health and pathophysiology of the tracheal mucosa is an important yet poorly understood aspect of respiratory medicine. Cilia, hair-like organelles important for mucociliary clearance, line the tracheal mucosa. Ciliary dysfunction leads to severe diseases such as primary ciliary dyskinesia and cystic fibrosis. Optical imaging can monitor ciliary function *in vivo*, *ex vivo*, and *in vitro* to understand the genesis of ciliary disease and potential treatment targets. Specifically, optical coherence tomography (OCT) has been used to quantify multiple parameters of ciliary motility in 2D and 3D. However, OCT inherently lacks information about the biochemical or metabolic state of cells. Optical metabolic imaging (OMI) quantitatively assesses cellular metabolism by imaging the autofluorescence intensities of endogenous metabolic co-enzymes nicotinamide dinucleotide (NADH) and flavin adenine dinucleotide (FAD). OMI probes the optical redox ratio (NADH intensity divided by FAD intensity), which is sensitive to the relative amounts of electron donors and acceptors within a cell. Ciliary function is highly dependent on ATP and therefore tightly linked to NADH and FAD levels through multiple metabolic pathways.

Here, we show that widefield epifluorescence OMI and OCT provide complementary information about the effects of cyanide on ex vivo mouse tracheae. Cyanide, a known inhibitor of oxidative phosphorylation, increases NADH and reduces FAD in the cytoplasm (increasing redox ratio) and decreases ciliary activity by limiting ATP synthesis. Cyanide treatment is a relevant proof-of-concept experiment because Pseudomonas aeruginosa, a key pathogen in cystic fibrosis and pneumonia in intensive care units, produce cyanide. Widefield OMI images captured using an epifluorescence microscope (Nikon, Ni-U, 4x air objective) show two distinct regions of the tracheal mucosa: areas containing cartilage rings and areas without cartilage (Figure 1). When the OMI images were quantified, both areas showed significant increases in redox ratio (n=5, $p<0.05$) after 20 minutes of 10mM sodium cyanide treatment. Cartilaginous regions had an increase of 55% (95% CI: [32% 79%]) compared to 38% in areas without cartilage (95% CI: [12% 63%]). We applied particle tracking velocimetry (PTV) with OCT (Thorlabs, Telesto II) to validate the impact of cyanide on ciliary activity. PTV measurements were made using 5µm polystyrene beads (Bangs Laboratories) added to the media above trachea prior to cyanide treatment, rinsed off, and then again added after 20 minutes of cyanide treatment. Bead motion was imaged over 21s at 28fps. Maximum intensity projections over 7s qualitatively show that particle velocity is reduced by cyanide treatment (Figure 2). Quantitative particle tracking performed with TrackMate (ImageJ/FIJI) showed cyanide significantly reduced the mean velocity of beads by 19% (n=4, $p<0.05$, 95% CI: [10% 28%]). These results demonstrate that OMI reflects changes in ciliary activity assessed with OCT. We believe that OCT and OMI provide complementary information to evaluate tracheal health without the need for exogenous dyes or destructive histology. This multimodality approach could be used to understand respiratory disease pathogenesis and improve drug development in preclinical models. In the future, similar methods could be used to monitor tracheal health in intensive care patients

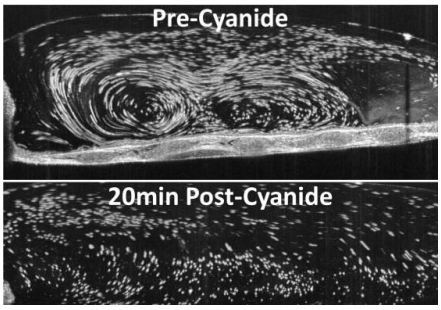

Figure 2 – Maximum intensity projections over 7s of bead motion shows reduction in cilia-driven fluid flow after cyanide treatment.

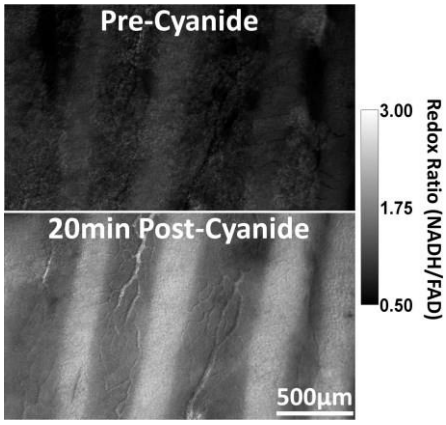

Figure 1 – Redox ratio (NADH/FAD) images show increase after cyanide treatment.