Quantification of Airway Remodeling in Normal and Asthmatic patients

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Abstract

Rationale: Airway remodeling is a fundamental feature of asthma, linking inflammation with airway hyperresponsiveness (AHR). Airway remodeling pertains particularly to the structural changes that occur in and around the trachea, bronchi and bronchioles. Remodeling involves all parts of the airway wall including the epithelial, submucosal, and smooth muscle layers as well as vascular structures. The asthmatic airway also shows evidence of fibrosis, with deposition of abnormal extracellular matrix (ECM) components in the basement membrane in particular collagen I. While it is known that the basement membrane is thicker in asthma, it is unknown whether this is a uniform thickening, or irregular in nature. In addition, the re-organization of collagen I components during remodeling is still poorly understood.

Methods: High resolution Aperio images of airways stained with Masson's Trichome from asthmatic (n=5) and non-asthmatic (n=5) subjects were used to analyze variations in basement membrane thickness. An image-analysis algorithm was designed to measure the standard deviation of thickness variations on optimized images of basement membranes. To analyze the remodeling of fibrillar collagens, multiphoton microscopy was used to acquire specific second harmonic generation (SHG) images. The morphology of the structures within the image was quantified based on 'Structure Tensors' method. This method not only calculates the overall dominant direction in a certain region of interest, it also provides a measure of how well the structures are organized in that direction.

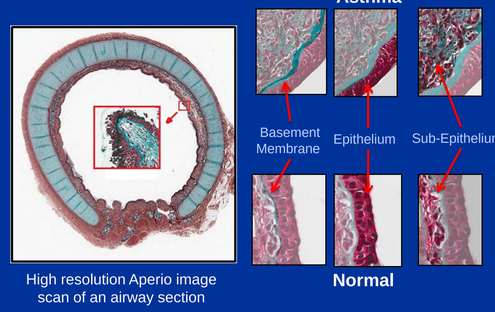
Results: Using our image analysis algorithm, we found that there was a trend towards thicker basement membranes in asthmatic airways. We found that the variation in basement membrane thickness was similar between asthmatic and non-asthmatic airways.
Using Structure Tensors method we determined the orientation coherency of collagen fibers in asthmatic airways was significantly lower than non-asthmatic airways (p= 0.0186). These data indicate that fibrillar collagen in asthmatic airways is less oriented.

Conclusion: Using multi-photon microscopy, we demonstrate for the first time that fibrillar collagen in the asthmatic airways is less orientated. Understanding the remodeling of fibrillar collagen as well as basement membrane modification is important as collagen structure plays an important role in ability of fibroblasts to maintain airway structure during homeostasis and repair. Future studies will determine the effect of collagen structure on fibroblast function.

Introduction

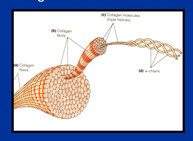
Asthma is the most common chronic condition in children worldwide. This chronic inflammatory disorder appears as airway hyperresponsiveness and inflammation and is often characterized by pathological modification of the airway structure.

The Structural changes are often called airway remodeling and include : epithelial changes, sub-epithelial fibrosis and deposition of abnormal extracellular matrix components in the basement membrane and in particular collagen.



High resolution Aperio image scan of an airway section

The collagen molecule subunit is a rod about 300 nm long and 1.5 nm in diameter, made up of three polypeptide strands, they twist together into a right-handed coiled coil to form a triple helix .



When imaging by Multiphoton Microscopy, non-centrosymmetric structures such as collagen exhibit a SHG peak and because it is shorter than the fluorescence spectrum, it can be separated and provide contrast for tissue imaging.

Hypothesis

We hypothesize that the structural orientation of fragmented collagen and the degree of variation in basement membrane thickness will be different in normal and asthmatic airways.

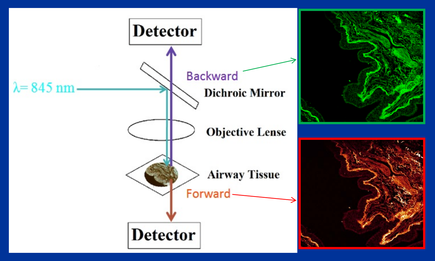
Specific Aim

The aim of this study was to compare fibrillar collagen arrangement and basement membrane thickness variations in normal and asthmatic airways and also to determine the effect of less oriented collagen on fibroblast function.

Methods and Procedures

MPEF and SHG microscopy

A mode-locked femto-second Ti:Sapphire Tsunami was used for the experiments. The laser output was attenuated using neutral density filters and the average power was below the damage threshold of the samples. The laser beam was focused on the specimen through a Lecia water immersion objective, 63 X/1.2 NA. Leica Software was used for the image acquisition. A short pass filter was used to prevent the scattering radiation from reaching the detector and a long pass dichroic beam splitter was used to separate SHG signal from the MPEF signal. SHG signal in forward direction was captured using a non-de scanned detector.



All SHG and MPEF spectral measurements were performed using the de-scanned PMT detector located inside the scan head. A series of individual images were collected using a narrow detection window with a width of 10 nm through the overall spectral capacity of the system and each image detected at this specific emission wavelength band provided a data point in the spectral graph.

Analysis

Thickness variations:

For thickness measurements, an image analysis algorithm based on the local thickness plugin in Fiji was designed to determine the average thickness and the thickness variations. The theory of the local thickness is for each point in the object, the diameter of the largest circle that fits inside the object and contains the point is considered to represent the local thickness of that point. Different parts of the object which are different in thickness are coloured relatively.

$$\tau(\vec{p}) = 2\max\left(\left\{r \mid \vec{p} \in sph(\vec{x}, r) \subseteq \Omega, \vec{x} \in \Omega\right\}\right)$$

Collagen fibers orientation:

Structural orientation coherency of collagen fibers was determined using the local orientation and isotropic properties (coherency and energy) of every pixel of the image derived from the structure tensor matrix that was defined for each pixel.
Structure tensors are a matrix representation of partial derivative information. In the field of image processing and computer vision, it is typically used to represent the gradient or "edge" information. It also has a more powerful description of local patterns as opposed to the directional derivative through its coherence measure.
The coherency parameter C which is an index between 0 and 1 , represents the structural orientation coherency. It is defined as the ratio between the difference and the sum of the tensor eigenvalues.
This coherency parameter was measured on 3 different regions of interest on 5 different spots on each sample.

Samples

	Gender	Average Age
Asthma	3f / 2m	20.8 yrs
Normal	2f / 3m	17.4 yrs
Total	10	19.1 yrs

Table 1. Information of the donors used (m=male f=female yrs=years).

Results

Figure 1: Determining the correct counter stain for collagen analysis with SHG imaging

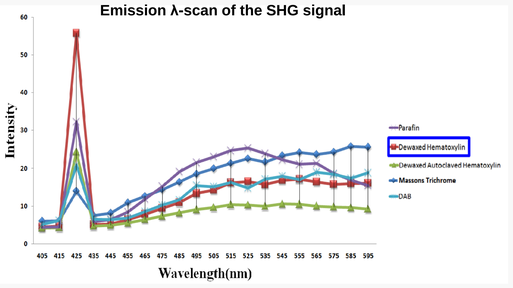
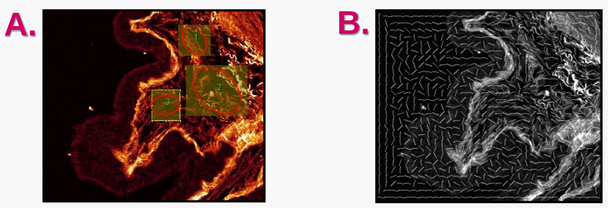


Figure 1: Immunohistochemistry studies require removal of paraffin by autoclaving and counter staining of biological tissues to identify different structures within patient samples. Human lung airway sections were stained to visualize tissue structures by light microscopy with Hematoxolin, Massons Trichrome and an anitbody labeled with DAB. These sections were then analyzed using a wavelength scan for the second Harmonic signal of Collagen 1 structures compared to autofluorescence. Our data demonstrate that only tissue sections stained with hemotaxolyn enable a strong SHG signal compared to autofluorescence.

Figure 2: Sub-epithelial collagen is more disorganized in asthmatic airways based on the structure tensor analysis



C.

Structure Tensors : $J = \begin{bmatrix} < f_x, f_x >_w & < f_x, f_y >_w \\ < f_x, f_y >_w & < f_y, f_y >_w \end{bmatrix}$

Local Dominant Direction : $\theta = \frac{1}{2}\arctan\left(2\frac{< f_x, f_y >_w}{< f_y, f_y >_w - < f_x, f_x >_w}\right)$

Coherency Parameter : $C = \frac{\lambda_{max} - \lambda_{min}}{\lambda_{max} + \lambda_{min}} = \frac{\sqrt{\left(< f_y, f_y >_w - < f_x, f_x >_w\right)^2 + 4 < f_x, f_y >_w}}{< f_x, f_x >_w + < f_y, f_y >_w}$

D.

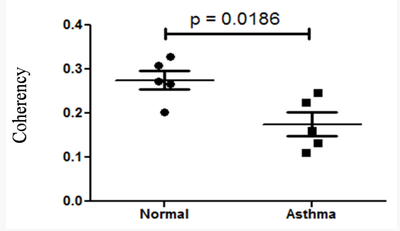
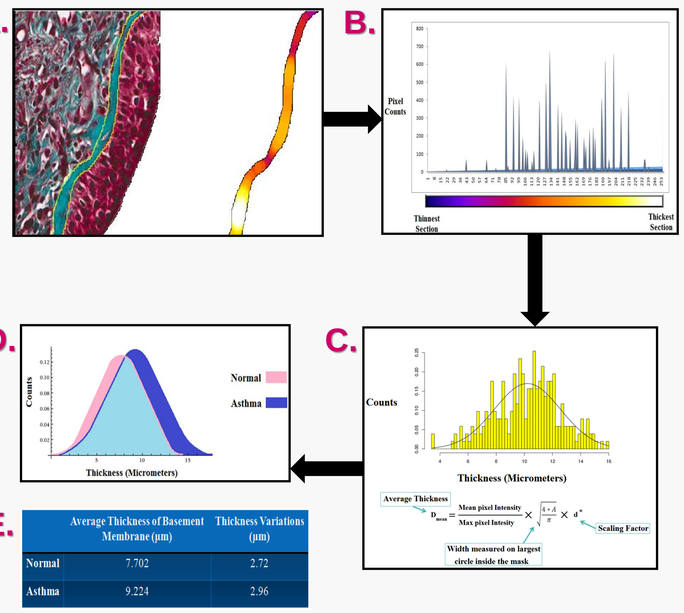


Figure 2: A) Forward scatter image of an airway where Collagen fibers are visualized as orange and the numbered circle are the different regions of interest analyzed within the sub-epithilium. **B)** Structure tensor vectors overlaid on the forward scatter image **C)** Structure tensor equations used for analysis **D)** Comparison of sub-epithelial collagen fibers orientation coherency in normal and asthmatic airways. By performing an unpaired t-test on collagen fiber orientation coherency, we demonstrate that asthmatic airways have significantly less organized collagen fibers compared to normal airways.

Figure 3: Basement membrane thickness variation is different in normal and asthmatic airways



E.

	Average Thickness of Basement Membrane (μm)	Thickness Variations (μm)
Normal	7.702	2.72
Asthma	9.224	2.96

Figure 3: A) Aperio image snapshot of an airway and the local thickness output of a traced basement membrane **B)** Colour histogram showing the frequency of different colours observed **C)** Numerical conversion of the thickness **D)** Normal distribution of the results **E)** Averages and variations of basement membrane thickness in normal and asthmatic. By using this algorithm, basement membrane average thickness and its variation was compared and it was seen that these terms are bigger in asthmatic airways.

Summary

In comparison, asthmatic airways have…

- Less structured orientation coherency of sub-epithelial collagen compared to non-asthmatic airways.
- Trend towards greater and more variable basement membrane thickness than non-asthmatic airways.

•Future Studies:

- ❖ Finishing analysis of the entire patients cohort (n=10 per group).
- ❖ Determining the ability of airway fibroblasts from asthmatic and non-asthmatic donors to remodel collagen 1 gels by developing a collagen 1 gel contraction bio-assay.

References

1. Bousquet, J., et al. "Asthma: a disease remodeling the airways." Allergy 47.1 (1992): 3-11.
2. Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. Curr Opin Allergy Clin Immunol. 2012 Feb;12(1):53-59.
3. Orgel, Joseph PRO, et al. "Microfibrillar structure of type I collagen in situ." *Proceedings of the National Academy of Sciences* 103.24 (2006): 9001-9005.
4. Rezakhaniha, Rana, et al. "Experimental investigation of collagen waviness and orientation in the arterial adventitia using confocal laser scanning microscopy." *Biomechanics and modeling in mechanobiology* 11.3-4 (2012): 461-473.
5. Dougherty, R., and Karl-Heinz Kunzelmann. "Computing local thickness of 3D structures with ImageJ." *Microscopy and Microanalysis* 13.S02 (2007): 1678-1679.