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MICROMECHANICS OF THE CONNECTIVE TISSUE MATRIX OF THE LUNG

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Abstract-**The number of macromolecules thought to play an important role in tissue rheology is small, including collagen, elastin and proteoglycans. Yet different tissues with similar constituents exhibit different rheological behavior. We hypothesized that the rheological behavior of the lung parenchyma arises from 1) the structural complexity of the collagen network within the alveolar wall and 2) the mechanical interaction between collagen and proteoglycans. In disease, active cellular remodeling alters the complexity of tissue architecture which changes its fractal dimension. We have developed a novel technique to visualize macromolecular complexes in the alveolar walls of a slice of intact lung tissue during macroscopic deformation. Collagen or elastin from normal and elastase-treated rat lungs were labeled and the rheology of the samples were assessed while the deformation of the macromolecules were imaged. The data suggest that 1) folding and relaxation of collagen may be important in the hysteretic behavior of the normal lung; 2) increased macroscopic hysteresis in emphysematous lungs is related to increased hysteresis during folding of alveolar walls; 3) proteoglycans stabilize the folding of the alveolar walls; and 4) the macroscopic power law stress relaxation may be related to the fractal organization of collagen within the alveolar wall.**
Keywords **– collagen, relaxation, complexity**

I. INTRODUCTION

The rheological properties of connective tissues play fundamental roles in the functioning of many organs, especially the lung. Yet virtually nothing is known about the molecular mechanisms contributing to the macroscopic hysteresis of the airway wall and lung parenchymal tissues. There are only a few types of macromolecules namely collagen, elastin and proteoglycans, that are thought to play an important role in tissue rheology. Nevertheless, different connective tissues can exhibit very different mechanical behaviors [1]. Thus, the mechanical behavior must critically depend on the organization of the macromolecules and their complex interactions. These interactions span an enormous length scale ranging from hydrogen bonds in a collagen molecule (subnanometer scale), to shear stress transfer between collagen fibers and proteoglycan matrix (micrometer scale), to mechanical interdependence between airways embedded in the lung parenchyma (millimeter scale). Likewise, there is a wide range of time scales in the mechanical behavior: the stress relaxation of lung tissue follows a power law over several decades of time. Such behavior is a reflection of complexity in structure and dynamics. Complexity can be characterized by the fractal dimension which is an integer number for simple Euclidean objects such as a straight line and can take fractional numbers for fractal objects [2].

Several mechanisms have been proposed to account for lung tissue viscoelasticity which include fiber-fiber interactions [3], polymer-like "reptation" [4] or complexity of the structure per se [5]. In this study, we hypothesized that the rheological behavior of connective tissues of the lung is an emergent property arising primarily from the structural complexity of the collagen network within the alveolar wall and the mechanical interaction between collagen fibers and proteoglycans.

II. METHODOLOGY

We have developed a novel and unique technique that enables us to visualize macromolecular complexes in the alveolar walls of a slice of lung tissue during macroscopic deformation mimicking breathing [6]. This technique allows us to study and quantify the structural complexity and the dynamics of the collagen network and its interaction with the proteoglycans. Briefly, thin tissue strips (5x5x0.3 mm) were sectioned from fresh isolated rat or mouse lungs before or after treatment with elastase. Collagen and elastin fibers were separately or simultaneously labeled with different color fluorophores using indirect immunofluorescence (monoclonal antibodies). During slicing and labeling, the tissue was kept as intact as possible in physiologic saline solution and viability was confirmed by cellular contraction in response to histamine challenge. The microstructure was then visualized under an inverted fluorescent microscope with a resolution of ~1 micron. The extracellular matrix was also visualized during uniaxial macroscopic deformation. Images were collected during both the loading and unloading phases of stress-strain curve measurements as well as during stress relaxation experiments.

At the level of the alveolar walls, segment lengths and angles between neighboring segments were recorded. At a higher resolution, individual fibers within the alveolar walls were detected. Analysis of the data included statistical evaluation of the folding and stretching of the alveolar walls. Within the alveolar walls, the curvature of individual fibers were measured and the fractal dimension of the collagen was determined at various macroscopic stretches. A network model was also developed for the interpretation of the results. The network consisted of elastic or viscoelastic line elements arranged in a square or hexagonal lattice. The network was stretched and internal configuration of the line elements was solved using simulated annealing.

III. RESULTS

The macroscopic mechanical behavior of the thin tissue slice was similar to that reported in larger tissue strips and whole lung. In particular, the stress-strain curve was nonlinear and showed hysteresis. The stress relaxation followed a power law as evidenced from the linear decrease of stress on a double logarithmic graph. Fig. 1 shows two images of the

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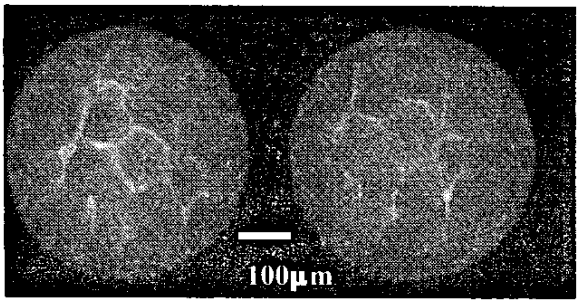

Figure 1. Same region of elastin labeled tissue at 0% (left) and 30% (right) macroscopic strain. Direction of strain is vertical.

same region of the tissue at 0% and 30% macroscopic uniaxial strains. The labeling is for elastin, however, at this magnification the images contour the alveolar walls. It is evident that the alveolar wall reorient and stretch. It was evident from double labeling images that the collagen and elastin were interwoven, where collagen appeared as distinct fiber-like structures, and elastin was seen to have a more diffuse mesh-like organization both in normal and diseased tissues. We also observed that the collagen fibers were wavy at low strains and the wavy fibers tended to become more straight with increasing deformation. During stretching, crimps along the collagen unfold and the collagen contributes progressively more to the developed stress in the tissue. This results in a decrease of the fractal dimension of the collagen from 1.2 at 0% strain to 1.1 at 20% strain. Figure 2 shows examples of the collagen network within the alveolar wall. These microscopic observations were correlated with tissue strip level mechanical measurements. Using digestion of proteoglycans, we found that the proteoglycans appear to play a stabilizing role in the deformation of the alveoli. In summary, the data suggest that 1) folding and relaxation of collagen may be important contributors to the hysteretic behavior of the normal lung; 2) increased macroscopic hysteresis in emphysema-like remodeled tissue is related to an increase in the hysteresis of the folding of alveolar walls; and 3) the power law stress relaxation may be related to the fractal organization of collagen within the alveolar wall. It is now tempting to speculate that the wavy nature of the collagen with its low fractal dimension (1.1 at 20% uniaxial strain) has an intimate relationship with the stress relaxation exponent which has a numerical value of 0.1. Following a step change in strain, the fractal dimension slightly decreases, but over time the crimps rearrange along the fibers as the system approaches equilibrium which results in a very slow power-law stress relaxation and the value of the relaxation exponent would primarily be determined by the fractal dimension of the wavy collagen.

IV. CONCLUSIONS

The macroscopic elastic and hysteretic properties of the lung connective tissue appear to be related to the complex

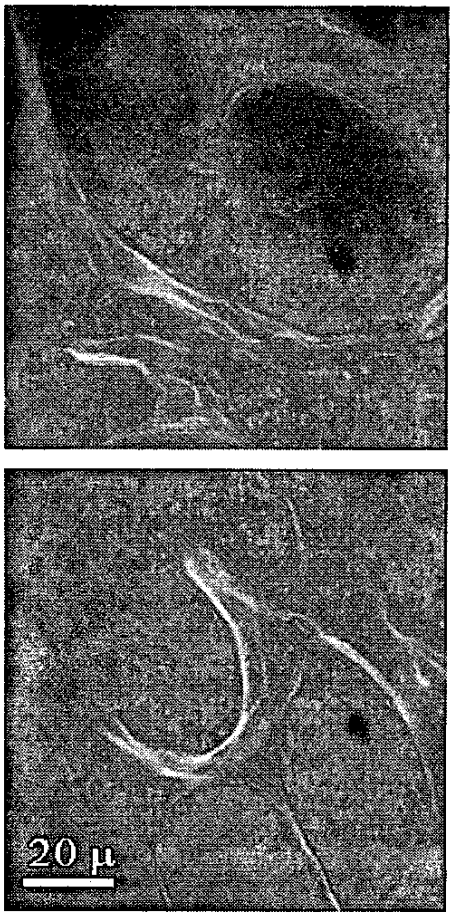

Figure 2. Fluorescently labeled collagen network in the alveolar wall at macroscopic strains of 0% (top) and 20% (bottom). The wavy nature of the fibers is more apparent at 0% strain. Direction of strain is vertical.

organization of the collagen and how the proteoglycans stabilize the folding of collagen and hence the alveoli during deformation.

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