

Quantification of the effects of alveolar surface tension modulation by magnetic resonance elastography (MRE).

Kieran P. McGee¹, Rolf D. Hubmayr², Yogesh K Mariappan¹, Zhonghao Bao³, David L. Levin¹, Rickey E. Carter⁴, and Richard L. Ehman¹

¹Radiology, Mayo Clinic & Foundation, Rochester, Mn, United States, ²Pulmonary and Critical Care Medicine, Mayo Clinic & Foundation, Rochester, Mn, United States, ³Information Technology, Mayo Clinic, Rochester, Mn, United States, ⁴Health Sciences Research, Mayo Clinic, Rochester, Mn, United States

Introduction: The alveolar air-liquid interface produces surface tension forces that increase the elastic recoil of the lung, facilitating expulsion of exchanged gases during the expiratory phase of respiration [1]. Alteration of these forces by either loss or over expression of pulmonary surfactant (the primary lipoprotein responsible modulation of surface tension) profoundly influences lung elastic recoil and lung function on both regional and global scales. Assessment of pulmonary surfactant is most commonly performed by means of bronchiolar lavage, a highly invasive and infrequently performed process. Magnetic resonance elastography (MRE) [2] is a novel method for quantifying and spatially resolving shear stiffness in biological tissues and has recently been demonstrated to quantify lung elasticity in both *in situ* [3, 4] and *in vivo* [5] imaging conditions. We hypothesize that MRE is capable of resolving on both global and regional scales the effects of loss of surface tension and by inference pulmonary surfactant within the lung. The purpose of this work is to test this hypothesis by comparing MRE-based estimates of shear stiffness in lungs with and without the alveolar air-liquid interface.

Materials and Methods: Ex vivo ¹H MRE was performed on six air and six fluid-filled ex vivo Sprague-Dawley (Harlan Labs, Indianapolis, IN) female adult rat lungs (figure f1) with two of the air-filled lungs being previously reported [6]. Fluid-filled lungs were obtained by creating pulmonary edema and involved placing the animal on a mechanical ventilator (Flexivent, Quebec CA) while under deep anesthesia for 20 minutes at a maximum airway pressure of 40 cm H₂O. Immediately following ventilation the animal was sacrificed. Air-filled lungs were obtained by surgical removal of the lungs *en bloc* following expiration of the host. Animal preparation protocols for both lung sets were in accordance with our Institutional Animal Use and Care (IACUC) guidelines. Prior to imaging the wet weight of each lung was measured in grams (Ohaus SP6001, Parsippany, NJ). For air-filled lungs, each lung set was degassed and then inflated to a pressure of 20 cm H₂O prior to imaging. The lungs were then deflated to 3 cm H₂O after which ¹H MRE imaging was performed. The lungs were then inflated to a pressure of 20 cm H₂O for one minute followed by deflation to 6 cm H₂O after which ¹H MRE imaging was repeated. This process was reproduced for additional inflation pressures of 9, and 12 cm H₂O. Fluid-filled lungs were imaged immediately following excision but did not undergo any additional pressure-volume maneuvers. Instead these lungs were imaged at pressures of 3 cm, 6 cm, 9 cm and 12 cm H₂O in sequential order.

MRE was performed on each lung set with the following parameters: FOV = 6cm, slice = 5 mm, k_x = 128, k_y = 64, TE/TR = 13/318 msec, shear wave motion encoding = 220 Hz, phase offsets = 4. Two additional sequences were also acquired at each inflation pressure. The first was a T₁-weighted 2D spin echo sequence (TE/TR = 10/600 msec, k_x = 192, k_y = 160, FOV = 8 cm, slice = 4mm) and was used to obtain volumetric information for each lung at each pressure while the second was a T₂*-weighted multi-echo gradient echo sequence (# echoes = 3, TE_{1,2,3} = 1.6, 2.6, 3.6 msec, TR = 12.5 msec, $\alpha = 10^\circ$, k_x = k_y = 128, FOV = 6 cm, slice = 5 mm) which was used to spatially resolve the physical density of the lung within each slice. Density maps were derived from the T₂* maps by solving for S₀ in the equation S = S₀e^{-TE/T2*} using an in-house software application followed by normalization of the S₀ map using the signal from a gadolinium (Magnevist, Berlex labs. Wayne, NJ) doped tube of normal saline and the approach described by Theilmann et al [7].

For both air and fluid-filled lungs shear stiffness was estimated using the principal frequency analysis (PFA) method [6] while the local frequency estimation (LFE) [8] method was applied to fluid-filled lungs following correction for physical density using the T₂*-derived density maps. For the PFA data, a hierarchical linear model was used to test if shear modulus was differentially affected by pressure changes in fluid-filled lungs (edema) versus air-filled lungs. Pressure was treated as a factor with four levels. The compound symmetry covariance pattern was used to describe the association of shear modulus readings within an individual animal lung (i.e., repeated measures analysis).

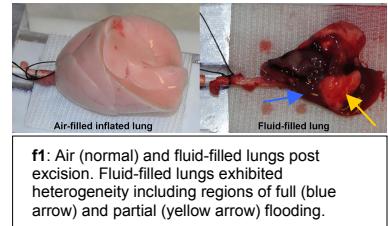
Results: Table 1 presents post-hoc tests for differences in shear stiffness between air and fluid-filled lungs at each pressure (P_{tp}) with P_{tp} being considered equal to the transpulmonary pressure. At all four pressures, the within group shear stiffness was statistically significantly greater for the air-filled compared to fluid-filled lungs. Figure 2 shows the density corrected (wet mass / volume) PFA estimates of shear stiffness averaged over all slices for the two lung groups as a function of P_{tp}. Air-filled lung shear stiffness increased with P_{tp} as expected [9] while fluid-filled lungs exhibit almost no increase in stiffness with P_{tp}. This latter effect is most likely due to the inability to move fluid plugs within airways at these relatively low inflation pressures. These data suggest that surface tension forces contribute to increased lung elastance as measured by MRE-derived shear stiffness estimates. Figure 3 show magnitude and density corrected LFE-derived elastogram images of a fluid-filled lung at four P_{tp} values. The magnitude images demonstrate significant heterogeneity and identify regions of full (yellow arrow) and partial (pink arrow) flooding. Partially flooded regions demonstrate an increase in shear stiffness with inflation pressure suggesting that these regions are undergoing active recruitment with increasing pressure. In contrast, fully flooded regions do not exhibit the same increase in shear stiffness with P_{tp} and were softer (lower shear stiffness).

Conclusions: These data identify that the shear stiffness of fluid-filled lungs are less than air-filled lungs and demonstrate that lung elastic recoil decreases in the absence of the alveolar air-fluid interface. Spatial resolution of changes in shear stiffness with P_{tp} as shown in f3 provide evidence that MRE-based estimates of shear stiffness can identify regions of lung capable of undergoing active recruitment during respiration and suggest that shear stiffness may be a surrogate marker for pulmonary surfactant content.

Acknowledgements: This work is supported by NIH Grants EB07593 & EB001981.

References:

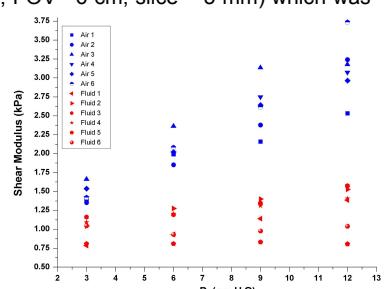
1. Levitzky, MG, *Pulmonary Physiology*, 2007: McGraw-Hill Medical
2. Muthupillai, R, et al *Science*, 1995. 269(5232): 1854-1857.
3. Mariappan, YK et al. in *Proc. ISMRM*, 2010.
4. Mariappan, YK, et al. In Press *Magn Reson Med*, 2011.
5. Mariappan, YK, et al. *J Magn Reson Imaging*, 2011. 33(6): p. 1351-61.
6. McGee, KP, et al. *Phys Med Biol*, 2011. 56(14): p. 4291-309.
7. Theilmann, RJ et al. *J Magn Reson Imaging*, 2009. 30(3): p. 527-34.
8. Manduca, A, et al in *Vis. Biomed. Comp.*, 1996, Springer. p. 63-68.
9. Lai-Fook, SJ, et al *J Appl Physiol*, 1976. 40(4): p. 508-13.



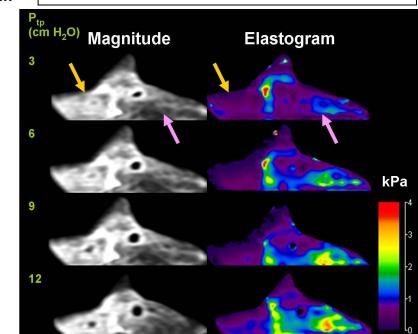
f1: Air (normal) and fluid-filled lungs post excision. Fluid-filled lungs exhibited heterogeneity including regions of full (blue arrow) and partial (yellow arrow) flooding.

| P _{tp} (cm H ₂ O) | Fluid-filled shear stiffness | | Air-filled shear stiffness | | Unadjusted p-value |
|--|------------------------------|--------|----------------------------|--------|-----------------------|
| | Mean (kPa) | SE | Mean (kPa) | SE | |
| 3 | 0.9896 | 0.1031 | 1.4626 | 0.1031 | 0.0036 |
| 6 | 1.0570 | 0.1031 | 2.053 | 0.1031 | < 0.0001 |
| 9 | 1.1675 | 0.1031 | 2.6125 | 0.1031 | < 0.0001 |
| 12 | 1.2897 | 0.1031 | 3.1212 | 0.1031 | < 0.0001 |

Table 1: Shear modulus for air and fluid-filled lungs at 4 inflation pressures.



f2: PFA-derived shear stiffness estimates for air (blue) and fluid-filled (red) lungs as a function of P_{tp}.



f3: magnitude and elastogram images of fluid-filled lung. The magnitude image exhibits regions of full (yellow) and partial (pink) flooding.