Multiscale Nonlinear Mechanics of Lung Extracellular Matrix

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A precise knowledge of the mechanical properties of the extracellular matrix (ECM) is critical to further our understanding of the cell-matrix interplay. Atomic force microscopy (AFM) is particularly suitable to study the mechanical properties of ECM at the microscale that cells sense the stiffness of their microenvironment [1]. Nevertheless, although many biological tissues including those of heart and lung are physiologically subjected to stretch, conventional AFM systems do not allow measurement of the stiffness of the sample at different stretch levels. We studied nonlinear micromechanical properties of lung ECM by means of AFM using a novel device fabricated with a 3D printer to stretch the ECM sample during AFM measurements. To compare micro and macroscale mechanics we also probed ECM by means of tensile tests. The study was carried out in lungs (n = 3) excised from healthy Sprague-Dawley rats. For AFM measurements, the left lobe was decellularized with sodium dodecyl sulfate 1% and triton X-100 0.1% and ~10 μm thick sections were cut with a cryostat. An ECM slice was adhered with genipin onto the flexible membrane of the stretching device. Force-indentation (F-δ) curves were recorded with a custom-built AFM at increased stretch levels (Fig. 1). Micromechanical Young's modulus (E) was computed by fitting the Hertz contact model to F-δ data. For tensile tests, peripheral parenchymal strips (~8×1×1 mm) were cut from the right lung lobe with a scalpel. Fresh strips were attached to a servocontroled actuator (Aurora Scientific) and stress-stretch (σ-λ) curves were recorded. Subsequently, the strips were decellularized and additional tensile tests were performed. The micromechanical Young's modulus of the strip was defined as $E = d\sigma/d\lambda$. Strip data were analyzed with the Fung's model which assumes that E increases linearly with stress. Under relaxed conditions, lung ECM exhibited a micromechanical stiffness of 14.03 ± 2.1 kPa (mean ± SD) and raised markedly with stretch. Macromechanical stiffness of the relaxed ECM was one order of magnitude lower (1.83 ± 0.88 kPa) and displayed a weaker stretch dependence. Interestingly, fresh parenchymal tissue strips composed of ECM and lung cells showed similar stiffness than the decellularized strips (relaxed ECM 2.02 ± 1.21 kPa) showing that lung tissue macromechanics is dominated by ECM. We have fabricated a novel device that allowed us to characterize ECM micromechanics with AFM at different levels of sample stretch. To the best of our knowledge, we report the first data of ECM micromechanical nonlinearity. Noteworthy, our device can also be applied to probe mechanical nonlinearity of many soft biological samples including cells. Our data reveal that lung ECM exhibits a marked stretch hardening behavior both at the micro- and macro-scales. Moreover, the higher stiffness obtained by AFM indicates that lung ECM macromechanics is determined by the local intrinsic mechanical properties of ECM as well as the 3D architecture of the lung tissue.

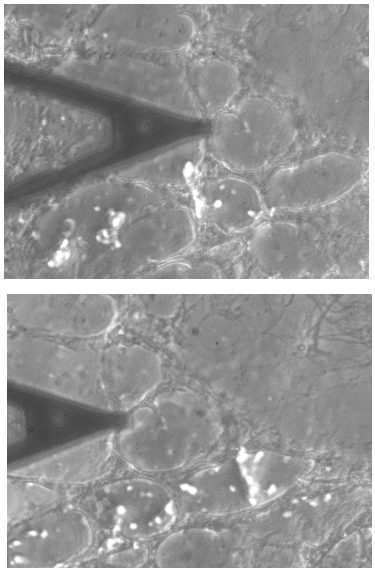

Figure 1. Optical image of a slice of lung ECM adhered on top of the device with AFM cantilever. ECM in unstretched condition (top) and at stretched state (bottom).

[1] Jorba I et al. J Cell Physiol. 232(1):19-26 (2017).