Characterization of the Mechanical Properties of Lung Adenocarcinoma

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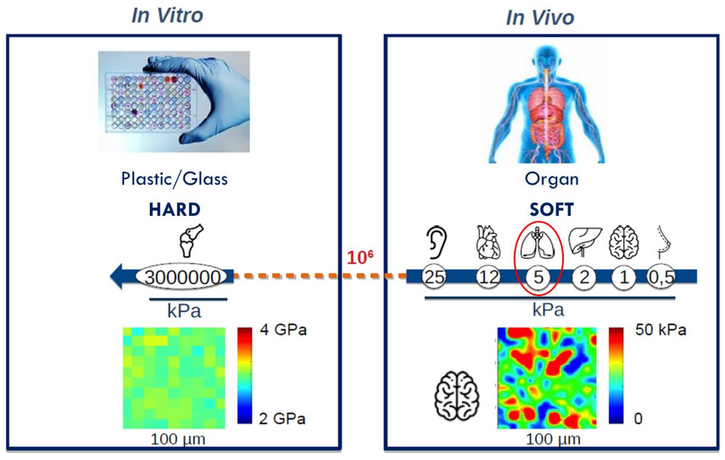
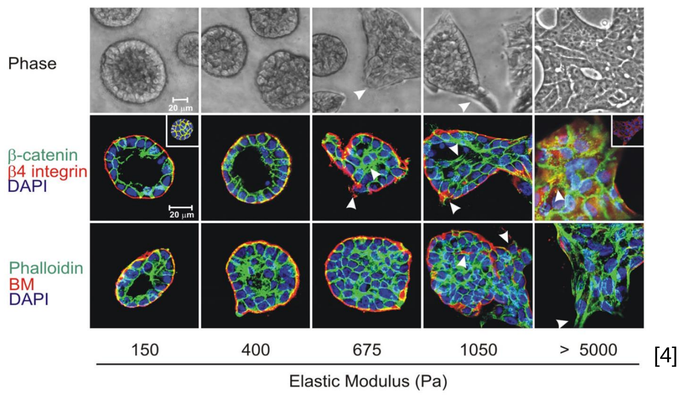
INTRODUCTION



Rigidity of ECM impacts several cellular functions [1-3]



[4]

Tumor breast tissue is 10x more rigid than normal tissue (1.5 kPa vs 150 Pa) [5]

↑ Aggressiveness
↑ Metastatic potential [6]

Hypothesis

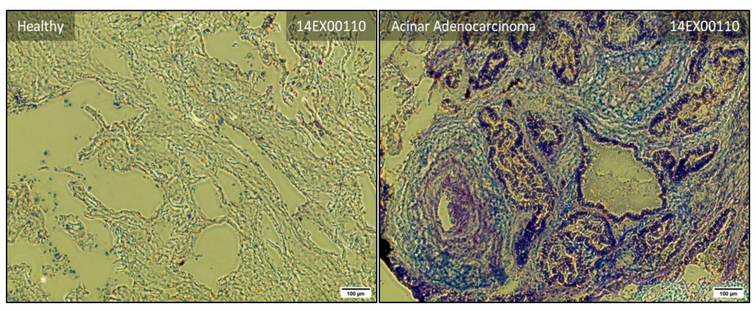
Mechano-mimetic culture substrates will preserve cell models phenotypes during passages and induce similar physiological behavior

Soft Substrate + **Rigidity Texture**

MATERIALS AND METHODS

Tissue Preparation

Frozen lung tissues were embedded in OCT compound and cut at 100 µm in thickness (for AFM measurements) and 14 µm thickness (for histological staining) using a cryostat (LEICA 3050S).
Tissue sections were deposited on special adhesion glass slides and stained for toluidine blue, a basic thiazine metachromatic dye with a strong affinity for acidic tissue components.



Selection of areas of interest

After histological staining, tissue sections stitched images were taken at 20x using an inverted Microscope IX83 Olympus to give support in selecting the tumor region during AFM measurements. A triplicate for each sample was used for AFM characterization.

Stiffness measurements with IT-AFM + Cell Hesion ® Module

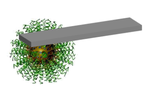
Young's moduli of lung tissues samples were measured on a JPK Nanowizard II AFM in the force mapping mode. A polystyrene sphere with 10 µm diameter to acquire 10x10 to 100x100 µm stiffness maps. A custom made algorithm was used to treat the processed curves using JKR model.

JKR

$$h = \frac{a_{JKR}}{R} - \frac{4}{3}\sqrt{\frac{a_{JKR} F_{ad}}{RK}}$$

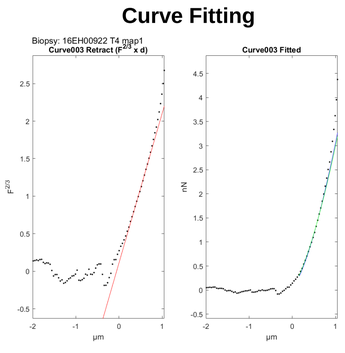
$$a_{JKR} = \frac{R}{K}\left(\sqrt{F_{ad}} + \sqrt{F_n + F_{ad}}\right)^{\frac{2}{3}}$$

$$F_{ad} = \frac{3\pi}{2}\gamma R$$



AFM spherical tip
10 µm Ø coated with F-127 Pluronic
Cantilever MLCT-O
D position
k=0.03 N/m

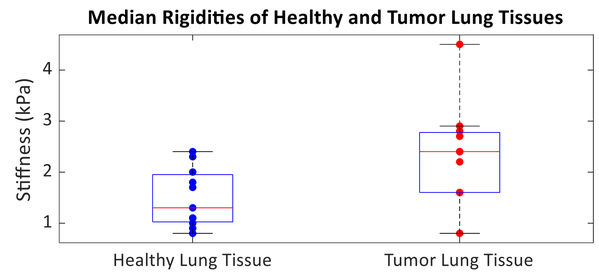
Curve Fitting



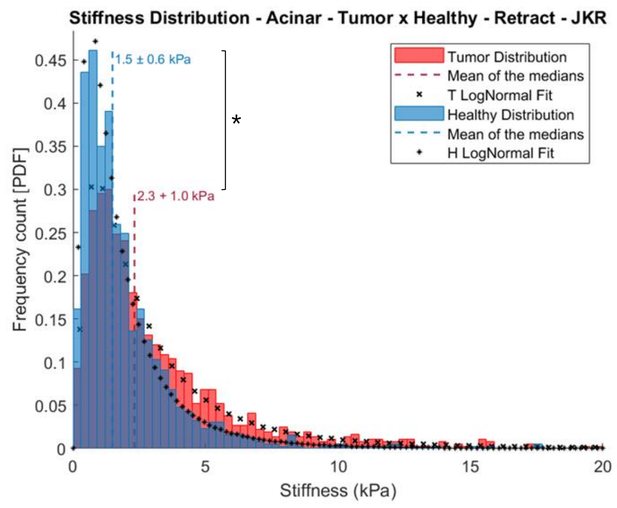
Human tumor lung tissue is stiffer than healthy lung tissue

First characterization of human tumor lung tissue at the microscale

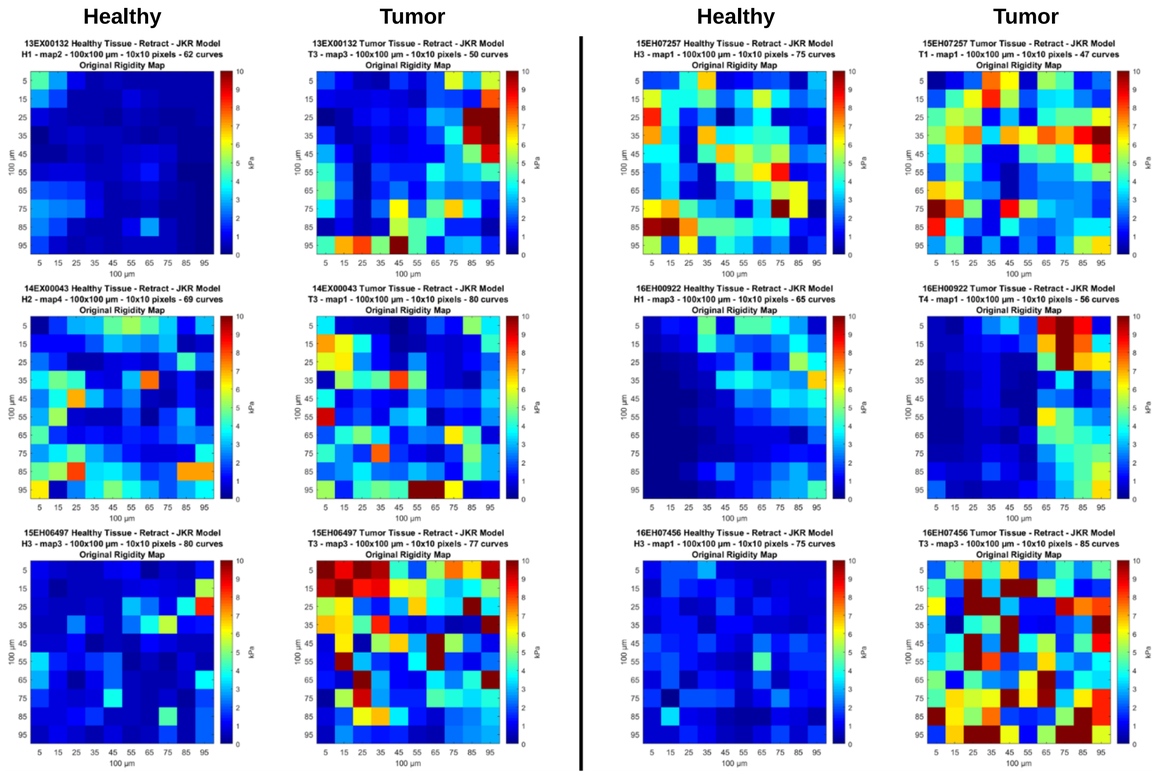
	Healthy	Tumor	Total
# Curves	17310	17187	34497
Maps	247	299	546
Pre-Treatment	8693	7643	16336
%	50%	44%	47%
Post-Treatment	1598	1536	3134
%	18%	20%	19%



- For each of the 11 patients, one healthy and one cancerous lung biopsy were obtained, resulting in a total of 22 samples
- Young's Modulus (*E*) for **healthy lung tissue** is consistent with the literature *E* = **1.5 ± 0.7 kPa** (mean of the medians) (*E*~1.4±0.4 kPa porcine lung [7], 1.5 kPa mice lung [8], human parenchyma 1.87±0.95 kPa [9])
- **Acinar tumor lung tissue** presented a mean *E of* **2.3 ± 0.9 kPa** (mean of the medians)



Tumor lung tissue presents more stiffness heterogeneity



Textured stiffness maps for all biopsies, one paired sample of each patient; colored bar from 0 to 10 kPa

CONCLUSIONS & PERSPECTIVES

- Healthy and tumor lung tissue samples were characterized using IT-AFM to observe if they differ mechanically and to obtain their stiffness distribution
- These novel findings about the microscale mechanical properties of lung adenocarcinoma gives strong indication that, besides their *de visu* difference, observed in histological analysis, their stiffness distribution also differ from healthy tissue
- A broader stiffness distribution indicates more heterogeneity, thus, considering the premise that lung adenocarcinoma tissue possesses regions rich in collagen type I [10] and vimentin [11] expression and can be associated with pulmonary fibrosis, these intrinsic characteristics corroborate for a stiffer environment
- Theses results give the parameters to design a gray-level mask that will be fabricated in the next phase of this work and enable future experimentations of cell culture models on rigidity textured polyacrylamide hydrogels

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