Ph.D. Annual report

Second Year

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Cycle: XXXVI
Year: 2021-2022
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Research and Activity:

Objective:
Cells and tissues in their physiological environment have well-defined mechanical properties, that are important to preserve organs and cell functionality. Different pathologies affect cell or tissue mechanics. For this reason, the biophysical communities considered cell mechanics a bio-marker of cell states.
In the last years, several groups proposed to identify diseased and healthy cells using stiffness measurement; recently, a few companies started using mechanical indentation as a primary diagnosis of cancer.
The nucleus is the largest organelle, containing the genetic material, and its mechanics regulate many important functions in cell life (Burridge 2019, Miller 2020). Variation of nuclear stiffness has been found in laminopathies, a vast class of disorders, including Emery–Dreifuss muscular dystrophy, lipodystrophy, leukodystrophy, progeria, diabetes (Fererra 2014, Apte 2016, Mu 2020) as well as in cancer cells (Zwerger 2011, Deville 2019, Fischer 2020). The knowledge in the field of nuclear mechanics is still limited. In my project, I am focusing on finding the optimal solutions for data acquisition and analysis in the study of the mechanical properties of cell nuclei. I will finally apply these technical results to nuclei from laminopathies.
Working at a higher scale level, in collaboration with Prof. Leonardo Ricotti, of the Scuola Superiore Sant'Anna in Pisa, I am investigating the stiffness of bovine cartilage, measuring cartilage mechanics before and after treatment with enzymes that degrade the extracellular matrix of the samples. I aim to correlate my data with that derived from other techniques to calibrate the results derived from a new tool based on ultrasound technology for diagnosis in vivo.

Experiment:
No one methodology is ideally suited for answering all biomechanical questions as the geometry, force magnitude, and time scale must match that of the physiological process under investigation (Hobson 2021). Among all techniques, AFM has proven to be powerful in the investigation of biological samples for these main advantages:
i) High spatial resolution
ii) Precise control of the applied forces and
iii) Applicability in liquid in close-to-physiological conditions to study living cells (Rigato 2015).

Cell nuclei:
In the first phase, we measured the mechanical properties of the cells' nuclei in the intact form inside the cells using two different AFM probes: standard sharp AFM tips and polystyrene beads with a diameter of 10µm. We noticed that a sharp probe frequently breaks the cell membrane during indentation, penetrating through it. This uncontrolled event generates a high indetermination in the evaluation of Young's modulus. The study continued by using a larger spherical probe that guarantee a softer contact with the sample. I developed dedicated software for data analysis in collaboration with the group of Prof. Massimo Vassalli at the University of Glasgow. The software considers the indenter shape and the local variation of the F-D curve slope to derive a tomography of the sample stiffness. The presence of inhomogeneity in the cell composition could bring a trace in the force-distance curve, typically a change in the local slope of the curve. The aim is to focus on these discontinuities, to quantify the inner cell mechanical properties, i.e., the nuclear mechanical properties.

In a second step, nuclei were extracted from the cells and studied in the isolated form Fig.1. The idea is to decouple the contribution of other cellular compartments. On the other hand, nuclei in a non-native environment can change their mechanical properties, due to the altered homeostasis, and the loss of connection with the cell cytoskeleton. Our studies show that the measured stiffness is softer for extracted nuclei Fig. 2. The question is still open: which method is providing more reliable results? Which one is more important, the affection induced by the presence of the cytoskeleton and other cell compartments, or the influence of the modified homeostasis on the properties of isolated nuclei? To solve this problem, I would like to employ a different technique for nuclear mechanics determination. I am planning to perform Brillouin Microscopy (BM); a technique that is emerging as the next-generation contact-less method to measure the mechanical properties also on soft hydrated materials. I contacted Dr. Silvia Caponi from the CRN in Perugia, an expert in Brillouin spectroscopy and microscopy, to visit her laboratory,

having the possibility to perform the experiments on my cell system. The final goal is to develop a reliable experimental and theoretical model to apply next to real diseased cells like Progeria or ADLD cells.

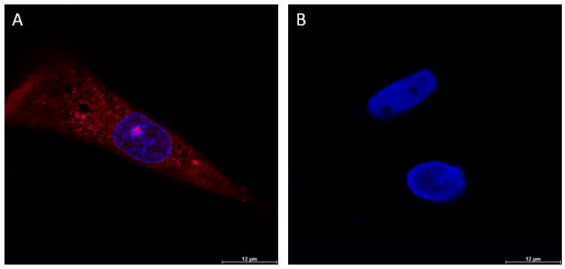

Figure 1: Confocal images after labeling nucleus with DAPI and Mitotracker. (A) Cell images showing nucleus and cytoplasm in blue and red respectively. (B) Obtained images after the nuclei extraction prove the existence of nuclei in absence of the cytoplasm.

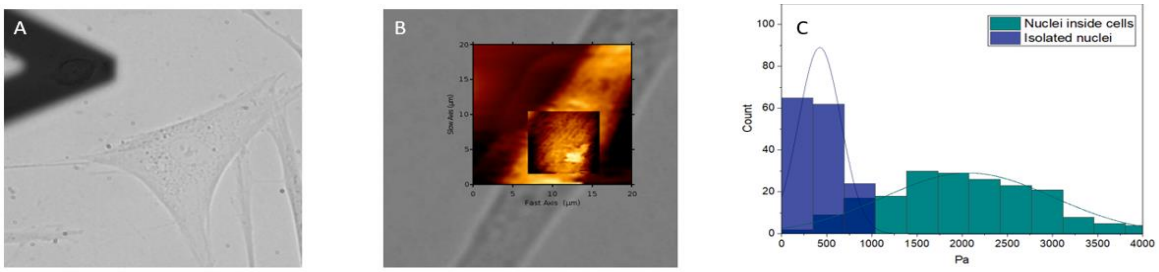

Figure 2: (A) Optical image of the inverted optical microscope integrated with the AFM, showing cells and the position of the AFM probe on the top-left. (B) Overlay of the optical image and AFM images. (C) Histogram result of the F-D curves obtained on the intact nuclei and isolated nuclei

Tissues:

I also studied the mechanical properties of tissues. This study is in collaboration with Dr. Leonardo Ricotti at the Scuola Superiore Sant'Anna, where they use a newly devised non-invasive instrument that exploits ultrasound as a probe, to measure the stiffness of cartilage in vivo. The cartilage samples are obtained from bovine and the stiffness is measured both with ultrasound and AFM technique as a standard source of comparison. I acquire F-D curves using standard pyramidal probes, acquiring maps of curves on several positions, to obtain a value of the cartilage Young's modulus derived from a relevant statistical analysis. After the first set of measurements on the untreated sample, cartilage is treated with two enzymes, trypsin, and collagenase. Trypsin is an enzyme involved in digestion that cut the protein polypeptide chain into smaller parts, while collagenase acts only on collagen, but with a high intent, destroying the extracellular matrix environment. Samples are treated for 2h and 6h with trypsin, and 6h and 24h with collagenase solution. The data obtained from the AFM analysis are compared with that derived from the new ultrasound tool, providing a base for the validation of the results generated with this new technique.

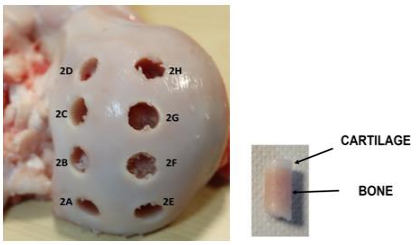

Figure 3: Cartilage samples obtained from the bovine.

Courses and Exams:

• Atomic Force Spectroscopy, Annalisa Relini	Unige	Pass
• Microscopic and Spectroscopic Techniques, Renato Buzio, Andrea Gerbi, Letizia Savio	Unige	Pass

Schools and Conferences:

- Advanced Microscopy Practical Workshop, November 29-December 3, 2021, Genova, Italy
- Focus On Microscopy conference, April 10-13, 2022 (online)
- AFM-BioMed school, July 11-15, 2022, Marseille, France
- AFM-BioMed conference, August 30-September2, 2022 (online)