ROLE OF DYNAMIC MECHANICAL LOADING ON CELL FATE AND BEHAVIOR

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Mechanical forces acting on human bodies at different levels, from whole body to individual organs, tissues, and cells, can potentially influence the growth and shape of every tissue and organ. Focusing the attention on the cellular level, each cell can sense mechanical cues, through a process known as mechanosensing and respond to these signals by translating mechanical stimuli into biochemical ones, through mechanotransduction. These mechanical cues can be either forces applied on the cell from the extracellular matrix (ECM), or intracellular forces generated in response to variations in ECM stiffness. Even if the physical pathway responsible to transmit these signals from the ECM to the nucleus is well known, how the mechanical cues are transduced into biochemical ones remains an open challenge.

This topic results of great interest since the mechanical cues are involved in cell proliferation, differentiation, survival, apoptosis and in pathology like cancers or neurodegenerative diseases, where the mechanosensing and mechanotransduction pathways are compromised.

Moreover, on the cell scale, the geometric form of cells and their biological functions are inherently correlated; particularly, there is an increasing evidence of the effects of substrate curvature on cell behaviour. In fact, the cells can discriminate between planar, convex, and concave surfaces; convex substrates are able to regulate cell proliferation, cell shape and locomotion, whereas the role of concave substrates on cell cycle is not fully understood. Then, there is a spatio-temporal dependence in the influence of cell response from its surrounding environment, which must be considered. In fact, in the body, cells especially those from blood, bone marrow, lungs, heart, or musculoskeletal tissues, are subjected to mechanical loading cyclically.

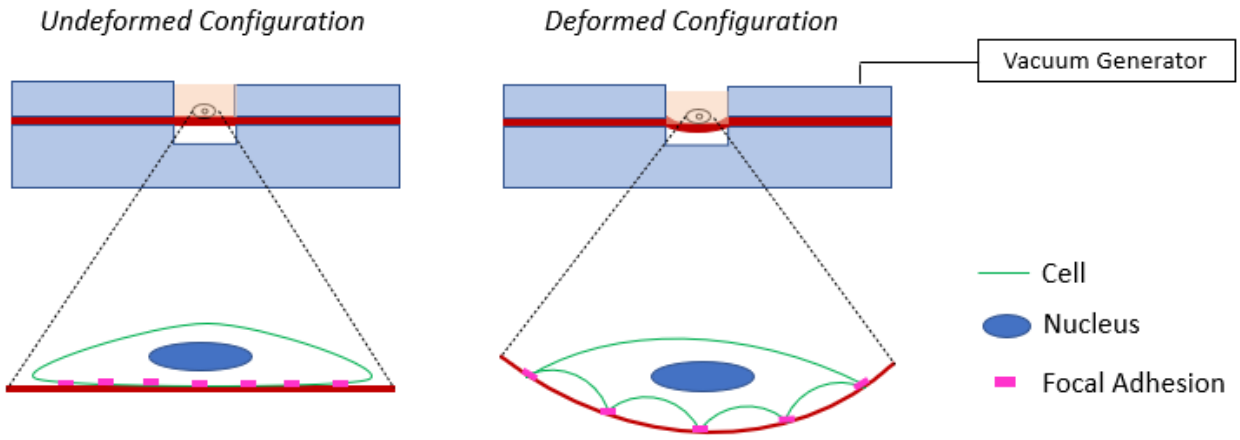
Considering all these aspects, the aim of this project consists in the design of a microfluidic device where a single cell is dynamically stretched. This device is designed to modify the curvature of the membrane where the cells are seeded at a frequency useful to observe the cell response in real-time for each cycle. To do that, the system is connected to a vacuum generator that modifies the pressure inside the micro-fluidic device by inducing a variation of the membrane curvature as consequence of the mechanical solicitation. Particularly, we are interested to study the effects of cyclic mechanical stimulation and, consequently, of variation of the membrane curvature, on cell morphology, focal adhesions organization, and, finally, cell differentiation, by analysing the activation of specific biomolecular pathways. The last ones could be studied analysing the flux of specific transcription factors from the cell cytoplasm to the nucleus. Moreover, to quantify the stresses and strains developed on the substrate surface during the mechanical solicitation, a specific 3D finite element model (FEM) can be developed using a software based on finite element approach called “Abaqus CAE”. Knowing the mechanical properties of the membrane material and the pressure variations applied during the experiments, the stress/strain on the material surface can be quantified. It is important to quantify these parameters, particularly on the part of the substrate in contact with the cells because these are the stresses/strains sensed by the cells themselves. Finally, knowing the cellular and nuclear morphology due to the mechanical solicitation, a 3D dynamic model of the cell adhered to the substrate in the different configurations could be formulated. So, a quantification of all stresses and strains developed on cellular and nuclear surfaces can be done.

During the first PhD year, a prototype of the microfluidic device was designed choosing as membrane material polydimethylsiloxane (PDMS), whereas the other layers were made with polymethylmethacrylate (PMMA). PDMS is a thermoset polymer that cannot be remoulded; on the contrary, PMMA is a thermoplastic polymer that can be melted and used in different fabrication processes. Even if PMMA and PDMS are two polymers with characteristics completely different, their combination permit to attach the PDMS substrate to a more rigid polymer, like PMMA, which ensures the mechanically solicitation only to the membrane where cells are seeded without deformation of other device layers. The sizing of this device before manufacturing was done using different simulation software: “*COMSOL”* and “*Abaqus CAE”*.

In the second year, the reproducibility of the microfluidic device design was tested fabricating different devices. As a result, the same response at a specific mechanical solicitation was recorded. To quantify stresses and strains developed on the

PDMS substrate during the mechanical solicitation, we are developing a specific 3D finite element model (FEM) using the software *Abaqus CAE*. It is important the quantification of these parameters, particularly for the part of the membrane in contact with cells because these will be stresses/strains sensed by the cells themselves.

The cell line choose is the adipose stem cells (ASCs) to study the role of substrate curvature dynamic variations on cellular and nuclear morphology, the re-organization of the focal adhesions, and if specific pathways, involved in cell differentiation, are activated. The cellular – nuclear morphology and the focal adhesions organization should be studied using the confocal microscopy technology. To study the activation of specific pathways involved in cell differentiation due to the mechanical solicitation, it should be useful to analyse the translocation of specific transcription factors from the cytoplasm to the nucleus like Yes-associated proteins (YAP), β-catenin and others. The techniques useful to this aim will be the fluorescence recovery after the photobleaching (FRAP) and the fluorescence resonance energy transfer (FRET). So, the cellular response to the cyclic mechanical stimulation will be studied during the final part of the second year and the third one of the PhD project.



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