Título: DETECTION AND CHARACTERIZATION OF MECHANICAL FORCES AND MOLECULAR DYNAMIC REARRANGEMENTS IN COLLECTIVE CELL MIGRATION

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Resumen: Mechanobiology studies the interplay between the cell and its environment to correlate cellular functions with physical forces. Cells have the ability to exert active forces on their surroundings and, in turn, are able to sense and respond to changes in mechanical properties of the extracellular matrix. This bi-directional communication controls many relevant biological processes, such as morphogenesis, tissue repair or cell migration, proliferation, and differentiation. Dysregulation of these highly coordinated mechanisms, results in the appearance of pathological processes such as cancer invasion, chronic wounds, or skin fragility disorders.
The inherent difficulty in measuring certain physical quantities such as force, in a biological system, has meant that most advances in this field are due primarily to a deeper knowledge of the biochemical processes that govern these mechanisms. A full understanding of mechanobiology, however, requires a combined study of biological and physical phenomena.
In this regard, a variety of techniques have been developed that have provided us with qualitative and quantitative data on the kinematics and dynamics of cell behavior. These methods in turn, are often complemented by theoretical models that decipher and predict biological behavior based on the laws of physics. Nevertheless, in the context of an organism, most tissues acquire a three-dimensional structure, so there is a

need for these measurement techniques to be applicable to 3D environments. Much remains to be developed and improved in this direction, as 3D measurement techniques still present many difficulties.
The present thesis reports the development of a new 2D force measurement technique based on a flexible structure of known mechanical properties. This cantilever-based force sensor is deformed by epidermal keratinocytes as they migrate, in a wound healing experimental setup. The resultant deflection of the structure is detected over time by image analysis to infer the forces exerted by the cells. According to the results obtained, an active fluid based theoretical model was formulated that represents the fundamental characteristics of the system and predicts the evolution of its behavior.
Thereby, we established a reproducible, easy to implement and affordable 2D force measurement tool. On the other hand, the proposed theoretical model, collects the essential ingredients needed to describe how a multicellular tissue modulates the force it exerts depending on the compliance of its external surroundings. One of its most advantageous features is that its reduced complexity allows for easier interpretation and possible incorporation into other more complex models.
This measurement technique was subsequently tested in a Kindler syndrome cellular model, which was generated using the CRISPR/Cas9 tool to produce the loss of kindlin-1 expression. The results obtained showed that, in our specific context, the force measurement tool is sensitive enough to detect a difference between the forces and mechanical work exerted by healthy cells and those exerted by cells exhibiting poor adhesion to the substrate.
Furthermore, we also studied the distribution of the cytoskeleton and cell adhesions of the migrating monolayer when interacting with the force sensor, to characterize how cells respond to this experimental environment.
Future perspectives focus on a deeper molecular and metabolic characterization of the cellular responses upon interaction with a compliant body and the adaptation of the force sensor to a 3D environment.