MODELING AND SIMULATIONS IN MECHANO-BIOLOGY

1.1 Introduction

Blood vessels are a prerequisite for normal behavior of the organism, but not just, they allow also tissue growth and repair as they provide nutrients, remove waste products and are means of transportation of cells to distant sites. Angiogenesis, namely the physiological or pathological formation of new blood vessels from other existing ones, is governed by the metabolic demands of the tissue. In response to increase in mass, new capillaries arise from the vascular bed in embryogenesis, wound healing, the growth and repair of normal tissues and in disease states such as diabetic retinopathy, rheumatoid arthritis, and tumor growth.

During hypoxia, inflammation or tumor growth, the release of angiogenic signals (including the Vascular Endothelial Growth Factor (VEGF)) causes the detachment of pericytes from the vessel wall, the loosening of inter-endothelial cell contacts and the increase of vascular permeability.

Numerous inducers of angiogenesis (VEGFs and others) are produced by tumor or inflammatory cells, accumulate in the extra cellular matrix (ECM) and eventually interact with receptors expressed on the endothelial cell (EC) surface. In particular EC response to VEGF is regulated in a complex fashion by distinct sets of inputs conveyed by vascular endothelial growth factor receptor 2 (VEGFR2) and different co-receptors, including integrin receptors. VEGFR2 is the main pro-angiogenic receptor expressed by endothelial cells, playing a pivotal role in neovessel formation during tumor progression .

1.2 Cellular interactions and the role of cell mechanics

The cellular response to ECM biophysical stimuli, including the mechanical ones, depends on the hierarchy of the mechanochemical systems that provide adhesion receptors (such as integrins), intracellular focal adhesions, cytoskeletal networks, and molecular motors. The integrated relationship between mechanics and dynamics thus defines the shape of the cell, the intensity of the generated forces and the consequent remodeling of the surrounding ECM (which changes in tumor growth).

In blood vessels, ECs stably adhere to the ECM. The ECM provides cells with mechanical support, but also influences their survival, differentiation, shape, polarity, and mobility. Integrin receptors provide cells with the possibility to interact and sense the ECM.

The expression of different integrins in various physio/pathological conditions provides cells with the required flexibility to interact with the ECM and coordinate cellular responses. Integrins link directly the basement membrane to the cell cytoskeleton and signal transduction, contributing both to mechanical support and cell migration, proliferation and survival. The coordinated action of restructuring the cytoskeleton and the activity of the motor proteins allows a continuous adaptation of the cell to the mechanical conditions of its micro-environment. A peculiarity of cancer cells is its resistance to mechanical stresses that originate from tumor growth, changes of ECM and oncotic pressure. To maintain biomechanical balance, such cells work to develop reaction forces to the received pulses: for example, the cytoskeleton network adjusts its intracellular voltage to respond to mechanical abnormalities. Through internal friction interactions, cells can store and dissipate applied mechanical energy in relation to deformation velocity.

1.3 Problem Statement

Despite the biochemical pathways that lead to the activation of VEGFR2 (transmembrane protein and pro-angiogenic receptor) are well know, the knowledge of the receptor dynamics on the cell membrane

remains limited. The polarization of endothelial cells is induced by stimulation with ligands (eg VEGF-a and Gremlin).
Integrins (transmembrane proteins) bind components of the surrounding ECM (e.g. fibrinogen) with the cytoskeleton. This bond triggers the propagation of intracellular signaling cascades that affects the cell's mechanical behavior.
Mathematical models may lead to scientific predictivity via computational simulations. Models are mainly based on balance equations, including mass, chemical reactions, energy, entropy, momentum and angular momentum, in agreement with solid thermo-chemo-mechanics. This type of work requires a rigorous thermodynamic framework for the necessity to create thermodynamically consistent constitutive models. Indeed, it is really important to understand, from a thermodynamic point of view, what has been done so far, because the only way to consciously extend the constitutive model to more complicated problems is to have full knowledge of the thermodynamic model used. For this reason I am studying in depth the links between the non-equilibrium thermodynamics in the mechanics of solids and in fluid mechanics.

1.4 Research Objectives and Approach

Aim of my PhD will be the identification of an approach to angiogenesis through the construction of a multiphysics model to describe the effects of different scaffolds on:

i) model for diffusion of VEGFR2 and low affinity integrins along the cellular membrane;
ii) the kinetic chemistry of the ligand-receptor reaction;
iii) mechanical adhesion and cell diffusion on a extracellular substrate rich in ligands, in finite deformations.
iv) a long-term goal will be to simulate and model the migration process of a tumor cell, which leads to tumor metastasis.

Goals will be achieved by the integration of data coming from experimental (SM and FRAP) and computational approaches (finite element code), as will be detailed below.

Receptor membrane dynamics can be studied by two complementary imaging approaches: by the ensemble or bulk fluorescence imaging and by the single-molecule (SM) imaging. Fluorescence recovery after photobleaching (FRAP) is the most famous of ensemble techniques. FRAP is an averaging method, i.e., the properties of a large number of fluorescent particles are averaged in time and space. The position of individual molecules can be followed in time and quantitatively described using SM imaging and tracking (single particle tracking, SPT).
Imaging experiments will be performed in the newborn “Imaging core facility” of the Preventive and Personalized Medicine Laboratory of the University of Brescia by prof. Stefania Mitola and Dr. Cosetta Ravelli..
Finally, it will be necessary to implement a finite element code (with the application of Abaqus Cae and Abaqus UEL or Deal II, simulations are going to be carried out), by which it is possible to construct numerical simulations’ model, along with a sensitivity analysis of the parameters, which will highlight the key factors controlling vascular morphology.

During my PhD work I would like to pursue also another aim, which is more theoretical and fundamental, but I feel is not at all marginal to the modeling activity I just described. This has presented itself as an opportunity during the study phase related to the modeling work. Indeed, thanks to the necessity to create thermodynamically consistent constitutive models, I had the possibility to devote myself to a theoretical study of non-equilibrium thermodynamics (NET) foundations, especially as they relate to the

different ways in which they have developed over the years in the solid mechanics community and in the fluid mechanics community. Considering that these communities have only recently begun to interact but still use different nomenclature and notation, I felt the need to attempt to connect the two approaches and identify a theoretical common basis. This part of my PhD work is still in a preliminary phase nevertheless, in addition to being stimulating from the scientific point of view, I feel it could be fundamental to verifying the effects that our thermodynamic approach may have on modeling. For this reason I think it is worthwhile to deepen this theoretical research activity and keep it in parallel with the modeling activity. In a more specific way, for example, I am focusing on the meaning that the term equilibrium assumes in continuum thermo-fluid-dynamics NET theories versus its meaning in continuum mechanics theories. A deep understanding the concept of equilibrium, in both cases, can facilitate the formulation of a common theoretical basis for NET in solids mechanics and fluid mechanics.