

PHD BIOENGINEERING – THESIS FINAL DEFENSE



PHD Student Federica Colombo

Advisor Prof. Marco Rasponi

Co-Advisor Prof. Alessandra Agresti

SAVE THE DATE

11.12.2020

h. 15:30

Aula Seminari

«Alario»

Online Teams

PHD Student Marco Stefanati

Advisor Prof. Josè Felix Rodriguez Matas

Co-Advisor Prof. Yvan Torrente



THESIS:

CHARACTERIZATION OF NF- κ B DYNAMICS IN A
MODEL OF TUMOR/MICROENVIRONMENT
INTERACTION USING A COMBINATION OF SINGLE-
CELL LIVE IMAGING AND MICROFLUIDICS:
INSIGHTS FROM MULTIPLE MYELOMA

THESIS:

MULTISCALE CHEMO-MECHANICAL MODELING
OF DYSTROPHIC SKELETAL MUSCLE
BIOMECHANICS

COMMITTEE MEMBERS

Prof. Felix Naef	Prof. Pablo Lamata	Prof.ssa Monica Soncini
Laboratory of Computational and Systems Biology, EPFL – Lausanne (CH)	Associate Professor Department of Biomedical Engineering King's College of London	Politecnico di Milano Dipartimento DEIB

SCHEDULE OF THE DAY

15:30 – 15:45	Committee Meeting
15:45 – 16:45 16:45 – 17:45	PhD Student Colombo Federica PhD Student Stefanati Marco Thesis presentation - Discussion
17:45 – 18:00	Committee meeting
18:00	Award Ceremony

PhD student: COLOMBO FEDERICA – XXXIII Cycle

Thesis title: Characterization of NF- κ B dynamics in a model of tumor/microenvironment interaction using a combination of single-cell live imaging and microfluidics: insights from Multiple Myeloma

Advisor: Prof. Marco Rasponi

Co-Advisor: Dr. Alessandra Agresti

Abstract:

NF- κ B is a family of transcription factors involved in cells survival, cytokines production and immune response. In physiological condition, NF- κ B resides inactive in the cytoplasm; extracellular signals activate NF- κ B that translocates into the nucleus and promotes the transcription of several genes, including its own inhibitors (negative feedbacks). Deregulation of NF- κ B is associated to the second most common haematological malignancy in Italy, Multiple Myeloma (MM). In this PhD project we aimed at characterizing, for the first time, NF- κ B dynamics in living MM and bone marrow stromal cells to better comprehend MM biology. A bioengineering approach with cutting-edge techniques, as quantitative single-cell live imaging and microfluidics, was developed for this purpose. NF- κ B dynamics were investigated by live cell imaging in p65 (subunit of NF- κ B) YFP knocked-in cells obtained by CRISPR/Cas9: p65 YFP MM.1S (myeloma) and p65 YFP HS-5 (bone marrow stroma). Both cells were subjected to static (autocrine/paracrine signaling) and continuous (autonomous signaling) inflammatory stimulation provided by TNF- α . Clinical anti-MM treatments were also employed to verify how they act on NF- κ B hyperactivation. We demonstrated that, upon static stimulation, stromal cells were able to resolve p65 nuclear translocation, while MM.1S cells tended to maintain a sustained p65 activation; in continuous inflammatory stimulation, both cells exhibited a comparable and more sustained p65 activation, meaning that extrinsic factors influenced p65 modulation differently in stromal and myeloma cells in static setting. Furthermore, to investigate how stromal cells-mediated paracrine signaling acts on NF- κ B in MM, a 2-layer custom-made microfluidic device was designed and validated. The first layer was composed of two symmetrical channels for 3D cultures of MM.1S and HS-5 in fibrin gels. The two separated 3D cultures can be connected using Doormat valves controlled by the top pneumatic layer. The most striking result obtained was the heterogeneous activation of p65 in myeloma cells once the paracrine factors from stromal inflammatory environment reached the MM compartment. This result was not achievable in 2D macroscopic co-culture, highlighting the importance of new user-friendly *in vitro* solutions to better mimic the tumor microenvironment.

PhD student: STEFANATI MARCO – XXXIII Cycle

Thesis title: MULTISCALE CHEMO-MECHANICAL MODELING OF DYSTROPHIC SKELETAL MUSCLE BIOMECHANICS

Advisor: Prof. Josè Felix Rodriguez Matas

Co-Advisor: Prof. Yvan Torrente

Abstract:

Muscular dystrophy is a pathology characterized by the lack of dystrophin in the skeletal muscle that causes progressive weakness and loss of muscle mass. The lack of dystrophin causes an increased frequency of degeneration/regeneration cycles of muscle fibers. This increase in the frequency of degeneration/regeneration cycles leads to incompletely regenerated fibers and gross alterations in the fiber ultrastructure related to the loss of muscle performance. The aim of this thesis is to build a comprehensive chemo-mechanical mathematical model for the dystrophic skeletal muscle of a biophysical basis and simulate the skeletal muscle contraction within the continuum mechanics framework. The main hypothesis behind this model is that alterations at the microscale i.e., fiber ultrastructure, are responsible for the loss of muscle performance, while muscle integrity at the macroscopic scale remains unaltered.

Chapter 1 presents a description of the structure and function of the skeletal muscle, the implications of muscular dystrophy on muscle function focusing on the Duchenne Muscular Dystrophy (DMD), the main progress towards the mathematical modeling of skeletal muscle reported in the literature, and outlines the main objectives of the project.

Chapter 2 develops a three-dimensional (3D) chemo-mechanical mathematical model of dystrophic skeletal muscle. This model is based on stress-strain mechanical data of the muscle and studies of changes in fiber structure and interaction aiming to shed light into the biophysical mechanisms regulating muscle contraction. The main supported hypothesis behind is that the myosin function does not underlie the weakness of the dystrophic muscle and, consequently, the active cross-bridge mechanisms inside myofibrils are not seriously affected by dystrophic disease. So, it is hypothesized that the loss of isometric contraction force and isotonic concentric contraction velocity is a mechanical problem resulting from an increase of muscle matrix stiffness, loss of fibers density, a decrease of lateral transmission force efficiency, non-uniform fiber distribution and myofibrillar structure misalignment. The developed model aims at testing which of these defects are responsible for the dystrophic muscle weakness, i.e. the reduction in isometric force and contraction velocity. The results confirm that the alterations in the fiber microstructure, specifically in the myofibril alignment within the muscle fiber, could be correlated with the reduction of contractile force and shortening velocity in the dystrophic fiber. The resulting model represents an innovative tool for researchers to predict muscle response under conditions that are not possible to explore in the laboratory and could be an important step in-silico trial to study DMD pathogenesis by providing insights into the underlying mechanisms of muscle response to force and bypassing the use of animal models.

Chapter 3 presents an evolution of the previous model by introducing a more detailed description of the muscle fiber ultrastructure to better explain the disorders associated with the DMD pathogenesis. The results confirm that changes in the muscle microstructure, i.e. the dispersion in myofibrillar orientations, disorders in sarcomere pattern, and fiber branching, have an important impact on the mechanisms regulating the dystrophic biomechanics. In particular, the reduction of active force in the dystrophic muscle is connected to the alteration of the myofibrils alignment within the single fiber, while the reduction of contraction velocity seems to be associated with the chaotic organization of the sarcomeres. So, these microstructural changes, caused by the lack of dystrophin, myofibrils misalignment, and sarcomeres disorganization, together with the myofibrils branching, synchronicity lack of the fibers and increased fibrosis, determine an important reduction of the muscle performance (i.e. isometric force and contraction velocity) in the dystrophic condition. The resulting model represents an original approach to account for defects in the muscle ultrastructure caused by pathologies as DMD.

Chapter 4 describes the experimental test performed on the healthy and dystrophic diaphragm to study the fiber-ECM interaction by looking into changes in the microstructure of the muscle during monoaxial loading, to formulate a much more accurate model of the tissue and to determine potential mechanisms of damage that compromise muscular functioning in DMD. The muscle was subjected to controlled mechanical deformation using a custom-made device developed and manufactured, and the muscle microstructure was analyzed through a particular microscope that has allowed the visualization of a single fiber. The results of the experimental tests confirm that the kinematics of the fiber-ECM interaction and passive lengthening of the dystrophic muscle are not altered by the pathology. This observation is in agreement with the results of the fiber ultrastructure model (Chapter 3) in which the difference between the healthy and dystrophic muscle lies mainly in the fiber microstructure, in particular in the dispersion of myofibrillar orientation and disorders in sarcomere pattern.

Chapter 5 gives the final discussion and future developments. This chapter also reports the results of an implementation of the proposed skeletal muscle model in the commercial software ABAQUS (Dassault Systèmes®) as a user subroutine for future applications with real muscle geometries.