Final Defense

PHD BIOENGINEERING – THESIS FINAL DEFENSE

PHD Student VALENTINA PARODI

Advisor Prof.sa Manuela Teresa Raimondi

Co-Advisor: Prof.ssa Jacchetti Emanuela

Co-Advisor: Prof. Dario Polli

SAVE THE DATE

25.01.2021
h. 10:00
Online MS Teams

THESIS: ***In vitro*** **assessment of mesenchymal stem cell differentiation directly on live cells adhering to the 3D Nichoid scaffold by label-free nonlinear optical microscopy**

COMMITTEE MEMBERS			
Prof. Michael Monagham	Prof. Filippo Causa	Prof. Giuseppe Chirico	Prof. Gabriele Candiani
Trinity College, Dublin, Ireland	Federico II University, Naples, Italy	Bicocca University, Milano, Italy	Dipartimento CMIC PhD Board Bioengineering Politecnico di Milano

SCHEDULE OF THE DAY	
10:00 – 10:15	Committee Meeting
10:15 – 11:15	PhD Student VALENTINA PARODI Thesis presentation - Discussion
11:15 - 11:30	Committee meeting
11:30	Award Ceremony

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PhD in Bioengineering

Final Defense

POLITECNICO MILANO 1863

PhD student: **VALENTINA PARODI – XXXIII Cycle**

Thesis title: ***In vitro* assessment of mesenchymal stem cell differentiation directly on live cells adhering to the 3D Nichoid scaffold by label-free nonlinear optical microscopy**

Advisor: Prof. Manuela Teresa Raimondi – Prof. Emanuela Jacchetti – Prof. Dario Polli

Abstract:

The advent of multi-modal multi-photon microscopy techniques based on the nonlinear light-matter interaction, highly changed the way to observe biological specimens in their unperturbed state. By exploiting intrinsic signal generation without exogenous contrast methodologies, a huge variety of information can be obtained from a living biological specimen for functional bioimaging. In this context, two-photon excited fluorescence (TPEF), second and third harmonic generation (SHG-THG), and coherent Raman scattering (CRS) microscopy techniques exploit different properties of nonlinear interaction enabling to scan thick and heterogeneous tissues. The advantages of using these techniques reside in the possibility to observe vital cellular and animal models in their unperturbed state, investigating physio-pathological conditions with a non-destructive approach with respect to traditional microscopy techniques.

In this Ph.D. thesis, multi-modal nonlinear microscopy applied to biology and to tissue engineering is presented and discussed. In the first part of the thesis, the physical principles behind each technique are described, and a collection of *in vitro* and *in vivo* studies made by nonlinear microscopy is discussed. Hence, the advantages and limitations of using these methods for the characterization of bioengineered systems are elucidated. Bioengineered three-dimensional *in vitro* culture systems represent a promising and valid intermediate step between *in vitro* and *in vivo* conventional testing, offering a non-invasive tool with specific and personalized feature to model a physio-pathological conditions with higher affordability with respect to cell monolayer (Chapter 2). Among these, the Nichoid, a miniaturized three-dimensional (3D) synthetic scaffold based its hypothesis on the mechanical conditioning of stem cells *in vitro* by emulating the native stem cell niche constraints reorganizing the cytoskeleton and thus, the nuclear membrane accessibility to gene-regulating transcriptional factors. This 3D scaffold is produced by two-photon laser polymerization of a hybrid organic-inorganic resin drop-casted on a glass cover-slide. In this Ph.D. thesis I presented an advanced polymerization process based on the addition of a spatial light modulator which allowed to increase the number of foci from one to six, thus reducing the microfabrication time, from 12 hours to 2 hours and half, without interfering with the scaffold functionality (Chapter 3). Then, the Nichoid was used as a model to study mesenchymal stem cells exposed to basal, adipogenic and chondrogenic media via microscopy observations comparing conventional observational approaches *i.e.,* cyto/histopathological assays and immunofluorescence with nonlinear multi-modal microscopy. Hence, coherent Raman microscopy (CARS) and second harmonic generation (SHG) allowed identifying selectively markers of phenotypic expression such as intracellular lipid storage in adipogenesis and extracellular matrix formation during chondrogenesis, without the need of fixation and staining. 3D and vital observations of unperturbed specimens revealed that Nichoids promotes adipogenesis while reducing chondrogenesis with respect to flat control substrates (Chapter 4).