

Board of examiners

Prof. dr. Agapios Sachinidis

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University of Cologne, Germany

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Liver Cell Biology Research Group
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Promoters:

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Prof. em. dr. Vera Rogiers

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Prof. dr. Joery De Kock, co-promoter

Department of *In Vitro* Toxicology and Dermato-Cosmetology
Vrije Universiteit Brussel



PhD in Pharmaceutical Sciences
2020-2021

INVITATION to the Public defense of

Alessandra NATALE

To obtain the academic degree of '**DOCTOR OF PHARMACEUTICAL SCIENCES**

Shift from 2D to 3D in the development of a human skin cell-derived hepatic in vitro model for toxicological applications

The defense will take place on **Wednesday, 14th October 2020 at 5 p.m.**

and will be organised online via Zoom meeting

accessible through the following link:

https://gf.vub.ac.be/redirects/PhD_defense_Alessandra_Natale.php

and in Auditorium Piet Brouwer

Faculty of Medicine and Pharmacy, Laarbeeklaan 103, 1090 Brussel

ADMITTANCE to the auditorium will only be granted upon presentation of the personal invitation from the PhD candidate.

Summary of the dissertation

Drug-induced liver injury (DILI) is a major threat to human health and is, together with absence of clinical efficiency, at the basis of a 90% failure rate in drug development. As preclinical data obtained using experimental animals do not adequately represent the human situation, in vitro models based on human cells are highly needed.

In this doctoral thesis the relevance of human-skin derived stem cells differentiated towards hepatic cells (hSKP-HPC) for toxicological screening has been highlighted. It could be shown that these cells cultured in monolayer can be used for investigating drug-induced phospholipidosis (DIPL). Upon exposure to amiodarone, which is known to induce phospholipidosis in humans, hSKP-HPC displayed typical hallmarks of DIPL, including phospholipids accumulation, lamellar body formation and changes in gene expression. As hSKP-HPC monolayers still showed an immature hepatic phenotype, the next objective was to try to enhance their maturation. Changes in intrinsic properties of undifferentiated stem cells in 3D spheroid cultures versus 2D culture conditions were explored. It was found that the 3D configuration of hSKP spheres plays a pivotal role in the preservation of the stemness signature. Consequently, 3D nano-scaffolds were generated by two-photon polymerization of biocompatible photopolymers to mimic the micro-architecture of hepatic cell plates in human liver and it could be demonstrated that these scaffolds supported homing and proliferation of hSKP.

Overall, these findings emphasize the biological importance of 'in vivo-like' conditions for culturing and differentiating human stem cells. Although further optimization is necessary, our results further pave the way to enhance hepatic maturation which is a prerequisite to reliably predict human-specific hepatotoxicity of new pharmaceuticals.

My sincere thanks go to Mrs Mireille Aerens for supporting my research!

Curriculum Vitae

Alessandra Natale acquired a Bachelor and Master's degree in medical biotechnology in 2013 at the University of l' Aquila (Italy). In 2014 she successfully obtained the Leonardo da Vinci Erasmus scholarship that allowed her to start an internship at the Vrije Universiteit Brussel (VUB) in Belgium.

In January 2016, she started a PhD program at the research group "In vitro Toxicology and Dermato-Cosmetology" (IVTD), under the promotorship of Prof. Robim Rodrigues, Em. Prof. Vera Rogiers and Prof. Tamara Vanhaecke and the co-promotorship of Prof. Joery De Kock.

Her research, supported by the 'Chair Mireille Aerens for Animal-free Alternative Methods Development' and 'Leefmilieu Brussel', was focused on the applicability of human-skin derived stem cells differentiated towards hepatic cells (hSKP-HPC) for toxicological screening and on the improvement of hepatic maturation using 3D culture systems. In the framework of a multidisciplinary project granted by the Fund for Scientific Research in Flanders (FWO), in collaboration with B-PHOT's Photonics Innovation Centre of the VUB-Faculty for Engineering and Polymer Chemistry and Biomaterials Research Group (PBM) at Ghent University, she established the basis for the development of a biomimetic 3D scaffold. Furthermore, Alessandra also obtained a Marie Skłodowska-Curie scholarship to conduct an internship at Inocure in Prague focusing on the fabrication of fiber scaffolds for cell culture by electrospinning. The work conducted during her doctoral thesis resulted in three publications as first-author and several co-publications in peer-review journals as well as the participation at several national and international congresses. In addition, Alessandra has supervised a master thesis project and assisted students of pharmaceutical sciences at the VUB during their practical and theoretical courses.