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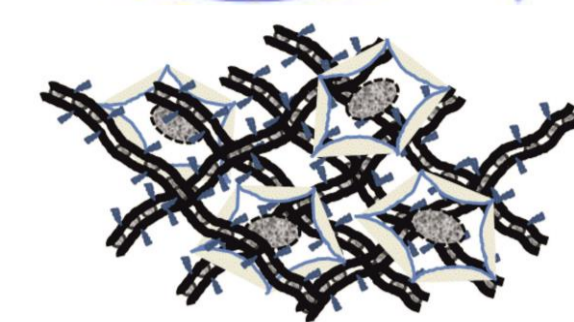
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Bio-Analyses of Antimicrobial Biomaterials for Wound Dressings – A Step Forward to Prevent Microbial Infection Diseases



POLITECNICO DI TORINO



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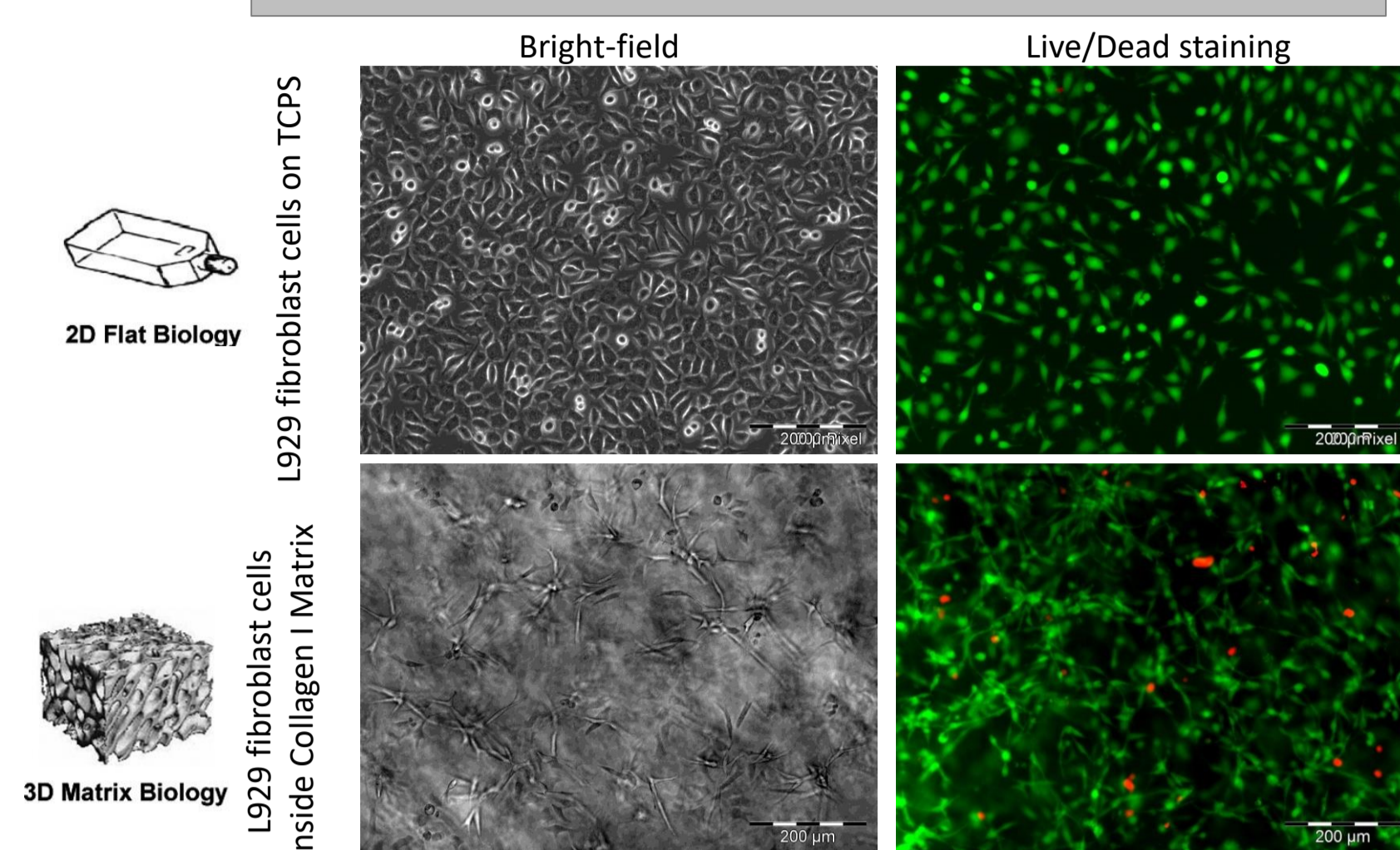
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Introduction: The aim of this study is the bio-evaluation of antimicrobial biomaterials intended for wound dressings. As a wound dressing will be in direct contact with the wound and therefore the skin cells, it should not only be antibacterial to prevent infection at wound site but also be non-toxic to the surrounding tissue. For this purpose, testing of the newly developed biomaterials will be performed using *in vitro* assays based on 2D cell culture (according to ISO 10993-5) & 3D cell culture systems as well as testing against microbial biofilm formation.

Therefore the first part of my PhD thesis is focused on the establishment of a suitable 3D skin model and, even more importantly, on the development of cyto-compatibility assay protocols suitable for the testing of material samples in this 3D system. In this study, we established a dermal layer 3D model using L929 fibroblasts. We used this model for trials and optimization of different methods of cytocompatibility analysis for example, Live/Dead staining as well as quantitative assays to measure the amount of viable and dead cells.

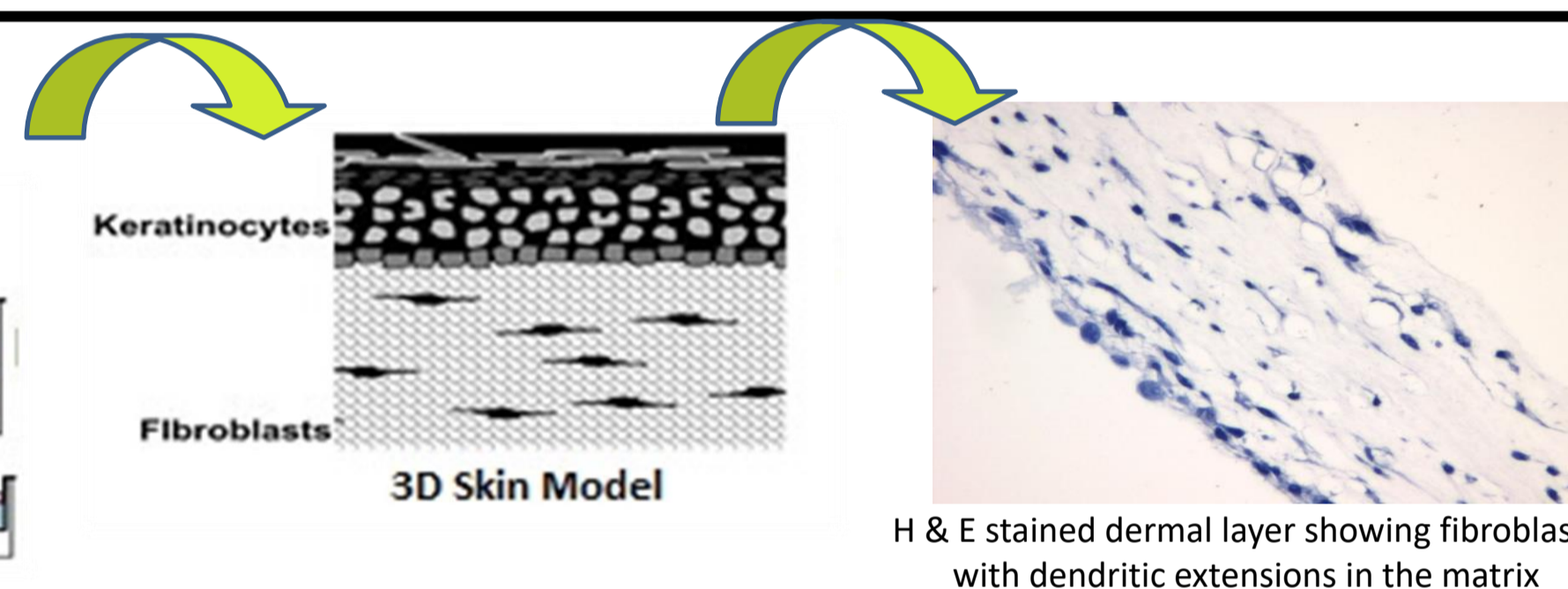
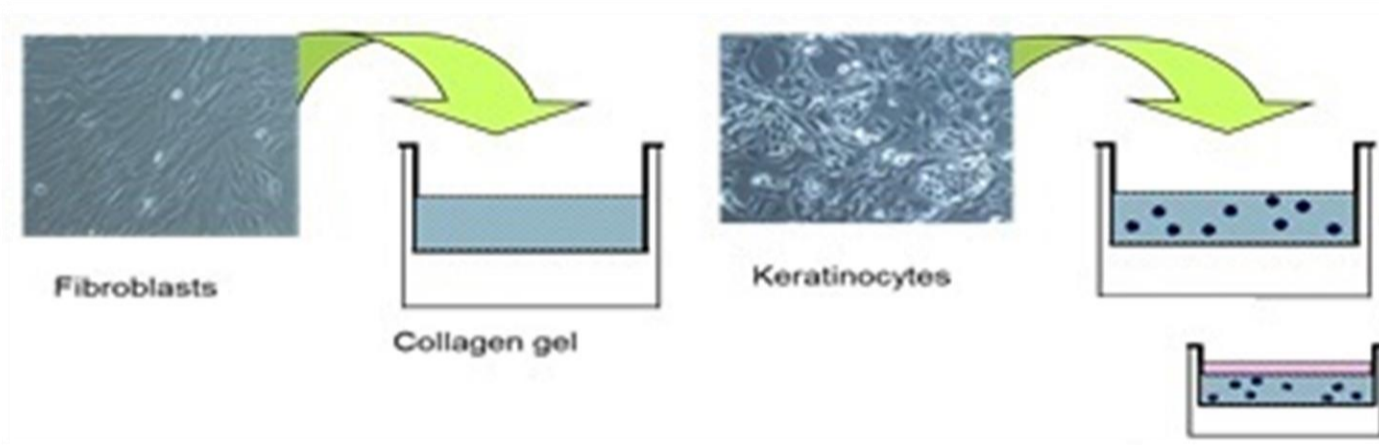
Visualization of Cells in 3D



Cells grown on conventional 2D surface have flattened morphology, while the cells in the 3D matrix show filopodia (thin cell membrane protrusions), and this morphology resembles the *in vivo* situation.

This evidence shows that Live/Dead staining is also applicable in 3D skin model, indicating that cells can directly be observed to assess morphological changes and sudden cell death as a result of interaction with biomaterials.

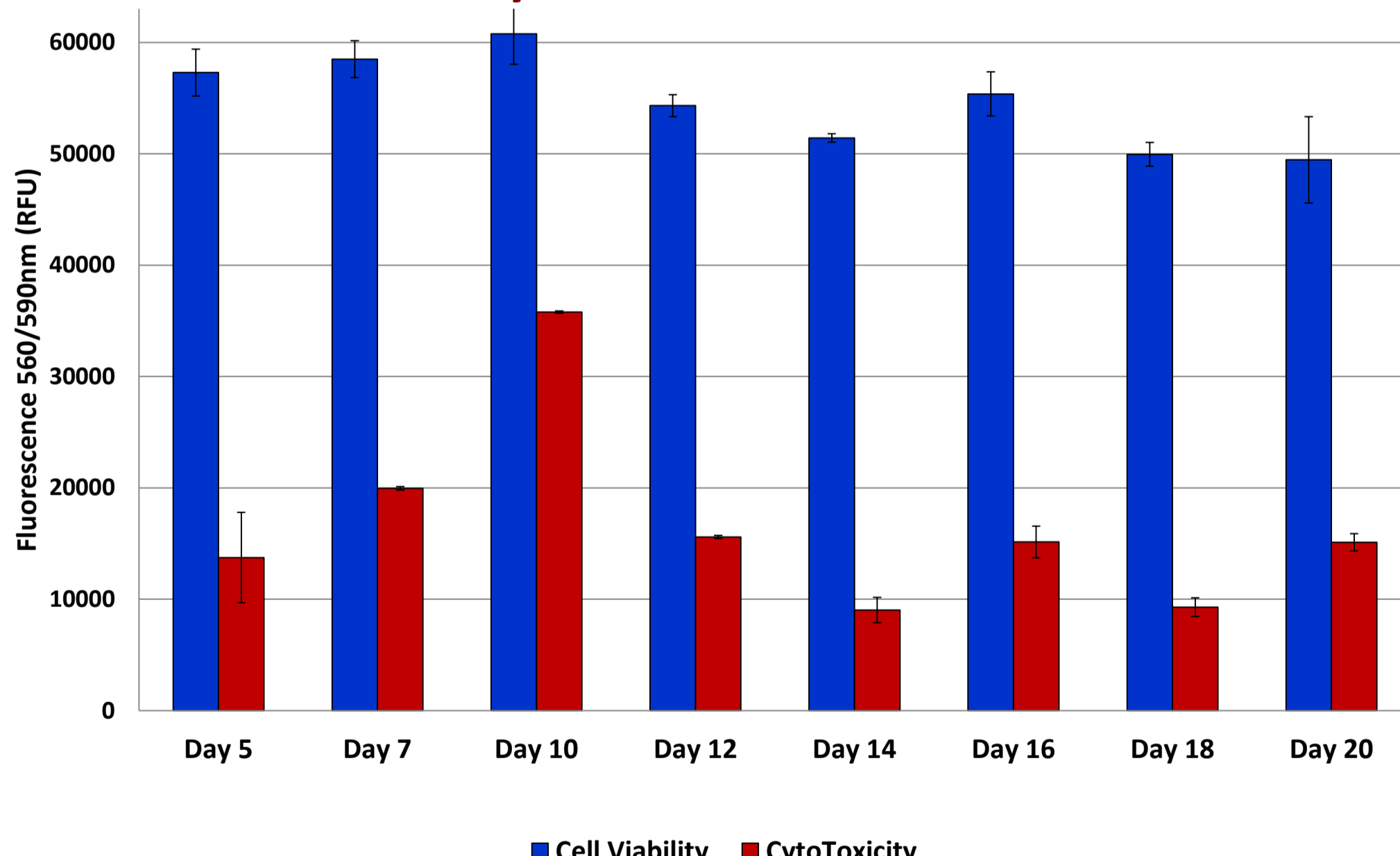
3D skin model Development



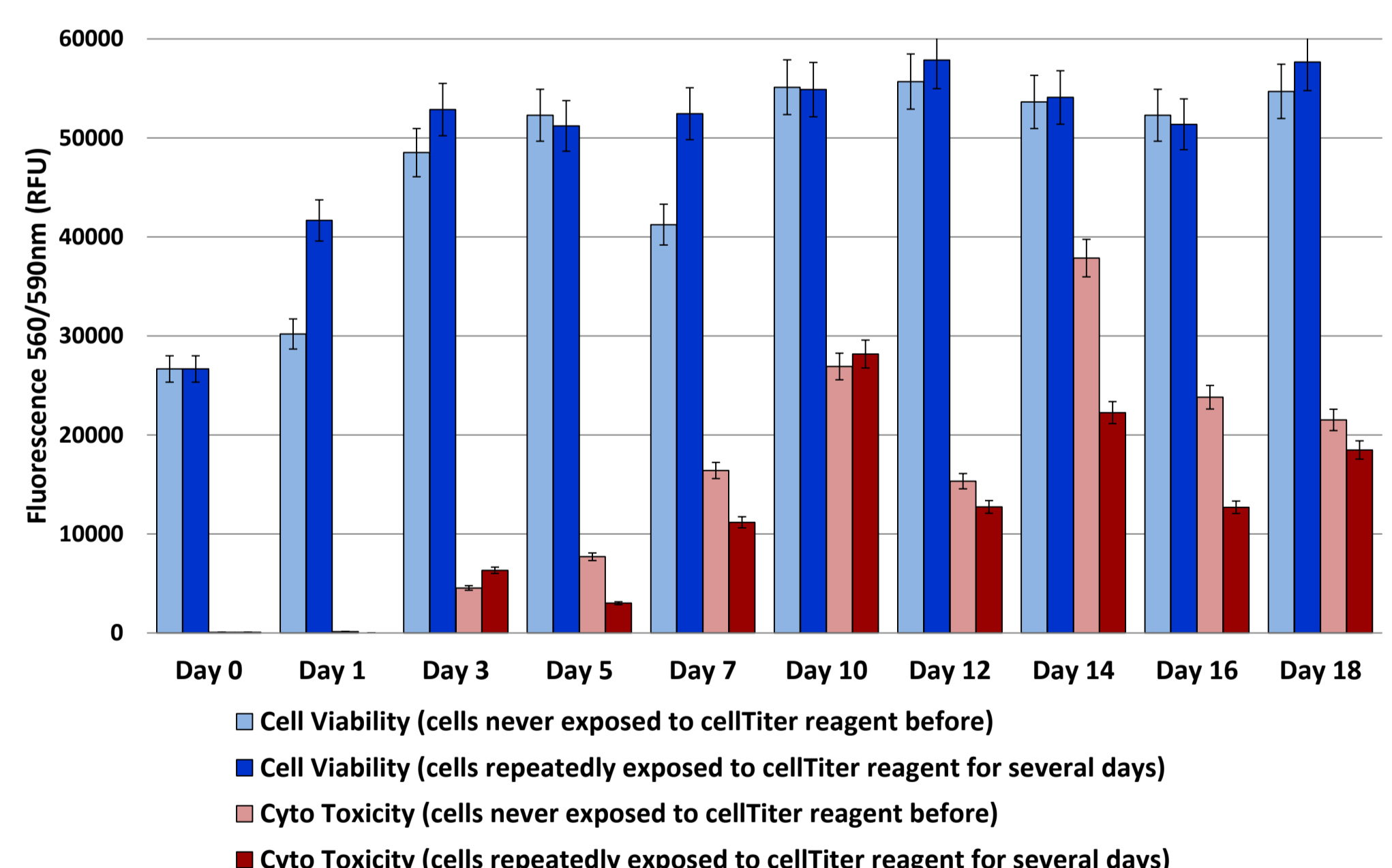
Scheme of the construction of 3D skin model: A collagen I gel (mimicking the extracellular matrix) is embedded with human dermal fibroblasts. After dermal fibroblasts contract and remodel the collagen matrix, keratinocytes are then seeded onto it. Tissues are then raised to an air-liquid interface to initiate skin-analogue tissue formation.

Development and Optimization of Quantitative Cytocompatibility Assays in 3D

Cell Viability and Cell Death of 3D Fibroblasts



Effect of Repeated Exposure of CellTiter Reagent on Cells in 3D



Both assays (CellTiter-Blue® and CytoTox-ONE™; www.promega.com) are adaptable to 3D system. Cells were viable for at least 20 days in matrix which is a prerequisite for such experiments.

Repeated exposure of cells to reagent for up to 18 days has no lasting toxic effect. Therefore this assay can be used as a method to monitor cell viability over an extended time frame.

Future Activity Plan: The knowledge deriving from this 3D model will be used to develop a human primary cell based *in-vitro* model (with human dermal fibroblasts and epidermal keratinocytes) able to mimic human skin. Then the development and optimization of bio-assays in this system will be performed to evaluate the cell compatibility of biomaterials.

Biofilm development and anti bacterial testing will be performed to evaluate the antibacterial properties. Work will start after meeting at the Department for Medical Microbiology RUB (also National Reference Center for gram-negative bacteria) on the 19th of October.

Schools/Workshops/Industrial trainings/Courses/Publications:

- HYMEDPOLY WINTER SCHOOL 1 and workshop: 8th–10th February 2016, “*Biodegradable polymers synthesis and functionalisation*” Politecnico di Torino, Turin, Italy
- HYMEDPOLY Meeting and Workshop: 19th–20th July, 2016, “*Biomaterials and cells interaction – new concepts of drug-free antibacterial therapies*”, University of Westminster, London, UK
- Workshop of the project *Smart Injectable Drug-Delivery systems for Parkinson’s and Alzheimer’s Disease Treatment (PAD-INJ)*: 19th -20th November, 2015, “*Engineered Biomaterials and Biomedical Devices in the Regenerative Medicine of the Nervous System*”, Doctoral School of Politecnico di Torino, Turin, Italy (Prof. Valeria Chiono and Prof. Maria Grazia Spillanti)
- BioPmed Seminar: 26th January 2016, “*Biological evaluation of Medical Device: ISO 10993*”, Bioindustry Park, Ivrea (Vicent Legay)
- Course: 11-13th November 2015, “*Advancement in the knowledge and the treatment of neurodegenerative diseases*”, Doctoral School of Politecnico di Torino, Turin, Italy, (Prof. Maria Grazia Spillanti)
- Course: 23rd November 2015, *Entrepreneurship*, Doctoral School of Politecnico di Torino, Turin, Italy, (Professor Erasmo Carrera and Dr. Marco Petrolo)
- Anticipated publication of the review article: “*Drug-free antibacterial polymers for biomedical applications*”, Journal: Biomedical Science and Engineering Page Press, (in progress)



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PhD Day- PhD Cycle 31- 4th October, 2016-Politecnico di Torino, Italy