Summary

This PhD Thesis focused on the development of a 3D *in vitro* skin model that could be used as a multipurpose platform to screen different pathological conditions and possible therapeutical approaches. At first, an accurate material selection has been done, focusing on gelatin methacryloyl (GelMA) synthesis and characterization, unveiling the possibility to tune its chemical and mechanical properties, putting it in a prominent position for producing *in vitro* models. Once assessed the potential of GelMA for the skin model development, the material was applied in 3D bioprinting, demonstrating the great influence that geometry can have on cell culture behavior. For this reason, efforts were put also in developing a technique to improve the printing resolution using a Carbopol-based sacrificial bath, successfully introducing the possibility to create vessels and complex structures.

Then, the GelMA-based 3D *in vitro* skin model started to be designed, including a dermal and an epidermal compartment. Its development was monitored up to 31 days and it was characterized in terms of epidermal barrier development and extracellular matrix deposition and remodeling, revealing the enormous potential of the model to grow and develop.

Once established the feasibility of the model for further and more complex investigations, three different pathological conditions were taken into consideration: inflammation, bacterial infection and viral contamination.

First, preliminary study on the possibility of electrostimulating the 3D *in vitro* skin model after its inflammation, to obtain an alternative therapeutical approach, were carried out. The electrical stimulation was intended to mimic or enhance *in vivo* endogenous electric fields, that naturally take place during wound healing. These preliminary results revealed the range of voltages that can be applied to the model without damaging GelMA matrix.

Next step has been wounding and infecting the 3D *in vitro* skin model with Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The infection was then treated with antibiotics and analysis of both bacteria and skin model response to infection and treatment followed. Results showed that the skin model was able to perform a barrier against bacteria. Moreover, it was able to react to wounding, infection and treatment in a complex way, in terms of extracellular matrix deposition and remodeling, inflammatory response, blood vessels recruitment attempt, antimicrobial peptides production and change in cellular behaviors, from epithelial to mesenchymal (EMT) and from fibroblasts to myofibroblasts (FMA).

To further evaluate the skin barrier effect against viruses, contamination of the 3D *in vitro* skin model surface with a Coronavirus was performed. A platform to test disinfection methods has been developped: both ethanol and an alternative strategy based on N-halamine modified cotton, were applied. Once again, the established skin barrier was performant, and the disinfection strategies were effective.

To address the need to be able to monitor both the development and pathological conditions of the 3D *in vitro* skin model, a non-invasive monitoring device has been developed, able to measure Trans Epithelial Electrical Resistance (TEER) through Electrical Impedance Spectroscopy (EIS).

To enhance the 3D *in vitro* skin model complexity, preliminary efforts to obtain a vascular compartment were made, taking advantage of KLISBio silk fibroin electrospun membranes, as support for endothelium development and witnessing great endothelial development, in particular in co-culture with the dermal compartment.

Finally, investigations were conducted to provide dynamic environments to the skin model. Biological tests on a silicon like 3D printable resin (TEGORad) were carried out, to candidate it as a possible material to develop 3D printed microfluidic platforms; in addition the design and development of an ALI perfusion bioreactor to obtain a macrofluidic system was accomplished.

It is worth notice that the all project aimed to follow the 3R principles, particularly the Replacement, *i.e.* substitute of living animals when *in vitro* or *ex vivo* alternatives are available, representing a significant step forward in creating physiologically relevant platforms for biomedical research.