SCAR ON CHIP: REPLICATION OF THE HUMAN SKIN WOUND HEALING PROCESS

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When a tissue is damaged (e.g., accident, disease, surgery) a complex biological wound repair process is triggered, known as ***wound healing*** that aims to restore the architecture, morphology and functionality of the injured tissue.
Wound healing is a complex mechanism that extends over different time and spatial scales and requires the precise recruitment and coordination of different cell populations through mechanical and biochemical regulation networks.
Wound healing is generally divided into four distinct but overlapping phases. The process begins with the hemostatic phase immediately followed by the inflammatory phase; after that, at the end of inflammation, with the proliferation phase we witness the generation of new temporary tissue that will replace the damaged one and that will mature during the remodeling phase.
New reconstituted and mature tissue will be present during the last two phases, but these stages are largely dependent on the inflammatory phase. The role of inflammation extends far beyond the protection of tissue damaged by infectious agents and the removal of damaged cells: the lesion causes inflammation, which in turn orchestrates wound healing and tissue regeneration.
In post-natal human tissues, due to the lack of regenerative ability, this process can end with the formation of a scar tissue that will not be able to replicate either the function or the structure of the normal one.
When the wound healing process is well organized and controlled, the inflammatory response resolves quickly, and normal tissue architecture is restored. However, if the wound healing is chronic or becomes unregulated, it can lead to the development of pathological fibrosis or scars, compromising normal tissue function and eventually leading to organ failure and death.
The formation of fibrotic tissue is characterized by excessive growth and hardening of the various tissues due to the disproportionate deposition of components of the extracellular matrix, especially of type I collagen. Of the latter, a microscopically reticulated network is created that not only causes a mechanical restriction, but the thickened tissue is weak and hypoxic. (Nguyen et al., 2009)
In addition, due to the strong similarities between the key features of wound healing and tumor development, including the activation of stem cells and myofibroblasts, increased cell proliferation, inflammation and neoangiogenesis, it is tempting to postulate that chronic injury can cause aberrant healing and a regenerative response that ultimately promotes the expansion and progression of the mutated cells. (Kuraishy et al., 2011)
In this regard, it is recognized how essential it is to intervene through new efficient therapies that assist during the healing process, that improve chronic or fibrotic wounds and that correct the inflammatory response.
Currently, numerous models both *in vitro* and *in vivo,* have been developed for this study and to conduct several tests of new therapeutic strategies capable of manipulating tissue repair. These include 2D cell culture, animal models and 3D exogenous models. Despite the benefits they have brought in understanding the different key pathophysiological mechanisms, these models have been shown to have some limitations. It is preferred to avoid animal models for ethical reasons, high maintenance costs and because, although mice and humans have almost the same types of cells, murine models do not summarize human physiopathology following a predominantly regenerative rather than reparative type pathway.
2D cell cultures, instead, are characterized by cells seeded on flat and hard substrates, not representative of the cellular environment. The biggest problem is that 2D cultures lack the extracellular scaffolding matrix (ECM), which establishes intercellular signals or networks and external dynamic stimuli that mimic the microenvironment *in vivo* and that instruct cells to grow, migrate, and differentiate. (Gottrup et al., 2000)
In the specific case of scar tissue, these aspects are important both because fibrosis is a biological process that happens at the extracellular matrix level and to evaluate cellular communication once the injury has occurred and how the immune system acts in response to it. For this reason, it is necessary to replicate *in vitro* an equivalent model of human skin to be damaged, to be put in communication with cells of the immune system and on which to perform preclinical pharmacological tests.

The use of exogenous three-dimensional equivalents has contributed to the knowledge of important information about cell-matrix interaction but, nevertheless, they are not able to reproduce the native environment of the tissue. The ideal is, therefore, to use human dermal 3D equivalents, 3D-HDE, produced with a bottom-up approach and composed of human fibroblasts embedded in their extracellular matrix, an endogenous matrix that has a functional importance in the regulation of different cellular processes. (Palmiero et al., 2010), (Imparato et al., 2013)

It has been shown, (Lombardi et al., 2017), that after induced physical damage, these 3D-HDEs undergo a series of cellular and extracellular events like those occurring in the native dermis and that will therefore lead to a correct reconstruction of the three-dimensional structure until the evolution of a scar.

Starting from these results, my PhD project is introduced which involves the design and fabrication of a microfluidic device that will summarize *in vitro* the main processes related to wound healing and that will bring numerous advantages including that of reproducing the hydrodynamic and mechanical environment to which cells are normally subjected.

In this work, dermal dendritic cells and Langerhans cells will be incorporated into the previously introduced 3D-HDE, and the immunocompetent model thus produced will be put into communication with a "fluidic lymph node" characterized by the presence of T cells.

In this way, we will be able to control and summarize the repair process and the inflammatory response but also to characterize both molecularly and mechanically a scar tissue and a normal tissue.

This new model of scar on chip tries to overcome the disadvantages of the models discussed above: medicine and research can be transformed by radically changing the methods of experimentation.

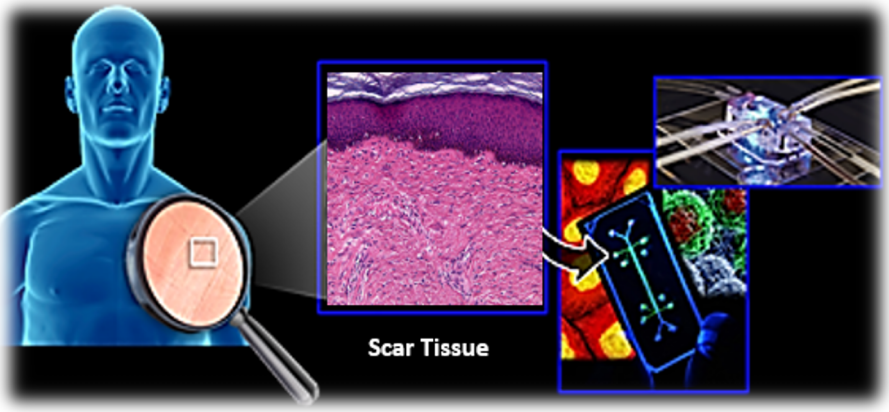
In this regard, the experiments of the first year of doctorate will involve the production of 3D-HDE on which human keratinocytes will be seeded to recapitulate a complete human skin model.

A repeatable and automated injury procedure will also be validated.

During the second year, a new microfluidic platform will be developed that will host the equivalent three-dimensional model thus produced and that will put it in communication with the T cells.

In parallel, the production protocol of the 3D-HDEs will be revisited to incorporate dermic and epidermal dendritic cells into them.

Finally, with the third-year experiments, we will try to test new pharmacological treatments that will aim to revert the state of the tissue from pathological to healthy.



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