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Electrostimulation of a 3D *in vitro* skin model to activate wound healing

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Abstract

The aim of the work is to propose a methodology for the stimulation of a 3D in vitro skin model to activate wound healing. The presented work is in the frame of the national research project, CronXCov, "Checking the CHRONIC to prevent COVID-19", devoted to understand how physiologic and inflamed skin on chip 3D models evolve upon a range of physical (*e.g.*, electrical, mechanical, optical) stimulations, over time. Thanks to the 3D modelling, using Next Generation Sequencing and the *network medicine* frame of analysis to process the data, we will systematically characterize the effects of the applied stimuli, offering new insight for the exploitation of wound healing.

Introduction

Wound healing is the physiological function granting return to homeostasis, and its forced elicitation is used in orthopaedics and dermatology to accelerate bones and burns healing.[1] For these approaches however, dosage is empirical and the systematic investigation of the optimal parameters for stimulation is lacking. Of note, endogenous electric fields occur naturally *in vivo* during wound healing and these are estimated to be 100–150 mV/mm at skin wounds.[2]

To shed light on these practices– in line with the 3R Principles (in particular, Replacement) – we considered an *in vitro* 3D skin model avoiding the use of *in vivo* animal models.

Materials and Methods

Samples. Samples have been produced with assessed methodology which technology continues to be exploited currently in a variety of applications.[3]

Electrostimulation. Stimulation has been released in the skin models by two needles stimulation by TENS machine. Stimulation is released by electrostimulator QiuTian Model SDZ II, and Tewa steel needles. We decided to stimulate in DC (like skin endogenous fields) and AC (pulsed stimulation) with various voltages.

Extraction. DNA and RNA were extracted using Quick-DNA/RNA™ Microprep Plus Kit from Zymo Research.

Results

In vitro model. We realized a 3D coculture model with fibroblasts and epithelial cells that matured in 14 days: we could visualize both the fibroblast embedded in the collagen matrix and the epithelial layer over them (Figure 1).

Electrostimulation. We stimulated the samples with the previously established parameters[2] without compromising cell viability (Figure 2).

Extraction. The extraction protocol for both DNA and RNA from the same sample has been optimized, obtaining a satisfactory yield and quality for both types of nucleic acids. This is a kye point for following investigations on the model biomimicry and healing efficacy.

Discussion and Conclusions

After 14 days the skin model reached maturity and cells were well stratified and differentiated, well mimicking the human skin. Sample perfusion with TNF-α will be considered to mimic an inflammatory state,[4] proxy for a variety of inflammation that affect patients requiring therapeutic stimulation of wound healing, and then the electrostimulation will be tested on normal and inflamed samples. In the future different conditions will be tested inflamed and

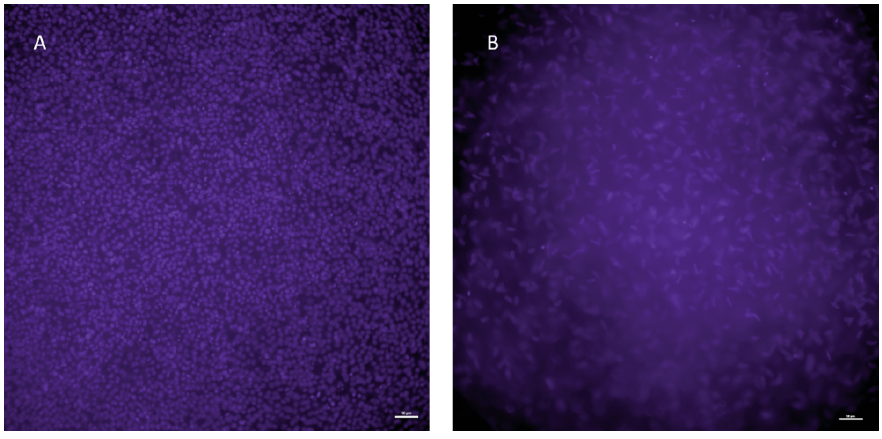

Figure 1. Fluorescence images of DAPI labelled A) epithelial layer and B) fibroblast immersed in the collagen matrix, day 14 (Bar scale 100 m).

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Key words: Skin model; *in vitro* model; wound healing; electrostimulation.

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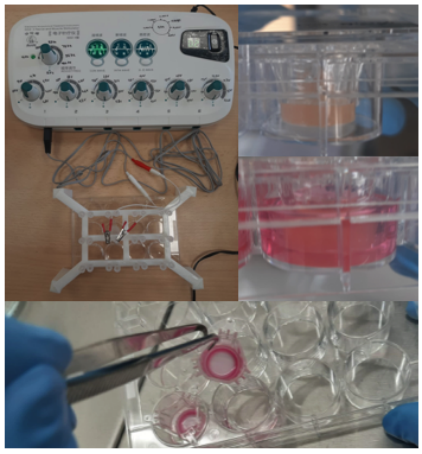

Figure 2. A) Experimental setting for samples electrostimulation. The needles holding has been ad hoc designed and 3D printed. B) The sample after cell seeding and before being immersed in the cell culture medium. C) The sample after being immersed in cell culture medium. D) The sample after ALI (Air Liquid Interface) culture, day 14.

controls, stimulated and non-stimulated, along three time points, according to the most appropriate literature: from baseline, to 1h for early gene activity[5] to 48h as the best compromise between model viability and efficacy of the stimulation.[6] As we managed to stimulate the samples without damaging them, we can say that we are obtaining a real *in vitro* model for wound healing that can be a good in *vitro* platform to characterize the effects of the applied stimuli for the development of various treatments, avoiding the use of animal models.

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