

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

Original

Electrostimulation of a 3D in vitro skin model to activate wound healing / Villata, Simona; Napione, Lucia; Baruffaldi, Désirée; Nardini, Christine; Frascella, Francesca; Pirri, Fabrizio. - In: BIOMEDICAL SCIENCE AND ENGINEERING. - ISSN 2531-9892. - ELETTRONICO. - 4 s1:(2021), pp. 119-120. (Intervento presentato al convegno III Convegno Annuale "L'era delle 3R: modelli in silico, in vitro e in vivo per promuovere la ricerca traslazionale" tenutosi a evento online nel 30 Settembre - 1 Ottobre 2021) [10.4081/bse.2021.200].

Availability:

This version is available at: 11583/2954805 since: 2022-02-07T16:10:02Z

Publisher:

PAGEPress

Published

DOI:10.4081/bse.2021.200

Terms of use:

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)

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

Simona Villata,^{1,2} Lucia Napione,^{1,2}
 Désirée Baruffaldi,^{1,2}

Christine Nardini,³ Francesca
 Frascella,^{1,2} Fabrizio Pirri^{1,2}

¹Dipartimento di Scienza Applicata e
 Tecnologia, Politecnico di Torino, Turin;
²PolitoBIOMed Lab, Politecnico di
 Torino, Turin; ³IAC Institute for Applied
 Computing, Consiglio Nazionale delle
 Ricerche (CNR), Rome, Italy

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 parameters² 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 key 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

Correspondence: Lucia Napione,
 Dipartimento di Scienza Applicata e
 Tecnologia, Politecnico di Torino, Turin;
 PolitoBIOMed Lab, Politecnico di Torino,
 C.so Duca degli Abruzzi 24, 10129 Turin,
 Italy.
 E-mail: lucia.napione@polito.it

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

Acknowledgments: Funding support by
 DEFLeCT, Food Drug Free and Smartest project
 funded by Regione Piemonte POR-FESR
 2014–2020.

Disclosures: The authors declare to have nothing to disclose.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico*, *in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.

Accepted for publication: 7 September 2021.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright: the Author(s), 2021

Licensee PAGEPress, Italy

Biomedical Science and Engineering 2021; 4(s1):200
 doi:10.4081/bse.2021.200

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

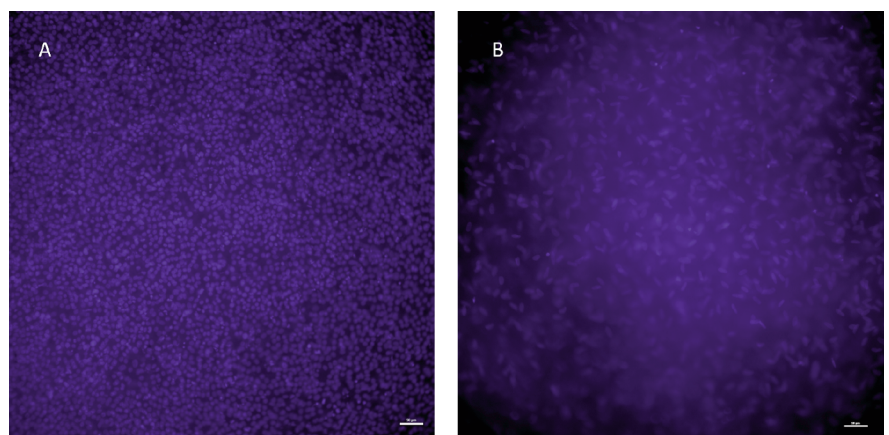


Figure 1. Fluorescence images of DAPI labelled A) epithelial layer and B) fibroblast immersed in the collagen matrix, day 14 (Bar scale 100 μ m).

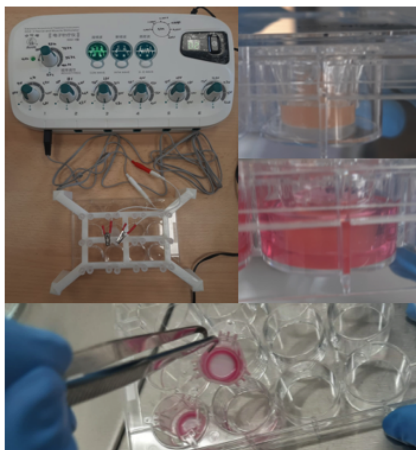


Figure 2. A) Experimental setting for samples electrostimulation. The needles holding has been ad hoc designed and 3D printed. B) The sample after cell seeding and before being immersed in the cell culture medium. C) The sample after being immersed in cell culture medium. D) The sample after ALI (Air 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 activity⁵ 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.

References

1. Maturo MG, Soligo M, Gibson G, et al. The greater inflammatory pathway-high clinical potential by innovative predictive, preventive, and personalized med-

ical approach. *EPMA J* 2019;11:1-16.

2. Song B, Gu Y, Pu J, et al. Application of direct current electric fields to cells and tissues *in vitro* and modulation of wound electric field *in vivo*. *Nat Protoc* 2007;2:1479-89.
3. Arnette C, Koetsier JL, Hoover P, et al. *In vitro* model of the epidermis: connecting protein function to 3D structure. *Methods Enzymol* 2016;569:287-308.
4. Wufuer M, Lee G, Hur W, et al. Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. *Sci Rep* 2016;6:37471.
5. Systemic wound healing associated with local sub-cutaneous mechanical stimulation. *Sci Rep* 2016;6:39043.
6. Jennings J, Chen D, Feldman D. Transcriptional response of dermal fibroblasts in direct current electric fields. *Bioelectromagnetics* 2008;29:394-405.