Development of 3D skin model and 3D skin infection model, as advanced testing tools for the bio-evaluation of antimicrobial biomaterials for wound healing.

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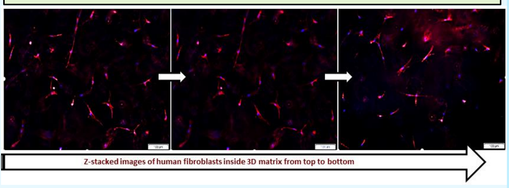
A bacterial colonized human skin equivalent (c-HSE)

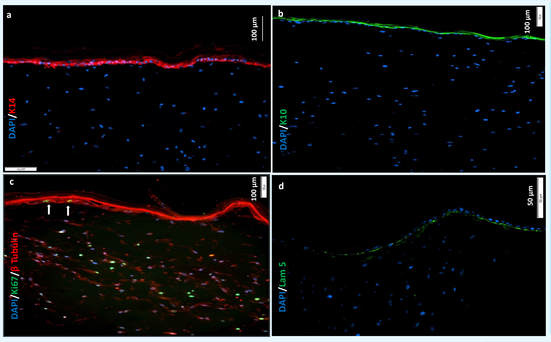
The aim of this study was the development of a human skin model and human skin wound infection model for the bio-evaluation of antimicrobial biomaterials intended for wound healing purposes. The three-dimensional *in vitro* models will represent advanced and complex systems to perform more reliable preclinical studies. These models will be employed for *in vitro* screening of both antibacterial activity as well as cytocompatibility of new biomaterials and wound dressings to optimize their *in vivo* performances. In the study, the 3D systems were developed and their structure was characterized. The antibacterial activity and cytotoxicity of a model wound dressing releasing Ag^+ was analyzed in the models.

3D Dermal Fibroblast Model



Optimizing the dermal part of human skin:
Z-stacked fluorescence images reveal filopodia-like protrusions, elongated morphology and uniform distribution of human fibroblasts at different planes within a Collagen-I (Col-I) matrix. Images show cell nuclei stained with DAPI and cytoskeletal F-actin filaments stained with Phalloidin. Scale bar=100 µm

Immunohistochemistry of the 3D Human Skin Model

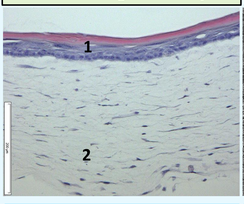


IHC verification of the Human Skin Equivalent (HSE):

(a) Keratin 14 (K14) red;
(b) Keratin 10 (K10) green;
(c) Ki67 (arrows) green;
(d) Laminin 5 (Lam5) green;

Cell nuclei are shown in blue stained with DAPI. Laminin 5 is used as a dermal-epidermal junction (DEJ) marker and appears as a thin green-stained line.

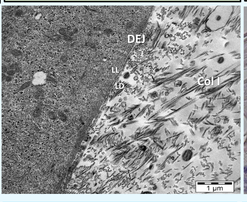
HSE: Histological Analysis



H&E stained cross section of *in vitro* HSE model:
- Epidermal layer (1)
- Dermal layer (2)

The epidermal layer (**1**) of the HSE has the characteristic structure: *Stratum corneum*, *St. granulosum*, *St. spinosum* and *St. basale*.

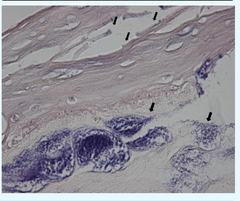
Ultrastructure Analysis



TEM image:
- Collagen-I fibres (**Col I**)
- Epidermal-dermal junction (**DEJ**).

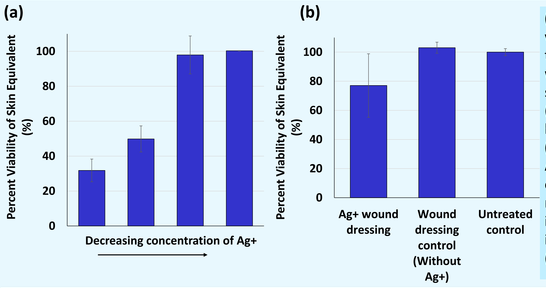
The **DEJ** is represented by two very thin layers, called *lamina lucida* (**LL**) and *lamina densa* (**LD**), mimicking exactly the DE-barrier of native human skin.

S. aureus-colonized HSE



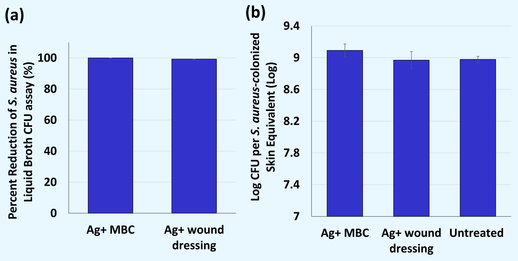
Inoculated bacteria (**blue**) adhere to dermal structures, migrate, proliferate, and colonize to establish spots of biofilms.
Big arrows: Bacteria located within a biofilm matrix inside the dermis;
Small arrows: Bacteria surrounding keratinocytes within the epidermis.

Cytocompatibility Analysis



(a) Primary human cells viability analysis applying the 3D-HSE. The model was exposed to a range of silver ion concentrations (Ag^+) for a period of 24 hours.
(b) A clinically applied Ag^+-releasing wound dressing served as model material and was tested in the 3D-HSE along with its control material (without Ag^+).

Antibacterial Analysis



(**a**) The graph compares the efficacy of *S. aureus* reduction by Ag^+ at MBC (recorded as per CLSI guidelines) and a clinically applied Ag^+-dressing in tryptic soy broth (TSB). **100% reduction of bacteria was observed.**
(**b**) The graph shows the inefficacy of the same antimicrobial Ag^+ at more realistic condition given by a 3D c-HSE.

Conclusion

With an increasing need for reliable *in vitro* test systems, we were successfully able to verify our advanced 3D models, to serve as a risk assessment platform for cytocompatibility and antibacterial properties.
Tissue culture models indicated an 'environmental effect' on cytotoxicity, with decreased sensitivity to Ag+ cytotoxicity for cells in 3D with respect to cells in 2D cultures.
The considerable variation in antibacterial activity was observed under different antimicrobial evaluation systems indicated that antibacterial ability of Ag^+ was highly dependent on wound extracellular micro-environment, that could affect Ag^+ availability.
Moreover, these results also suggested the critical considerations to be taken into account while deciding the use of Ag and Ag-dressings for wound care strategies.
HSE and c-HSE have great potential to develop even more complex skin models for testing skin treatment strategies, as these models allow the study of skin-pathogen interactions, and of novel targets for designing new antibacterial agents.

Acknowledgement

This work was supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie Grant agreement No. 643050 (HyMedPoly Project).