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Development of 3D skin model and 3D skin infection model, as advanced testing tools for the bio-evaluation of novel antimicrobial biomaterials for treating infected wounds

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EU 7th amendment (Dir. 2003/15/EC) of the "Cosmetics Directive" (76/768/EEC) has required the replacement of animal trials with reliable *in vitro* tests for assessing cutaneous resorption since 2009. *In vitro* bio-evaluation would be more accurate using biomimetic 3D testing systems respect to 2D cell cultures. In this work, *in vitro* 3D human skin equivalent (HSE) and wound infection model (c-HSE) were developed and used for testing commercially available Ag-dressings, then compared to 2D cultures.

Initially murine *in vitro* dermal construct (MDC) and human *in vitro* dermal construct (HDC) were generated, and different viability assays were optimized for 3D system [1]. Then, in-house reconstructed human epidermis (RHE) and reconstructed murine epidermis (RME) [2] were developed with the aim to understand the relevance of outcomes obtained from human- and animal-based systems reducing the animal trials. The human skin equivalent (HSE) having both dermal and epidermal compartments was obtained by optimizing 3D cell culture conditions based on serum/animal component-free and fully-defined media to obtain epidermal differentiation mimicking as closely as possible native human skin (NHS). Skin infection model (c-HSE) was created by full-thickness incision and colonization with *S. aureus.*

To validate the 3D systems, Ag+ and several commercially available Ag-dressings were bio-evaluated using HSE and c-HSE. On the other hand, cell monolayer cultures (based on primary cells and cell lines) were used as 2D cytocompatibility evaluation systems. These materials were also evaluated for their antibacterial activity against a range of clinically relevant pathogens using different growth culture conditions including simulated wound fluid (SWF). Moreover, additional efforts were addressed to the bio-evaluation of novel drug-free biomaterials intended for infected wound healing applications.

Histology showed characteristic well-differentiated epidermal layers. Immunohistochemistry for proteins' tissue-distribution analysis showed Ki-67 & K14 positive basal keratinocytes; K10 positive supra-basal keratinocytes; loricin, filaggrin, & involucrin positive sub-corneal & corneal keratinocytes; and laminin 5 positive basement membrane at dermal-epidermal junction. TEM revealed the ultrastructure of basement membrane demonstrating lamina lucida, lamina densa, regular hemidesmosomes, and anchoring fibres. The epidermal compartment showed abundant of intracellular keratin filaments, desmosomal connections, and tight junctions between keratinocytes. SEM revealed the interwoven network architecture of ECM with embedded dermal fibroblasts. The contact angle of 82.5°±8.9° demonstrated the barrier function of HSE with respect to 90.0°±5.1° for NHS. This model represented a fundamental construct with highly resembling features to that of NHS. Histology of c-HSE demonstrated bacterial aggregations and early biofilm formation while TEM revealed interesting features including *S. aureus* induced ECM degradation, fibroblasts/keratinocytes cell lysis, bacterial internalization by keratinocytes, and dissociating epidermal layers. Grape-like bacterial clusters were found encased in extracellular polymeric substance at wound site. The c-HDC results suggested that co-existence of *S. aureus* & *P. aeruginosa* have a significant impact on bacterial colonization and pathogenicity in wounds. The bio-evaluation outcomes were different in 2D monolayer vs. 3D HSE based cell culture systems, and conventionally used microbial methods vs. advanced c-HSE system.

With an increasing need for reliable *in vitro* testing systems, we were successfully able to establish closely mimicking and verify our advanced 3D models, to serve as a risk assessment platform for cytocompatibility and antibacterial properties.

References

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