Bioengineered, xenogen-free 3D human skin equivalents (HSE) as wound infection models

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Introduction

Soft tissue- and skin-related infections are great challenges in public health. Novel and alternative strategies to combat wounds colonized with resistant bacteria are urgently needed. Furthermore, many of the commercially available and clinically applied antimicrobial dressings are showing not the results as guaranteed. Shortening the time from lab developments to promising clinical applications and to bioevaluate already CE-marked products more reliable and reproducible without abusing unnecessarily test animals bioengineered, xenogen-free 3D human skin equivalents (HSE) as wound infection models are highly demanded.

Experimental Methods

Dermal and epidermal compartments were established by embedding human primary fibroblasts (NHDF) in recombinant human collagen type I (rhColl-I) hydrogels and then seeding human primary keratinocytes (NHEK) on it to generate the epidermis. The cultural conditions were optimized to obtain closely mimicking *in vivo* skin. Therefore, the biomechanical properties of different NHDF/rhColl-I combinations and clinical human dermis samples were tested and compared applying nanoindentation (Piuma, Optics11). Skin wound models with defined wound depths were created with a novel developed programmable punch device and colonized with relevant skin infectious bacteria e.g. *S. aureus* at wound site, to generate an *in vitro* skin infection model. The novel 100% xenogen-free human skin, wound and colonized wound models were fully characterized by histopathological methods, confocal microscopy, TEM analysis and nanoindentation. The infection model was validated by applying different antimicrobial wound dressings, testing cyto- and immunocompatibility and antimicrobial properties.

Results and Discussion

Different combinations of cell numbers (NHDF) and rhColl-I compositions led to dermis constructs with different biomechanical properties significantly influencing fibroblasts' metabolic activity and gene expression. "Instable" dermis constructs inhibited the cultivation and development of regular NHEK layers and prevent the formation of characteristic multilayered epidermal structures. With specific combinations, uniform distribution and filopodia like morphology of NHDFs stable 3D HSEs could be achieved. Morphology studies and uniform distribution of fibroblasts at different planes inside the dermal compartment were analysed by confocal imaging. Immunohistology and TEM were used to visualize the correct establishment of basement membranes (laminin 5 expression) and dermal-epidermal junctions. Proliferating NHEKs showed keratin 14 and keratin 10 in the corresponding layers. The establishment of standardized wounds and infected wound models is necessary for the reliable and reproducible analysis of antimicrobial wound dressings, their validation and comparison with each other. Figure 1 is representing a microscopic analysis of the HSE wound model after 24 h of incubation with Staph. aureus showing bacteria establishing colonies of different sizes within the dermis at wound site dissolving the matrix and surrounding layers of keratinocytes. Four different clinically applied silver-containing wound dressings were used to investigate differences in cytocompatibility and antimicrobial efficacy. The comparison of the evaluation results between common standardized cell culture and microbio tests with the 3D HSE wound infection model revealed significant differences regarding cytotoxic concentrations of the active component, here Ag^+ ions, and the antimicrobial activity against susceptible described bacteria under more realistic conditions.

Conclusion

With our bioengineered, 100% xenogen-free 3D human skin equivalent it was possible to bioevaluate clinically applied silver-containing wound dressings and to demonstrate the significant differences of the materials' cytocompatibility and antibacterial properties in a more realistic biological microenvironment. This model as many other 3D tissue equivalents seems promising for its application in diverse areas of biomaterials research, including cytocompatibility evaluation, drug testing, wound healing and skin infection.

References
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