S. aureus Colonized Human Skin Equivalent - In Vitro Bio-evaluation Tool for Antibacterial Polymers: ESR14

Drug-Free Antibacterial Hybrid Biopolymers for Medical Applications

S. aureus Colonized Human Skin Equivalent - *In Vitro* Bio-evaluation Tool for Antibacterial Polymers: ESR14

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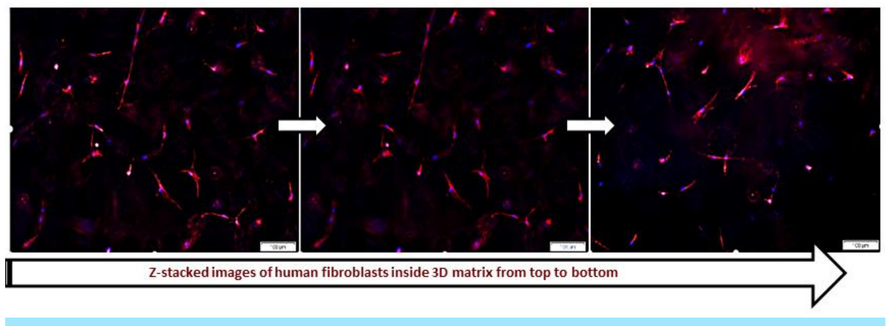
A bacterial colonized human skin equivalent (c-HSE)

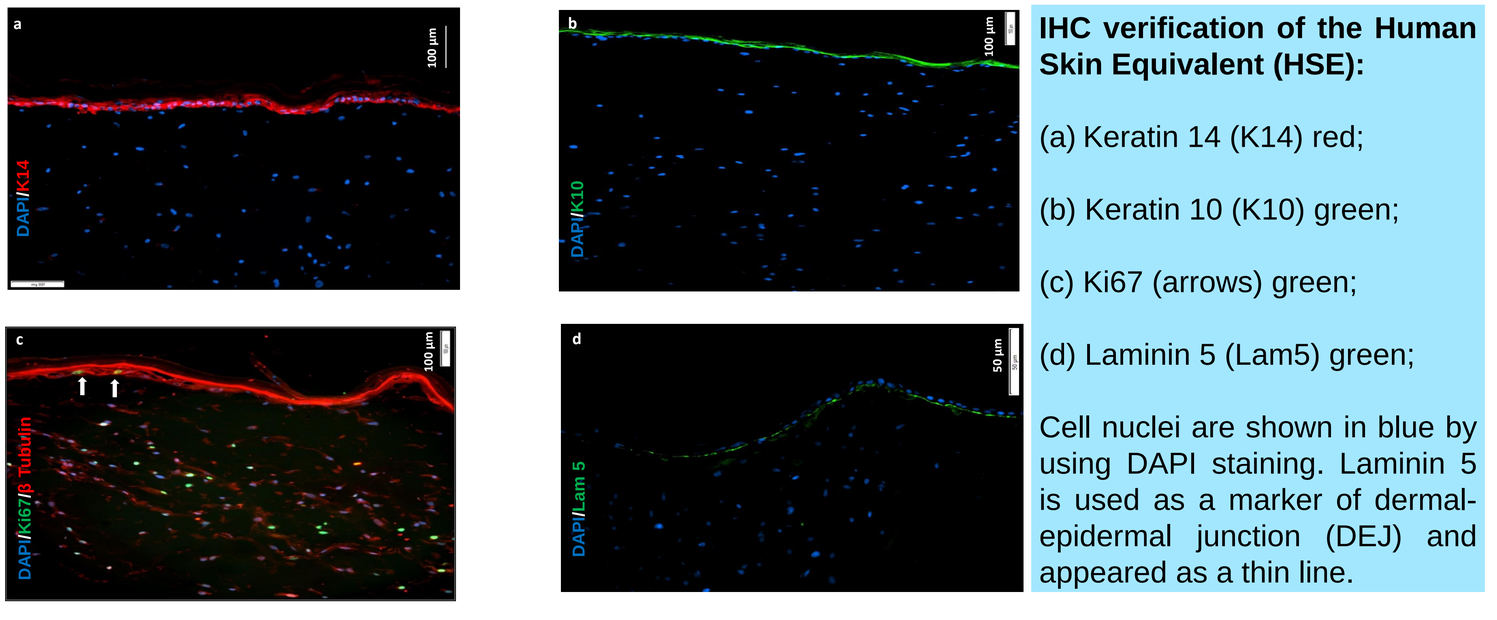
The aim of this study was the development of a human skin model and human skin wound infection model for the bio-evaluation of antimicrobial biomaterials intended for wound healing purposes. The three-dimensional *in vitro* models will represent advanced and complex systems to perform more reliable preclinical 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 imaging revealed the filopodia like morphology and a uniform distribution of human fibroblasts at different planes inside a Col-I matrix. Fluorescent microscopic images show cell nuclei stained with DAPI and cytoskeletal F-actin 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;

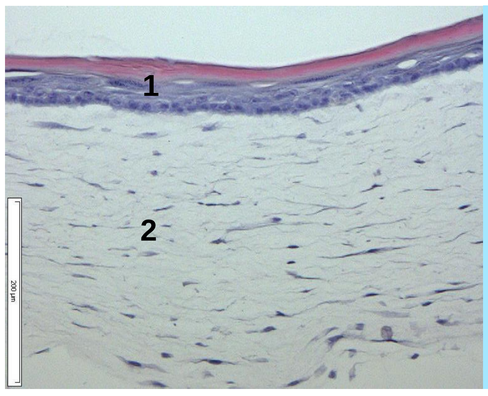
(b) Keratin 10 (K10) green;

(c) Ki67 (arrows) green;

(d) Laminin 5 (Lam5) green;

Cell nuclei are shown in blue by using DAPI staining. Laminin 5 is used as a marker of dermal-epidermal junction (DEJ) and appeared as a thin line.

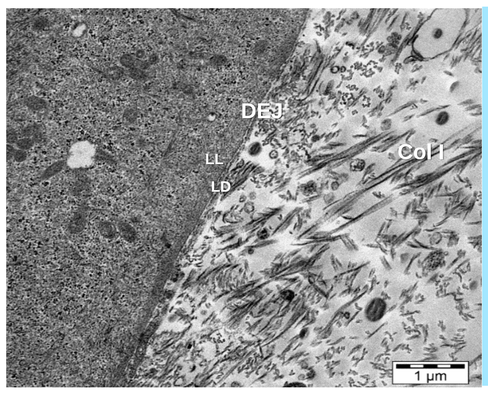
Histological Analysis of the HSE



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

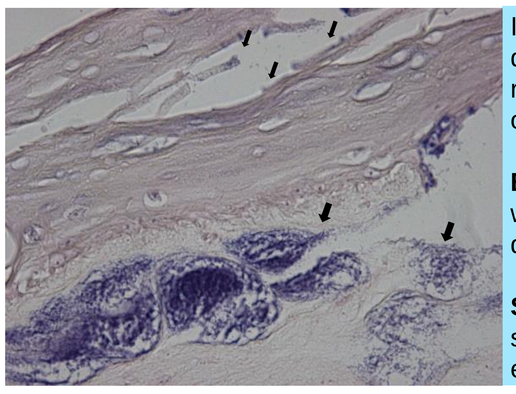
The HSE epidermis has a characteristic structure: Stratum corneum, granulosum, spinosum and basale.

Ultrastructure Analysis



TEM image:
- Collagen-I fibres (Col I)
- Epidermal-Dermal Junction (DEJ).
- DEJ presents lamina lucida (LL) and lamina densa (LD).

S. aureus-colonized HSE

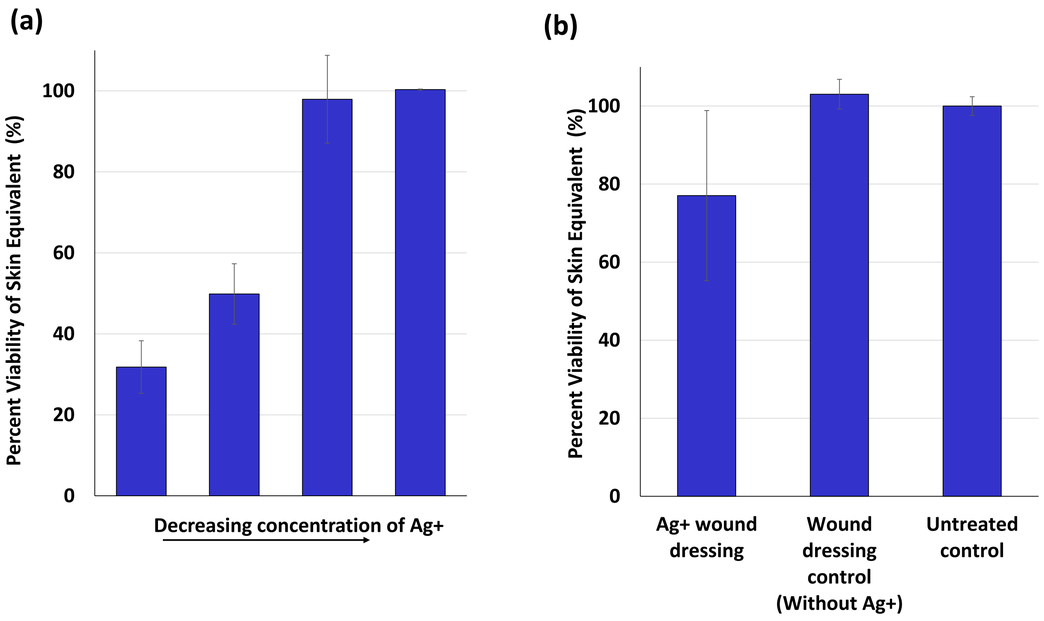


Inoculated bacteria adhere to the dermal surface, colonize, and replicate to make large structures of biofilm.

Big arrows: Bacteria located within a biofilm matrix inside the dermis.

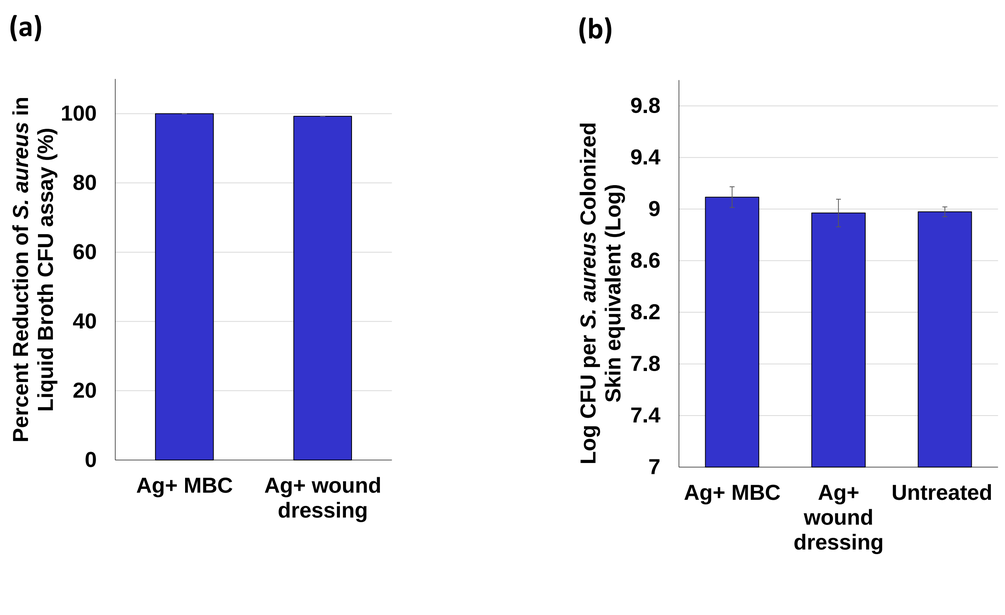
Small arrows: Bacteria surrounding keratinocytes in epidermis.

Cytocompatibility Analysis



Cell viability measuring in the 3D system. The 3D skin model was exposed to a range of silver ion concentrations (Ag^+) for a period of 24 hours.
A commercially available Ag^+ releasing wound dressing served the purpose of a model material and was tested in a 3D system along with its control material (without Ag^+).

Antibacterial Analysis



The graph demonstrates the treatment of infected skin equivalents with a commercially available Ag^+ releasing wound dressing. Skin equivalents were infected with *S.aureus* and thereafter, Ag^+ releasing wound dressing or Ag^+ in PBS was applied onto the skin equivalents.

Conclusion: Development of colonized human skin equivalent (c-HSE); Risk assessment platform for cytocompatibility evaluation; Efficacy assessment of antibacterial materials; Comparison of 2D vs. 3D systems; Understanding "Host-Pathogen Interaction"; Development of complex skin models.

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