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

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S. aureus Colonized Human Skin Equivalent - In Vitro Bio-evaluation Tool for Antibacterial Polymers / Idrees, Ayesha; Viebahn, Richard; Ciardelli, Gianluca; Chiono, Valeria; Salber, Jochen. - (2018). ((Intervento presentato al convegno Drug-free antibacterial technology for medical applications tenutosi a The Hauser Forum, Cambridge nel 14 December, 2018.

*Availability:*

This version is available at: 11583/2722662 since: 2019-03-06T15:06:25Z

*Publisher:*

Not applicable

*Published*

DOI:

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## 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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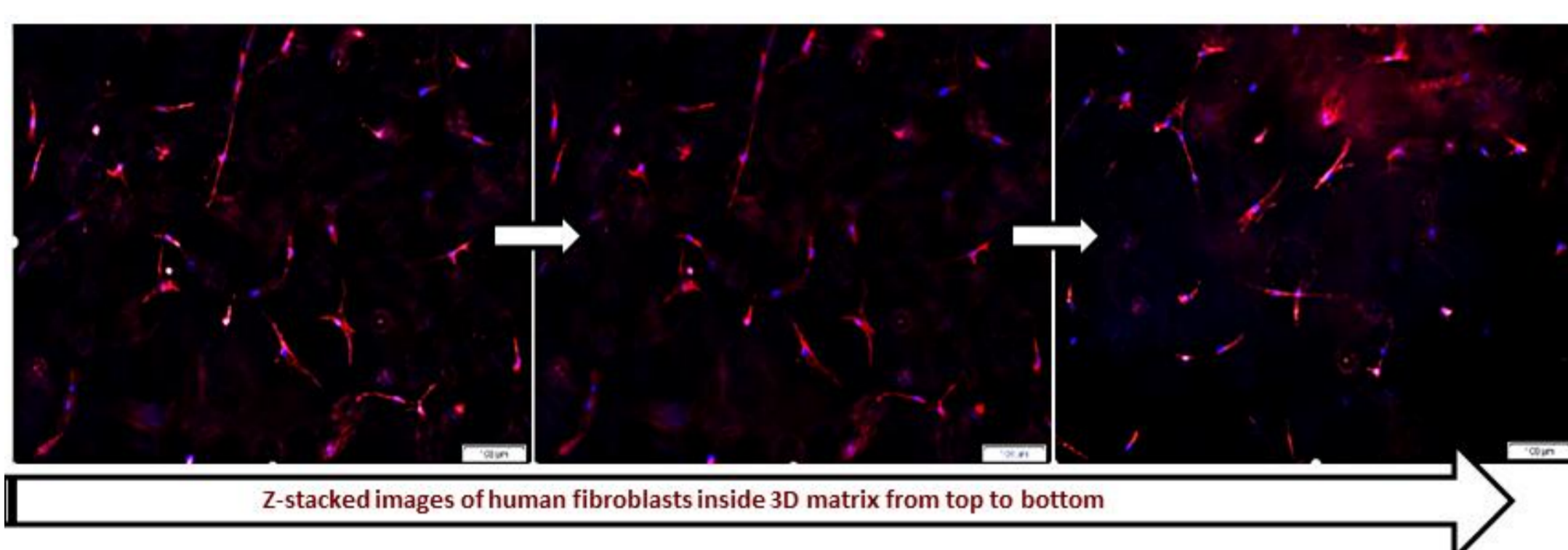
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#### A bacterial colonized human skin equivalent (c-HSE)

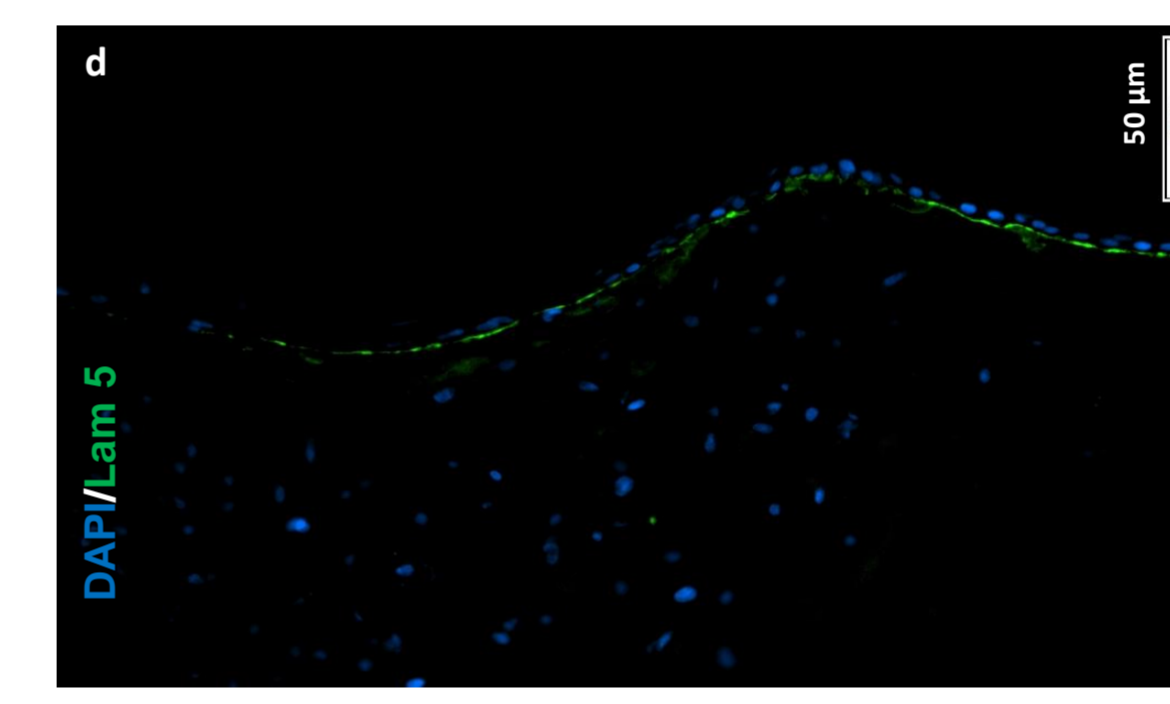
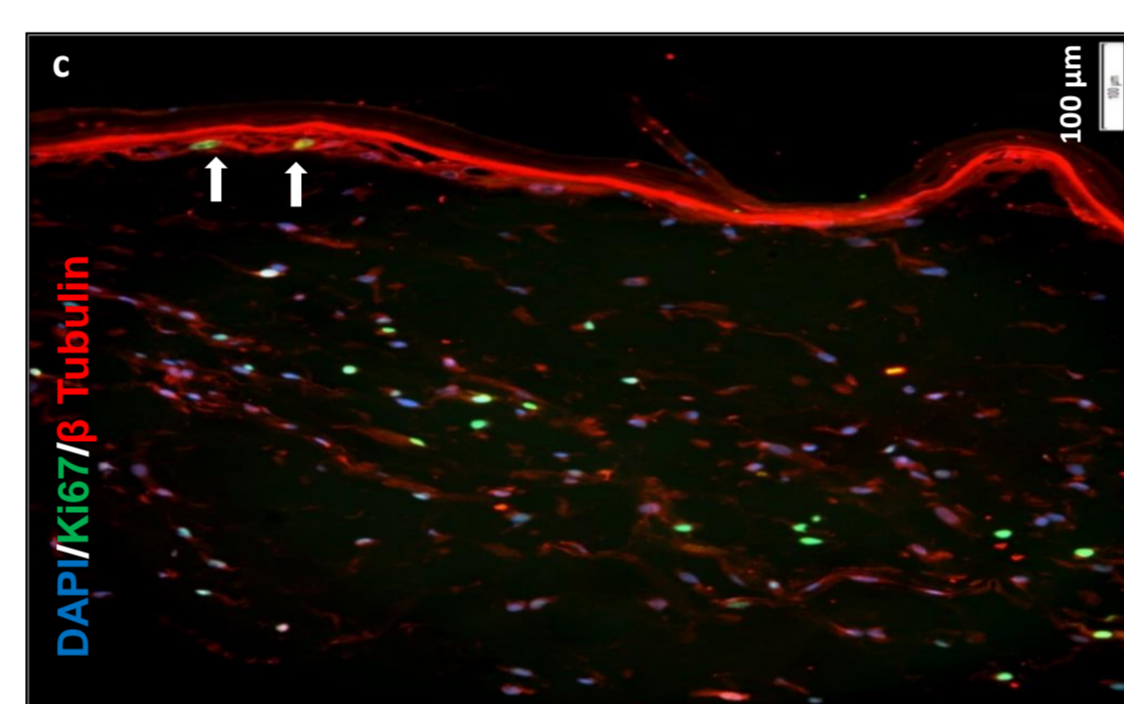
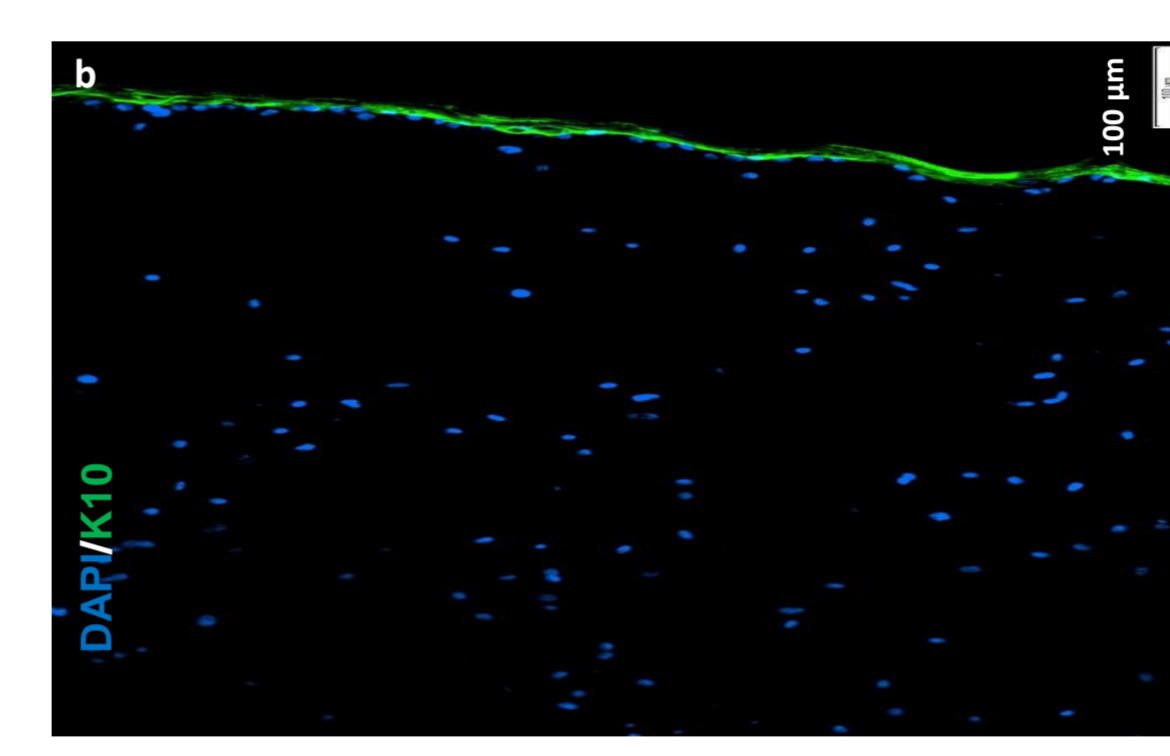
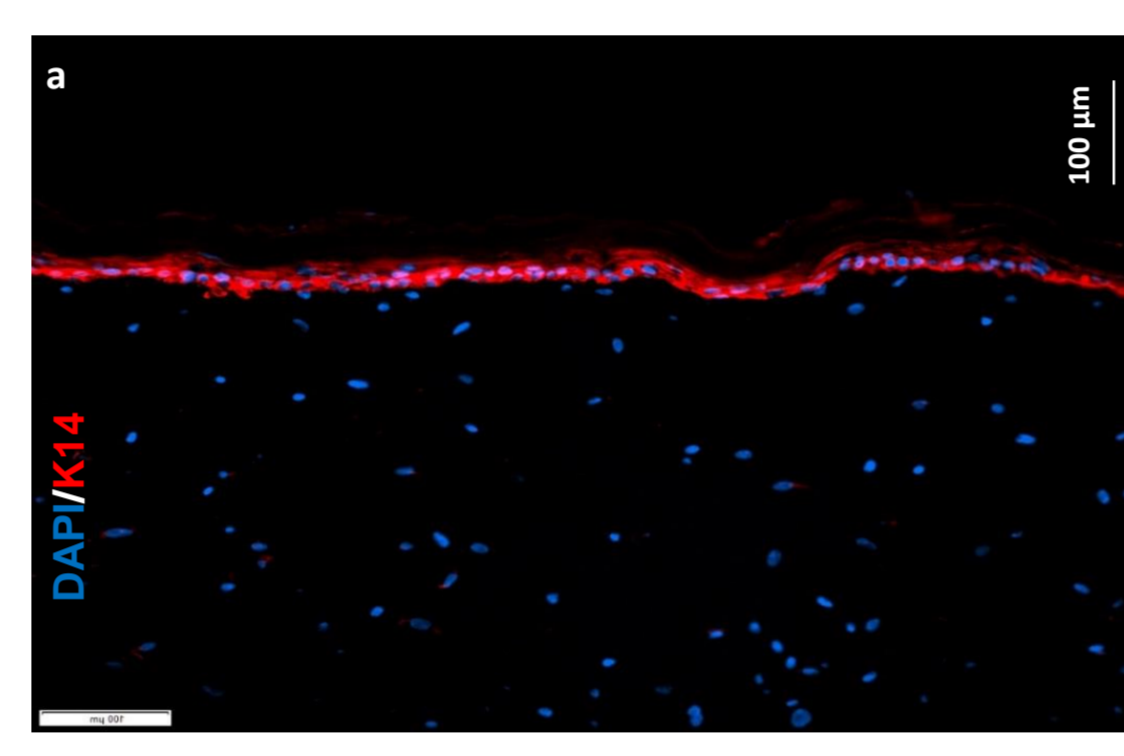
The aim of this study was the development of a human skin model and human skin wound infection model for the bio-evaluation of antimicrobial biomaterials intended for wound healing purposes. The three-dimensional *in vitro* models will represent advanced and complex systems to perform more reliable preclinical 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<sup>+</sup> 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

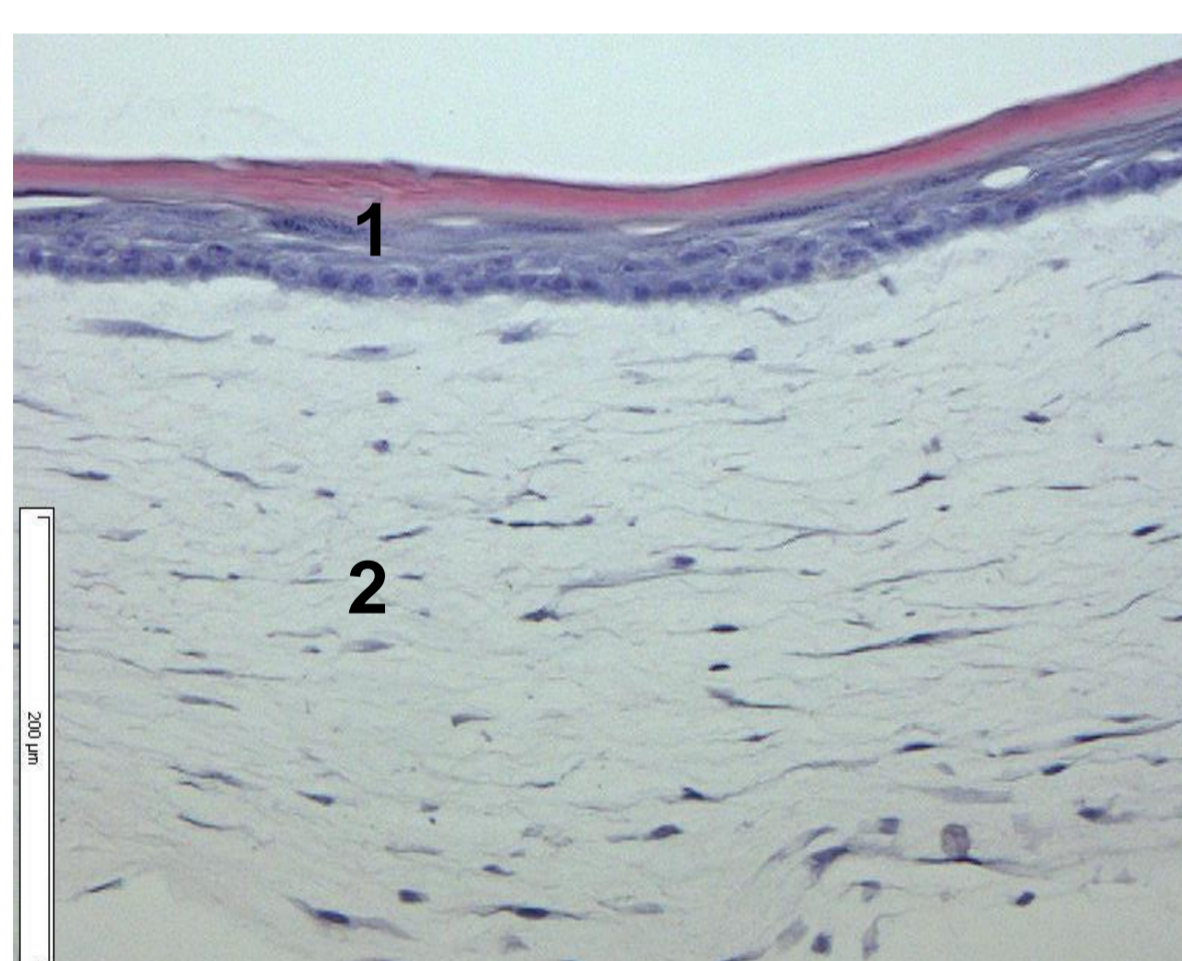


#### IHC verification of the Human Skin Equivalent (HSE):

- (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

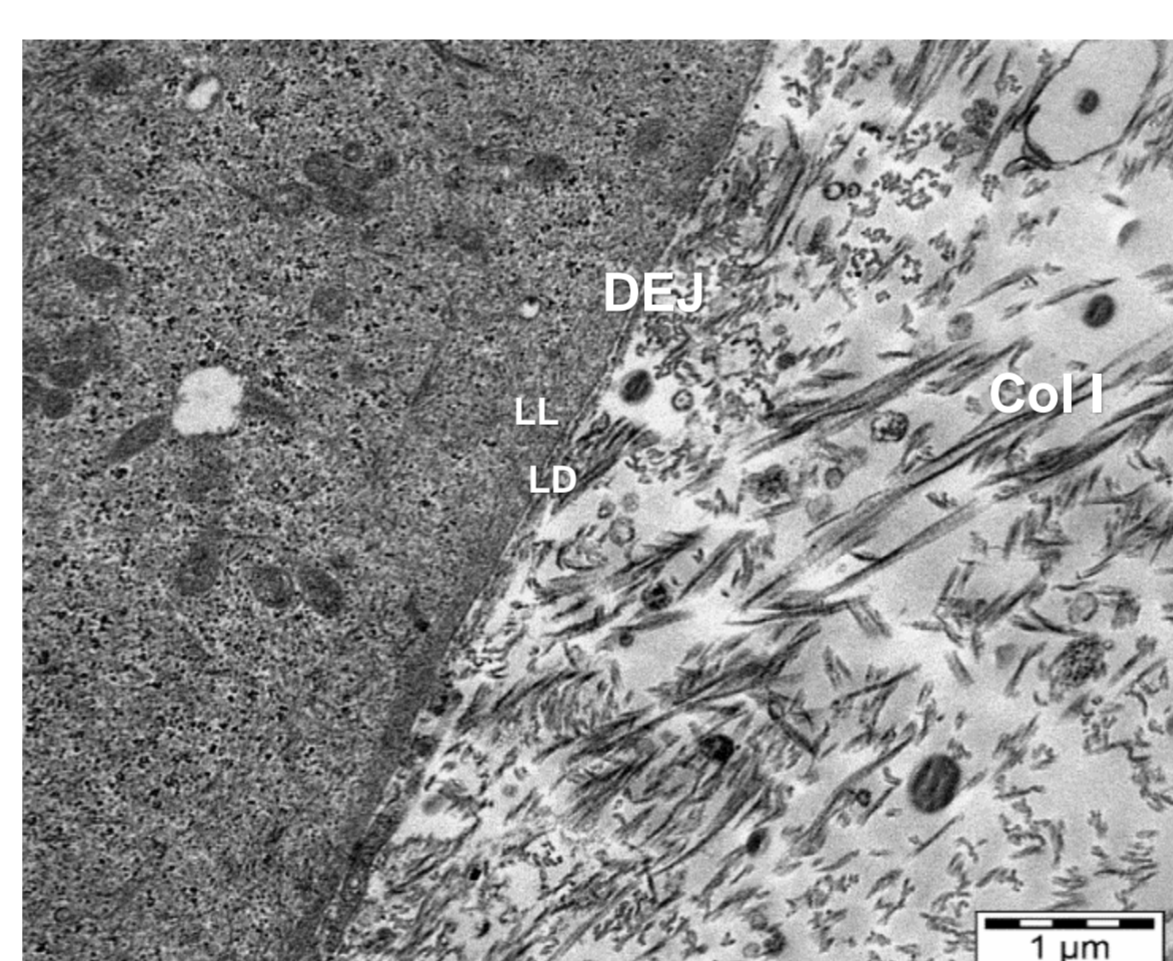


H&E stained cross section of *in vitro* HSE model:

- Epidermal layer (1)
- Dermal layer (2)

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

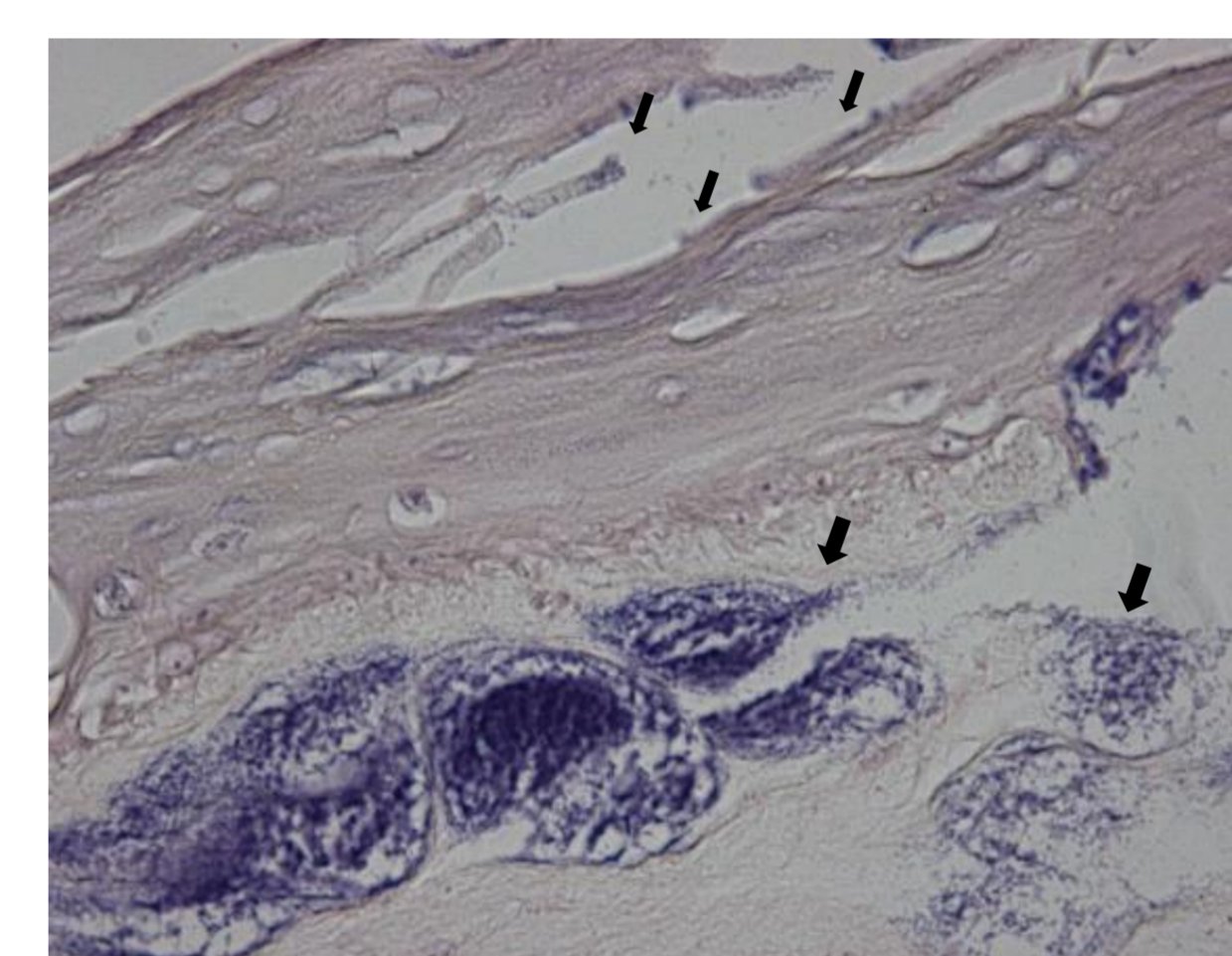
#### Ultrastructure Analysis



TEM image:

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

#### *S. aureus*-colonized HSE

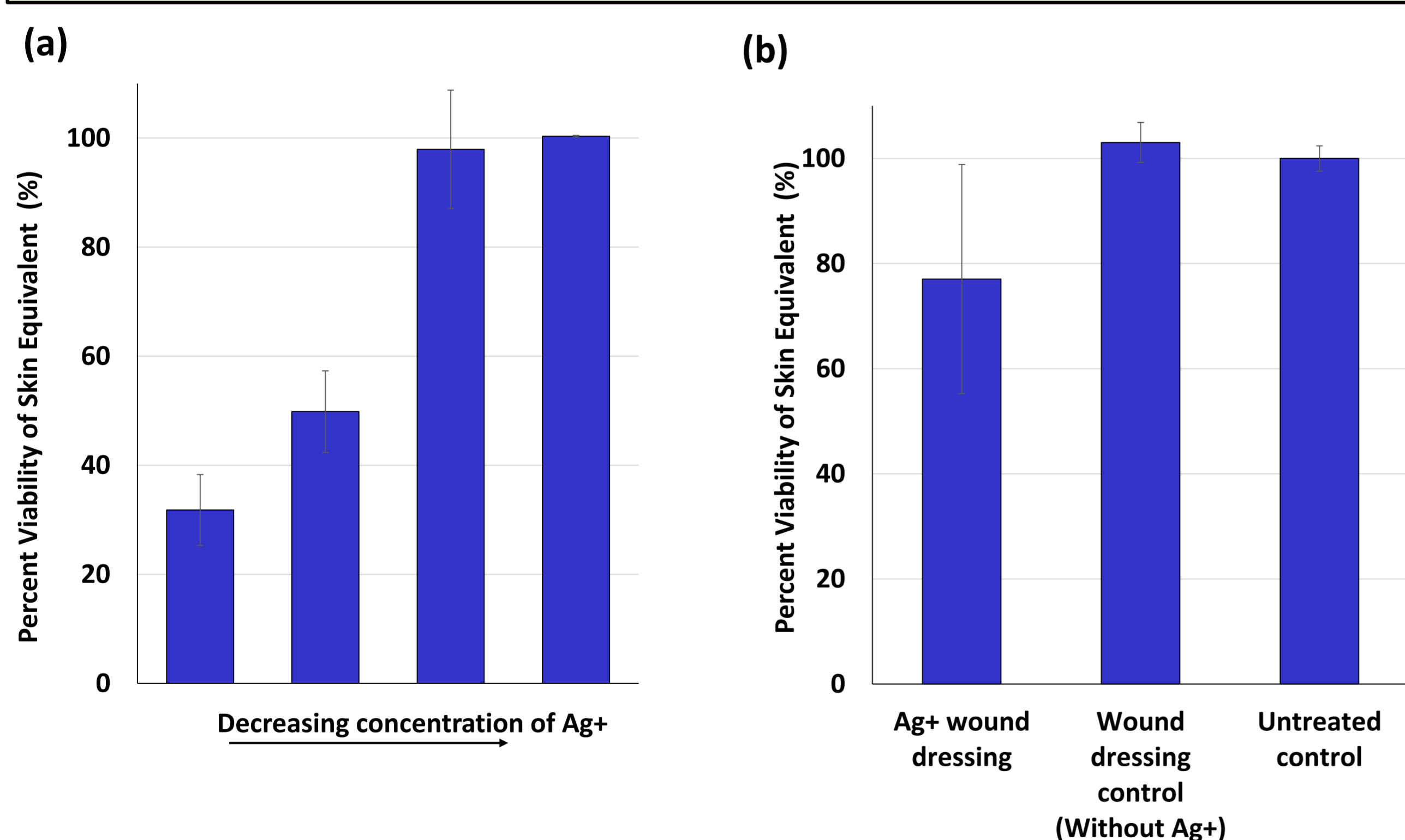


Inoculated bacteria adhere to the dermal surface, colonize, and replicate to make large structures of biofilm.

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

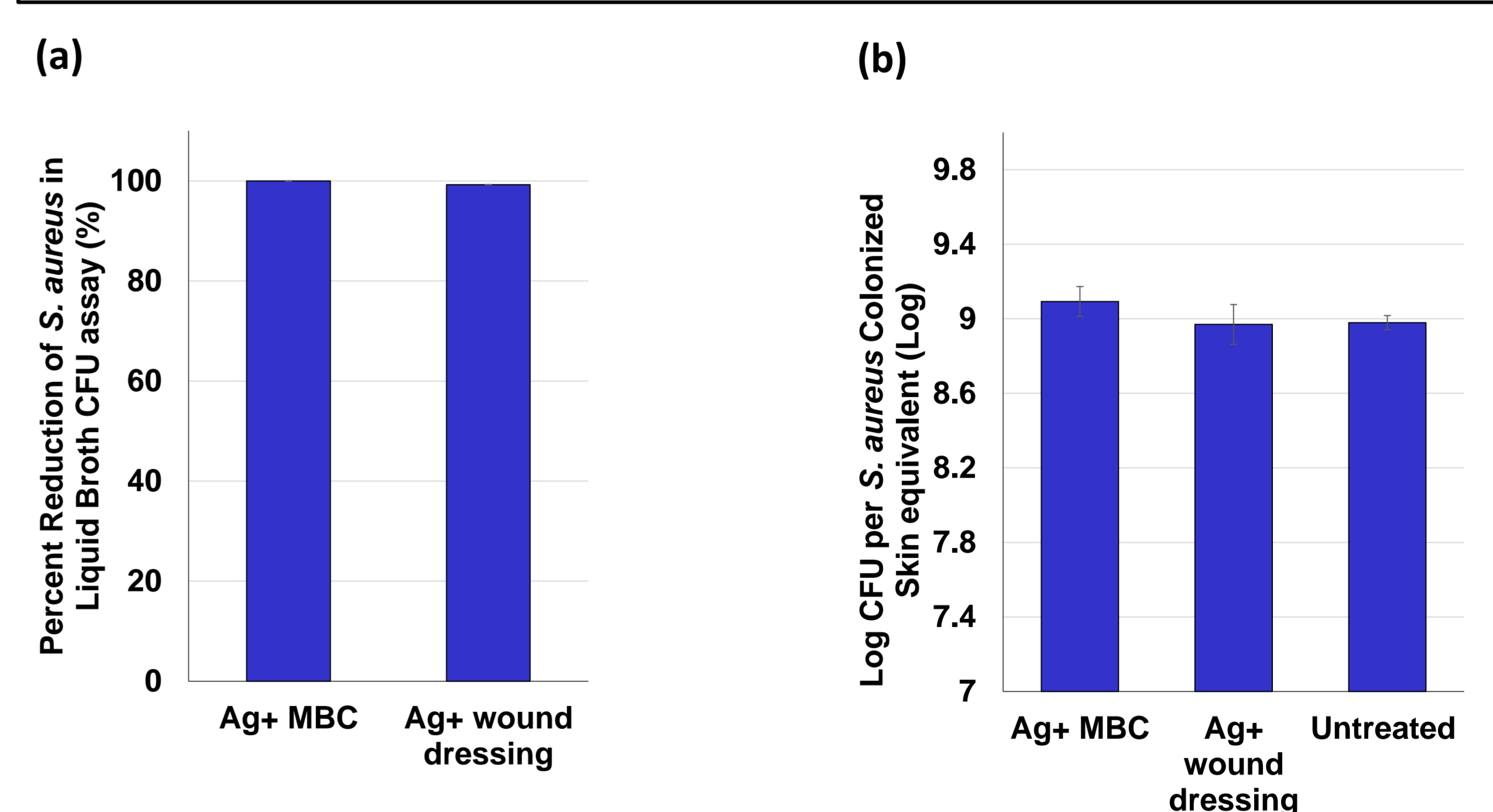
**Small arrows:** Bacteria surrounding keratinocytes in epidermis.

#### Cytocompatibility Analysis



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

#### Antibacterial Analysis



The graph demonstrates the treatment of infected skin equivalents with a commercially available Ag<sup>+</sup> releasing wound dressing. Skin equivalents were infected with *S. aureus* and thereafter, Ag<sup>+</sup> releasing wound dressing or Ag<sup>+</sup> 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.



HyMedPoly has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement number 643050.

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