Bacterial infection and wound healing in an *ex vivo* human skin model

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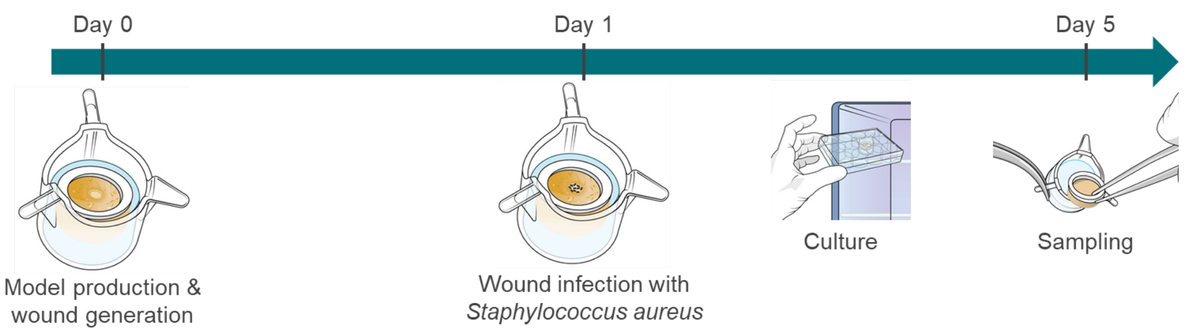
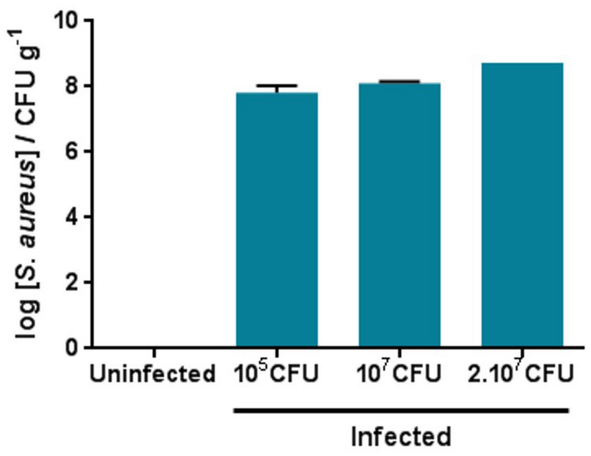
Skin possesses excellent regeneration properties that allows its rapid healing upon dermal injury. If wounds fail to heal in an orderly and timely manner, chronic ulcers (e.g. diabetic foot ulcers, venous leg ulcers, pressure ulcers, etc.) develop. Efficient treatment of chronic wounds remains a global challenge. Next to wound debriding or hyperbaric oxygen therapy, a myriad of topical therapies and dressings are available to the clinicians with very few having shown their effectiveness in promoting wound repair. The integration of nanomaterials into wound healing bandages is believed to be one possibility to overcome some of the current limitations. To test the potential of these innovate wound healing dressings, wound infection models are needed. Current research on wound infections is mainly conducted on animal models, which often limits direct transferability to human and poses ethnic issues. Some of these limitations can be overcome by using an *ex vivo* skin model, HypoSkin®, human skin discarded from cosmetic surgery.

Experimental Workflow



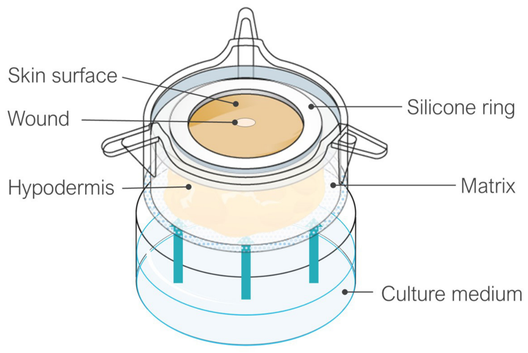
WOUND PRODUCTION AND INFECTION OF HYPOSKIN® MODEL.
Wounded HypoSkin® models were produced and shipped. Bacterial infection was initiated by dropping *S. aureus* (10^5 to $2x10^7$ cfu) solutions on the wound and keeping the skin samples in culture medium without antibiotics at 37°C for 4 days. The wound was then analyzed for the presence of infection.

Wound infection with *Staphylococcus aureus*

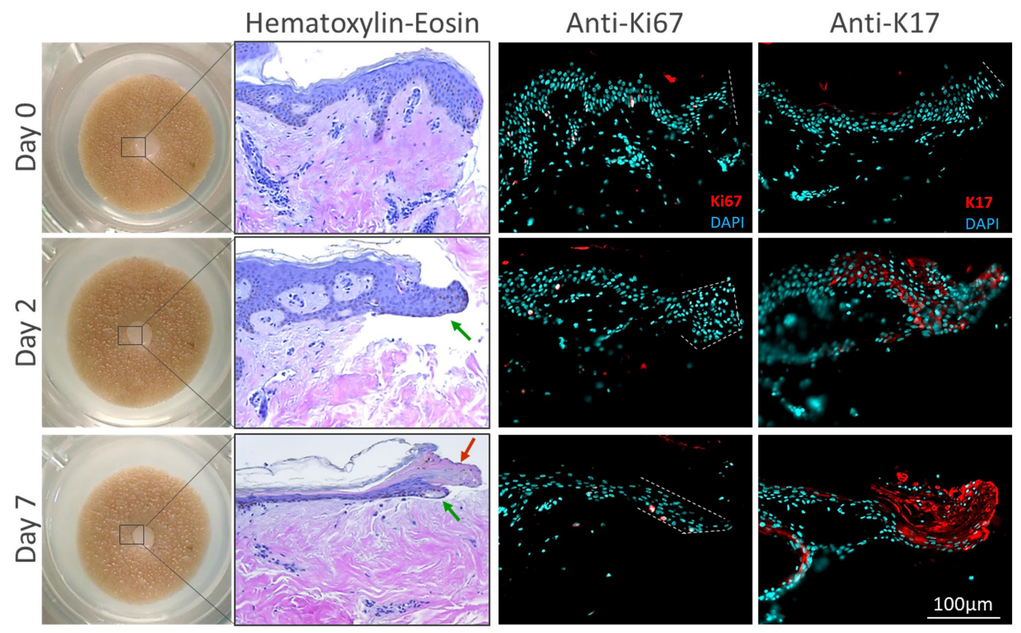


SKIN CONTAINING *S.AUREUS* BACTERIA
Number of bacteria colony forming units (CFU) in skin infected for 4 days by different concentrations of *S. aureus* is showed on the graph. No bacteria were detected on uninfected skin (control). The detected amount of bacteria in infected skins was ≈$1x10^8$ CFU/g of skin.

Wound Healing Processes occuring in Hyposkin® Model

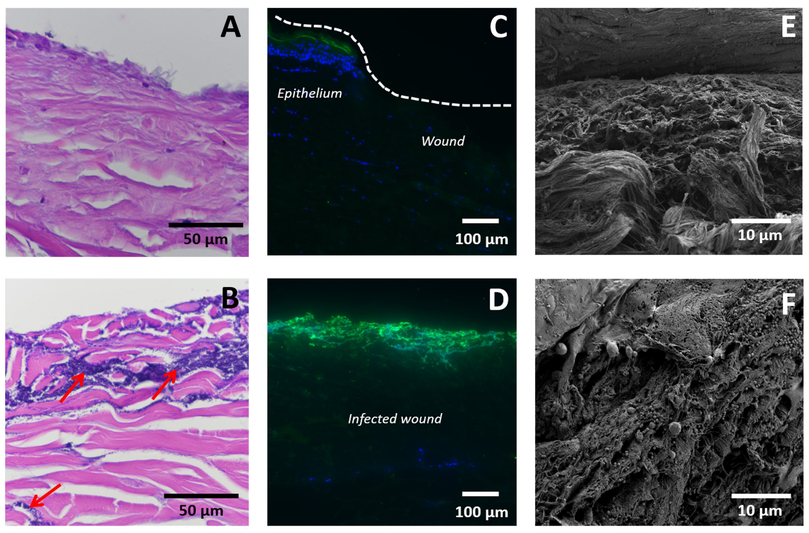


A HUMAN *EX VIVO* MODEL SUITABLE FOR BACTERIAL INFECTION
HypoSkin® is an *ex vivo* human skin model with epidermis, dermis and hypodermis. The skin explant is embedded in a proprietary gel-like matrix with epidermal surface left in direct contact with the air.
To generate a wound, a defect of a controlled diameter is performed to remove all the epidermis and upper part of the dermis. A silicon ring is adhered on the skin surface to prevent lateral bacteria leakage.
The system is mounted into cell culture inserts and maintained in standard cell culture conditions.



WOUNDED HYPOSKIN® RECAPITULATES SOME ASPECTS OF WOUND HEALING
At day 0, the edge of the wound was a clear cut of the epidermis. From day 2, an epithelial tongue appeared and migrated until day 7. A crust was also visible at day 7. Cells in the migrating epithelial tongue were proliferating (Ki-67 positive staining) and expressing K17 which is a specific marker of keratinocytes migrating over the wound bed.

Histological Observations of Infected Wounds



A – C – E = Non infected wound

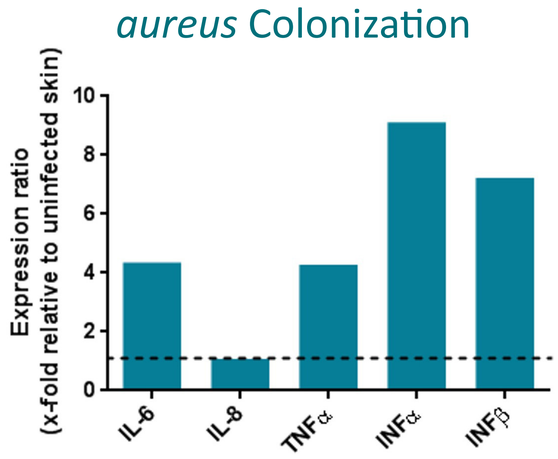
B – D – F = Wound infected with *S. aureus*

BIOFILM DEVELOPMENT ON THE WOUND (A-B)
Hematoxylin and eosin staining shows the structure of the wound (dermis in pink) and bacteria (dark purple). A *S. aureus* biofilm developed over 4 days of infection in the wound.

***S. AUREUS* SPECIFIC BIOFILM (C-D)**
S. Aureus was detected on tissue sections by using a specific antibody. High level of green fluorescence indicates the presence of *S.aureus* within the wound.

BACTERIAL COLONIZATION OF SKIN TISSUE (E-F)
Scanning electron microscopy analysis shows presence of bacteria among collagen fibers.

Skin Inflammation in Response to *Staphylococcus aureus* Colonization



***S.AUREUS* INDUCES INFLAMMATORY RESPONSE**
The analysis of pro-inflammatory cytokines expression by qPCR on total skin lysates shows an increase of cytokines expression levels after 4 days post-infection with *S.aureus*, confirming the initiation of inflammatory responses after bacterial infection.

We demonstrate that Wounded HypoSkin® model is suitable to study wound infection:

- Skin wound healing processes occur
- Specific bacteria biofilm is formed in the wound bed
- *S. aureus* infection triggers inflammatory responses in the model

This model presents the ability to evaluate topical therapies and dressings in prophylactic administration, to prevent wound infection, or in therapeutic treatment, for infected wounds.