



INFECTIOUS DISEASES (BACTERIAL, FUNGAL, VIRAL, PARASITIC, INFESTATIONS)

COLONIZED HUMAN RECONSTRUCTED EPIDERMIS AS A SUITABLE MODEL TO IDENTIFY NEW ANTI-MICROBIAL ACTIVITIES

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Introduction: The skin is colonized by bacteria and yeasts most of which are harmless or even beneficial. *Staphylococcus aureus* and *Streptococcus pyogenes* are among the most common contaminating bacteria and show an innate resistance to antibiotics, disinfectants, and clearance by host defenses.

Objective: In vitro Reconstructed Human Epidermis (RHE) infected with *S. aureus* and *S. pyogenes* has been developed to evaluate the anti-microbial and anti-adhesive efficacy of disinfectants and detergents as direct interference with the bacterial burden and adhesion.

Materials & Methods: The RHE surface (0.5 cm², 17 days differentiation) was colonized with 2*10⁶ CFU/tissue of bacterial suspension (*S. aureus* MRSA ATCC 33591 or *S. pyogenes* ATCC 19615). After 4h, the treatment with disinfectants was performed for 15 min and, at different time-points, the sample was processed in order to separate the apical compartment (non-adherent bacteria) from the homogenate (adherent bacteria) and perform a bacterial viable count on both fractions.

Results: The bacterial burden measure revealed that the treatment of 15 minutes with a chlorhexidine-based product completely inhibited bacterial viability in the apical compartment, whereas a low fraction of adherent viable cells was detected in the tissue homogenate, showing that opportunistic pathogens increase their resistance to disinfectants by adhering to the tissue (protecting themselves through the formation of biofilm matrix).

Conclusions: Studying anti-microbial activity on reconstructed tissues instead of bacterial suspensions is a good strategy to assess the efficacy in a more realistic model that mimics the real site of infection and helps to discriminate the higher bacterial resistance due to the adhesion to epidermis.

