Colonized Human Reconstructed Epidermis as a Suitable Model to Identify new Anti-microbials

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Introduction

The skin is colonized by bacteria and yeasts most of which are harmless or even beneficial, others are pathogenic. *Staphylococcus aureus* is one of the most common contaminating bacteria able to easily form a biofilm on epidermal surface and it has been shown that its presence cause delays in the healing process (1). Other pathogen bacteria, such as *Streptococcus pyogenes,* also colonize both skin and respiratory surfaces, and frequently presents clinically adverse events, such as strep throat or impetigo (2-3). These biofilm-associated bacteria show an innate resistance to antibiotics, disinfectants, and clearance by host defenses (4-5).

Materials & Methods

The RHE surface (0.5 cm^2, 17 days differentiation) was colonized with the bacterial suspension (*S. aureus* MRSA ATCC 33591). After 4h, the colonized tissues were treated with a chlorhexidine-based product for 15 min and, at different time-points (15 min, 2h and 24h), the tissues were 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 (**Figure 1**).

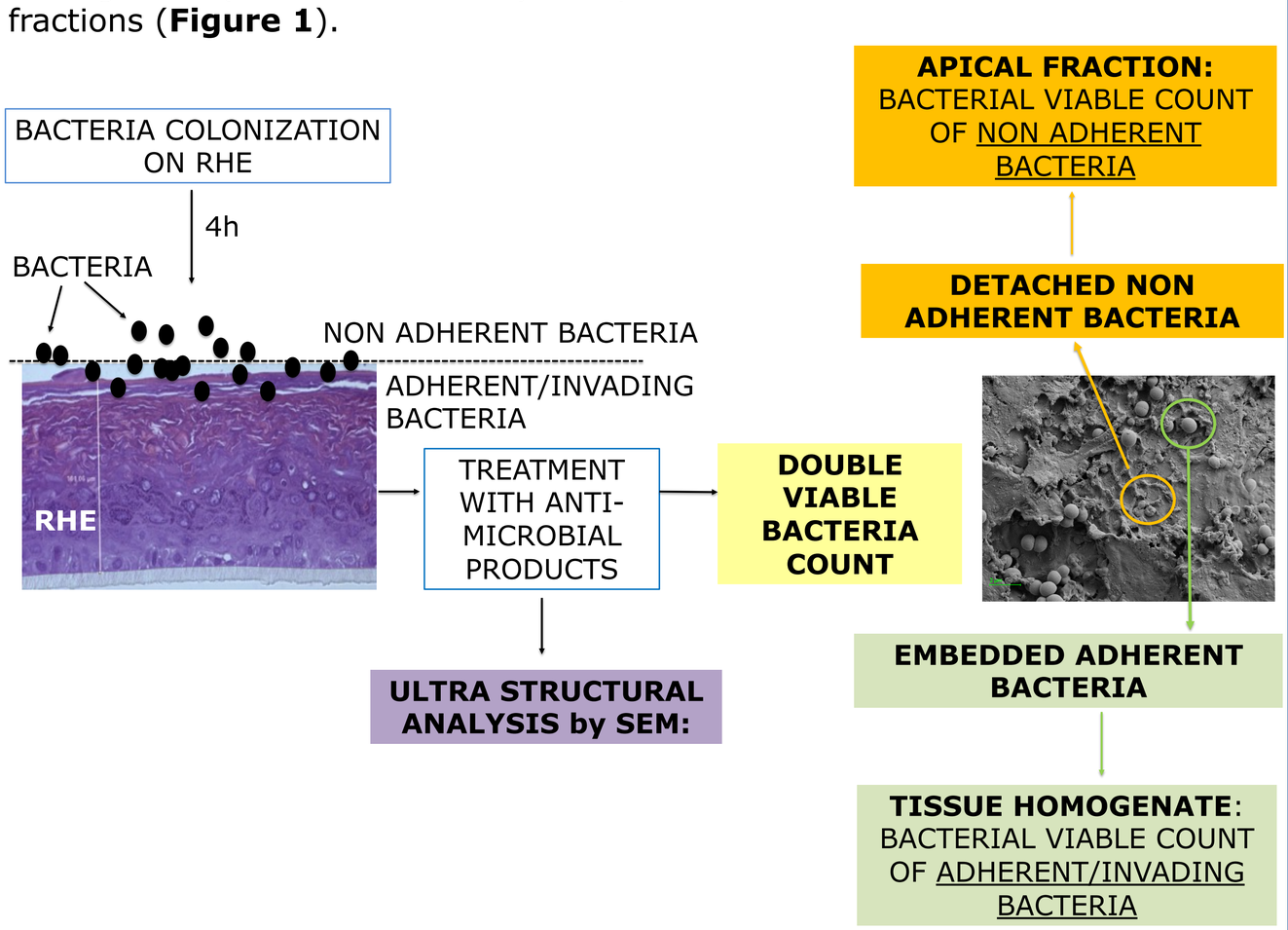


Figure 1. Experimental design for RHE colonization, treatment, double viable count procedure and SEM analysis on colonized RHE.

Objective

To develop an *in vitro* experimental procedure based on the use of Reconstructed Human Epidermis (RHE) infected with *S. aureus* or other pathogen bacteria to evaluate:
anti-microbial and anti-adhesive efficacy of disinfectants and **detergents** for their direct interference with the bacterial burden and adhesion.

DOUBLE VIABLE COUNT

The bacterial burden measure revealed that the 15 min-treatment with a chlorhexidine-based product completely inhibited bacterial viability in the apical compartment (**Figure 2A**), 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)(**Figure 2B**).

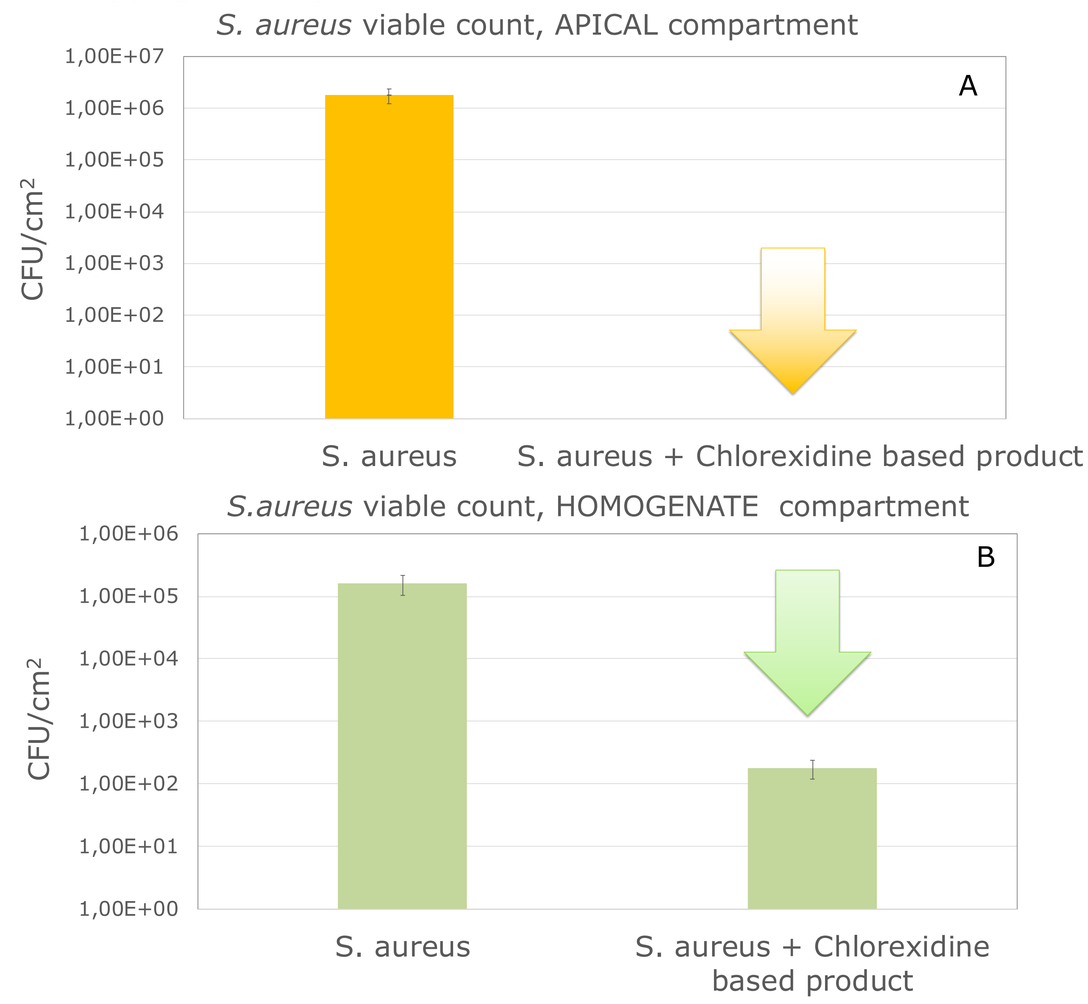
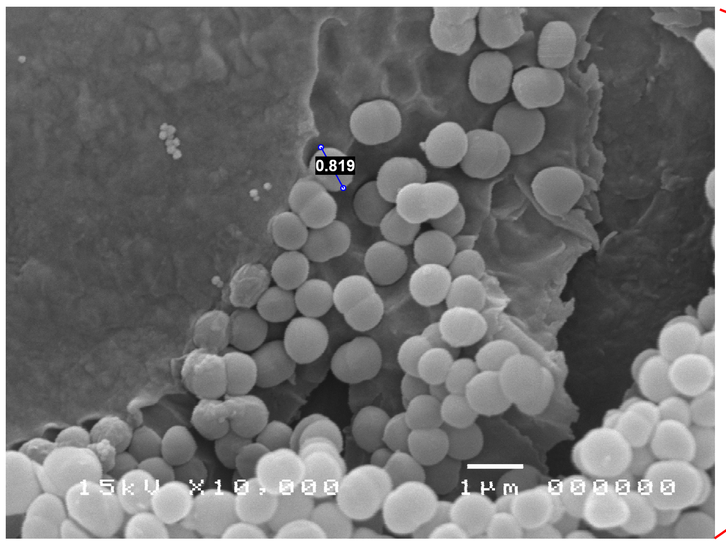
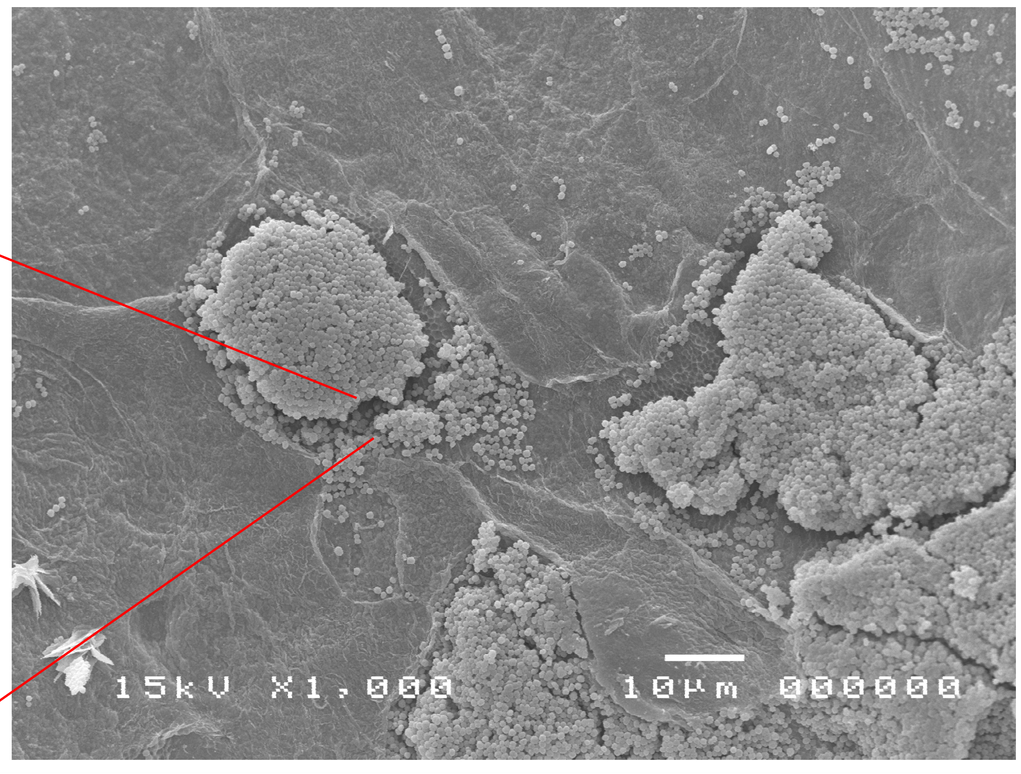


Figure 2. Antimicrobial activity of a **chlorhexidine-based product** evaluated on RHE colonized with *S. aureus* after **15 min** of treatment. Bacterial viable count in apical (A) and homogenate (B) compartment.

ULTRASTRUCTURAL ANALYSIS: INTERACTION BETWEEN SKIN AND BACTERIUM

The RHE colonized with *S. aureus* can be analyzed also by SEM imaging to get insights of BACTERIA ADHESION, VIABILITY, PHENOTYPE, DISTRIBUTION, MORPHOLOGY, BIOFILM FORMATION.
The colonized RHE model, thanks to the close bacteria-epidermal surface interaction, allows to determine the influence of anti-bacterial or anti-biofilm products on bacterial infections.





Conclusions:

Studying anti-microbial activity on reconstructed tissues has many advantages compared to anti-microbial activity studies performed on bacterial suspensions:

- The use of colonized *in vitro* tissues models allows to assess the anti-microbial efficacy in a more realistic model that mimics the real site of infection
- The presented strategy helps to discriminate the higher bacterial resistance due to the adhesion to epidermis.

Viable bacteria count and SEM analysis of colonized reconstructed tissues can be effectively used to assess product efficacy and screen anti-bacterial formulations.

References

1. Schneider-Lindner V et al. (2007) Antimicrobial drugs and community-acquired methicillin-resistant *Staphylococcus aureus*, United Kingdom. Emerg Infect Dis. 2007 Jul;13(7):994-1000
2. Klonoski JM et al. (2014) Vaccination against the M protein of *Streptococcus pyogenes* prevents death after influenza virus: *S. pyogenes* super-infection. Vaccine 32:5241-9.
3. Larru B and Gerber JS (2014) Cutaneous bacterial infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes* in infants and children. Pediatr Clin North Am. 61:457-78.
4. Holland DB et al. (2008) Microbial colonization of an in vitro model of a tissue engineered human skin equivalent- a novel approach. FEMS Microbiol Lett, 279 (2008) 110-115
5. Archer N. et al. (2011) *Staphylococcus aureus* biofilms Properties, regulation and roles in human disease**.** Virulence 2:5, 445-459; 2011