



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

ASSESSMENT OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BIOFILM DYNAMICS ON IN VITRO MODEL OF WOUNDS

S. Balzaretto⁽¹⁾ - *S. Del Prete*⁽²⁾ - *M. Meloni*⁽¹⁾

Vitroscreen, In Vitro Research Laboratory, Milan, Italy⁽¹⁾ - *Service Biotech Srl, Biothecnology, Naples, Italy*⁽²⁾

Introduction: Biofilms represent a key challenge being associated with failure in acute wounds healing and in wound chronicity. The skin microflora component *Staphylococcus aureus* is involved in wound infections and harbors a tightly regulated genetic complex to produce polymeric matrix to develop biofilm.

Objective: This study aimed at developing an in vitro model of biofilm on lesional skin, by colonization of Human Reconstructed epidermis (RHE) with *S. aureus*. This model was used to compare reference products and evaluate their efficacy in destroying or preventing the biofilm.

Materials & Methods: The RHE surface (0.5 cm², 17 days differentiation) was gently scraped and colonized with *S. aureus* MRSA ATCC 33591. Prevention model: immediately or after 24h from inoculum, 30 µL of product were applied on the epidermis, and re-applications were performed after 24h. Biofilm eradication: after 48h from inoculum, the treatment was performed and repeated after 52h and 72h.

The tissues were fixed in glutaraldehyde 1.2% and processed for scanning electron microscopy (SEM).

Results: At 24h, the bacteria were mostly enclosed in the EPS matrix and, at 48h, the biofilm structure was clearly visible and bacteria integrated in the EPS matrix with visible fimbriae. At 72h, the cells were shrouded in a dense and mature glycocalyx.

The reference products induced morphological changes, increased the ratio of planktonic cells over the encapsulated forms, both in the prevention and in the eradication model.

Conclusions: The colonized system developed on RHE allowed a clear understanding of *S. aureus* biofilm dynamics on a biologically relevant surface, thus purchasing a model of investigation for anti-microbial and anti-biofilm efficacy on wounds models.

