Assessment of Methicillin Resistant *Staphylococcus aureus* (MRSA) Biofilm Dynamics on *in vitro* Model of Wounds

S. Balzaretti[1], S. Del Prete[2], M. Meloni[1]
[1]VitroScreen, Milan (Italy) – [2]Service Biotech srl, Naples (Italy)

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Introduction:

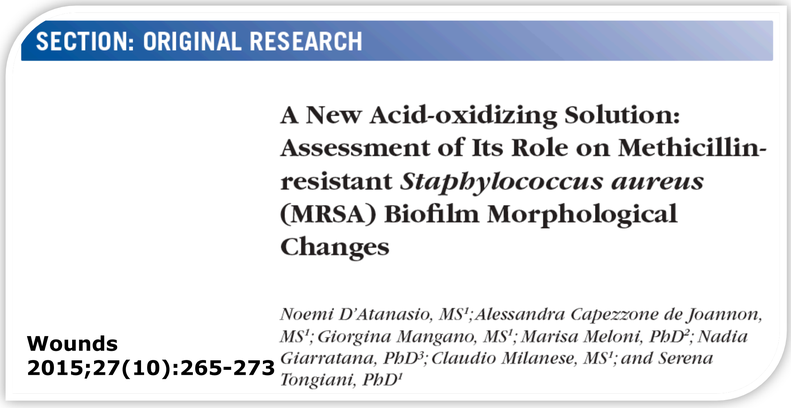
A biofilm is an assemblage of microbial cells associated with a living organism or tissue and enclosed in a matrix of polysaccharide material. The skin microflora component *Staphylococcus aureus* is involved in wound infections and harbors a tightly regulated genetic complex to produce polymeric matrix to develop biofilm. Biofilm-associated bacteria show an innate resistance to antibiotics, disinfectants, and clearance by host defences. These properties likely contribute to the persistence and recalcitrance to treatment of biofilm infections. Therefore, biofilms represent a key challenge being associated with failure in acute wounds healing and in wound chronicity.

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 cm^2, 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 model: 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.

SEM images of colonized RHE tissues after treatment with reference products and new acid oxidizing solutions. Products effects on bacteria and RHE model are also summarized.


SECTION: ORIGINAL RESEARCH

A New Acid-oxidizing Solution: Assessment of Its Role on Methicillin-resistant *Staphylococcus aureus* (MRSA) Biofilm Morphological Changes

Noemi D'Atanasio, MS[1]; Alessandra Capezzone de Joannon, MS[1]; Giorgina Mangano, MS[1]; Marisa Meloni, PhD[2]; Nadia Giarratana, PhD[3]; Claudio Milanese, MS[1]; and Serena Tongiani, PhD[1]

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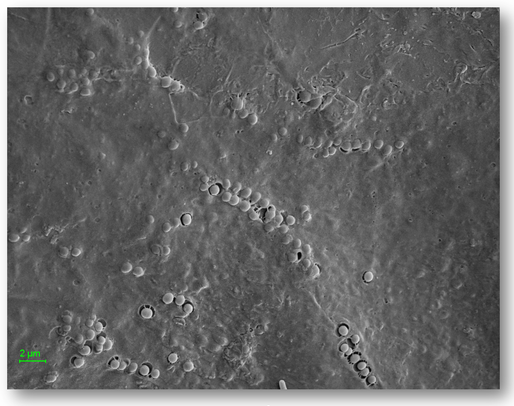
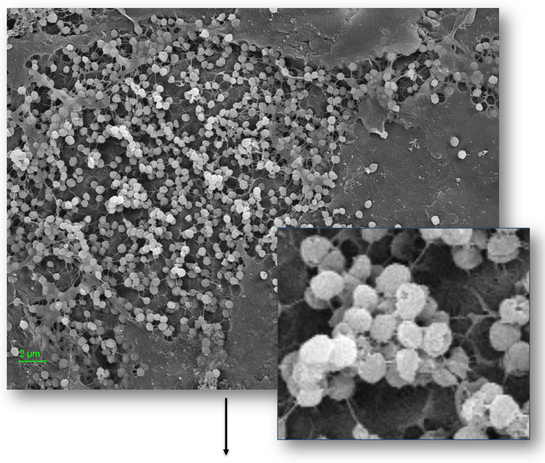
3 days *S. aureus* control



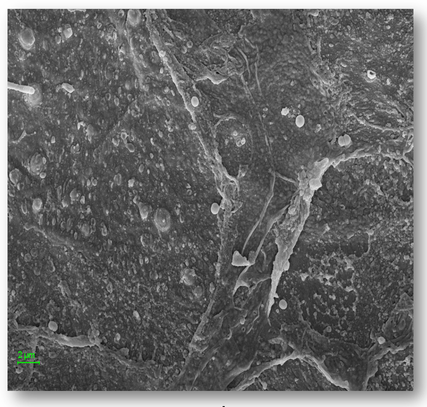
- **Dense biofilm**
- **Cocci are embedded in the matrix and protected**

Betaine and polyhexanide: strong antimicrobial - toxic



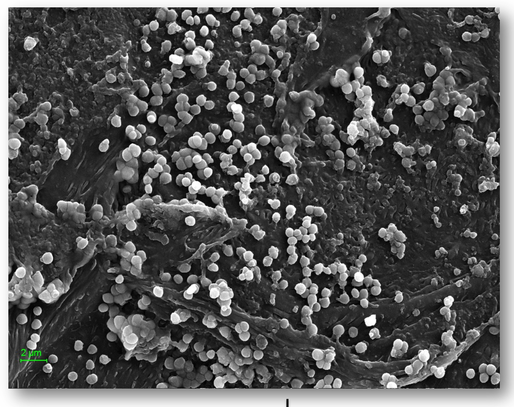
- **Dissolution of the biofilm**
- **Smaller cocci with damaged cell wall**

Hypochlorous acid and sodium hypochlorite: low antimicrobial but aggressive on RHE



- **Detachment of the bacterium**
- **Dehydration**
- ***Stratum corneum* damages**

Acid-oxidizing solution: safe for the skin, able to modify bacterial phenotype into planktonic

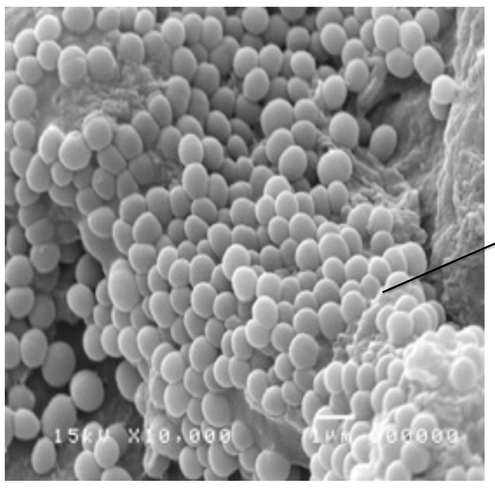


- **Single free bacteria or small aggregates**

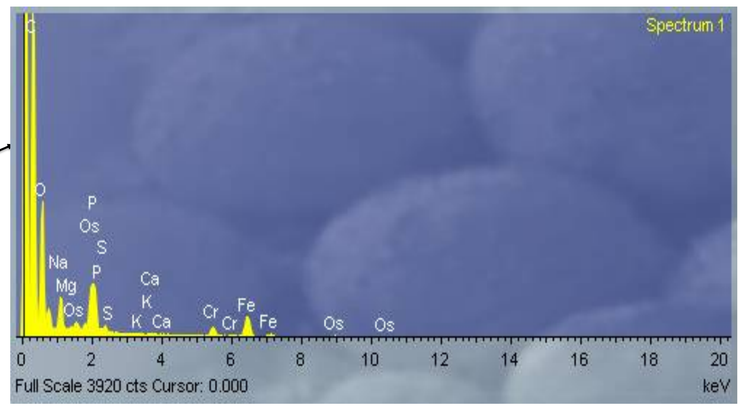
INTERACTION BETWEEN A MOLECULE AND THE BACTERIAL BIOFILM: MICROANALYSIS

The **SEM ultrastructural analysis** can be combined to the **microanalysis**: it is a qualitative analysis on a selected area of the sample that gives information on the **elementary composition of both the organic or inorganic components** present on the tissue surface or on the bacterial surface/biofilm.

SEM image



MicroAnalysis



The microanalysis detects the electron-dense microelements on a selected area.

Applications:
- ❖Finding selective markers (elements) of biofilm development
- ❖Identifying particular elements present in the product which interact with the bacteria (i.e. electrostatic interactions)

References
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Conclusions:
The **colonized wounds system** developed on RHE allowed a comprehensive and deep understanding of *S. aureus* biofilm dynamics on a living epidermal tissue better recapitulating the bacterial feature and providing a more predictive tool, compared to in tubo assays, to assess antimicrobial efficacy.