TARGETING *S. AUREUS* BIOFILM FORMATION TO REDUCE THE OCCURRENCE AND THE SEVERITY OF FLARES-UP IN CHILDREN WITH ATOPIC DERMATITIS

Callejon S.[1], Gayraud F.[2], Chavagnac-Bonneville M.[2], Sayag M.[2] and Trompezinski S.[1]

[1]NAOS ILS, Research and Development Department, Aix-en-Provence, France; [2] NAOS, Research and Development department, Lyon, France.

INTRODUCTION

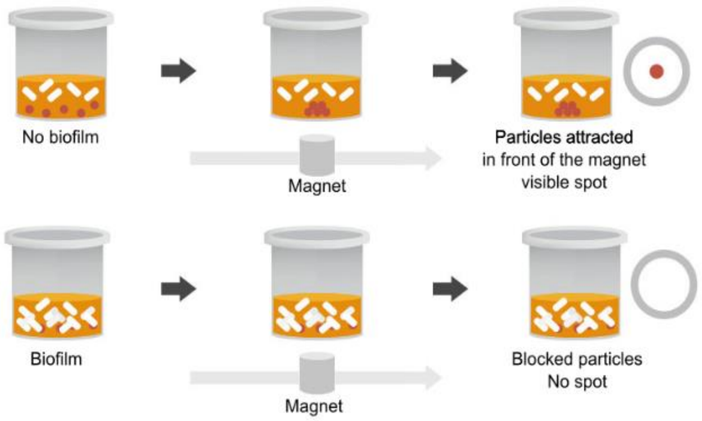
Atopic dermatitis (AD) is an inflammatory chronic dermatosis characterized by a marked dysbiosis with a decline of microbiota diversity. During AD flare-ups, biofilm-growing *Staphylococcus aureus* was described as the major colonizer in the skin lesions and its production strength was recently correlated with the disease severity[1,2]. This suggests that the biofilm-growing *S. aureus* plays a major role in the chronicity and severity of AD. Therefore, the aim of this study was to evaluate the prevention of *S. aureus* biofilm formation of both the patented active agent, called Skin Barrier Therapy (SBT) and a dermo-cosmetic balm (containing SBT), that was known to reduce the occurrence of relapses and the severity of AD in children[3].

MATERIALS & METHODS

In microplates, bacterial suspensions of *S. aureus* strain (CIP 4.83; 106 CFU/mL) were mixed and incubated at 37°C for 4 hrs. with SBT (from 0.1 to 0.00005%) or for 4 or 6 hrs. with the balm (500 mg/mL).

For SBT, the prevention of *S. aureus* biofilm-growing was evaluated by the BioFilm Ring Test. The principle is based on the capacity of bacteria to immobilize paramagnetic microbeads when forming a biofilm at the surface. After magnet contact, free magnetic microbeads (TON004; 10µL/mL) are concentrated in the center forming a ring, whereas those blocked by sessile cells remain in place (**Figure 1**). After adding a contrast liquid, the microplate was magnetized for 1 min on the block-test to reveal the mobility of the microbeads. Image acquisition and analysis of the microplate was performed using BFC Elements 3.0 software.

Figure 1: *BioFilm Ring Test principle*



For the dermo-cosmetic balm, to recover the biofilm phase, the microplate was sonicated after addition of PBS. The "biofilm" supernatants were then diluted and counted after filtration (0.2µm) on membranes. The agar plates were incubated at 37°C for 24 hrs. and then counted.

RESULTS

Figure 2: *Efficacy of SBT on S. aureus proliferation as a biofilm after 4 hrs. by BioFilm Ring Test*

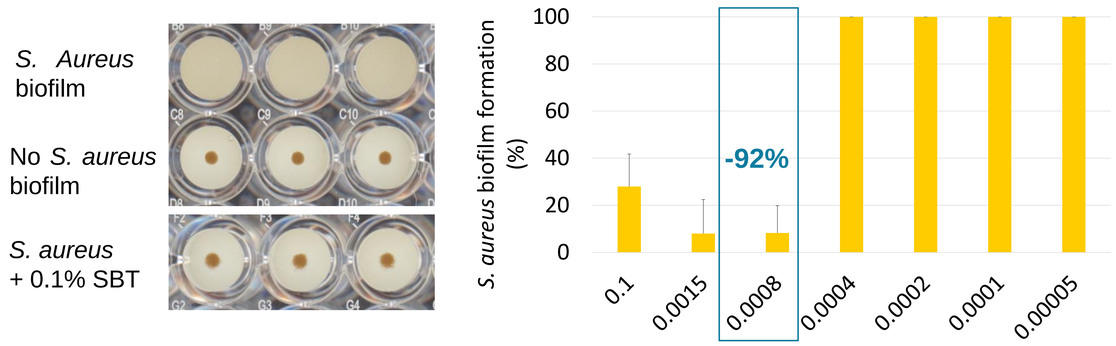
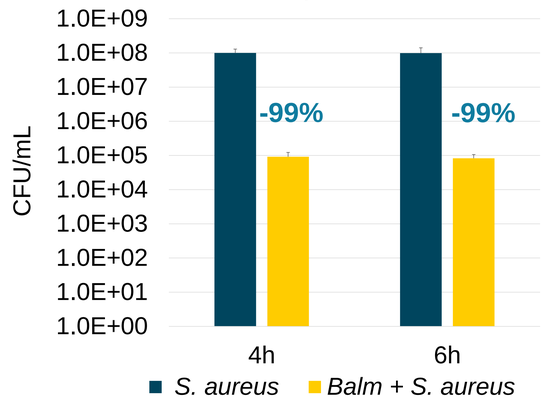


Figure 3: *Efficacy of the balm on S. aureus proliferation as a biofilm by the enumeration technique*



The SBT presented an inhibitory activity of 92.0% at 0.0008% (w/v) on *S. aureus* proliferation as a biofilm after 4 hrs (**Figure 2**). In presence of the balm, the *S. aureus* proliferation as a biofilm was reduced by 3 log at both 4 and 6 hrs. compared to the controls, corresponding to a reduction of -99.9% (**Figure 3**).

These effects were not due to bacterial toxicity of the product because no difference of the proliferation was observed between 4 and 6 hrs.

DISCUSSION

These results showed that the SBT confers to the dermo-cosmetic balm inhibition properties on *S. aureus* biofilm formation. A previously published study on the balm showed that after 6 months of application of this dermo-cosmetic balm in children with mild AD, 76% of patients did not relapse and the time-to-relapse increased (59 ± 11 days) compared to the emollient base (39 ± 12 days)[3]. In addition, the severity of the relapses had decreased by -49% compared to -15% in the emollient base group. Therefore, these clinical results could be explained by the ability of the balm to prevent *S. aureus* from growing as a biofilm. In conclusion, the SBT demonstrates a great contribution in a dermo-cosmetic balm to reduce the flare-ups occurrence and severity in AD.

[1]**Di Domenico *et al.* Sci Rep. 2018, 8:9573**
[2]**Di Domenico *et al.* Microorganisms. 2019, 7:301**
[3]**Gayraud *et al.* J Cosmet Dermatol. 2015, Jun;14(2):107-12**