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## **TARGETING *Staphylococcus aureus* BIOFILMS: *IN VITRO* ASSESSMENT OF A NOVEL BIOPOLYMERIC MEMBRANE**

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Biofilm-associated skin wound infections delay healing and cause increased pain. Effective treatment of biofilm infections requires a high and sustained concentration of antimicrobials at the infection site. In a previous stage a biopolymeric antibiotic-anesthetic membrane (BAAM) containing sodium hyaluronate (H), sodium alginate (A), ciprofloxacin (C), lidocaine (L) and glycerol (G) was developed. The aim of this study was to evaluate the antibiofilm activity of the BAAM components, both individually and in combination, against a clinical strain of *S. aureus*. The inhibition of biofilm formation was determined using crystal violet (CV) staining, while disruption of mature biofilms was assessed through viable bacteria count with plate counting. Additionally, structural characteristics of treated biofilms were evaluated using scanning electron microscopy (SEM) imaging. Statistical analysis was performed using one-way ANOVA. The CV assay results showed that all treatments containing C inhibited *S. aureus* biofilm formation with the same significance as C alone ( $p < 0.05$ ). Treatments H, L and G did not produce significant inhibition of biofilm formation. Additionally, A exhibited biofilm inhibitory activity comparable to C ( $p < 0.05$ ). The quantification of viable cells in mature biofilms showed that both C alone and in combination with the other components (AHCLG) produced a bacterial death in *S. aureus* biofilms of 28.75% and 27.82%, respectively, with no statistically significant difference between these effects. However, A showed no activity against the established biofilm. Several polysaccharides, such as alginate, present in the extracellular matrix of biofilms, play key roles in adhesion and stabilization processes. Due to these properties, many of these polysaccharides showed inhibitory activity against biofilms through various mechanisms, including surface modification, inhibition of cell adhesion or blocking carbohydrate-protein interactions. These mechanisms might explain the inhibitory, but not disruptive, effect that A exhibited on *S. aureus* biofilms. In line with these results, SEM images revealed that both C and AHCLG reduced biofilm mass in the samples and caused morphological alterations in bacterial cells: some of them showed irregular cell surface, with large invaginations. The antibiofilm activity of BAAM components, both individually and in combination, was evaluated. The results demonstrated that C significantly inhibited biofilm formation and disrupted mature

biofilms, and its combination with other BAAM components did not interfere with its activity. Additionally, A inhibited biofilm formation with the same significance as C, presenting a novel and beneficial effect beyond its primary role in the membrane. These results demonstrate the potential of BAAM not only to prevent biofilm formation but also to disrupt established biofilms, which could have significant implications for the treatment of chronically infected wounds.

Palabras clave: Biofilm – Biopolymeric membrane – Infected wound – *Staphylococcus aureus*