Effect of electroporation and photodynamic therapy (PDT) on *Staphylococcus aureus* biofilm matrix

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Abstract: Microbial resistance has been one of the most health problems worldwide, causing a higher number of morbidity and mortality especially among immunodepression patients. Consequently, promoting an economic and social burden. Thus, is urgent the finding new strategies to overcome this situation that is mainly related to biofilm infections due to its ability to produce an extracellular polymeric matrix (EPS) that hampers the antibiotic effects. One of the main microorganisms related to these infections is *Staphylococcus aureus*. Thus, the combination of treatments such as PDT and electroporation is an option that showed greater results than the literature once was ab
le to inhibit almost 100% of the biofilm and reduced its metabolic activity as well as the EPS matrix.

Keywords: biofilm, infections, aPDT, electroporation, methylene blue

Introduction

Currently, biofilms have been the cause of a wide variety of infections in the human body, reaching 80% of all microbial infections [1]. The bacteria Staphylococcus aureus is a leading cause of hospital-acquired infections. The biofilms present specific properties such as the extracellular polymeric substance (EPS), which increases the resistance to antimicrobial treatments [1]. Thus, the development of new approaches is urgent, and antimicrobial photodynamic therapy (aPDT) has been shown as a promising candidate. aPDT involves the synergistic combination of a photosensitizer (PS), molecular oxygen and visible light of an appropriate wavelength in order to produce highly reactive oxygen species (ROS), which leads to the oxidation of several cellular components [2]. Even though this therapy showed be efficient to attack many components of the biofilm, the EPS hampers the PS access to the deeper biofilm cells, promoting the re-grow of the microorganism community [2]. Therefore, to overcome this problem, combining the aPDT with a promising approach, such as electroporation (EP) is necessary. The EP may enhance the permeability of the EPS-biofilm, allowing the PS to reach the deeper cells and consequently, the aPDT can completely disrupt the biofilm.

Thus, the aim of this work was to evaluate the synergism between aPDT and EP against the S. aureus biofilm, detecting, mainly, the effect of this on the *S. aureus*-EPS components (proteins and carbohydrates).

Material e Methods

Biofilm formation: The *S. aureus* biofilm was formed on cellulose membrane using the:
The method described by Kim et al [3], with slight modifications.
aPDT and Electroporation: The S. aureus biofilm was incubated with 1 mg mL-1 of methylene blue (MB) for 15 minutes and
subsequently the electroporation was performed by the following parameters: two electric pulses at 1000 Vcm−1, 50μs long, 1-Hz frequency. After, the biofilm was irradiated by a LED (red light - 630 nm) for 20 minutes. Control studies were performed. Cell viability was determined using XTT assay and the biofilm structure was visualized by scanning electronic microscopy (SEM), as described by Chandra et al [4].
EPS analysis: Before the biochemical analysis, the EPS was extracted using NaOH treatment and the proteins and carbohydrates were determined by Bradford and anthrone methods, respectively [5].

Results and discussion:

Table 1 shows the survival index (SI) of S. aureus biofilm through the measurement of its metabolic activity (XTT assay). The viability of S. aureus after only aPDT treatment or only EP was around 45.4% and 93.1% respectively, while the synergism between them promoted a significant decreasing in the SI of the bacteria biofilm (~5.08%). This synergic effect can be visualized in the Figure 1, showing *S. aureus* biofilm before (control) and after the treatment that significantly decreased the number of cells, caused a morphologic damage to the bacteria and eliminated the presence of EPS.

Table 1: *S. aureus* biofilm survival index (SI). Carbohydrates and proteins content of EPS extracted from *S. aureus* biofilm.

Conditions	*Survival index* (%)	*Proteins* (µg/mL)	*Carbohydrates* (µg/mL)
Control	100±0.50	123.1±2.5	78.9±3.8
Light only (630nm)	95.5±0.2	120.3±1.5	73.6±1.2
MB(1mg mL^{-1})	98.6±1.2	122.1±1.0	72.8±2.3
aPDT	45.4±1.0	30.8±5.0	15.5±4.2
EP	93.1±1.1	118.5±2.0	71.9±2.8
aPDT + EP	5.08±0.8	10.2±2.8	3.9±3.1

In addition, aPDT+EP reduced 91.71% and 95.05% of proteins and carbohydrates present in the EPS extracted from *S.aureus*
biofilm. The effect of the red light or MB alone did not caused *S. aureus* biofilm reduction, as the EP only condition.

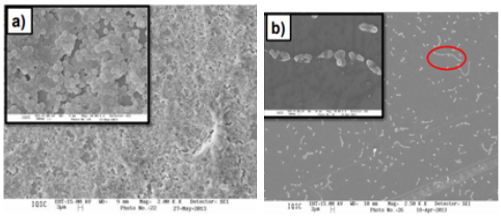

Figure 1. *S. aureus* biofilm before (a), and after treatment of aPDT + EP (b)

The main results heightened permeability likely facilitated the efficient penetration of the photosensitizer (PS) into the deepest layers of the biofilm, reaching the underlying cells. As a result, this synergistic interaction between electroporation and antimicrobial photodynamic therapy (aPDT) could have substantially intensified the effectiveness of aPDT by targeting the biofilm's bottom layer and its associated deeper cells [4,5]. The therapy targets various components of the biofilm, such as proteins, lipids, and nucleic acids found within the biofilm matrix. It inhibits the growth of cells within the extracellular polymeric substance (EPS) as well. Recent advancements in the development of new photosensitizers (PSs) [6] aim to increase the production of reactive oxygen species (ROS). Combining aPDT with other therapies, particularly pulsed electric fields (PEF), has been instrumental in achieving improved biofilm inhibition. PEF has demonstrated antimicrobial properties by triggering extensive chemical reactions when electric fields are applied. This treatment approach not only ruptures the microorganisms' membranes but also generates reactive compounds, including free oxygen, hydrogen, hydroxyl, and hydroperoxyl radicals.

By specifically targeting the bottom layer of the biofilm and its associated deeper cells, this combined strategy has the potential to significantly enhance the outcomes of biofilm treatment. Further studies and investigations are warranted to validate and optimize the utilization of electroporation in conjunction with aPDT for effective biofilm eradication.

Conclusions

We may suggest that the EP possibly increased the EPS permeability allowing the PS to reach the biofilm bottom layer and consequently the deeper cells, intensifying aPDT effect.

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