SYNERGISM BETWEEN aPDT and PULSED ELECTRIC FIELDS: AN INNOVATIVE STRATEGY TO OVERCOME BIOFILM INFECTIONS

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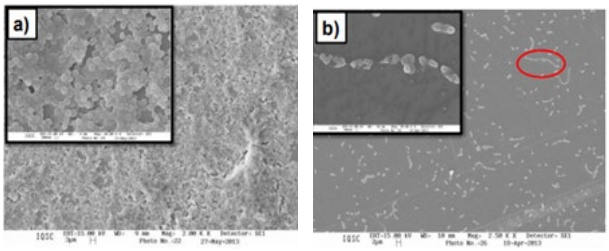
Currently, biofilms have been the cause of a wide variety of infections in the human body, reaching 80% of all microbial infections [1]. The bacteria *Staphylococcus aureus* is a leading cause of hospital-acquired infections. The biofilms present specific properties such as the extracellular polymeric 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 to produce highly reactive oxygen species (ROS), which leads to the oxidation of several cellular components [2]. Even though this therapy showed to be efficient to attack 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, it is necessary to combine the aPDT with a promising approach, such as electroporation (EP). 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. This works aimed 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).

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 decrease in the SI of the bacteria biofilm (~5.08%) (Table 1). This synergic effect can be visualized in Figure 1, showing *S. aureus* biofilm before (control) and after the treatment that significantly decreased the number of cells, caused morphologic damage to the bacteria and eliminated the presence of EPS. 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 cause *S. aureus* biofilm reduction, as well as the EP alone.

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±2.58	78.9±3.8
Light only (630nm)	95.5±0.25	120.3±1.58	73.6±1.2
MB(1mg mL^{-1})	98.6±1.20	122.1±1.03	72.8±2.3
aPDT	45.4±1.02	30.8±5.03	15.5±4.2
EP	93.1±1.10	118.5±2.05	71.9±2.8
aPDT + EP	5.08±0.85	10.2±2.81	3.9±3.1

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



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.

References

[1] Jamal, M., et al., J Chin Med Assoc. 81, 7-11 (2018).
[2] de Melo, et al., Exp Rev Anti-Infect Ther. 11, 669-693 (2013).
[3] Kim, Y.W., et al., Biof Microb, 1, 15016 (2015).
[4] Chandra, J., et al., J Dent Res, 80, 903-8 (2001).
[5] Wu, J. and C. Xi, Appl Environ Microbiol, 75, 5390 5 (2009).