Elektroporacijos ir fotodinaminės terapijos poveikis *Staphylococcus aureus* bioplėvelės matricai

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

Wanessa Melo[1], Viktorija Juscenko1[1] and Arunas Stirke[1]
[1]Laboratory of Bioelectric, Department of Functional Materials and Electronics, State Research Institute Center for Physical Sciences and Technology, Savanorių pr. 231, 02300 Vilnius, Lithuania

wanessa.melo@ftmc.lt

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 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, 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.

Thus, the aim of this work was 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).

Methods:

Biofilm formation: The *S. aureus* biofilm was formed on cellulose membrane using 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. The cuvettes used fitted with electrodes with 0.1 cm between embedded aluminum plates. After, the biofilm was irradiated by a LED (red light - 630 nm) for 20 minutes. Control studies were performed. Cell viability was determined by using XTT assay and the biofilm structure visualized by scanning electronic microscopy (SEM), both as described by Chandra el al [4].

EPS analysis: Before the biochemical analysis, the EPS was extract using NaOH treatment and the proteins and carbohydrate were determinate 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. 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.

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

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.

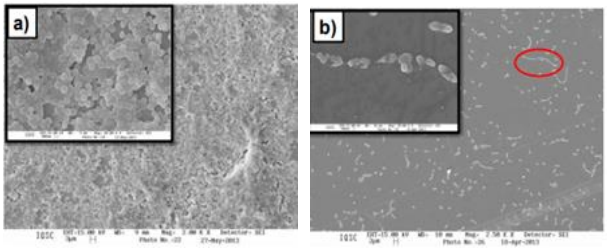

Figure 1. *S. aureus* biofilm before (a) and after treatment of aPDT + EP (b)

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).