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Advanced microscopy applied to the study of photodynamic mechanism in bacteria

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Abstract
Photodynamic therapy (PDT) is an emergent technique used in clinical treatments for several pathologies, in which the photoactivation of photosensitizer molecule leads to cell death. Our project investigates the photodynamic effect of Protoporphyrin IX (PpIX), one of the currently most used photosensitizers, in prokaryotic cells, in order to explore its use in sterilization. Atomic Force Microscopy (AFM) and Wide Field Microscopy (WFM) were used to evaluate PDT effects on X.fastidiosa cells.

Key words:
Protoporphyrin, Photodynamic therapy, bacteria

Introduction

First PDT results date from 20th century. Since then, many applications of this therapy have been developed. Nonetheless, in the last decade the interest in antimicrobial effects of PDT has been growing, since its mechanism is based in reactions of the photosensitizer that lead cells to death[1]. The project in question studies the effect of PpIX in bacteria, *Xylella fastidiosa*. PpIX presents fluorescence when excited by blue light and is activated (i.e., starts the reactions that kills cells, likely by the production of reactive oxygen species) when excited by red light. Based on this property, experiments showed PpIX inside animal cells; more recent studies show that this photosensitizer localizes specifically on mitochondria.[2] This project investigates the interaction mechanism between PpIX molecule and bacteria cells, using microscopy techniques, to probe the morphological and structural properties of the cell membrane.

Results and Discussion

One of our first goals centered on finding evidences of the presence of porphyrin in the cell membrane, otherwise the photodynamic effect is not possible. Thus, in order to avoid more complex interactions due to cell metabolism, we used bacteria grown in a medium with low nutrient concentration, in stress state. The photosensitizer molecule PpIX was added to such cell cultures, which were compared with a control sample with no exposition to PpIX. Those samples were analyzed by WFM and AFM. The control sample presents very low autofluorescence, while the excitation with blue light warrants a large fluorescence signal if PpIX is present on the cell membrane. Figure 1 shows that individual cells and biofilms on the sample exposed to the photosensitizer show a large fluorescence signal as expected if the molecule couples to the bacterium membrane. AFM images suggest that membrane lysis may occur.

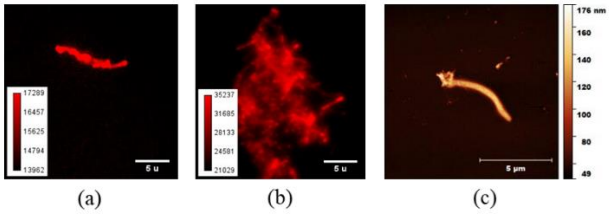

Image 1: (a) Image from WFM of control sample; (b) Image from WFM and (c) Image from AFM both of sample with porphyrin.

Once the presence of PpIX on cells was determined, a second experiment was used to find the best parameters to activate the photosensitizer. Different times of treatment (PpIX incubation/excitation) were tested using WFM fluorescence levels to determine the best parameters for this process.
These parameters were then applied for a different experiment, now in a growth medium rich in nutrients. In general, similar results (WFM signal levels) were obtained, showing the presence of PpIX on the cell membrane. However, the spatial distribution of the fluorescence signal shown in WFM images is different, suggesting that the photosensitizer molecule couples on a specific and limited region of the bacterium in this case.

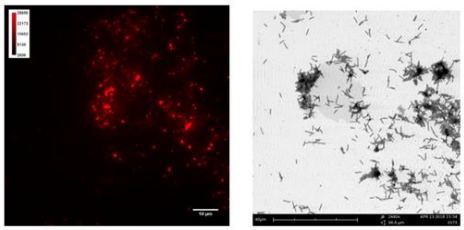

Image 2: Images of the sample with PpIX in growth medium rich in nutrients from: (a) Fluorescence microscope and (b) Scanning electron microscope.

Conclusions

Based on the experiments we can conclude that PpIX couples to the bacterial cell membrane; the best treatment conditions to be used on the samples were also determined. Furthermore, differences on PpIX spatial distribution related with the growth medium used were observed, but further experiments are necessary to understand this result. AFM images suggest cell lysis due to the presence of PpIX. However, more experiments are necessary in order to test cell viability after treatment.

Acknowledgement

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[1] B.W. Genderson, T.J. Dougherty, Review article – How does photodynamic therapy work? *Photochemistry and photobiology*, **55,** (1992), *145-157*.

[2] E.R Reis et al., *Comparative study of phototoxicity of protoporphyrin IX synthetic and extracted from ssp Rattus novergicus albinus rats toward murine melanoma cells. European Biophysics Journal* (2018).