Primary cellular targets of MB-APDT in bacteria and yeast

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Antimicrobial photodynamic therapy (APDT) is a promising tool to counterattack the emerging threat of drug-resistant pathogens. The mechanisms of action of APDT are often discussed as a generalized oxidation of all cellular structures. However, since some APDT-produced reactive oxygen species (ROS; *e.g.* $^1O_2$ and ·OH) present diffusion-limited reactivity towards biomolecules, cell damage should be mostly co-localized with photosensitizer accumulation site. Hence, understanding photosensitizer accumulation and the most damaged cellular structures in the nanoscale can bring important insights to the photophysical, photochemical and biochemical mechanisms of photodynamic therapy. In this study, we developed an experimental strategy to investigate which are the primary cellular targets for methylene blue (MB) mediated APDT in pathogenic yeast (*Candida albicans*), Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) bacterial cells. We used a series of advanced microscopy techniques, as well as electrophoresis and optical spectroscopy analysis to understand what are the main photosensitizer interaction sites and the predominant ultrastructural damages. Our data suggest that MB-APDT mainly degrades intracellular components (e.g. proteins and nucleic acids) while surface structures, such as cell membrane and wall, are minimally affected. Therefore, we concluded that even though ROS can react with virtually any biomolecule, photosensitizer interaction sites tend to locally concentrate most oxidative damages even in single-compartment cells such as bacteria.