Detection of intracellular ROS formation in *Candida albicans* during antimicrobial photodynamic therapy

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One of the challenges for scientific *in vivo* research is the detection of reactive oxygen species (ROS), because it requires methods and tolls specific for a deep investigation of biologic process. 2,7-dichlorofluorescein (DCF) in its diacetate form offers an interesting option to investigate the general oxidative stress induced in cells and tissues. The purpose of this study was to demonstrate, in real-time, the intracellular production of ROS in *Candida albicans* during normal metabolism, under oxidative stress induced by external hydrogen peroxide and photodynamic antimicrobial therapy (PAT) using methylene blue as a photosensitizer. A suspension of *C. albicans* was incubated with a solution of 10μM of DCFH-DA for 15 min in dark conditions allowing the DCFH-DA to penetrate the cell membrane and be hydrolyzed by intracellular esterases to form the nonpermeable DCFH. After incubation time, the cells were washed, centrifuged and the supernatant was removed. The cells were then re-suspended in a buffer solution to withdraw all DCFH-DA not adhered to the microorganism. A second suspension of *C. albicans* was incubated as described above, and received 100μL of hydrogen peroxide at 1M, after 1 minute. Another suspension of microorganisms received for 10min. 60μM of MB in buffer solution followed by irradiation using a red laser with output power of 40mW and fluence of 60J/cm^2. For the fluorescence microscopy analyze, the cells were placed in glass slides and an optical and a fluorescent microscopy image was captured each minute for 8 minutes. The images were analyzed using the software Image J. Our results suggest that the use of microscopy fluorescent images is a fast and easy way to quantify ROS production inside live microorganisms and allow a real-time investigation.