Evaluation of photodynamic action over extracellular slime and bacterial biofilm

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Photodynamic antimicrobial therapy (PAT) may become a useful clinical tool to treat microbial infections. On clinical practice, microbial biofilm would be the main target for PAT. Therefore, the aim of our work was to verify the damage caused by photosensitization over bacterial biofilm via atomic force microscopy (AFM) and scanning electron microscopy (SEM), looking for structural changes that might occur on extracellular matrix after PAT. For AFM images, cells culture were grown until a stationary phase to reach a concentration of approximately 10^8 cells/mL, allowing the production of extracellular slime in a biofilm-like structure. The cells, including the extracellular matrix, were put in a microscopy slide and its structure was observed using AFM; subsequently, a water solution of methylene blue at 60μM was applied over the cells and a pre-irradiation time of 3 minutes was waited and followed by illumination with a diode laser (λ=660nm, output power 40mW, 3 minutes, fluence 180J/cm^2, beam diameter 0.04cm^2). The same cells were observed and the images stored. A second set of experiments was performed with biofilm developed on dental root canal; the biofilm was imaged via SEM before and after PDT with the same parameters as abovementioned. The results showed alterations on cellular scaffold and mostly on the extracellular slime. The slime is targeted by the photosensitizer, and after irradiation a destruction of the matrix was observed. SEM images showed that the matrix after PAT disappeared allowing the visualization of the dentin tubules, while the AFM images showed a marked reduction of the slime. These findings indicate that extracellular matrix seems to be the primary target for the photosensitizer, therefore, it protects the cells, acting as a physical barrier between photosensitizer and cells and also reacting with the reactive oxygen species.