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Background: The aim of this study is to test the application in photodynamic therapy protocols of different laser wavelengths in combination or not with different photosensitizing dies on Candida albicans biofilms with or without a synthetic killer decapeptide (KP).

Study: For this study, C. albicans SC5314 was grown on Sabouraud dextrose agar plates at 30 °C for 24 h. Cells were added to RPMI 1640 buffered with MOPS and cultured on resin discs of polymethyl methacrylate. A previously described synthetic killer decapeptide (KP) was used in this study. The fungicidal activity of KP, associated or not with PDT protocol applications, on C. albicans biofilms adhered to acrylic discs was evaluated *in vitro* by the XTT assay. For scanning electron microscopy (SEM), 5 ml of yeast cell suspension were put onto resin discs within each well. Protocols were realized with three different wavelengths and colors: red diode and toluidine blue, blue-violet diode and curcumin and green diode and erythrosine. Laser irradiation has been performed in continuous mode at a fluency of 10 J/cm^2.

Results: The most effective dye, when used without laser, was curcumin that showed a significant effect on C. albicans biofilm in comparison with the untreated control ($p < 0.0001$), but also in comparison with toluidine blue and erythrosine ($p < 0.0001$). In the comparison with the untreated control, the application of red diode laser with or without toluidine in combination with KP treatment showed a statistically significant result ($p < 0.0001$), but the combination of dye and KP defined the same significant result without laser application ($p < 0.0001$). In the comparison with the untreated control, the application of blue diode laser with curcumin in combination with KP treatment showed a statistically significant result ($p = 0.0006$), but the combination of dye and KP defined the same significant result without laser application ($p = 0.0001$). In the comparison with the untreated control, only the application of green diode laser without erythrosine in combination with KP treatment showed a statistically significant result ($p = 0.0002$).

Conclusion: The combination of laser light and right color for the used wavelength, together to the use of a synthetic killer decapeptide (KP) may change dramatically the results in terms of antimicrobial effect.

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APPLICATION OF PHOTODYNAMIC THERAPY WITH LED AND METHYLENE BLUE IN STREPTOCOCCUS MUTANS IN THE PRESENCE OF GLUCOSE- *IN VITRO* STUDY

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Background: Streptococcus mutans is a microorganism associated with tooth decay; many genes that express adhesion, biofilm formation, extracellular polysaccharide, carbohydrate uptake and acid tolerance regulate its cariogenic properties. S. mutans inhabits a complex biofilm and it produces a large amount of exopolysaccharides to promote adhesion and enabling transport pumps. Photodynamic therapy involves the use of a photosensitizer (PS), which is absorbed by specific cells followed by irradiation with visible light, resulting in cell death. The aim of this study was to investigate antimicrobial photodynamic therapy on Streptococcus mutans in the presence of glucose.

Study: Streptococcus mutans was grown in brain heart infusion (BHI) at 37 °C for 48 h. Inocula were prepared with pure colonies, which were suspended in phosphate buffered saline (PBS) with and without 50 mM glucose. One-hundred micromolar methylene blue was used as photosensitizer and the experiments were performed with groups (control, irradiated with LED, FS without irradiation, and PDT 30, 60, and 120 s). Colony form units were counted and statistically analyzed (one-way ANOVA and Tukey 5%).

Results: The irradiation as well as the photosensitizer in the dark did not cause cell death. In contrast, in experiments without glucose, PDT caused cell death proportional the amount of light used. The more light, the higher the inactivation of S. mutans and after 2 min a reduction of 7 orders of magnitude (100%) was observed. In experiments with glucose cell death was observed even increasing the radiant exposure.

Conclusion: We concluded that PDT is a viable solution for inactivation of S. mutans in suspension, and that the presence of glucose activates efflux pumps in the bacterial cell wall, and it drastically reduces the effect of PDT.

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SINGLET OXYGEN IS MORE EFFECTIVE THAN RADICALS TO KILL C. ALBICANS BY aPDT

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Background: Antimicrobial photodynamic therapy (aPDT) is based on the combination of a photosensitizer, light and oxygen. In Brazil, methylene blue (MB) is a well-known photosensitizer due to its low cost and effectiveness. However, MB has its photochemical action mechanism modulated by its aggregation state, i.e., depending on the vehicle in which MB is used, it has a tendency to occur as monomers or dimers. Is was already shown that while monomers generates Type II reactions (singlet oxygen) dimers triggers Type I reactions (radicals).

Study: In order to evaluate if one of the photochemical mechanisms is more effective to kill Candida albicans, methylene blue in different states of aggregation was evaluated in planktonic culture. Besides the photobiological analysis assessed by counting CFU/mL, in which MB concentrations (0–100 μg/ml), incubation interval (1–20 min) and irradiation interval (0–30 min) were tested, the dimer-monomer ratio was determined by absorption spectroscopy.

Results: The results showed no statistically significant differences between the irradiation intervals used (0–30 min) and between the incubation intervals (1–20 min). The MB lowest concentrations (10 and 20 μg/ml) were the most effective for Candida albicans inactivation. This result breaks the paradigm that the highest MB concentrations are related to greater effectiveness. This observation is associated with MB physicochemical properties in aqueous medium and the photochemical mechanism. At the concentrations of 10 and 20 μg/ml the dimer/monomer ratio is approximately 0.40, while at higher concentrations, 50 and 100 μg/ml, the ratio increases to 0.50 and 0.64, respectively.

Conclusion: Thus, at the most effective concentrations,, i.e., the lowest ones, the amount of monomers is greater than dimers and consequently, the singlet oxygen generation is bigger than