ANTIMICROBIAL EFFECT ON *CANDIDA ALBICANS* BIOFILM BY APPLICATION OF DIFFERENT WAVELENGTHS AND DYES AND THE SYNTHETIC KILLER DECAPEPTIDE KP

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This *in vitro* study aimed to test the application of different low fluence laser wavelengths with and without proper photosensitizing dyes on *Candida albicans* biofilm with or without a synthetic killer decapeptide (KP).
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1. Introduction

Fungal infections, mainly those caused by *Candida albicans*, have an important impact on human health particularly because of the growing number of immunocompromised hosts [1]. Systemic mycoses, associated to a poor outcome, can often occur in these patients, and mortality due to invasive candidosis is estimated up to 40% [2].
The main objective of this study was to apply different laser wavelengths at a low fluence in combination or not with proper photosensitizing dyes on in vitro *C. albicans* biofilm mimicking the in vivo infection. In addition, the previously described antibody-derived decapeptide KP, endowed with anticandidal activity [3], was also used in combination with laser and dyes application.

2. Materials and Methods

Microbial strain and culture conditions for biofilm formation
The reference strain *C. albicans* SC5314, selected for its ability to form biofilm, was grown on Sabouraud dextrose agar (SDA) plates at 37°C for 24 h. Cells were suspended in RPMI 1640 medium buffered with 3-(N-morpholino)propanesulfonic acid (MOPS) at a concentration of 106 cells/ml using the McFarland turbidity standard. Yeast suspensions were cultured in flat-bottomed 96-wells plates (100 µl/well).
Plates were incubated 18 h at 37°C on an orbital shaker at 180 rpm to allow biofilm formation. After 18 hours, plates were checked for the homogeneity of biofilm growth under optical microscope (Zeiss, Model Axiovert A1, Germany) at 4× and 10× magnification.

3. Results and Discussion

Application of red, blue and green lasers alone did not kill *C. albicans* cells, nor combined treatment with toluidine blue, red laser and KP did. On the contrary, combined treatment with blue laser, curcumin and KP, as well as green laser, erythrosine and KP led to death most *C. albicans* cells.
Evaluation of *C. albicans* biofilm inhibition was realized in this study by means of the XTT assay, a method for quantitative evaluation of metabolic activity that is not affected by inter-operator variability and is easier to perform than the CFU assay. Moreover, literature highlighted the limitation of CFU count for *C. albicans* biofilms as multicellular fungi, while presenting a large biomass compared to single yeast cells.

4. Conclusion

In our study, curcumin and erythrosine at the chosen concentrations proved to be able to significantly reduce C. albicans biofilm viability even without KP or laser association The potential advantages of APDT in terms of reduced costs, scarce side effects, low overdose risk and unlikely resistance induction, are compatible with the optimization of APDT protocols in the future

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