The cell biocompatibility of human pulpal fibroblasts after photodynamic therapy

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Introduction: The persistence of bacterial infection of dentin and pulp tissue caused mainly by the difficult to adequately achieve cavity sealing is the main factor of reduced clinical success of pulp conservative treatments, *i. e.*, pulp direct capping (Coehn, 2004). As an alternative to the usual removal of the infecting organisms by drilling and preserve more tissue, an innovative proposition is to kill the organism *in situ* (Wilson, 2004). If PDT *in vivo* could eradicate bacteria from carious lesions, better pulp healing and less traumatic treatments would be performed. Therefore, the purpose of this study was to verify the cytotoxicity of this therapy *in vitro* using PDT parameters similar to those that may be applied in a clinical setting.

Methods: *Cell Culture:* Primary cultures of human pulp fibroblasts were obtained from a sample of ten (n=10) third molars scheduled for extraction conformed to a protocol approved by Ethic Committee from Universidade Federal de Minas Gerais. The cells were cultured in Dulbecco´s Modified Eagle´s Medium supplemented with 20% of fetal bovine serum, 1% penicillin, 1% streptomycin and 0.2% fungizone. Cells were grown to confluence and only 3 from 5 passages were used in this study. *PDT:* Pulp fibroblasts were seeded (1×10^{-5}) in 96-well plates and tetraplicate cell culture was exposed to a PDT protocol considering methylene blue (MB) (Chimiolux, Aptivalux, Belo Horizonte/MG) as the photosensitizer in three different concentrations (12.5, 25.0 and 50.0 µg/mL). The pre-irradiation time (PIT) varied 3 from 5 minutes for each concentration of MB. The cells were than sensitized by a white light source from a 400W tungsten filament lamp (Curing Light 3M Espe®, USA) during 1minute, divided in 2 applications of 30s and an interval of 30s between then to prevent excessive heat. Controls were (1) untreated cells, (2) cells exposed to light in the absence of MB, and (3) treated with MB for 3 and 5 minutes but not exposed to light. *Measurement*: Cell viability was evaluated by the Neutral Red (NR) assay and the mitochondrial activity was determined using the 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) microculture tetrazolium assay (Invitrogen, São Paulo/SP). The absorbance of each well was measured at 540 nm for NR and 570 nm for MTT. *Statistical Analysis:* For this experimental study, each combination of assay mean optical density values were evaluated by one-way analysis of variance (ANOVA). Comparisons between groups were performed by a Tukey test.

Results: Light alone had no effect on cell viability and mitochondrial activity in all groups. MB alone had little effect on proliferation (less than 9.5% of reduction) and on cell viability (NR) (less than 23%). Exceptional result was obtained for 50 µg/ml concentration in the MTT assay, which significantly increased the cell proliferation ($p<0.05$).

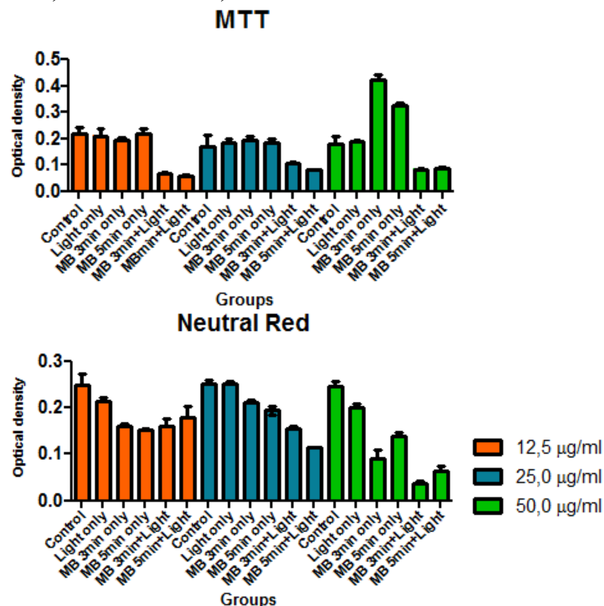

Fig.1. Control: No Light/No MB; **Light only:** No MB; **MB 3 min only:** MB 3 min/No light; **MB 5 min only:** MB 5 min/No light; **MB 3 min + Light:** PDT; **MB 5 min + Light:** PDT.

The opposite result was observed by NR assay, where this concentration reduced the cellular viability ($p<0.05$). These results could be due to the strong blue color of the highest concentration of MB tested interfering with color of MTT assay. MB and exposed light groups reduced significantly cell activity compared with control ones in all assays. For MTT assay, concentrations of 12.5µg/ml, 25µg/ml (only 3 minutes of PIT) and 50µg/ml were significantly different, with mitochondrial activity reduction ranging 40 to 70% for tests groups. These results were confirmed by NR assay, where all concentrations, except 12.50 µg/ml (only PIT of 5 minutes), were statistically different from controls with reduction of 36 to 85%. Comparing these groups with MB only group no significant results could be found in all tests, at any concentration. The difference in PIT did not influenced the results in all groups ($p>0.05$). In accordance to this work, Ribeiro *et al.* (2010) tested a kind of PDT in MDPC-23 odontoblasts-like and L929 gingival fibroblasts cells and found that this therapy caused intense toxic effects to both cell lines evaluated.

Conclusions: PDT protocol tested in this study caused severe cytotoxic effects in human pulp fibroblasts primary cell cultures. Although these cells are more susceptibility for *in vitro* cytotoxicity tests, further studies are necessary to find a PDT protocol able to kill cariogenic microorganisms selectively without affecting host cells, before indicating this therapy for clinical appliance.

References: (Coehn, S. Pathways of the pulp. Elsevier, 2007.), (Wilson, M. Photochem Photobiol Sci. 2004; 3:412-18.), (Ribeiro, APD. Cell Biol Intern. 2010; 34:343-51.).

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