Application of Low Level Laser on skin cell lines

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INTRODUCTION: Lasers have emerged as powerful tools for tissue engineering. To examine cellular growth, and cell to cell interactions, *in vitro* skin models have been developed combining two major cell types of skin, keratinocytes and fibroblasts. The main objective of the study is to construct 3D skin model for photodynamic therapy (PDT) investigation in skin cancers. Prior to using PDT on skin models, monolayer cultures are first used to investigate optimal dose of light and concentration of PDT drugs to be used for skin cells. In the current study, proliferation of cells (keratinocytes and melanoma), was evaluated for the possibilities of cancerous cells recovering after PDT treatment using low level laser light.

METHODS: Human skin keratinocytes (CRL2310) and melanoma (UACC62) cells were grown in their respective growth medium (Keratinocyte Basal Medium and RPMI medium) supplemented with growth factors (Whitehead Scientific). The cultures were incubated at 37°C with 5% CO_2 humidity. Cells were trypsinized using a 0.25% (w/v) trypsin-0.05% EDTA solution (Whitehead Scientific) and counted in a haemocytometer.

Photosensitization of cells

Cells were seeded into 24 well tissue-culture plates at a density of 3.0×10^3 cells/well (keratinocytes) and 1.5×10^3 melanoma cells in 1 ml of KBM or RPMI medium, and allowed to attach for 48 hours before being washed twice with 2ml PBS, then photosensitized by the addition of serum-free medium without additives but only 10 µg/ml mixed-sulfonated zinc phthalocyanine (ZnPcSmix). Control wells contained medium without ZnPcSmix. Plates were incubated at 37°C in 5% CO_2 in the dark for 18 hours followed by irradiation with a diode laser with output power of 45 mW, with power density of 33 mW, at a wavelength of 672 nm for 30s. The diameter of the laser beam was 1.32 cm to give a laser dose of 1 J/cm^2. Following irradiation, cells were cultured in their respective medium and returned to the incubator for further 72 hours. Cell morphology of treated and untreated cells were observed using an inverted microscope and digitally recorded.

RESULTS: Morphology of CRL2310 and UACC62 cells in culture was monitored under the inverted microscope at 32x magnification. The results indicated that addition of ZnPcSmix to normal human keratinocytes and cancerous melanoma cells exposed to 1 J/cm^2 diode laser, showed no proliferation of cells indicated by changes in cell morphology and detachment of cells from the surface of the flask, see Figure 1 (proliferation) and Figure 2 (no proliferation).

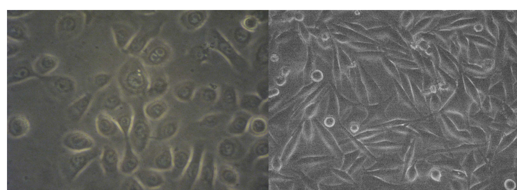

Fig. 1: Untreated keratinocytes (left) and melanoma cells (right).

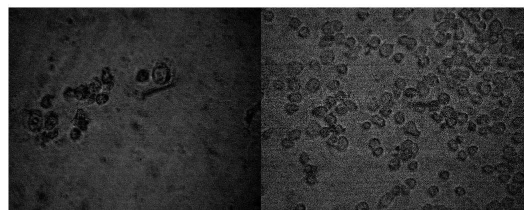

Fig. 2: Keratinocytes (left) and melanoma cells (right), 72hrs after PDT treatment

DISCUSSION & CONCLUSIONS: The results in Figure 2 (left) and (right) show that there is no proliferation of cells but cell death in PDT treated cells 72hrs post PDT treatment. Therefore low level laser can be a relevant medical device for treatment of skin diseases and tumors.

REFERENCES: [1]Fabris, C., Soncin, M., Miotto, G., Fantetti, L., Chiti, G., Dei, D., Roncucci, G. and Jori, G (2006) *Journal of Photochemistry and Photobiology Biology* **83**: 48–54. [2]Oleinick, N. L. and Evans H. H (1998) *Cellular Targets and Mechanisms Radiation Research* 150(5): S146-S156.

ACKNOWLEDGEMENTS: This project was made possible by the CSIR, National Laser Centre; Prof. Heidi Abrahamse, Laser Research Group, University of Johannesburg; Ms N Kolesnikova, CSIR, Biosciences and Prof. Tebello Nyokong, Department of Chemistry, Rhodes University.