Impact of Varying Intensities of Blue-Light Exposure on 3T3 cells

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INTRODUCTION: There is the need to develop a compatible sterilisation method for hybrid biomaterials. High-intensity blue light in the 405 nm region has been shown to be an effective bacterial decontamination method [1], to cause no noticeable damage to the gross structure of type-I collagen monomer (when treated at 10 mW/cm^2) [2], and to have no noticeable effect on 3T3 cell viability, growth rate, redox state or lactate dehydrogenase (LDH) leakage (at 1.0 mW/cm^2) [2]. The purpose of this research was to investigate the effect of varying the blue-light intensity on the 3T3 cell response parameters.

METHODS: 3T3 cells, at a seeding density of 2 x10^4 cells/cm^2, were exposed to the blue-light source at intensities of 10, 1 and 0.1 mW/cm^2, for 1 hour. Cell responses were measured for up to 4 days post treatment using the MTT and neutral red (NR) microplate assays, LDH leakage and the intracellular levels of reduced glutathione (GSH) and protein.

RESULTS AND DISCUSSION: At treatment intensities of 0.1 and 1 mW/cm^2 there was no significant negative effect on any of the response parameters. For example, MTT was 150 ±4% of control cells, NR was 102 ±1%, LDH leakage 70 ±4% and GSH 112 ±8% 1 day after treatment with blue-light at 0.1 mW/cm^2. Figure 1 shows that, in contrast, treatment with 10 mW/cm^2 had a negative effect on cell responses 1 day after treatment.

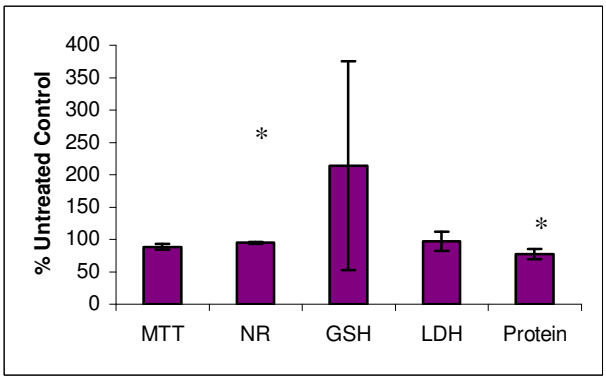

Fig. 1: Effect of blue-light treatment at 10 mW/cm^2 on 3T3 cell response parameters at 1 day post-treatment. Statistical analysis was carried out using ANOVA followed by Dunnett's test, at the 95% level.

A small drop in viability after 1 day was observed but was found only to be significant using the NR assay. Treatment at 10 mW/cm^2 had no significant effect on LDH leakage, therefore it does not appear to compromise cell membrane integrity. The most notable effect of blue-light treatment at 1 day was on intracellular levels of GSH, where an increase was observed (0.030 ±0.022 GSH/mg protein compared to 0.014 ±0.004 GSH/mg protein for the untreated control). It is known that blue-light causes excitation of endogenous porphyrins, generating light-induced reactive oxygen species (ROS). The increased GSH levels observed suggest that the blue-light at 10 mW/cm^2 results in the production of ROS and induces a state of oxidative stress within the cells. This effect was reversible, and by 2 days post treatment the GSH levels were comparable to those of the untreated control (0.038 ±4 and 0.044 ±4 GSH/mg protein, respectively), providing evidence of recovery. The cell growth rate also showed evidence of recovery post-treatment with all control and treated cultures reaching confluence at day 3.

CONCLUSION: Blue light treatment at intensities of 1 mW/cm^2 and lower has no significant affect on 3T3 cell response parameters. This finding together with the lack of effect on type I collagen suggests that blue light shows excellent potential to be utilised as a sterilisation method for hybrid biomaterials.

REFERENCES: [1] M. Maclean, S MacGregor, J Anderson et al (2008) *FEMS Micro Let* **285**(2): 227-232. [2] S. Smith, M. Maclean, S. MacGregor et al. (2009) *IFMBE Proceedings* **23**: 1352-1355.

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