Effect of HINS light on the contraction of fibroblast populated collagen lattices

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INTRODUCTION: High intensity narrow spectrum (HINS) light has been shown to have bactericidal effects on a range of medically important bacteria[1]. HINS technology could potentially be useful as a method for disinfecting medical implants, tissue engineered constructs and wounds. The fibroblast populated collagen lattice (FPCL) was used as an in vitro model to investigate the effect of HINS light on the wound contraction phase of wound healing.

METHODS: Collagen lattices (0.3% (w/v) type I rat tail collagen) were seeded with 3T3 mouse fibroblasts cells at a density of $2.5x10^4$ cells/cm^2 and allowed to contract freely. FPCLs were treated with HINS light at 0.1, 1, and 10mW/cm^2 for 1 h, equivalent to a dose of 0.36, 3.6 or 36 J/cm^2 respectively. The contraction of FPCLs was measured prior to, and for up to 7 days following treatment. At 24, 48 and 120 h post treatment, cells were counted using the MTT assay, after using collagenase to release the cells from the lattices. At these same time points, FPCLs were stained with propidium iodide (PI) and acridine orange (AO) to assess cell viability by fluorescence microscopy.

RESULTS: Figure 1 shows that no significant difference was observed between contraction rates of untreated FPCLs and those treated at 0.1mW/cm^2 and 1mW/cm^2. FPCLs treated at 10mW/cm^2 stopped contracting immediately and did not recover significantly within 7 days.

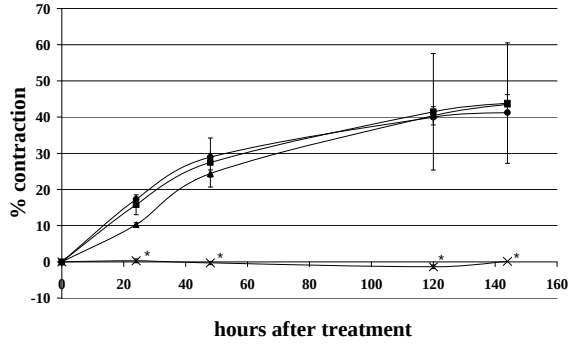

*Fig. 1: Effect of HINS light on the contraction of FPCLs. Treatments were carried out, at 0.1, 1 and 10mW/cm^2 (squares, triangles and crosses respectively) for 1 hour. Control is indicated by circles. The percentage contraction, of the FPCL area, was calculated from the point of treatment. *indicates significant difference from control at each time point (P>0.05, ANOVA followed by Dunnett's test)*

The MTT assay results show that for up to 120 h post treatment, there was no significant difference in cell number when treating FPCLs at 0.1mW/cm^2 and 1 mW/cm^2 (see Figure 2). Treatment at 10mW/cm^2 for 1 h caused an approximate 80% decrease in cell number after 24 h. By 120 h post treatment, cells in FPCLs treated at 10mW/cm^2 did not show significant recovery.

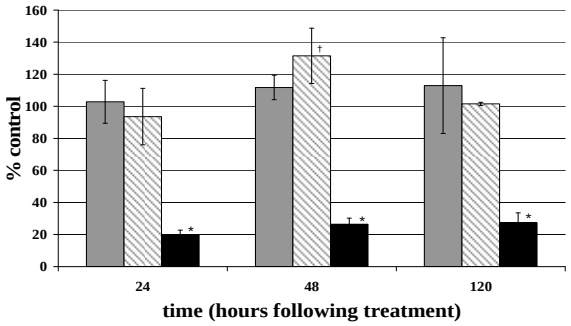

*Fig. 2: MTT assay results for FPCLs treated at 0.1, 1 and 10mW/cm^2 (grey, shaded and black respectively) at 24, 48 and 120 hours post treatment. *P<0.5, comparing between treatments at each time point, †P<0.5, comparing each individual intensity over the time points (ANOVA followed by Fisher's test for both comparisons)*

Microscopic assessment of cell viability using PI and AO staining confirmed these results.

DISCUSSION & CONCLUSIONS: The results show that HINS light treatment at values of 0.1 and 1mW/cm^2 have no detrimental effect on FPCL contraction. This technology has considerable potential to augment efforts to disinfect medical devices, tissue engineered constructs and implants.

REFERENCES: [1] M. Maclean, S.J. MacGregor, J.G. Anderson, et al (2008) FEMS Microbiol Lett Aug;285(2):227-32

ACKNOWLEDGEMENTS: RM is supported by the ESPRC Doctoral Training Centre (DTC) in Medical Devices.