The effects of laser irradiation on proliferation in osteosarcoma cell lines (MG63 and U2OS)

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The effect of laser irradiation on proliferation of two different osteosarcoma cell lines (MG63 and U2OS)

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INTRODUCTION

Low level laser therapy (LLLT) has been used as a treatment in many clinical conditions with particular application for pain, post-injury inflammation and tissue healing. The action of laser therapy is based on the absorption of light by tissues, generating a series of modifications in cell metabolism. Once absorbed, the light can modulate cell biochemical reactions and stimulate mitochondrial respiration, with the production of molecular oxygen and ATP synthesis. These effects are known to increase the synthesis of DNA, RNA and cell-cycle regulatory proteins, thus promoting cell proliferation.

The effects of laser irradiation have also been investigated in malignant cells. From a physiotherapy perspective, this is an important investigative path to follow particularly when considering the safety and efficacy of LLLT in the oncology population of patients who may have indications for LLLT but for whom the treatment may be precluded by the proximity of an existing tumor or a relevant past history of cancer.

While there appears to be no evidence that LLLT has a carcinogenic effect, there is accumulating evidence to suggest that LLLT has a biostimulatory effect in tumor cells. However, the exact mechanism by which laser acts on malignant cells is not fully understood and, for many, the use of LLLT as a treatment modality is still controversial.

In order to progress our understanding of the physiological processes and clinical parameters involved in the application of laser therapy, and to determine the responses of malignant bone cells to laser irradiation, we investigated the dose-response effects of 670nm, 780nm and 830nm laser on cell proliferation in two different osteosarcoma (MG63 and U2OS) cell lines *in vitro*.

METHODS

Cell lines were grown and passaged using standard aseptic tissue culture protocols. For laser irradiation, 96-well plates were seeded with $1x10^4$ cells in fresh growth medium, then incubated for 24hr at 37°C in 5%CO_2. A single dose of laser was applied (670nm, 780nm and 830nm at 30mW) (Figure 1) at the intensities of 0.5, 1, 5 and 10 J/cm^2. Cell proliferation was assessed 24hr after treatment by colorimetric spectrophotometry.

STATISTICAL ANALYSIS

Data are described as means and standard deviation (SD) of the mean percentage difference of irradiated cultures compared to control cultures. A 2-way ANOVA test was used to assess the significance of differences between the percentage of increase or decrease of irradiated groups compared with negative (untreated) controls, and Duncan´s test was used to identify the differences. A *p*-value of 0.05 was set for determining statistical significance.

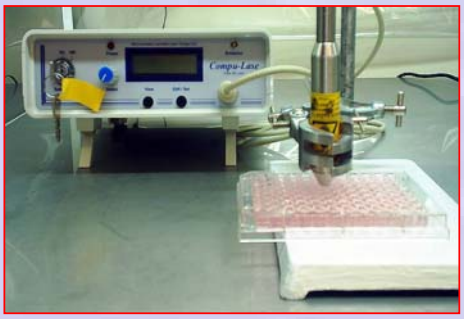

Figure 1: Laser irradiation of cell cultures

RESULTS

MG63 osteosarcoma cells: When compared to non-irradiated controls, the results demonstrated that MG63 osteosarcoma cell proliferation increased significantly after 670nm laser (at 5 J/cm^2), and 780nm laser irradiation (at 1, 5 and 10 J/cm^2) ($p \leq 0.05$), but not after 830nm laser irradiation (Table 1 and Figure 2).

Table 1: Mean percentage increase in osteosarcoma (MG63) cells after exposure to laser irradiation.

Wavelength	Dose			
	0.5 J/cm^2	1 J/cm^2	5 J/cm^2	10 J/cm^2
830nm	105% ± 6%	104% ± 5%	104% ± 5%	104% ± 3%
780nm	95% ± 6%	107% ± 5%*	127% ± 6%*	114% ± 4%*
670nm	104% ± 3%	101% ± 2%	109 ± 4%*	ND

*Statistically significant compared to untreated controls designated as 100%; ($p \leq 0.05$); ND: not done.

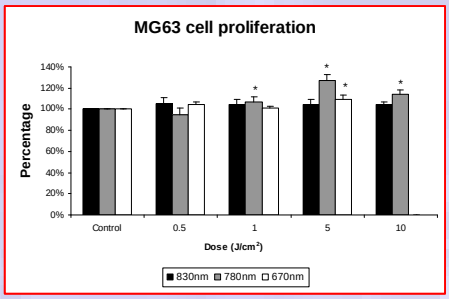

Figure 2: The effect of laser on proliferation of osteosarcoma (MG63) cells *in vitro*. *Statistically significant compared to untreated controls designated as 100%; ($p \leq 0.05$).

U2OS osteosarcoma cells: U2OS cell proliferation was significantly increased after irradiation with all laser wavelengths used, and at all doses ($p \leq 0.05$) (Table 2 and Figure 3).

Table 2: Mean percentage increase in osteosarcoma (U2OS) cells after exposure to laser irradiation.

Wavelength	Dose			
	0.5 J/cm^2	1 J/cm^2	5 J/cm^2	10 J/cm^2
830nm	111% ± 4%*	134% ± 8%*	119% ± 5%*	126% ± 3%*
780nm	113,5% ± 4%*	107% ± 4%*	107% ± 3%*	111% ± 5%*
670nm	114% ± 2%*	108% ± 3%*	123 ± 4%*	ND

*Statistically significant compared to untreated controls designated as 100%; ($p \leq 0.05$); ND: not done.

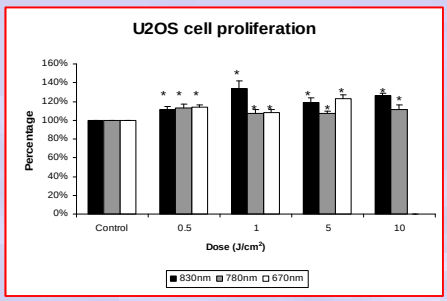

Figure 3: The effect of laser on proliferation of osteosarcoma (U2OS) cells *in vitro*. *Statistically significant compared to untreated controls designated as 100%; ($p \leq 0.05$).

DISCUSSION

The results of this study are consistent with previous work demonstrating that the response of some cells to LLLT is dose-dependent. Additionally, this study adds to the literature regarding wavelength-specificity of LLLT.

There is support for a dose / wavelength-specificity model for the MG63 osteosarcoma cell line, where 780nm and 670nm laser irradiation produced a stimulatory effect on osteosarcoma cell proliferation, particularly at moderate to high doses. However, the 830nm did not have any effect on MG63 cell proliferation.

Significantly, U2OS osteosarcoma cell line proliferation was stimulated by all wavelenghts and all doses employed in this study compared to negative (untreated) controls.

These findings support the work by Werneck *et al.* (2005) who found a statistically significant increase in proliferation of laryngeal carcinoma cells after 685nm and 830nm laser irradiation; and Coombe *et al*. (2005) who found no effect of 830nm laser on osteosarcoma cell proliferation.

The regulatory mechanisms of LLLT on tissues were not tested in this research and still remain unclear. However, such mechanisms are likely to involve photochemical signaling, with laser light enhancing cell proliferation through changes in mitochondrial physiology, with subsequent effects on RNA synthesis. Such effects may alter the expression of various cell regulatory proteins (Stein *et al*, 2005) and contribute to the results noted in our research and that of others.

The results observed in our study may be due to cell specific effects of LLLT as well as the LLLT wavelengths and doses utilised. Differences in the biochemical physiology of the MG63 osteosarcoma line compared to the U2OS osteosarcoma line may account for the results and as such have important implications for careful selection of laser parameters in clinical application with particular attention to histological findings. Our group continues to investigate the mechanisms and effects of LLLT on malignant cell lines.

CONCLUSION

The results of the present study demonstrate that laser irradiation has stimulatory effects on osteosarcoma cell proliferation. However, these data highlight that different osteosarcoma cell lines have varying responses to different types of laser and parameters utilised. Further investigations are required to investigate possible response mechanisms that may explain the outcomes obtained when examining laser irradiation of cultured cells. Such future studies will undoubtedly contribute to a better understanding of the safety and efficacy of LLLT in clinical oncology.

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