**LASER INDUCED THERMAL STRESS AND THE HEAT SHOCK RESPONSE IN NEURAL CELLS.**

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**Introduction.**

The Ho:Yag laser offers a minimally invasive method to ablate tissue with precision. Although experimental foraminooplasty has been carried out (Hafez et al 2001), the cellular effects of the heat generated are, as yet, unexplored.

When cells are exposed to adverse conditions such as heat and metabolic poisons, they respond by synthesising a characteristic group of proteins termed ‘heat shock proteins’, HSP (Lindquist 1986). One of the proposed functions of HSPs are the protection of cells against a wide variety of environmental stresses (Santoro 2000). Laser induced cellular heat stress can thus be represented by studies of heat shock proteins.

Neural tissues are in close proximity to areas being ablated by the laser. As the primary aim of foraminooplasty is to preserve the integrity of neural tissues, the effects of laser induced thermal stress on these cells thus needs to be elucidated.

Experimental foraminooplasty has shown the temperature of surrounding tissue during lasing to be 37°C when saline irrigation is incorporated into the procedure (Hafez et al 2001).

In this work, we thus aim to develop and study a cellular model both of the lasing interface and the thermal effects of the laser respectively.

**Materials and Methods**

Schwann cells were harvested from the sciatic nerves of 4-6 week old adult Wistar rats following euthanisation.

*Preparation of neural tissue for culture.*

Following its dissection, the epineurum was stripped off the nerve before being dissociated using collagenase/disase. Prior to use, the cells were phenotyped using fluorescence immunocytochemistry for S100, a marker for neural tissues.

*Exposure of cells to heat stress, via heated medium.*

Two experimental groups were exposed to heated medium. Nine sterile 6 well plates were used, with cells being seeded at a density of 9x10^4 per well, and incubated in DMEM for 48 hours (until confluence). 1.5 mls was the standard volume for medium changes.

The first group consisted of four 6 well plates. Medium was changed in three of the four plates, being replaced with medium at 45°C, 55°C and 65°C respectively with a control group at 37°C. Subsequent to the medium change, the culture plates were incubated in a precalibrated incubator at the temperature of the medium for 15 minutes.

The second group consisted of five 6 well plates, a control group and four six well plates, in which the original incubation medium was replaced with medium heated to 45°C. The culture plates were incubated for different periods of time: 5, 10, 15 and 20 minutes.

*Exposure of cells to heat stress via a laser source.*

Cells were cultured to confluence on microscopy slides. They were then exposed to a laser source, Holmium:YAG laser, 2.1 m. at a setting of 0.01kJ set of 1.0 joule/pulse and 10 pulses/sec/sec.

*Assessment of Viability & Heat Shock Immunocytochemistry.*

Using an inverted light microscope, cells were examined morphologically. Cell viability was calculated by counting the cells that excluded trypan blue within a set area. Subsequent to treatment, the cells were processed using antibodies to HSP to test for the presence of HSP.

**Results**

![Figure 1a. Effect on cell viability of exposure to different temperatures for 15 minutes.](image1)

![Figure 1b. The effect on cell viability of exposure to 45°C for increasing lengths of time.](image2)

![Figure 2a. Effects of direct lasing, showing a distinct border, with HSP expression (Fig 2a). The phenotype was confirmed with S100(Fig 2b).](image3)

![Figure 2b.](image4)

**Discussion.**

As well as outlining the effect of heat stress on neural cells (as may be encountered in foraminooplasty), this study has also shown a potential role for HSP in the studying laser generated heat stress.

The dose dependent manner of the results elucidated a negative correlation between the intensity of the stressor in 1a and the level of cell viability. A negative correlation also existed between the length of exposure and cell viability, 1b.

Laser ablation caused the expression of HSP, but also exerted mechanical effects, resulting in the shockwave induced detachment of cells in the neighbouring area. This caused a distinct border to be delineated, 2a. This suggets that as well as the thermal factor, the shockwave effect also has to be considered when regarding the the mechanism of action of the Ho:YAG laser in clinical use. It also helps to define the volume of tissue that may be subject to irreversible thermal stress during laser ablation.

Based on this work, a cellular model of the action of the Ho:YAG laser has been developed.

**References**

