Influence of cold atmospheric plasma treatments on mammalian cells

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There is growing interest in the application of cold atmospheric pressure plasma treatments in wound healing. The objective of this study is to compare the effect of the plasma treatment on cell survival and metabolic activity of two different mammalian cell lines **-** Chinese hamster ovary (CHO) and osteoblast (MG63) cells. Parameters evaluated included cell survival, viability and metabolic activity. In addition to comparing the effect of the helium plasma on the two types of cell a further objective was to quantitatively compare how treatment duration, input voltage and frequency affected the cell response.

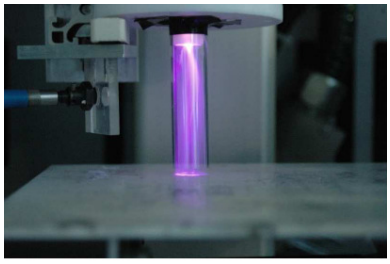

Figure 1 Atmospheric Pressure Plasma Jet formed in the 6 cm long quartz applicator

The helium atmospheric plasma jet (Figure 1), was generated using a duel-pin parallel tungsten electrode source. This source was powered using a C2000 Redline generator. The electrode pins were spaced 12 mm apart and positioned at one end of a quartz reactor cylinder of length 6 cm and nozzle exit area of 2 cm2. Applied voltage and frequency were varied between 50-300 Volts and 50-450 kHz respectively. The helium flow rate was kept constant at 10 L/min and treatment times were in the range 10 to 360 seconds. The jet to substrate distance was fixed at 15 mm. The mammalian cell lines investigated were CHO and MG63 which were both cultured in the appropriate medium. Treatments were performed on cells attached to polystyrene 6-well plates, with a diameter of 36 mm. Prior to the plasma treatment the medium was removed. Note that no deleterious effect was observed for cells with medium removal (no plasma treatment) for a period of up to 30 minutes.

The effect of the plasma was evaluated with respect to cell number, viability, cell cycle, ATP and apoptosis. The early stages of the research focused on quantifying the effects of varying treatment times on cellular responses. As expected cell death increased with increased plasma exposure times up to the 360 seconds investigated in this study. For all treated well plates a significant level of cell viability was observed 24 hours post treatment in culture. This study indicated that for the treatment conditions used, the most significant effect of the plasma was to reduce the level of cell proliferation. The effect of increasing the plasma power (voltage and frequency) resulted in an accumulation of cells within the G2 phase of the cell cycle process i.e. prevents mitosis. Analysis of apoptosis induction was carried out using Annexin V assay and flow cytometery, the results of which demonstrated a distinct progression of viable cells towards the apoptotic phase. Finally, using a Roche ATP Bioluminescence Assay Kit, a marked increase in ATP production within the cells was detected with increasing plasma power.
This study also indicated that MG63 cells were more sensitive than CHO cells to plasma exposure. Both cell types exhibited a dose dependent relationship with the intensity of the plasma applied.

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