

The Viability of Primary Human Skin Fibroblasts Exposed to Heat: The Lethal Dose for 50% Death is 48 °C

Elissa Henderson, Margit Kempf, Emily Jones, Sara Kong, Ella Pearson, Anastasia Kearns, Leila Cuttle

BACKGROUND

The majority of burns research to date has focussed on the impact to *in vivo* skin tissue, with the impact at the cellular level only examined following biopsy of the injury. Research has not yet been able to establish an understanding of the cellular mechanisms that occur with application of heat at increasing temperatures.

AIM

To understand the cellular mechanisms that occur in heat affected cells, determine the temperature at which cells begin to die and understand the relationship between temperature and cell viability.

MATERIALS & METHODS

Cell Culture: Primary human fibroblasts were isolated from surgical skin discards, cultured until fully confluent and seeded into a 6-well plate.

Heat dose exposure: A water bath was heated to the required temperature and the 6-well plate placed in the water bath for 1 hour and then recovered at 37°C for 1 hour.

MTT Assay: Following recovery, cells were washed with DMEM and MTT solution added. The plate was agitated for 20 minutes at 37°C. MTT was aspirated and replaced with DMSO in Glycine, agitated for 10 minutes and triplicate aliquots placed in a 96-well plate and the OD measured at 540nm.

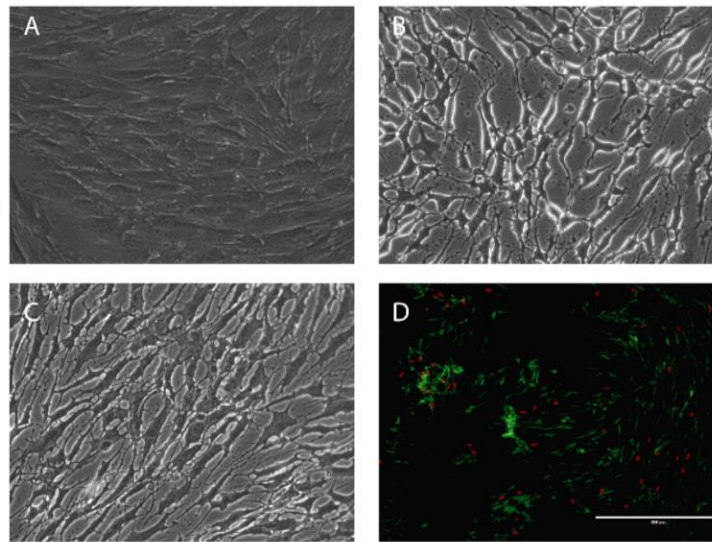
Live/Dead stain: Following recovery, media was aspirated and washed with phenol red free DMEM. An aliquot of Live/Dead reagent added to each well, agitated and viewed under a fluorescence microscope.



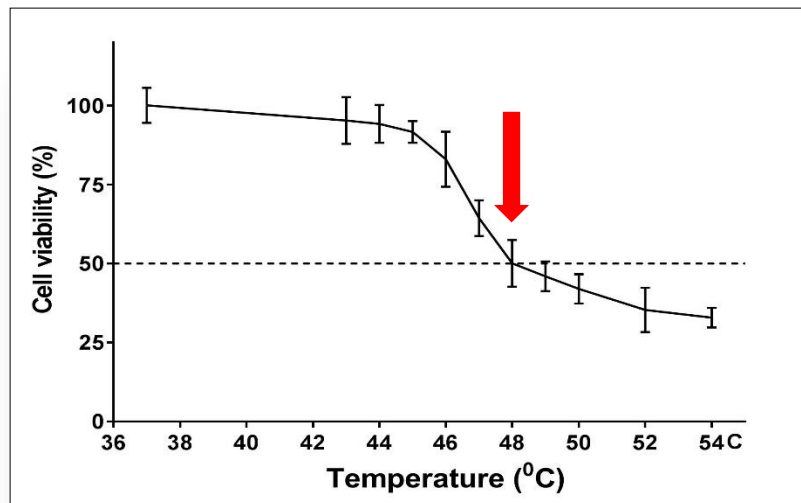
RESULTS

Cell morphology: Upon application of 48°C heat, cells appeared to lose adherence with a visibly expanded nucleus. With application of 54°C heat, disintegration of both the nuclear membrane and plasma membrane is visible and large, sparse areas between cells are visible.

MTT Assay: Results indicate a reduction in cell viability across all heat dosed cells. The decrease in cell survival occurs slowly up to 46°C with a sharp decline in cell viability between 46°C and 48°C



(A) Unstained 37°C control. (B) Unstained fibroblasts following 48°C heat application for 1 hour with 1 hour recovery. (C) Unstained fibroblasts following 54°C heat application for 1 hour with 1 hour recovery. (D) Live/Dead stain following 52°C heat application.



CONCLUSION

Thermal injury has previously been reported to occur at 43°C. This finding was supported in this study, however, there was only a marginal reduction in cell viability at this temperature (cell viability of 97%). The results from this study show that the Lethal Dose 50 for 1 hour of heat exposure is 48°C.