Derivation of hepatocytes from pancreatic progenitor cells and their use in a novel drug toxicity screening platform

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INTRODUCTION

Reactive oxygen species (ROS) are useful massaging molecules in cellular physiology, but cause damage to protein, DNA and lipid once their levels overwhelm the body's ability to deal with them. As a result, there is a great need to fully understand the roles of ROS in pathology associated with many organ systems. Hepatocytes are often used to study the potentially toxic effects of drugs on the liver. However, due to the limitation in obtaining primary human tissues it can be difficult to obtain reliable toxicity data.

Recently, B13 (rat pancreatic) cells have been converted to B13H (Hepatocyte-like) cells by pre-treatment with dexamethasone. This discovery offers the potential to grow fully functioning hepatocytes 'on-demand'.

AIMS

1) To trans-differentiate rat B13 pancreatic cells to B13H hepatocyte cells by pre-treating cells with dexamethasone.

2) To determine the ability of B13 H cells to produce cytotoxic superoxide (O_2^-) following exposure to ethanol, using direct real-time electrochemical sensing technology.

3) To further study the ability of a known O_2^- scavenger, superoxide dismutase (SOD) to control superoxide production.

Differentiation of B13 to B13H cells

B13 cells were seeded at 100,00 cells per well, in a 24 well plate in the presence of low glucose DMEM spiked with 10nM dexamethasone and incubated at 37°C and 5% CO_2 for 4-8 days to allow for differentiation

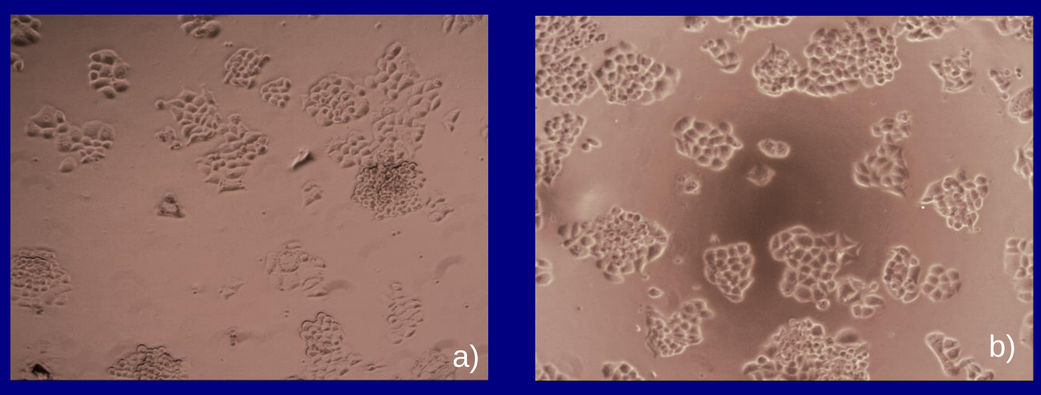
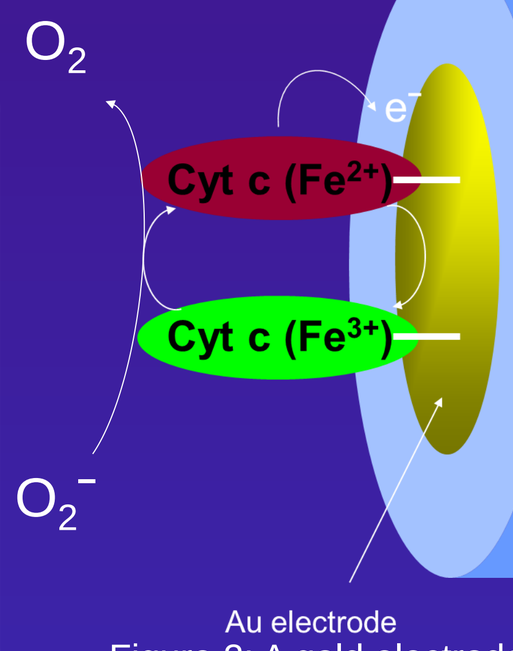


Figure 1a) Rat Pancreatic B13 cells ; b) Rat pancreatic B13 cells after 4 days pre-treatment in culture medium containing 10nM dexamethasone.

Electrochemical detection of O_2^-



DTSSP linker molecules covalently bonded cytochrome *c* (cyt *c*) to the surface of a gold electrode. When held at a working potential of +100mV against a Ag/AgCl reference, O_2^- reduced cyt *c* to its Fe^{2+} oxidation state. Cyt *c* was then reoxidised at the electrode surface to generate a current that was proportional to the rate of O_2^- production.

Figure 2: A gold electrode modified with N-acetylcysteine that measures O_{2-} production

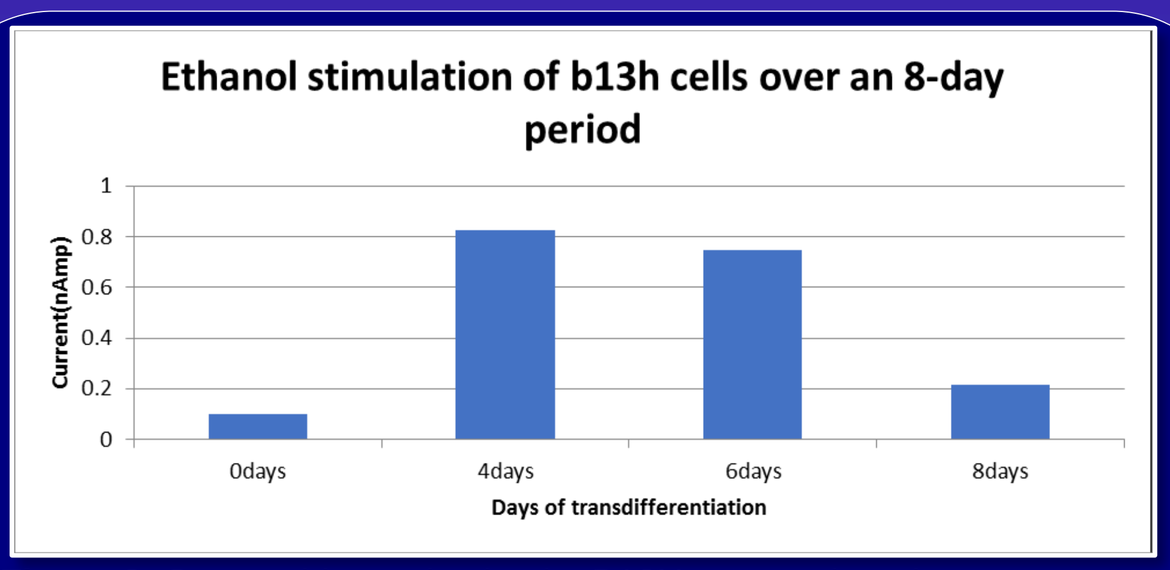


Figure 3: Superoxide production by B13H cells undergoing trans differentiation over an 8 day period. Data shows optimum O_2^- response to ethanol stimulation occurred at day 4 and becomes subsequently lower in day 6 and day 8.

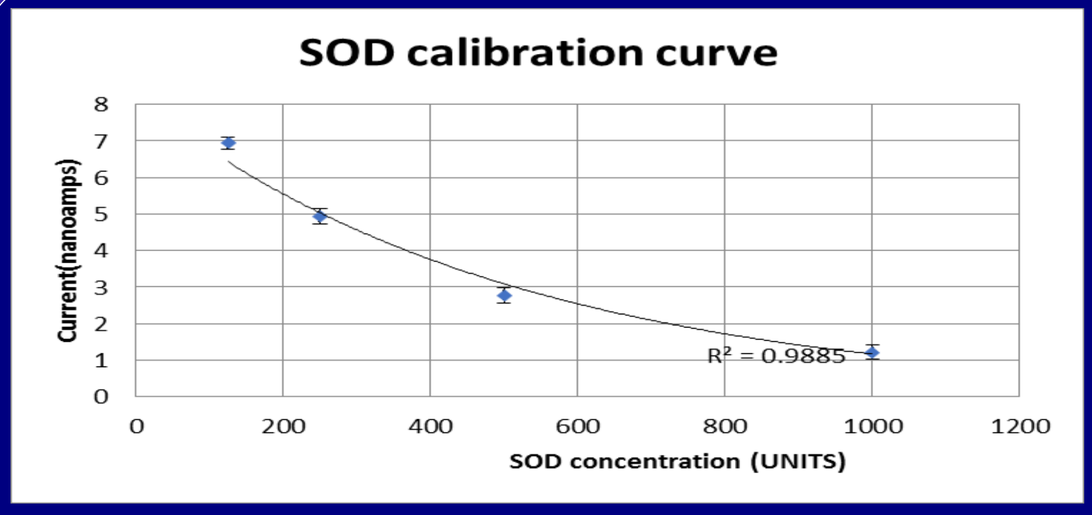


Figure 4: Increasing SOD concentration decreases the production of O_{2-} following stimulation with 5% ethanol. B13H cells had been incubated in culture media containing dexamethasone for 4 days prior to the experiment.

DISCUSSION

The use of direct, real-time electrochemical measurements to determine the production of O_2^- from B13H cells following stimulation by ethanol is a novel technique. The response was not seen in B13 cells prior to trans-differentiation. The production of O_2^- by B13H cells was confirmed by using SOD which eliminated detection of the free radical.

Acute ethanol exposure leads to the metabolism of ethanol by dehydrogenase enzymes producing NADH as a by-product. NADH may feed electrons to the mitochondrial electron transport chain leading to an increase in O_2^- production.Tthis may explain the increase in O_2^- response in B13H cells following ethanol stimulation.

CONCLUSION

The data shows clear morphological and biochemical differences between B13 and B13H cells. This 10-week summer project contributed to the on-going research in further characterisation of the nature and causes of these differences.

This research provided data to support the the development of a potential alternative source of human liver cells that could be used by the pharmaceutical industry for toxicity tests.

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