Investigating novel micro-bubbles for improved treatment of type 1 diabetes

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Introduction

- Diabetes mellitus is a debilitating disease that affects 3.2 million people in England
- 10% of the NHS budget is spent on diabetes equating to £1 million every hour
- Type 1 diabetes (T1D) is an autoimmune disease where insulin producing β-cells are destroyed in the pancreas leading to patients becoming dependent on insulin therapy
- The cause of T1D is unknown but there are several factors that are thought to contribute such as environment, genetic susceptibility, childhood infections etc.
- Short term effects are hypoglycaemia which may result in diabetic coma; or hyperglycaemia which may cause ketoacidosis
- Long-term diabetes can cause severe nephropathy, heart disease, blindness and amputation

Current treatments in T1D

In 1922 Banting discovered insulin, which revolutionised treatment.

- Insulin therapy- Insulin injections are required to maintain blood glucose concentration at normal level
- Whole pancreas transplantation - Invasive surgery with 30% of patients needing reoperation and a 5% mortality rate
- Islet transplants- Islets are isolated from a donor pancreas and implanted into the liver

Islet Allograft

This treatment (Fig.1) is used for people who suffer from severe hypoglycaemia unawareness; these usually occur after 15+years of diabetes.

Advantages

- Non-invasive procedure
- Eliminates severe hypoglycaemia episodes and restores awareness
- Improved control of blood glucose levels with potential for insulin independence
- Improved quality of life

Disadvantages

- 2-3 transplants are required for treatment and with only 1/3 of pancreases being transplantable, 4-9 pancreases are needed per person
- Each isolation costs up to £13,000
- Requires a high islet mass/kg due to a high attrition rate
- Isolation is stressful for the islets due to hypoxia, enzymes, mechanical force and temperature

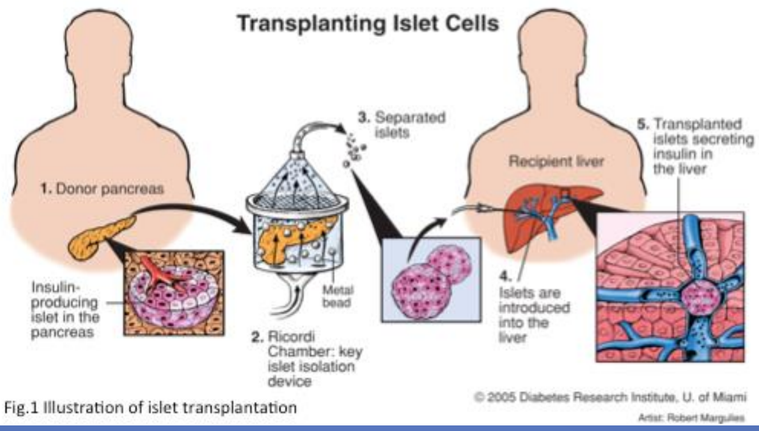

Fig.1 Illustration of islet transplantation

My project

Extended exposure to periods of warm and cold ischaemia during transport and isolation negatively impacts islet isolation outcomes. Emerging technologies have demonstrated the potential for maintenance of tissue oxygenation during organ preservation prior to islet isolation which has led to improved yields. It is anticipated that if adequate tissue oxygenation could be maintained during the isolation process itself; that further reductions in cell stress and death could be achieved. This work aims to explore the initial feasibility of one possible method for oxygen delivery during islet isolation in an attempt to consistently improve yields and enhance the rate of conversion to transplantable preparations.

Aims

- Investigate the use of novel oxygen micro-bubbles in β-cell (MIN6) culture to study cell toxicity of the compound and try to establish optimal micro-bubble concentration.
- Work with engineers to generate 'stable' micro-bubbles capable of oxygenating the MIN6 cells in culture.
- Use Hoechst and propidium iodide (PI) staining to image and quantify cell number and assess viability.

Micro-bubble production

- L-alpha Phosphatidyl-choline stabilised oxygen micro-bubbles were produced using a microfluidic T-junction device (below).
- The polymer (A) was infused into the microfluidic T-junction device (C) by a syringe pump; whilst gas (B) was pumped in from a supply to produce micro-bubbles (D) at the gas/liquid interface as shown in Fig.2

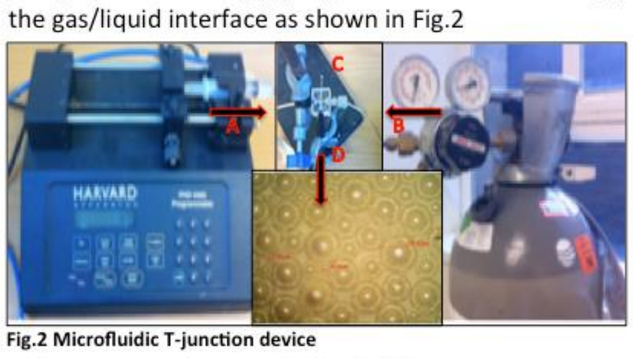

Fig.2 Microfluidic T-junction device

Cell culture

- The MIN6 cell line has been derived from a transgenic mouse
- Adherent MIN6 cells were cultured at 37°C in 5% CO_2 using an established cell culture protocol until 80% confluent
- Culture medium (DMEM+15% FBS) was changed every 2-3 days and viewed under light microscope for confluence
- To split: cells were trypsinized, washed and divided into new T75-flasks. For an experiment cells were trypsinized, washed, stained with trypan blue, and counted in a haemocytometer for viability assessment before use
- Work was conducted in microbiological safety cabinets (class II) and aseptic techniques were used throughout to prevent contamination

Testing protocol

- For this work we used MIN6 cells from passage 29. Cells were plated at a density of 25,000 cells/well in a 12-well plate
- Plates 1 and 2 were incubated under normoxic conditions at 37°C in 5% CO_2 for 48 h with a media change at 24h to replace the media along with the micro-bubbles or vehicle volume. Note that a 1% concentration of vehicle is going to be a much greater amount than that present in 1% micro-bubbles. Due to shortage of micro-bubbles only single wells for 5% and 10% were cultured with micro-bubbles for 48 h
- Plate three was incubated under hypoxic conditions 37°C, 5% CO_2, 1% O_2 for 24 h after media change

Normoxic culture

Plate 1

media control	vehicle 1%	vehicle 5%	vehicle 10%
media control	vehicle 1%	vehicle 5%	vehicle 10%
media control	vehicle 1%	vehicle 5%	vehicle 10%

Plate 2

media control	micro-bubbles 1%	micro-bubbles 5%	micro-bubbles 10%
media control	micro-bubbles 1%	micro-bubbles 5%	micro-bubbles 10%
media control	micro-bubbles 1%	micro-bubbles 5%	micro-bubbles 10%

Hypoxic culture

Plate 3

media control	N/A	N/A	N/A
media control	N/A	N/A	N/A
media control	N/A	N/A	N/A

Results

Hypoxic culture (negative control)

- Viability appears high, but the average number of cells per well remains low.

Vehicle

- Cells incubated with 1% vehicle had a 7.4% increase in average viability from the media control, this concentration of vehicle is equivalent to a much higher percent of micro-bubbles.
- 5% and 10% have a similar viability to media control

Micro-bubbles

- All 48h micro-bubble concentrations showed increased viability compared to the control (range 3.8-4.4%).
- The 24h micro-bubble well also showed an increase in average viability compared to media control (range 4.2-6.4%), and slightly higher compared to 48h.

Microscopy

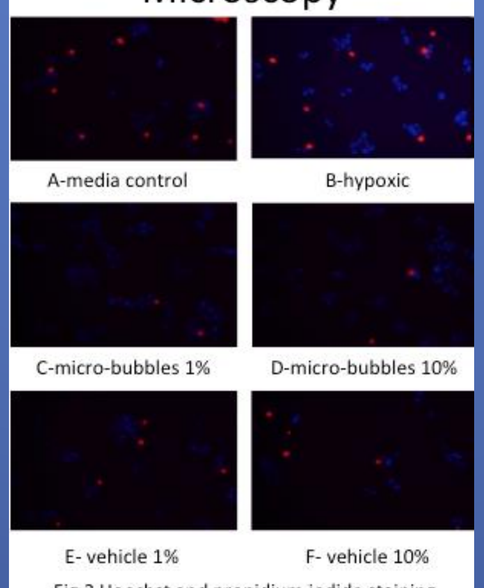

Fig.3 Hoechst and propidium iodide staining

A comparison of cell viability by propidium iodide staining

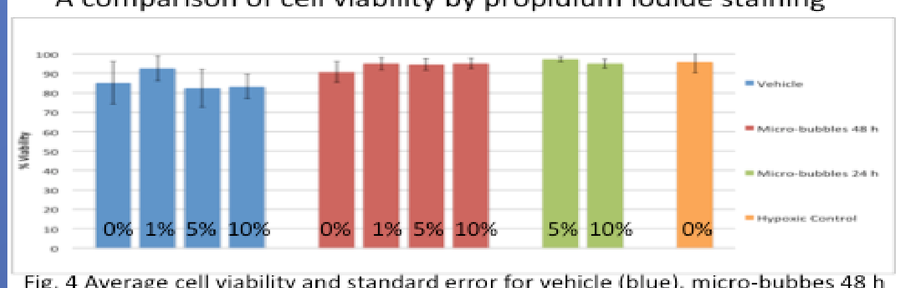

Fig. 4 Average cell viability and standard error for vehicle (blue), micro-bubbes 48 h (red), micro-bubbles 24 h (green) and hypoxic (orange)

Average cells per well

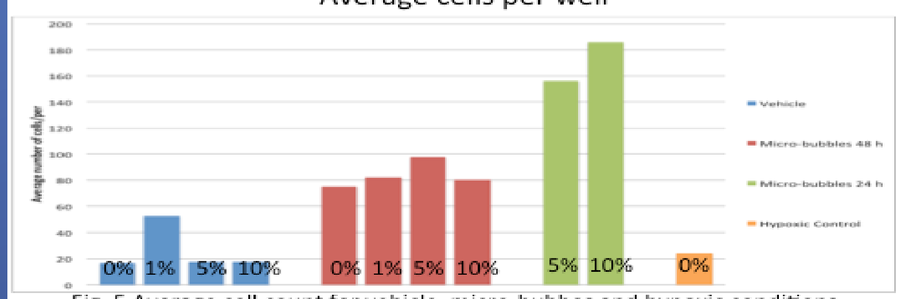

Fig. 5 Average cell count for vehicle, micro-bubbes and hypoxic conditions

Discussion

- 1% vehicle exhibited a higher viability than the media control, 5% vehicle and 10% vehicle, this suggests that the compound may have a beneficial effect at a moderate concentration
- Micro-bubbles have a greater effect on viability at 24h, which suggests there is a critical time point for a maximum viability
- The micro-bubbles do not appear to be severely toxic to the MIN6 cells and may provide a beneficial impact at certain concentrations. However, this is preliminary work and further repeats need to be carried out
- The hypoxic cells have a high viability, but a low cell count. Dead cells may have detached and been removed during the media change, meaning most of the cells that were stained had survived in the low oxygen condition

Conclusions and future directions

- At low concentrations, micro-bubbles and vehicle show no impact on cell viability, however further work will need to be done to optimise micro-bubble manufacture and improve stability
- Future experiments will further optimise the impact of micro-bubbles in maintaining cell viability
- Impact of micro-bubbles on cell function will be investigated in both hypoxic and normoxic conditions in pseudo-islets and human islets

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