MRI Studies of In-vitro Perfused Human Pancreatic Islet Cell Activation.

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Introduction: Recent progress in clinical islet transplantation have increased the need for the development of a dependable means to characterize islet product after it is isolated from a donor pancreas and before it is infused into an islet transplant recipient. The further development of endocrine replacement therapy for the treatment of diabetes depends on the availability of high quality islets and addressing unresolved issues such as functional islet mass engraftment rate, cell apoptosis, and immunorejection. We are in the process of optimizing techniques to determine the quality of isolated pancreatic islets by assessing their - functionality, integrity, cellular identity and viability using high-resolution dynamic perfusion based MR imaging. Manganese (Mn) enhanced MRI (MEMRI) is presented here as a tool to image isolated perfused human pancreatic islets. Glucose stimulated influx of calcium into β-cells precedes insulin release. When present during stimulation, extracellular Mn^{+2} can enter β-cells through the voltage gated calcium channels and its accumulation alters T1 relaxation times[1]. This results in increased T1 weighting contrast of activated versus non-activated β-cells without negatively impacting glucose sensitivity, calcium dynamics and insulin secretion. This study focused on assessing human islet functionality by developing a novel dynamic perfusion flow based MR Imaging.

Materials and Methods: *Islets:* Human islets, isolated from cadaveric donors were obtained from the Department of Surgery at the University of Illinois at Chicago and ICR in accordance with the University of Illinois and the University of Chicago IRB protocols. *Perfusion Set-up:* An original design for islet oxygen measurement [2] was modified for use in the MRI scanner (Fig 1). Custom-made porous glass frits (0.45 mm OD X 1 mm thick) cut from polyethylene sheets (Small Parts Inc., FL) were placed inside the 5 mm diameter NMR tube bordering human pancreatic islets cushioned between two thin layers of cytopore beads (Amersham Biosciences, NJ). The top frit kept the beads and islets from flowing out and the bottom frit served as a resting base.

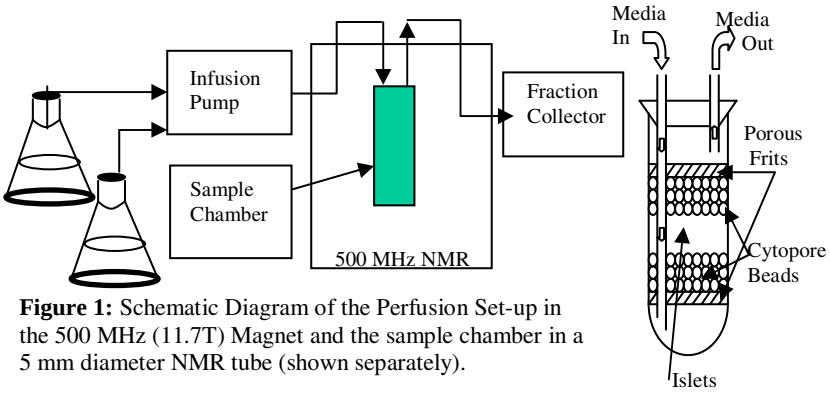

Figure 1: Schematic Diagram of the Perfusion Set-up in the 500 MHz (11.7T) Magnet and the sample chamber in a 5 mm diameter NMR tube (shown separately).

Perfusion media flowed from beneath the islets at a flow-rate of 250µL/min, for minimum islet motion, supplied by a thin capillary tube that ran through both the frits and opened just below the bottom frit above the bottom of the NMR tube. The fraction collector samples were later used for insulin measurements via ELISA assay tests. *Non-stimulated Islets (Pre-flow images):* Islets were perfused with Krebs Ringer Buffer (KRB) solution with 75µM Mn & 2mM glucose for 20 mins. *Stimulated Islets (Post-flow images):* Islets were switched to treatment with KRB with 75µM Mn & 14 mM glucose. *MR Parameters:* Spin echo (SE) sequences were used to obtain spin density, T1 and T2 weighted images. All experiments were performed in a 56-mm vertical bore 11.7T magnet using a Bruker DRX Avance Spectrometer (Bruker, Billerica, MA). The typical imaging parameters were: TR/TE=400/8 ms, Mx=128, NEX=20, Slice thickness=0.3 mm, FOV=0.45mm.

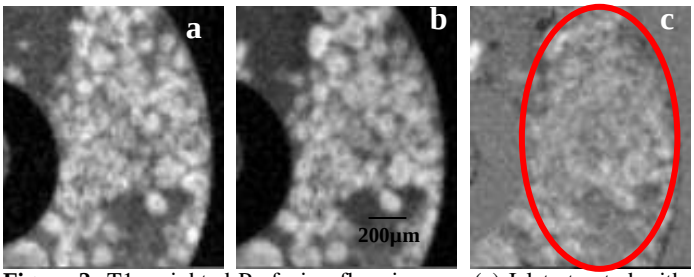

Figure 2: T1 weighted Perfusion flow images. (a) Islets treated with low glucose (2mM) & Mn. (b) Islets treated with high glucose (16mM) & Mn. (c) Subtracted image showing Mn enhancement due to stimulation by high glucose.

Results and Discussion: The difference in signal-to-noise ratio (SNR) is clearly visible in a population of human islets with this dynamic perfusion flow study. The post perfusion flow image results, as seen in Fig 2(b), demonstrate an increase in contrast with respect to the pre-flow images as in Fig 2(a). The islet per islet difference (between pre and post-flow) in contrast is very clear in the subtracted image, as seen in Fig 2(c), obtained by subtracting the pre-flow image from the corresponding post-flow image. A consistent reduction in T1 values of 300 msec (SD +/- 50 msec) and no significant change in T2 values were seen in activated versus non-activated islets (data not shown). An overall SNR improvement of 30 % (SD +/- 5%) shown in Fig 3 was obtained with islets treated with 75-µM Mn concentrations for 20 mins flow and similar results were obtained for islets treated with 50-µM Mn for 40 mins. Islets were recovered post-perfusion and tested for viability with trypan blue.

Conclusion: A novel non-invasive method using a dynamic perfusion flow based method in the NMR environment implementing MEMRI to assess human ß-cell functionality was described and demonstrated in this research. These results demonstrated the possibility of obtaining high-resolution MR images and T1 activation maps of human pancreatic islets. The concentration of Mn required for image enhancement did not inhibit ß-cell function. Beyond proof-of-concept, careful MR characterization of isolated human islets was also performed for further optimization of the imaging technique when applied in vivo.

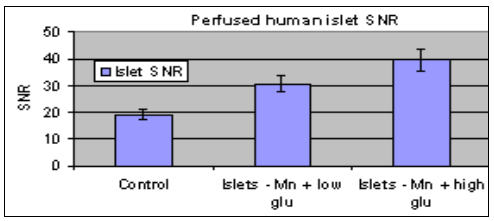

Figure 3: SNR graph showing enhancement in human islets perfused with high glucose. (n=5)

References:

1. Gimi et Al. Functional MR Microimaging of Pancreatic β-Cell Activation. Cell Transplantation. 2006.
2. Sweet et Al. Regulation of ATP/ADP in Pancreatic Islets. Diabetes. 2004.