MR Microscopy of Pancreatic Islets of Langerhans

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Introduction

Diabetes Mellitus is one of the most pervasive and devastating diseases of the endocrine system, with 140 million patients worldwide and World Health Organization estimates of 300 million diabetics by 2025 [1]. At the heart of the disease is a loss of functionality in the islet cells of the endocrine pancreas, the basic biological unit responsible for the maintenance of insulin and glucose levels in blood. The small size and deep-sited location of the human islet has made both *in vivo* and *in vitro* imaging of this structure, particularly the insulin-secreting β cells, very challenging. As a result, many questions have been left unanswered concerning the general architecture and organization of the human islet as well as the pathological progression of diabetes (i.e. longitudinal alterations in β cell mass, islet function, endocrine signaling and lymphocyte infiltration). This presentation describes the first successful application of ultra-high field magnetic resonance (MR) microscopy to the evaluation of micro-encapsulated human islets *in vitro*. Utilizing a magnetic field strength of 17.6 T, MR microimages were acquired at isotropic resolutions of less than 9.4 µm. These microimages display significant heterogeneity in the human islet and demonstrate the utility of various MR contrast mechanisms (e.g. diffusion, T_2 relaxation and T_2^* relaxation) in analyzing the makeup of this important structure.

Methods

Viable human islets were harvested from *cadaveric pancreata* at the University of Miami. Deemed unacceptable for human transplantation, these samples made available roughly 5000 islet equivalents per shipment for research endeavors. Within 48 hours of harvesting, these islets were encapsulated in low molecular weight alginate/poly-L-lysine/alginate (APA) microbeads [2] that were generated through the use of an electrostatic bead generator (Nisco Engineering, Zurich, Switzerland).

On the day of examination, 2-6 beads immersed in Dulbecco's Modified Eagle's Medium were loaded into a capillary having outer/inner diameters of 700/530 µm. MRI data were acquired using a 17.6-T vertical widebore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. This capillary was placed within a homebuilt solenoidal microcoil having a diameter of 850 µm. Coupled with ultra-high magnetic fields, these small RF solenoids, which are susceptibility-matched to reduce field perturbations, greatly improve the sensitivity of the MRI experiment [3]. Given the size of the alginate beads (400-µm diameter) and length of the microcoil (> 1 mm), several beads were analyzed simultaneously in an unperfused state.

A series of MR microimages were acquired from normal micro-encapsulated human islets using spin echo (SE) and gradient echo (GE) sequences to develop diffusion-, T_2- and T_2^*-weighted contrasts. A modified SE sequence (TE/TR = 30/2000 ms) was used with balanced bipolar gradients [4] to provide water diffusion-weighted images (b value = 1000 s/mm^2) at an in-plane resolution of 9.38x9.38 µm and slice thickness of 60 µm with a TR of 2 s and TE of 25 ms in 1.14 hours. T_2-weighted images were acquired with the same SE sequence without diffusion weighting and with a TE of 40 ms. Additionally, a GE sequence (TR = 150 ms) was acquired at an isotropic resolution of 9.38 µm and with TEs of 10 and 20 ms in 2.75 hours.

Results and Discussion

Figures 1-4 display images of encapsulated normal human islets with T_2, T_2* and diffusion weighting. Regardless of the dominant contrast mechanism, the MR microimages of the human islets show heterogeneity throughout the volume of islet. With heavy T_2 weighting, islet images demonstrate defined patches of low signal result from an increase in the fraction of bound water within the cytoplasms of individual cells or cell clusters. Given a comparison to histological sections, it would be tempting to assign these circular regions to individual cells; at this time, however, that assertion cannot be confirmed. Regardless, the contention that low signal areas in T_2-weighted images correspond to at least cell clusters is lent further support by diffusion-weighted images, which display hyperintense regions within the islet that result from localized regions of lowered water diffusion. Again, circular regions can be found within the islet on diffusion-weighted images, but with contrast opposed to similar regions in T_2 images. Additionally, T_2- and diffusion-weighted images consistently show two other features across all human islet samples. First, a dark ring demarcates the periphery of the entire islet on T_2 images. This ring marks the transition of the image from the homogeneous encapsulating alginate bead to the heterogeneous human islet and appears to define a transitional border around the islet in which the molecular motion of water is affected. Second, within the interior of the islet, there appear to be interconnected "canals," which are evident as hyperintense regions in T_2 images and dark areas in diffusion-weighted images. Based partially on histological comparison, it would appear that these areas correspond to interstitial spaces within the human islet that contain reticular fibers and function as part of the duct system of the endocrine pancreas. A 3D rendering and coregistration of T_2 and DW images is shown if Figure 5. Figure 4 displays a single slice of a GE dataset acquired at 17.6 and 11 T. These GE microimages confirm the features described previously for T_2- and diffusion-weighted images but provide even higher spatial resolutions and amplify structural contrast in certain cases. Again, heterogeneous circular regions within the islet are identifiable but at smaller dimensions. In several cases, these circular regions are defined by very distinct ring-like structures that would appear to be related to cellular membranes. Also, the interconnected interstitial/duct network that was previously identified for T_2-weighted images is more strongly delineated in the high resolution T_2*-weighted images. A new feature of the GE images is the appearance of hot spots within the islets. These hyperintense regions would appear to arise from the magnetic susceptibility-induced field perturbations, possibly originating from discontinuities caused by changes in the density or organization of membranes and connective tissue.

Conclusions

These images of the human islets of Langerhans demonstrate that high resolution details can be obtained utilizing MR microscopy *in vitro*. The three native contrast mechanisms that have been evaluated clearly demonstrate the heterogeneous nature of this vital endocrine structure. Ongoing work is focused on quantify these contrast parameters and identifying the source of the heterogeneity (i.e. assigning the apparent clusters to specific endocrine cells). Current research also is evaluating whether these contrast parameters might be useful in verifying cellular destruction caused by toxins or immunological agents. Potentially, MR microscopy may be useful in characterizing or monitoring isolated islet viability prior to human transplantation.

References 1. World Health Organization. Fact Sheet 138. Revised April 2002. http://www.who.int/inf-fs/en/fact138.html. 2. Lim and Sun. Science. 10. 1980. 3. Webb and Grant. J. Mag. Reson.B. 113. 1996. 4. Hsu et al. J. Mag. Reson.B. 109. 1995.

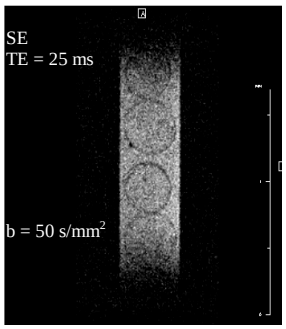

Fig 1. Low T_2W image

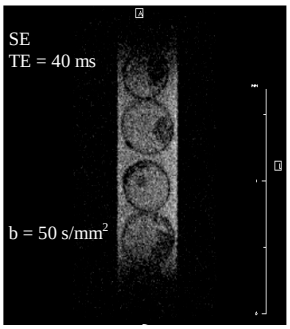

Fig 2. High T_2W image

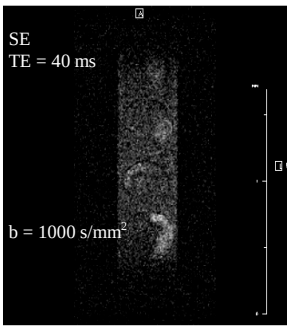

Fig 3. High DW image

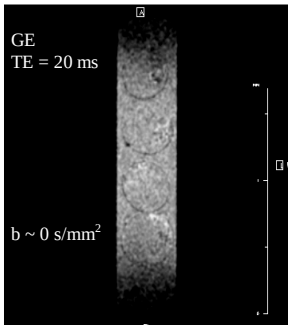

Fig 4. T_2*W image

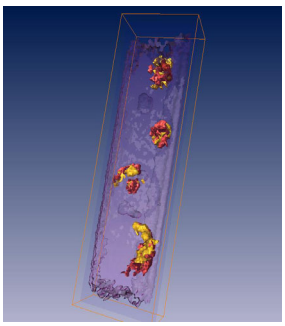

Fig 5. 3D Rendering of T2W (red) and DW (gold)