Developing magnetocapsules for immunoprotection and MR tracking of pancreatic islets using clinically approved materials

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Introduction: For patients with type I diabetes mellitus (TIDM), islet transplantation provides a moment-to-moment fine regulation of insulin that is unachievable by exogenous insulin injection. Effective glycemic control is critical in reducing the end-stage complications of TIDM and for this reason islet transplantation has received considerable attention in recent years. Early reports have described insulin-independence success rates ranging from 23-90%[1]. This wide range is largely due to the immunosuppressive regimen employed as most immunosuppressive regimens are highly toxic to isolated islets. For this reason, a means to immunoisolate islets allowing engraftment free of chronic immunosuppressive therapy is needed. One plausible approach is microencapsulation in which individual islets are surrounded with a thin shell that is impermeable to antibodies and other arms of the host's immune system but is permeable to insulin, nutrients, electrolytes, oxygen, and metabolic waste. Small-scale clinical trials have given impressive evidence of the potential of encapsulated cell therapy. Nevertheless, basic questions such as ideal transplantation site, best means of delivery and long-term survival of such grafts are only beginning to be addressed. If microcapsules could be visualized after implantation, their functionality and biodistribution could be evaluated more effectively. Rather than conventional labeling of cells directly, which may possibly affect their function, we have developed clinical formulations of alginate-based microcapsules that incorporate Feridex® (an FDA-approved SPIO formulation in an off-label application).

Methods: The protocol we have developed for synthesizing magnetocapsules is based upon the classic poly-L-lysine (PLL)-alginate protocol utilized for microencapsulation developed by Lim and Sun[2]. The procedure essentially involves using an electrostatic droplet generator to create 350 μm alginate/islet microcapsules. These microspheres are collected in a solution of $CaCl_2$ that complexes with the alginate to form stable capsules. A layer of PLL is then applied followed by an outer coating with a dilute concentration of alginate. In standard alginate-PLL microcapsules, the positively charged amino group of the lysine molecule interacts with the negatively charged carboxyl and hydroxyl groups of the uronic acid (basic unit of alginate). In addition to a polycationic stabilizer for microcapsules, PLL has traditionally been used as a cationic transfection agent utilized for labeling cells with Feridex[3]. As Feridex is known to complex with PLL through electrostatic interactions[4], we modified the traditional synthesis of capsules by adding a Feridex incubation step following initial incubation of capsules in PLL. Feridex incubation conditions were optimized to maximize iron labeling and cell viability. After an incubation period of 2 hours in saline with 200 μg Feridex per ml, magnetocapsules were found to have an iron content of 1.82 ng of Feridex per capsule as determined by a ferrozin-based iron assay. Following Feridex incubation, capsules were rinsed in saline and a final layer of alginate was applied (Fig. 1). This protocol was utilized to magnetoencapsulate human islets and murine βTC-6 insulinoma cells (Fig. 2a).

Results: Permeability of capsules was determined by incubation for 48 hours with fluorescently labeled lectins of varying molecular weight. Traditional microcapsules and magnetocapsules were both found to be permeable to fluorescent lectins <75kD but were found to be impermeable to lectins >150kD, thus blocking antibodies while allowing penetration of smaller nutrients and secretion of insulin. Using a microflurometric assay to selectively tag live cells with Newport Green and dead cells with propidium iodide, the viability of magnetoencapsulated human islets and βTC-6 cells (fig. 2b) was shown to have no statistically significant difference from that of controls over a two week period. Using a commercially available ELISA, the insulin secretory response of magnetoencapsulated islets was compared against non-treated islets. Using the FDA-approved test for bioequivalence (TOST) with a threshold value of 6% and α=.05, the insulin secretion from magnetoencapsulated islets was found to be bioequivalent to secretion from control islets over a two-week period in culture. Further, individual magnetocapsules could be visualized with Gradient Echo imaging in an agarose phantom on a Philips 3T XMR scanner (an interventional scanner being used for cell injections in our institute) (Fig. 3). Single magnetocapsules could also be visualized 24 hours post-transplantation in the peritoneal cavity of a mouse with Spin Echo imaging on a Bruker 9.4T scanner (Fig. 4).

Discussion: *In vitro* comparison of traditional microcapsules and magnetocapsules did not reveal any appreciable difference in permeability of capsules or viability and insulin secretory response of encapsulated β-cells. We have characterized the biophysical properties of these novel magnetocapsules and have demonstrated their MR detectability. Magnetocapsules thus show potential in islet cell transplantation and potentially other cell therapeutic applications, and have the necessary components to become clinically translatable.

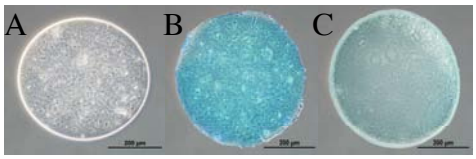

Figure 1: Magnetocapsule layers A) alginate B) alginate+ PLL+Feridex (with Prussian Blue staining to detect iron) C) alginate+PLL+Feridex+alginate

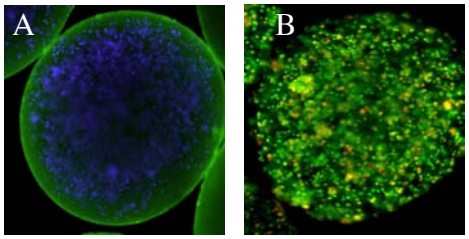

Figure 2: Magnetoencapsulated βTC-6 cells. A) Anti-dextran to detect Feridex in capsule (green); DAPI to detect cell nuclei (blue). B) Newport Green (viable) and propidium iodide (dead) cells.

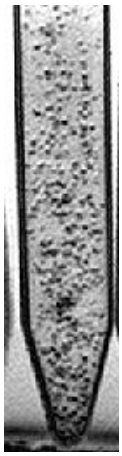

Figure 3: Gradient Echo (TR/TE: 20/10 ms, flip angle: 20°) of capsules suspended in agarose acquired on a Philips 3T XMR scanner.

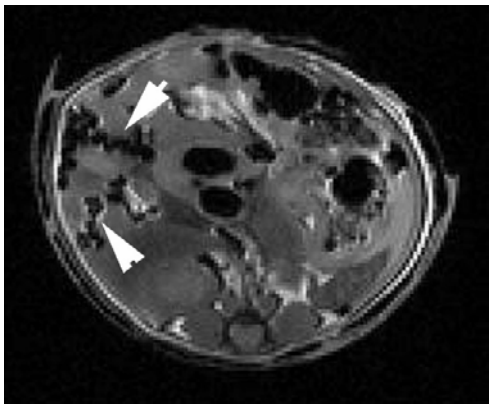

Figure 4: Spin Echo imaging at 9.4T of a mouse 24h post transplantation of 500 magnetocapsules. Single capsules can be detected as hypointense areas (white arrows) FOV: 3x3cm, resolution: 117x235 μm, slice thickness: 800 μm, flip angle: 30°, TR/TE: 1,500 ms/15 ms.

References: 1) Ault A. The Lancet. 361, 2054, 2003. 2) Lim F et al., Science. 210, 908, 1980. 3) Frank JA et al., Radiology 228, 480, 2003. 4) H. Kalish et al., Magn. Reson. Med. 50, 275, 2003.