Multifunctional fluorocapsules for ^{19}F MRI, immunoprotection, and oxygenation of transplanted pancreatic islet cells.

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Target Audience: Those working in the field of pancreatic beta islet imaging and ^{19}F MRI.

Purpose: Transplantation of pancreatic beta islets is currently a promising treatment of type I diabetes. Encapsulating the islets inside semi-permeable, biocompatible microcapsules provides immunoprotection for islet grafts while allowing the passage of insulin, glucose, oxygen, and other metabolites. However, the grafts typically experience hypoxia immediately after transplantation. The capsules may degrade inside the recipient and subsequently expose the islets to the host immune system. Here we synthesized fluorocapsules by encapsulating perfluoropolyether (PFPE) emulsions. PFPE is a bioinert compound that was previously studied for oxygen carrier applications[1]. Our aim is to test whether PFPE agents will function as an oxygen sink to support the survival of the islet grafts and whether we can image intact versus ruptured fluorocapsules using ^{19}F MRI.

Methods: PFPE emulsions were synthesized by sonicating an equal volume mixture of 2.5% w/v lecithin and perfluorocarbon at 50% amplitude. Islets and PFPE particles were encapsulated inside alginate/protamine sulfate/alginate microcapsules (12% v/v PFPE/ml of alginate). To evaluate the benefit of PFPE in hypoxia, we serially measured the insulin secretion levels of unencapsulated human islets and encapsulated islets without or with PFPE in a low-oxygen culture (O_2 level ≤ 1%) using an ELISA. We intraperitoneally implanted 5000 encapsulated human islets with or without PFPE (1 islet/capsule) into diabetic NOD/ShiLtj mice (n=4), and monitored the plasma human C-peptide levels over time. To rupture the capsules, 3000 fluorocapsules were incubated at 37°C in 0.9% NaCl+10 mM HEPES containing alginate lyase (0.75 U/ml) for 2 days. Treated capsules were then passed through a 25G needle. Intact and ruptured capsules were loaded into 5-mm NMR glass tubes for imaging. A reference standard made of PFPE in agar with a known amount of ^{19}F atoms was included to quantify the number of ^{19}F atoms in the samples. The phantoms were imaged using a vertical Bruker 17.6T MRI using a birdcage resonator that can be dual tuned to ^{1}H and ^{19}F. Images were obtained by a fast spin echo sequence (RARE; TR/TE= 1000/6 ms; RF=8, Resolution: 128x128; Slice thickness=1 mm, and NA=4).

Results: A human islet encapsulated inside a fluorocapsule with an apparent smooth surface is shown in **Fig. 1A**. When cultured in a hypoxic condition, the inclusion of PFPE enhanced the therapeutic function of fluoroencapsulated islets compared to unencapsulated islets and encapsulated islets without PFPE, as indicated by the levels of secreted C-peptide (**Fig. 1B**). Although islet grafts typically do not survive in severe xenotransplantation paradigms, the circulating C-peptide levels of the implanted islets were still detectable up to 33 days post-implantation when PFPE was co-encapsulated. In contrast, the C-peptide was undetectable 14 days after the transplantation without PFPE (**Fig. 1C**). As low as one fluorocapsule phantom could be imaged by ^{19}F MRI (**Fig. 1D**). The total amount of ^{19}F atoms in a single intact fluorocapsule and in a cluster of 10 fluorocapsules were calculated as $1.04x10^{19}$ and $1.26x10^{20}$, respectively. The numbers of ^{19}F atoms per image slice of intact and ruptured capsules were $5.99x10^{20}$ and $3.66x10^{20}$, respectively. When ruptured, the ^{19}F MR signals of the fluorocapsules thus decrease by about 61% due to the release of PFPE (**Fig. 1E**). Statistical analysis was performed using a two-tailed Student's t-test with unequal variances for a statistical significance of $P<0.05$.

Discussion and Conclusions: PFPE co-encapsulation improved the survival and therapeutic function of islet grafts, presumably by increasing the oxygenation of islets. Fluorocapsules could be visualized as "hot spots" by ^{19}F MRI with single capsule detection capability *in vitro*. This method can potentially be used to non-invasively image the integrity of implanted fluorocapsules and hence to indirectly monitor the immunoprotection offered by the capsules. **References:** [1]Riess, et. al. *Vox Sang* 61:225-239 (1991). This study was supported by MSCRFII-0161-00

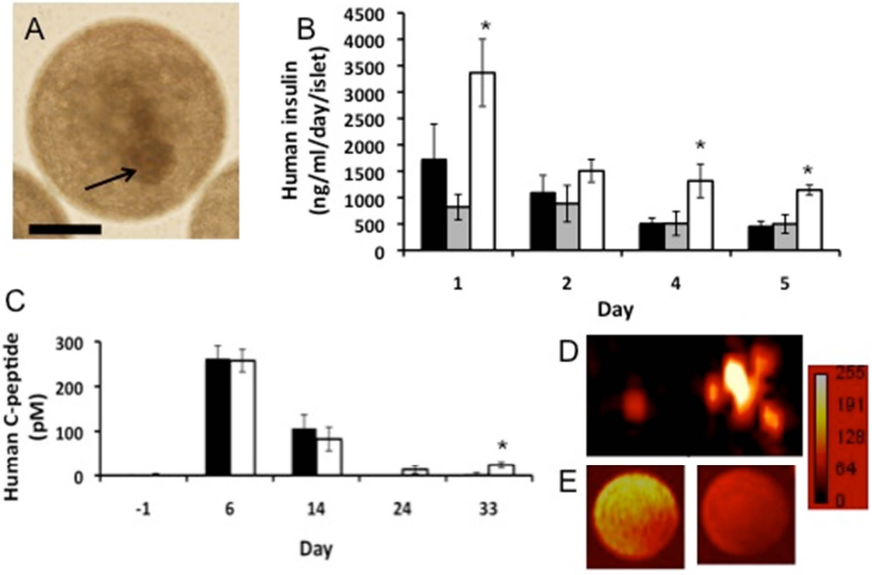

Figure 1: (**A**) Microscopic image of a human islet (arrow) encapsulated inside a fluorocapsule. Scale bar: 200 µm. (**B**) Human insulin secretion levels of unencapsulated islets (black bars), encapsulated islets without (grey) or with PFPE emulsions (white) in hypoxic culture (O_2 level ≤ 1%). Asterisk indicates the days when the insulin levels of encapsulated islets were statistically significantly different from unencapsulated islets ($P<0.05$). (**C**) Circulating C-peptide levels of encapsulated human islet grafts inside diabetic NOD/ShiLtJ mice (n=4) without (black bars) or with (white) PFPE. Asterisk indicates the day when the levels became statistically different ($P<0.05$). (**D**) Axial ^{19}F MR images of a single (left) and 10 (right) fluorocapsules embedded inside 2% agarose. (**E**) Axial ^{19}F MR-images of intact (left) and ruptured (right) fluorocapsules inside 5-mm NMR tubes.