Fluorocapsules for Improved Function, Immunoprotection, and Visualization of Cellular Therapeutics with MR, US, and CT Imaging¹

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Purpose:

To develop novel immunoprotective alginate microcapsule formulations containing perfluorocarbons (PFCs) that may increase cell function, provide immunoprotection for xenografted cells, and simultaneously enable multimodality imaging.

Materials and Methods:

All animal experiments were approved by an Institutional Animal Care and Use Committee. Cadaveric human islet cells were encapsulated with alginate, poly-L-lysine, and perfluorooctyl bromide (PFOB) or perfluoropolyether (PFPE). In vitro viability and the glucose-stimulation index for insulin were determined over the course of 2 weeks and analyzed by using a cross-sectional time series regression model. The sensitivity of multimodality (computed tomography [CT], ultrasonography [US], and fluorine 19 [¹⁹F] magnetic resonance [MR] imaging) detection was determined for fluorocapsules embedded in gel phantoms. C57BL/6 mice intraperitoneally receiving 6000 PFOB-labeled ($n = 6$) or 6000 PFPE-labeled ($n = 6$) islet-containing fluorocapsules and control mice intraperitoneally receiving 6000 PFOB-labeled ($n = 6$) or 6000 PFPE-labeled ($n = 6$) fluorocapsules without islets were monitored for human C-peptide (insulin) secretion during a period of 55 days. Mice underwent ¹⁹F MR imaging at 9.4 T and micro-CT. Swine ($n = 2$) receiving 9000 PFOB capsules through renal artery catheterization were imaged with a clinical multidetector CT scanner. Signal intensity was evaluated by using a paired t test.

Results:

Compared with nonfluorinated alginate microcapsules, PFOB fluorocapsules increased insulin secretion of encapsulated human islets, with values up to 18.5% (3.78 vs 3.19) at 8-mmol/L glucose concentration after 7 days in culture ($P < .001$). After placement of the immunoprotected encapsulated cells into mice, a sustained insulin release was achieved with human C-peptide levels of 19.1 pmol/L $\pm$ 0.9 (standard deviation) and 33.0 pmol/L $\pm$ 1.0 for PFPE and PFOB capsules, respectively. Fluorocapsules were readily visualized with ¹⁹F MR imaging, US imaging, and CT with research- and clinical-grade imagers for all modalities.

Conclusion:

Fluorocapsules enhance glucose responsiveness and insulin secretion in vitro, enable long-term insulin secretion by xenografted islet cells in vivo, and represent a novel contrast agent platform for multimodality imaging.

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Immunoisolation techniques, such as microencapsulation, have shown considerable potential for preventing immune-mediated cell destruction (1). Microencapsulation provides a semipermeable membrane that prevents the passage of antibodies but allows for passage of cell-derived therapeutic factors (2). Despite this semipermeability, encapsulation methods are often hindered by their inability to provide sufficient oxygen for long-term graft survival (3). Studies have shown that while nonencapsulated islets are completely vascularized after transplantation (4), encapsulation prevents the neovascularization process and thus fresh flow of oxygen, which can eventually lead to graft necrosis (5).

A potential means of increasing local oxygen tension in a graft is through the incorporation of perfluorocarbons (PFCs)—hydrocarbons in which most of the hydrogen atoms have been replaced with fluorine (6). PFCs have therefore been utilized in numerous biomedical applications, including use as blood substitution agents (7) and gas-permeable wound protectants (8). PFCs exhibit a high capacity for dissolving respiratory gases, with oxygen solubility in PFCs being 20 to 25 times greater than that in water or blood plasma (9). Furthermore, unlike hemoglobin, PFCs cannot chemically bind to oxygen and, as a result, release oxygen much more efficiently.

We hypothesized that encapsulated islet cells with coembedded PFC could benefit from such an increased oxygen tension in the immediate proximity of cells. Moreover, PFCs have been utilized

as imaging contrast agents for computed tomography (CT) (10), ultrasonography (US) (11), and magnetic resonance (MR) imaging (12–15), alone and in combination (16). To this end, we have tested two PFC preparations, perfluorooctyl bromide (PFOB) and perfluoropolyether (PFPE). Compared with PFOB, PFPE is preferred for use as a fluorine 19 (^{19}F) MR imaging contrast agent because all its fluorine atoms are chemically equivalent, which yields a single peak at nuclear MR spectroscopy. This is in contrast to the ^{19}F nuclear MR spectrum of PFOB, where eight resonance peaks are observable, one for each chemically equivalent ^{19}F nucleus. However, the presence of bromine may provide radiopacity and make capsules visible at CT.

By making encapsulated islets visible in a noninvasive manner, one could potentially monitor the capsule distribution, ideal transplantation site, and the best means of graft delivery in real-time by using imaging guidance. This has previously been demonstrated for islets encapsulated in magnetocapsules, where superparamagnetic nanoparticles were used to provide MR visibility (17). The purpose of our study was to develop novel immunoprotective alginate microcapsule formulations containing PFCs that may improve glucose responsiveness (insulin secretion) and simultaneously enable multimodality imaging.

Materials and Methods

Some authors (B.P.B., D.L.K., A.A., J.W.M.B.) hold intellectual property on contrast material-containing microcapsules for cell therapy. Two authors (A.A., J.W.M.B.) are consultants for SurgiVision (Irvine, Calif). One author (A.A.) is the founder of Surefire Medical

Implication for Patient Care

- In patients with type I diabetes, the transplantation of fluorocapsules may enhance cell function and enable tracking of encapsulated cells with three conventional clinical imaging technologies.

(Atlanta, Ga). One author (B.P.B.) is a Pfeiffer Foundation Fellow.

Preparation and Physicochemical Characterization of Fluorocapsules Containing Human Islets

A total of 1.97 g/mL PFOB (Sigma-Aldrich, St Louis, Mo) and 1.88 g/mL PFPE (Exflur, Round Rock, Tex) were emulsified (20% vol/vol) in a mixture of 5% lecithin (Sigma-Aldrich), 2% safflower oil (Sigma-Aldrich), and water by using sonication at 40% power. Size and zeta potential were determined by using dynamic light scattering and laser Doppler anemometry, respectively (Zetasizer 3000; Malvern Instruments, Southborough, Mass). Size measurements were performed at 25°C at a scattering angle of 90°.

To make fluorocapsules, the emulsions were mixed with 2% (w/v) ultrapurified sodium alginate (Protanal HF; FMC Biopolymers, Haugesund, Norway) and 20% (v/v) emulsified PFC. By using a three-way stop cock, homogeneous suspension was made through repeated transfer from one syringe to the other. Fresh human cadaveric islets were provided by the National Islet

Advances in Knowledge

- Fluorocapsules can increase insulin secretion of encapsulated islets (up to 18.5% in vitro) and enable prolonged insulin secretion in vivo for at least 55 days.
- Fluorocapsules with encapsulated human islets can be visualized with three clinical modalities, fluorine 19 MR imaging, US imaging, and CT, with research- and clinical-grade imagers for all modalities.

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Abbreviations:

PFC = perfluorocarbon

PFOB = perfluorooctyl bromide

PFPE = perfluoropolyether

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See Materials and Methods for pertinent disclosures.

See also Science to Practice in this issue.

Cell Resource Center Distribution Program. Islets were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, Calif), supplemented with 10% fetal calf serum (Invitrogen) and 1% penicillin-streptomycin-L-glutamine (Invitrogen), in a humidified incubator at 37°C and a 5% CO₂ atmosphere. Before encapsulation, human cadaveric islets were passed through a 20-gauge needle. Cells, adjusted to 400 islet equivalents per milliliter, were suspended in the ultrapurified sodium alginate and PFC emulsion mixture. The cell-alginate-PFC solution was passed through a needle at 200 mL/min by using a nanoinjector pump (Harvard Apparatus, Holliston, Mass) and an electrostatic droplet generator (Spellman, Hauppauge, NY). Droplets, representing islet cells surrounded by the first layer of alginate, were collected in a Petri dish containing 100 mmol/L CaCl₂ in 10 mmol/L HEPES buffer and then were washed three times. Gelled droplets were suspended in 0.05% poly-L-lysine (22–24 kDa, Sigma-Aldrich) for 5 minutes to cross-link the alginate. Droplets were washed and resuspended in 0.15% alginate (Keltone HVCR; Monsanto, St Louis, Mo) for 5 minutes and then were washed again.

In Vitro Characterization of Encapsulated Human Islets

Encapsulated human islets were cultured in standard RPMI 1640 medium (Invitrogen), supplemented with 10% fetal calf serum (Invitrogen) and antibiotics, and incubated at 37°C and 5% CO₂. The viability of encapsulated islets was determined by using Newport Green and propidium iodide (Invitrogen) staining. The permeability of PFC microcapsules was determined by incubation with fluorescent lectins of varying molecular weights for 48 hours in culture. The lectins used were wheat germ agglutinin (molecular weight = 36 kDa), *Maackia amurensis* lectin I (molecular weight = 75 kDa), *Ricinus communis* agglutinin I (molecular weight = 120 kDa), and elderberry lectin (molecular weight = 150 kDa) (Invitrogen).

To assess the glucose responsiveness of encapsulated islets, PFC microcapsules were incubated in RPMI

medium with 3 mmol/L glucose raised to 8 mmol/L. Secreted C-peptide concentrations were assessed (B.P.B.) at day 0, 7, and 14 after encapsulation by using an ultrasensitive human C-peptide enzyme-linked immunosorbent assay (Alpco Diagnostics, Salem, NH).

Fluorocapsule Transplantation Studies in Mice

All animal experiments were approved by our Institutional Animal Care and Use Committee. Fluorocapsules were transplanted into the peritoneal cavity of normal C57BL/6 mice (Charles River, Frederick, Md). Fluorocapsules were either 6000 PFOB labeled ($n = 6$) or 6000 PFPE labeled ($n = 6$), containing an average number of one human islet per capsule. PFOB provides radiopacity through the presence of bromine, and PFPE provides a single peak for ¹⁹F MR imaging. Control C57BL/6 mice for each group (PFOB [$n = 6$] and PFCE [$n = 6$]) were transplanted with 6000 empty microcapsules (ie, not containing cells). To test the functionality of encapsulated islets, blood samples were taken (B.P.B.) every 3–5 days through the tail vein during a 55-day period for measurement of serum human C-peptide (ie, insulin secretion) by using an ultrasensitive human C-peptide enzyme-linked immunosorbent assay kit (Alpco Diagnostics).

Fluorine 19 MR Imaging with Preclinical Imagers

In vivo MR imaging of mice transplanted with fluorocapsules was performed by using several experimental imagers. For high-magnetic-field applications, a 9.4-T MR imager (Bruker BioSpin, Karlsruhe, Germany) was used, and it was equipped with a slotted tube radiofrequency resonator (9.4 T), tunable to both ¹⁹F and hydrogen 1 (¹H) frequencies. In vitro MR imaging was performed in a 5-mm nuclear MR tube at 11.7 T for single capsule detection by using the standard proton probe (Bruker). A standard T2-weighted spin-echo pulse sequence was used for proton imaging (repetition time msec/echo time msec, 1500/15; field of view, 3 × 3 cm; image matrix, 128 × 64; 1-mm section thickness; number of

signals acquired, one), and a fast spin-echo sequence was used for fluorine imaging (same geometric parameters and 1080/47 [effective]; image matrix, 64 × 32; number of signals acquired, 64). Images were processed by using open-source software (DICOM Viewer; OsiriX Foundation, Los Angeles, Calif), and three-dimensional images were generated by using imaging software (Amira 4; Visage Imaging, San Diego, Calif).

Fluorine 19 MR Imaging with a Clinical Imager

For ¹⁹F MR imaging, one to 50 PFPE capsules were placed in 0.2-mL polymerase chain reaction tubes (VWR, Buffalo Grove, Ill) and covered with 4% gelatin. This phantom was imaged by using a clinical 3.0-T imager (Tim Trio; Siemens, Erlangen, Germany). To this end, a custom fluorine phased-array coil with four coil elements was used. Scout proton MR images were obtained by using a surface coil (Body Matrix; Siemens) followed by manual shimming by using the body coil. The custom fluorine coil was then used to obtain fluorine images by using a three-dimensional true fast imaging with steady-state free precession pulse sequence with the following parameters: 4.1/2.1; number of signals acquired, 32; 1.5 × 1.5 × 2.0-mm voxel size; field of view, 672 × 250; 1002-Hz bandwidth; 70° flip angle; and an acquisition time of about 12 minutes.

Micro-CT Imaging

Images of tubes containing fluorocapsules and mice with transplanted fluorocapsules were obtained by using a scanner (XSPECT; Gamma Medica, Northridge, Calif). For each scan, 1024 projections were obtained at 1° for a full 360° rotation. Scanning was performed in a clockwise direction with an x-ray tube-to-detector distance of 269 mm and an x-ray tube-to-center of rotation distance of 225 mm. Images were reconstructed by using standard back-projection techniques, and the images were reconstructed into axial sections with 1024 × 1024 pixels per section. Images were processed by using open-source software (DICOM Viewer; OsiriX Foundation), and three-dimensional

images were generated by using imaging software (Amira 4; Visage Imaging).

Fluorocapsule Transplantation and CT Imaging in Swine

Experiments were performed in two healthy swine (40–45 kg) (Thomas D. Morris, Reisterstown, Md). The renal arteries were catheterized with a 5-F cobra catheter (Cook, Bloomington, Ind) by using a femoral artery percutaneous puncture, and 9000 PFOB fluorocapsules per kidney were delivered intraarterially. All CT examinations were performed 2 hours after microcapsule injection by using a multidetector CT scanner (Somatom Volume Zoom; Siemens) with the following imaging parameters: 130 kV; 100 mAs; rotation time of 1.0 second; pitch of 2; section thickness, 500 μm ; image size, 512×512 . To evaluate the in vivo detectability of the fluorocapsules (B.P.B., A.A.), the scanning range covered the diaphragm to the pelvic floor. Images were processed and evaluated for attenuation by using open-source software (OsiriX Foundation).

US Imaging

To compare the relative echogenicity of unlabeled microcapsules against PFOB capsules (B.P.B.), an imaging software package (Image J; National Institutes of Health, Bethesda, Md) was used to compare the relative signal intensity of 10 representative US images of a single unlabeled or PFOB-labeled microcapsule. To evaluate the detectability of fluorocapsules with US imaging (B.P.B.), five PFOB microcapsules were transplanted into an explanted swine kidney through a 20-gauge needle. Sonography was performed with a probe (L25E 13-6 MHz; SonoSite, Bothell, Wash) and US system (Micromaxx; SonoSite). Gray-scale imaging was performed with a center probe frequency of 6.00 MHz, a dynamic range of 55 dB, and a persistence setting of two. Gray-scale gain was adjusted for baseline imaging. Segmentation and three-dimensional reconstruction for all imaging was performed by using imaging software (Amira; Visage Imaging).

Statistical Analysis

Statistical testing (D.L.K.) of in vitro capsule viability over time and between

Figure 1

Figure 1: (a) Light microscopic image of a PFOB fluorocapsule containing an encapsulated human islet. (Original magnification, $\times 400$.) (b) Newport Green and propidium iodide viability staining of a human islet 7 days after encapsulation in a PFOB fluorocapsule. (Capsule was dissolved to allow for direct fluoroscopic observation of human islet.) (Original magnification, $\times 400$.) Bars = 150 μm .

capsule types was performed by using a cross-sectional time series regression model (ie, panel data, by using a generalized least-squares estimator with a fixed- or random-effects model for between-regression estimations) (Stata, version 11; Stata, College Station, Tex). Comparisons were performed by using both fixed- and random-effects modeling and, if no difference was seen, then the random-effects model was used. Data were clustered by capsule batches where appropriate to eliminate bias due to correlated samples. Panel data for viability were first evaluated for the effects of labeling and time of sampling. If effects were found, further evaluation of the interaction of labeling and time of sampling was performed, and results were reported if statistically significant. Insulin secretion with 3-mmol/L and 8-mmol/L glucose medium was analyzed in a similar manner by using cross-sectional time series regression analysis. In addition, glucose responsiveness was calculated as the difference between 8-mmol/L and 3-mmol/L insulin levels at each time point. Glucose responsiveness determined in vitro and C-peptide levels determined in vivo were evaluated by using cross-sectional time

series regression analysis as reported for viability above.

Attenuation on CT images between kidney containing PFC capsules and nonembolized kidney was evaluated by using a paired *t* test (B.P.B.). A *P* value less than .05 was considered to indicate a statistically significant difference. Data were reported as means $\pm$ standard deviations.

Results

Physiochemical and Biologic Properties of Fluorocapsules

For PFPE nanoparticles, the zeta potential was $79.6 \text{ mV} \pm 14.7$, with an average particle diameter of 169.4 nm. For PFOB nanoparticles, the zeta potential was $72.8 \text{ mV} \pm 3.0$, and average particle diameter was 213.4 nm. Fluorocapsules exhibited a microscopic appearance similar to that of conventional unlabeled alginate capsules and contained on average one human islet (Fig 1a). Viability staining with Newport Green and propidium iodide demonstrated that, at 7 days after encapsulation, the average viability was greater than 90% for all capsule types (Fig 1b). Fluorocapsules

Figure 2

Figure 2: Graphs show in vitro and in vivo functionality of fluorocapsules containing human islets. (a) Viability of human islets encapsulated with PFOB (black bars) or PFPE (striped bars) or of those without PFC (open bars). (b) Glucose-stimulation index (the increase in insulin secretion from 3-mmol/L to 8-mmol/L glucose medium) of human islets encapsulated with PFOB (black bars) or PFPE (striped bars) or of those without PFC (open bars). (c, d) Mouse serum human C-peptide levels after intraperitoneal transplantation of (c) 6000 PFPE or (d) 6000 PFOB fluorocapsules with (●, $n = 6$) or without (○, $n = 6$) encapsulated human islets. Error bars = standard deviation.

were uniform in size, with an average diameter of 350 μm .

The addition of PFCs may potentially alter the size-selective permeability of the alginate microcapsules. Permeability assessment by using fluorescent lectins with varying molecular weights revealed no appreciable difference in permselectivity. The fluorocapsules were permeable to fluorescent lectins with molecular weights less than 75 kD but were impermeable to lectins with molecular weights greater than 120 kD (ie, suitable for blocking antibody penetration

[immunoisolation] while allowing inflow of nutrients and free diffusion of islet-secreted insulin).

Islet Viability and Glucose Responsiveness

The viability and glucose-stimulation index of human islets encapsulated with PFOB or PFPE or of those without PFC were assessed at day 1, 7, and 14 after encapsulation. No difference in viability between PFOB and PFPE capsules was detected ($P = .39$) (Fig 2a). Capsule viability declined significantly at days 7

(0.91 ± 0.02 , $P < .001$) and 14 (0.88 ± 0.03 , $P < .001$) relative to day 1 (0.92 ± 0.03) for all capsule types. Labeling of capsules with PFCs did not appear to alter this trend of viability decline over the course of time. However, PFOB and PFPE capsules had an overall better viability than unlabeled capsules (0.91 ± 0.02 for PFOB vs 0.92 ± 0.03 for PFCE vs 0.88 ± 0.03 for unlabeled capsules, $P < .001$).

In vitro insulin (C-peptide) secretion was similar between PFPE and PFOB capsules (1.45 ± 0.21 for PFCE

vs 1.41 ± 0.24 for PFOB, $P = .34$) and between PFOB and control capsules (1.43 ± 0.18 for control capsules, $P = .64$) at 3-mmol/L glucose concentration. Insulin release increased when capsules were transferred from 3-mmol/L to 8-mmol/L glucose medium (Fig 2b). At 8-mmol/L concentration, there was a significantly increased insulin secretion in PFOB capsules (3.78 ± 0.40) relative to PFPE (3.45 ± 0.28 , $P < .001$) and control (3.19 ± 0.25 , $P < .001$) capsules. Overall, insulin release significantly decreased at day 7 and day 14 relative to day 1 in both low glucose (3-mmol/L) medium and high glucose (8-mmol/L) medium. When comparing PFOB to PFPE capsule formulations, glucose responsiveness (change in insulin release when capsules were transferred from 3- to 8-mmol/L glucose medium) indexed at day 1 of PFOB capsules (2.45 ± 0.37) declined only in PFPE capsules at day 7 (1.88 ± 0.267 , $P < .02$). Glucose responsiveness at other time points in PFPE and unlabeled capsules was indistinguishable from PFOB capsules at day 1.

After transplantation of 6000 PFOB- or PFPE-encapsulated human islets (with an average number of one islet per capsule) into the peritoneal cavity of normal mice, serum human C-peptide levels were $19.1 \text{ pmol/L} \pm 0.9$ and $33.0 \text{ pmol/L} \pm 1.0$ for PFPE- and PFOB-encapsulated islets, respectively (Fig 2c, 2d), whereas levels were $-1.21 \text{ pmol/L} \pm 1.9$ for PFPE capsules and $-1.55 \text{ pmol/L} \pm 1.2$ for PFOB capsules without islets (eg, control capsules). The C-peptide levels in PFPE- and PFOB-encapsulated islets remained relatively constant over the course of 50 days after transplantation (not significant). However, C-peptide levels were significantly higher in PFOB-encapsulated islets than in PFCE-encapsulated islets ($P < .001$).

Imaging of Fluorocapsules

Results of in vitro ^{19}F MR imaging of 350- μm -diameter PFPE fluorocapsules with an 11.7-T imager demonstrated the ability to depict single capsules (Fig 3a). After transplanted into the peritoneal cavity of mice, fluorocapsules were clearly visible at in vivo ^{19}F MR imaging and, when overlaid on anatomic ^1H MR im-

Figure 3

Figure 3: (a) In vitro proton T2-weighted MR image (left) and corresponding ^{19}F MR image (right) at 11.7 T of PFPE fluorocapsules in a standard 5-mm glass tube. (The capsules were introduced in a 500- μm capillary tube.) (b) In vivo ^{19}F MR image at 9.4 T of a mouse after intraperitoneal transplantation of 2000 PFPE fluorocapsules. Overlay of the ^{19}F image (pseudocolor) on the ^1H image (gray scale) is shown. (c) Fluorine 19 MR image of PFPE fluorocapsules obtained with 3.0-T clinical system. From left to right, images are of gel phantoms containing one, two, five, 10, 15, 25, and 50 capsules.

ages, were easily distinguishable from the surrounding tissue (Fig 3b). By using an optimized true fast imaging with steady-state precession sequence, individual PFPE fluorocapsules were clearly detectable at 3.0 T (Fig 3c). With micro-CT imaging, individual PFOB fluorocapsules were visible in vitro (Fig 4a–4c) and in vivo when transplanted into the peritoneal cavity of a mouse (Fig 4d, 4e). By using a clinical-grade CT imager, PFOB fluorocapsules were also readily distinguished after transplantation into the kidney of a pig (Fig 4f). Of note, the contrast in the right kidney appears primarily in the collecting system and likely represents residual iohexol, whereas the contrast in the embolized left kidney appears as wedgelike streaks outside of the collecting system. The average attenuation from the embolized kidney in the plane of scan represented in Figure 4f was significantly increased ($P < .05$) at $203 \text{ HU} \pm 64$ compared

with the nonembolized kidney parenchyma with an average attenuation of $74 \text{ HU} \pm 24$. By using US imaging, individual PFOB fluorocapsules were visible both in vitro (Fig 5a–5c) and ex vivo after transplantation into the kidney of a pig (Fig 5d, 5e). The relative intensity values for unlabeled and PFOB capsules were 40.21 ± 3.87 and 87.66 ± 6.23 , respectively.

Discussion

Previous methods for tracking cellular therapeutics with MR imaging have been limited to direct labeling of cells with contrast agents (12,18) or a reporter gene method (19,20). By encapsulating cells in contrast material-containing alginate microcapsules, we can increase the overall payload of contrast agent, while providing immunoprotection of cells against a host-versus-graft rejection. Previously, we developed radiopaque (21)

Figure 4

Figure 4: (a–c) From left to right, CT images show one, five, and 10 PFOB fluorocapsules embedded in gelatin. (d) In vivo micro-CT image of a mouse after intraperitoneal transplantation of 2000 PFOB fluorocapsules (arrows). (e) Three-dimensional reconstructed image demonstrates that radiopacity of PFOB fluorocapsules (green) can be distinguished from bone. (f) CT image (using a clinical system) of 9000 PFOB fluorocapsules transplanted unilaterally into the left swine kidney. Residual iohexol in renal collection system from angiography prior to transplantation is visible in right kidney.

and MR-visible capsules (17) by using barium or bismuth sulfate and superparamagnetic iron oxide particles, respectively. Here, we developed novel fluorocapsules and demonstrated that they can be used to encapsulate human cadaveric islets while enhancing insulin secretion and enabling detection with three clinically applicable imaging modalities. Compared with PFOB, PFPE is preferred for use as a ^{19}F MR contrast agent because all its fluorine atoms are chemically equivalent, which yields a single peak at nuclear MR spectroscopy. This

is in contrast to the ^{19}F nuclear MR spectrum of PFOB, where eight resonance peaks are observable, one for each chemically equivalent ^{19}F nucleus. Thus, as a ^{19}F MR contrast agent, PFPE has a higher intrinsic sensitivity because all fluorines are spectrally equivalent, but, because of the lack of bromine, it lacks radiopacity and thus cannot be used as a CT contrast agent. In contrast, PFOB fluorocapsules proved to be detectable at CT. In addition, because PFOB is visible at US (11,22), it more than doubles the echogenicity of an unlabeled microcapsule.

PFCs have been shown to act as an oxygen sink facilitating an increase in gas exchange. For this reason, PFCs have been used as successful blood substitution agents in clinical trials because of their high oxygen solubility coefficient and ability to maintain high oxygen partial pressures for extended times. Furthermore, these agents can serve as oxygen “reservoirs” for harvested organs in pancreas transplantation studies (23–27). Both the fluorinated agents PFPE and PFOB are attractive in terms of theoretical safety because they are

Figure 5: US images show (a) one and (b) 15 PFOB fluorocapsules embedded in gelatin. (c) Graph shows the relative echogenicity of unlabeled microcapsules against PFOB capsules for 10 representative US images of a single unlabeled or PFOB-labeled microcapsule. The relative signal intensity values for unlabeled and PFOB capsules were 40.21 ± 3.87 and 87.66 ± 6.23 , respectively. Inset is example of representative picture for unlabeled and PFOB-labeled microcapsules. US images show (d) needle in kidney of swine and (e) transplantation of PFOB microcapsules at previous position of needle.

biologically inert and are not broken down. Both agents leave the body in a gaseous phase by exhalation. PFOB is marketed both as LiquiVent (Alliance Pharmaceuticals) (28), an oxygen-carrying liquid drug, and Oxygent (Alliance Pharmaceuticals), a blood substitution agent.

Khattak et al (6) initially demonstrated that adding a PFC emulsion into alginate microcapsules containing HepG2 cells resulted in a significant increase in cell viability. Although our incorporation of PFCs in alginate microcapsules did not show a statistically significant increase in the viability of encapsulated cells *in vitro*, insulin secretion was found to be improved. This enhanced cell function is likely the result of an increased oxygen concentration within the capsules, which can be measured at ^{19}F MR imaging (29). For clinical procedures, such as islet cell transplantation, this is vital because these capsules with PFC reservoirs could

“recharge” themselves by picking up O_2 from plasma that perfuses through the matrix. This concept has been applied to emulsified fluorocarbons currently being used for surgical applications (30). Furthermore, our data also support the notion that incorporation of PFCs into microcapsules does not affect islet response to glucose stimulation. By acting as oxygen sinks, PFC-containing microcapsules may have broad implications for increasing the responsiveness and function of many other cell types.

Previous research suggests that PFOB, in addition to increasing local oxygen concentration, may have an enhanced effect on immunosolatory properties of alginate capsules. The immunosolatory ability of alginate microcapsules currently results from a microporous matrix that prevents passage of relatively large antibodies and complement, while allowing for passage of standard

metabolites. *In vitro* studies have shown that PFOB reduces cytokine release by human alveolar macrophage and reduces oxidative injury in rat pulmonary artery endothelial cells (31). Furthermore, neutrophil adhesion to lung epithelial cells and subsequent cytolysis are inhibited by PFOB during the activation with a proinflammatory stimulus (32), and PFOB reduces neutrophil adhesion to activated endothelial cells (33). For these reasons, PFOB-loaded alginate capsules could further reduce rejection of transplanted cellular therapeutics in immunocompetent hosts.

A final potential advantage of incorporating PFCs in microcapsules, demonstrated here, was that it can provide a means of tracking and delivering cells by using imaging modalities such as CT, MR, or US imaging. X-ray- and US-guided procedures are currently the preferred method for minimally invasive interventions for cellular therapy. For this reason,

PFOB microcapsules could prove an ideal vehicle for targeted delivery of cellular agents, such as was previously demonstrated for superparamagnetic iron oxide-labeled capsules (17). Furthermore, because microcapsules containing PFCs are detectable at MR imaging, follow-up examinations with ^{19}F MR imaging may be performed while avoiding radiation exposure.

Analogous to radionuclide tracers, fluorine has no endogenous signal at in vivo imaging and is therefore an attractive MR contrast agent. Ahrens et al (12) estimated that the sensitivity of MR imaging visualization is in the order of 2×10^5 PFPE-labeled dendritic cells (containing approximately 5.2×10^{12} fluorine spins) per voxel at 11.7 T. This number agrees with an estimated number of 7500 PFPE-labeled neural stem cells at a slightly lower field strength of 9.4 T (15). As individual fluorocapsules incorporate orders of magnitude more contrast agent than single cells, the detection of single PFPE fluorocapsules is possible both with a research-grade 11.7-T, as well as clinical-grade 3.0-T, MR imager. Single microcapsule detectability at 3.0 T makes PFPE fluorocapsules ideally suited for clinical applications when precise tracking of microcapsules carrying a therapeutic agent is required.

In conclusion, by exploiting the inherent properties of PFCs, “smart” microcapsules can be synthesized, which increases local oxygen content and hence cell function for improved islet cell engraftment. Furthermore, the semipermeability of microcapsules enables immunoprotection and sustained cell function as demonstrated in our xenograft model of transplanting human islets into mice. The addition of fluorine has the added advantage of enabling monitoring of local oxygen tension noninvasively with ^{19}F MR imaging. Moreover, these fluorinated capsules are suitable for cell tracking with three clinically applicable imaging modalities (CT, US, and MR imaging). As multimodality imaging suites, such as hybrid x-ray/MR imaging systems, become more prevalent, capsules containing multimodality contrast agents, such as PFOB, could be used for accurate transplantation into the body

and subsequently monitored for functionality by using ^{19}F MR imaging. A possible limitation may exist in long-term stability of the capsules once grafted because very little is known about the host-versus-graft tissue reaction. However, by encapsulating brominated and fluorinated contrast agent, we open the door to noninvasive monitoring of capsule integrity and capsule rupture.

We have demonstrated the ability to depict fluorocapsules with clinical US, CT, and MR imagers. For clinical applications, such as islet cell transplantation in those with type I diabetes, this would allow researchers and clinicians to accurately deliver, monitor, and improve the function of transplanted, encapsulated insulin-producing cells. Finally, fluorocapsules may have a broader effect because they could be applied to other forms of cell therapies.

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