Published in final edited form as:

NMR Biomed. 2013 July ; 26(7): 850–859. doi:10.1002/nbm.2894.

Microencapsulated cell tracking

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Abstract

Microencapsulation of therapeutic cells has been widely pursued to achieve cellular immunoprotection following transplantation. Initial clinical studies have shown the potential of microencapsulation using semi-permeable alginate layers, but much needs to be learned about the optimal delivery route, *in vivo* pattern of engraftment, and microcapsule stability over time. In parallel with noninvasive imaging techniques for ‘naked’ (i.e. unencapsulated) cell tracking, microcapsules have now been endowed with contrast agents that can be visualized by ¹H MRI, ¹⁹F MRI, X-ray/computed tomography and ultrasound imaging. By placing the contrast agent formulation in the extracellular space of the hydrogel, large amounts of contrast agents can be incorporated with negligible toxicity. This has led to a new generation of imaging biomaterials that can render cells visible with multiple imaging modalities.

Keywords

microcapsules; cell therapy; diabetes; islet cell transplantation; MRI; ultrasound imaging; computed tomography; contrast agent

USE OF MICROENCAPSULATION FOR CELLULAR IMMUNOPROTECTION

The advent of cell therapy has created hope and expectations that, in the not too distant future, certain diseases may be treated in ways that were not previously possible. Stem cell therapy has been the focus of attention, with clinical targets ranging from prevalent diseases,

such as diabetes (1), stroke (2) and myocardial infarction (3), to less life-threatening conditions, e.g. androgenic alopecia (4) and erectile dysfunction (5). Although autologous stem cell transplantation may become the method of choice with the use of the patient's own induced pluripotent stem cells, most current transplantation paradigms rely on allografting. Often, donor cells are scarce, and an improper match of major histocompatibility antigens may cause acute graft failure. In islet allotransplantation studies for the treatment of type I diabetes mellitus, success can only be obtained by using very stringent immunosuppression regimens (6), which, in turn, may lead to serious complications (7). In addition, the recent success in the use of xenotransplants mandates well-controlled protocols of immunosuppression or immunoprotection (8–10).

Immunoisolation of islets provides a means to reduce or avoid immunosuppressive therapy altogether. In the most commonly used method of immunoisolation, microencapsulation (11), individual islets are surrounded with thin alginate membranes that are permeable to insulin and metabolites, but are impermeable to native antibodies. Small-scale preclinical (12) and clinical (13,14) trials, including the commercial development of a porcine xenotransplant (15), have provided evidence of the potential of microencapsulated islet cell therapy for the treatment of type I diabetes mellitus. Alginate microcapsules have also found cellular therapy applications beyond replacement of the pancreas. This includes, but is not limited to, the use of encapsulated hepatocytes for the treatment of fulminant liver failure (16), choroid plexus-derived epithelial cells for Huntington's disease (17), glial cell-derived neurotrophic factor-engineered myoblasts for Parkinson's disease (18), endostatin-engineered cells for tumor therapy (19), cellular cardiomyoplasty (20), antibody-secreting hybridoma cells for immunostimulation (21), and angiogenic monocytes (22) and mesenchymal stem cells (MSCs) (23) for the treatment of ischemia. Of note is that, in addition to their clinical use for islet encapsulation, microcapsules have been used in patients for the treatment of inoperable pancreatic cancer with kidney epithelial cells overexpressing the cytochrome P450 enzyme, also known as NovaCaps[®]. These cells locally activate systemically administered ifosfamide. The tumors of four patients regressed after treatment, and those of the other 10 individuals who completed the study remained stable. Median survival was doubled in the treatment group in comparison with historic controls, and the 1-year survival rate was improved three-fold (24).

Despite the multiplicity of recent studies using microencapsulation therapy, basic questions, such as the best mode of delivery, initial engraftment and tissue biodistribution, and whether or not the capsules remain intact over time, remain largely unanswered (25). If microcapsules could be imaged noninvasively after transplantation, a number of these questions could be better addressed. This review focuses on recent studies that have been successful in visualizing microencapsulated cells with multiple modalities, i.e. MRI, computed tomography (CT)/X-ray imaging and ultrasound (US) imaging.

MICROENCAPSULATION AND LABELING WITH CONTRAST AGENTS

Microcapsules are most commonly prepared from hydrogels, as these materials provide a highly hydrated environment in which cells are physiologically embedded, are characterized by an overall softness and flexibility, reducing mechanical friction in the host tissue, and enable a high degree of capsule permeability for low-molecular-mass metabolites and nutrients (26). Microcapsules can be synthesized according to three different methods: (i) matrix core/shell microcapsules, manufactured by gelling alginate droplets in a solution containing a bivalent ion (Ba^{2+} or Ca^{2+}), followed by a surface treatment with a polycation, often followed by the addition of a second layer of alginate; (ii) liquid core/shell microcapsules, produced by dropping an islet cell suspension containing bivalent ions into an alginate solution; and (iii) cells-core/shell microcapsules (i.e. conformal coating). The

first method, exemplified by using alginate as hydrogel and poly-L-lysine (PLL) as cationic cross-linker, has been by far the most widely used technique (11). Figure 1 shows a schematic representation of this synthesis. In brief, the cells of interest are first mixed with 2% w/v ultrapurified sodium low-viscosity guluronate (LVG) alginate. This mixture is passed through a needle using a nano-injector pump. Droplets, representing cells surrounded by the first layer of alginate, are collected in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer containing 100 mM CaCl₂ or 20 mM BaCl₂, and then washed three times in 0.9% saline. The gelled droplets are then suspended in 0.05% w/v cross-linker [PLL or protamine sulfate (PS)] for 5 min to cross-link the first layer of alginate. The droplets are washed with 0.9% saline and resuspended in 0.15% w/v low-viscosity mannuronate (LVM) alginate for 5 min, and then finally washed with 0.9% saline. The rationale for using two different alginate species is that the inner LVG provides superior mechanical strength, whereas the outer LVM alginate is less immunogenic.

Despite their reported use to successfully restore euglycemia in numerous diabetic animal studies, traditional alginate–poly-L-lysine–alginate (APLLA) microcapsules are not 100% biocompatible and have shown to be able to initiate both a foreign body and immune response (27–33). PS is a pharmaceutical-grade compound primarily composed of L-arginine, and can be found in nearly every hospital as a heparin antagonist. It has been used as a transfection agent with a much less toxic profile than conventional polycationic agents (34), and as a superparamagnetic iron oxide (SPIO) labeling agent to render cells MR visible (35). Our experience has shown alginate–protamine sulfate–alginate (APSA) formulations to have superior properties in terms of mechanical strength and cell viability (36,37).

Microcapsules can be made visible by adding contrast agents to the primary alginate layer in the step 1, such as the radio-opaque agents barium or bismuth sulfate (23,38) and gold (36,39) for detection by CT/X-ray modalities, SPIO for detection by ¹H MRI (39–43) or perfluorocarbon (PFC) emulsions for multimodal detection by ¹⁹F MRI, X-ray and US imaging (44). The entire synthesis can be completed within 2 h (45).

MICROCAPSULES LOADED WITH RADIO-OPAQUE CONTRAST AGENTS: X-CAPS

There have been a limited number of studies on X-ray and CT cell tracking, primarily because of the low sensitivity of radio-opaque contrast agents and their potential cytotoxicity. It would be highly desirable to identify a robust method that allows the monitoring of radio-opaque cells using X-ray imaging. Image-guided delivery of therapeutic agents is now a routine clinical procedure, with the current primary imaging modality being X-ray angiography. However, the ability to visualize the target tissue, monitor any nontarget distribution and provide real-time validation of the treatment endpoint is currently not feasible with current X-ray-based imaging strategies. Therefore, CT guidance is also being explored as an alternative imaging modality for the guiding and monitoring of therapeutic entities. CT images provide a new and attractive alternative to conventional X-ray approaches for targeted drug delivery. Over the last decade, significant progress has been made in the use of CT to guide various interventions (46,47). With recent advances in imaging, these systems now combine the advantages of three-dimensional CT imaging with live X-ray imaging of all organs in one examination and on a single system. These developments warrant the development of microcapsules containing radio-opaque agents or 'X-caps'.

X-caps can be prepared by mixing LVG alginate in the step 1 with 5% w/v barium sulfate or 5% w/v bismuth sulfate solution (38,45). These radio-opaque capsules have a characteristic white appearance for barium sulfate capsules (Ba X-caps) or yellow for bismuth sulfide

capsules (Bi X-caps) (Fig. 2). When compared with unlabeled (non-radio-opaque) microcapsules, both Ba X-caps and Bi X-caps have equal permeability to fluorescent lectins with different molecular weights. The X-caps are permeable to fluorescent lectins of <75 kDa, but not to lectins of >120 kDa, i.e. they block antibodies, whilst allowing the penetration of smaller nutrients and the secretion of insulin ($M_w \sim 5$ kDa) when containing pancreatic islet cells (38).

Radio-opaque capsules have been successfully imaged in mice and rabbits (23,38). Single Ba X-caps and Bi X-caps were clearly identified *in vivo* after transplantation into the peritoneal cavity of mice, and after intramuscular injection into the hind limb of rabbits. No immediate toxicity was noted, both for the cells and host tissue. X-caps containing MSCs have recently been employed to stimulate arteriogenesis and angiogenesis in a rabbit model of peripheral arterial disease. Using an endovascular peripheral arterial disease hind limb ischemia model (48), rabbits were randomized to receive MSC-X-caps, empty X-caps or unencapsulated MSCs in the ischemic thigh, 24 h post-occlusion. Immediately after MSC transplantation and 14 days later, digital radiographs acquired on a clinical angiographic system demonstrated persistent visualization of the X-cap injection sites with contrast-to-noise retained. Using a modified thrombolysis in myocardial infarction frame count, quantitative angiography demonstrated a 65% improvement in hind limb perfusion or arteriogenesis in MSC-X-cap-treated *versus* empty X-cap-treated animals (Fig. 3) (23). Post-mortem immunohistopathology of vessel density by anti-CD31 staining demonstrated an 87% enhancement in angiogenesis in X-cap-MSC-treated *versus* empty X-cap-treated animals. Thus, X-caps containing pancreatic islet cells or MSCs represent one of the first X-ray-visible cellular therapeutics.

MICROCAPSULES WITH INTRINSIC RADIO-OPACITY WITHOUT THE ADDITION OF CONTRAST AGENTS

A library of microcapsule formulations has been studied to determine the optimal capsule formulation for cell transplantation, using barium ions for gelation and clinical-grade PS, a new cationic capsule cross-linker (37). LVG alginate microcapsules are best gelated in a 20 mM BaCl₂ solution (step 2). Barium capsules are intrinsically radio-opaque because barium ions are more electron dense than calcium ions. Hence, they can be imaged *in vitro* and *in vivo* with micro-CT without the addition of contrast agents (Fig. 4) (37). The radio-opacity of these microcapsules is stable for at least 15 months *in vitro*, whereas APSA capsules gelated with calcium ions and APLLA capsules are not visible on micro-CT. *In vivo*, barium microcapsules subcutaneously engrafted in mouse abdomen can be readily visualized by micro-CT. In addition, these capsules are mechanically stronger and support higher encapsulated cell viability relative to APLLA capsules. Human islets encapsulated inside barium microcapsules exhibited a sustained morphological integrity, viability and biological function for at least 24 days in culture, with secreted human C-peptide concentrations of 0.2–160 pg/mL/islet (37). In contrast, unencapsulated islets broke into single cells after several days in culture and stopped functioning after 21 days post-culture. Less than 1 nM of barium is bound to the capsules. This concentration is much lower than the potentially toxic concentration of free barium ions [5–10 mM (49)].

MICROCAPSULES LOADED WITH SPIO CONTRAST AGENTS: MAGNETOCAPSULES

SPIO particles have been widely used for cell tracking in preclinical animal models and, to date, no less than seven clinical trials have been performed (50). As they are composed of biodegradable iron, they are considered to be safe for human use. Shen *et al.* (51,52) initially

mixed ferrofluids with alginate, which they termed magnetized alginate that is MR visible. When SPIO nanoparticles (e.g. Feridex[®], 2.24 mg of iron/mL of alginate) are mixed with the LVG alginate solution in step 1, it is possible to synthesize magnetocapsules (40,43,45). Single capsules can be visualized as hypointensities at 3 T employing a routine clinical set-up (i.e. using a clinical scanner and wrist coil) as capsules with a positive contrast for the capsule surface using inverse recovery with on resonance (IRON) (53) water suppression imaging (Fig. 5). Importantly, following capsule rupture induced by mechanical glass bead treatment *in vitro*, their hypointensity is much reduced, which could possibly be further exploited to probe the disintegration of capsules *in vivo*. Following intra-abdominal transplantation, magnetocapsules containing murine pancreatic β cells have been successfully used to restore normoglycemia in streptozotocin (STZ)-induced diabetic mice (40). These capsules are able to immunoprotect human islets xenografted in normal swine for short periods of time, without any immunosuppression. When injected intraportally, liver function remains normal, except for a transient increase in portal pressure over a period of approximately 30 min (41). The magnetic susceptibility induced by these capsules can be quantified using phase map cross-correlation detection and quantification, which automatically identifies the localization of engrafted magnetocapsules, estimating their volume, magnetic susceptibility and magnetic moment (42).

MICROCAPSULES LOADED WITH PFC CONTRAST AGENTS: FLUOROCAPSULES

¹⁹F MRI cell tracking, originally developed by Ahrens *et al.* (54), enables so-called ‘hot spot’ MRI (55,56) as the host tissue lacks endogenous fluorine. With the fluorine as a tracer molecule, the interpretation of the signal is much more straightforward than with the use of MR contrast agents that affect the ¹H signal indirectly. Originally developed in the 1970s (57), ¹⁹F MRI has seen a re-emergence in a wide variety of biomedical applications (58).

The first example of the encapsulation of PFCs into alginate microcapsules was demonstrated by Noth *et al.* (59). Here, the overall rationale was to determine *in vivo* the partial oxygen pressure (pO_2) inside the capsules at different implantation sites (kidney capsule, abdomen). This has been recently taken one step further using perfluorooctyl bromide to fabricate PFC emulsions. To this end, 0.12 g/mL of perfluorooctyl bromide is emulsified in a mixture of 5% w/v lecithin and 2% v/v safflower oil using a sonicator at 40% power on ice. The emulsions (20% v/v) are then mixed with LVG alginate in step 1 (44,45).

The addition of PFCs serves a dual purpose: It not only allows multimodal imaging (60), but also offers a potential means to increase the local oxygen tension in a graft, believed to be of critical importance to maintain cell viability. To this end, PFCs have been developed for numerous biomedical applications, including their use as a blood substitution agent (61) and as a gas-permeable wound protectant (62). PFCs exhibit a high capacity for the dissolution of respiratory gases, with oxygen solubility in PFCs being 20–25 times greater than that in water or blood plasma (63). With regard to encapsulated cells, Khattak *et al.* (64) demonstrated that the addition of a PFC emulsion into alginate microcapsules containing HepG2 cells resulted in a significant increase in cell viability. This enhanced cell function is probably the result of an increase in oxygen concentration within the capsules, which can be measured with ¹⁹F MRI (59). For clinical procedures, such as islet cell transplantation, this is vital, as capsules with PFC reservoirs could ‘recharge’ themselves by picking up O₂ from plasma that perfuses through the matrix.

Fluorocapsules have been recognized (65) as a versatile method to track transplanted encapsulated islet cells with multiple imaging modalities, i.e. ¹⁹F MRI, US imaging and CT, on both research- and clinical-grade scanners (Fig. 6) (44). Embedding islet cells in

fluorocapsules can increase their insulin secretion (up to 18.5% *in vitro*), and, as a xenograft, can enable prolonged insulin secretion *in vivo* in mice for at least 55 days (33.0 ± 1.0 pmol/L human C-peptide).

MICROCAPSULES LOADED WITH GOLD AND GADOLINIUM CONTRAST AGENTS: GADOLINIUM–GOLD (GADOGOLD) CAPSULES

GadoGold nanoparticles can be prepared by reacting GdCl_3 solution with Au@DTDTPA (DTDTPA, dithiolated diethylenetriaminepentaacetic acid) colloidal solution to chelate Gd^{3+} to gold nanoparticles (66). For the synthesis of GadoGold microcapsules, GadoGold nanoparticles are suspended in LVG alginate at a concentration of 17 μg of gold and 1.5 μg of gadolinium/mL of LVG alginate (36,45). Similar to fluorocapsules, GadoGold capsules have been recognized (67) as a versatile method to track transplanted encapsulated islet cells with multiple imaging modalities. They can be visualized by T_1 -weighted MRI (providing positive contrast, even at a field of 9.4 T), CT and US imaging (Fig. 7). For liver and other transplantation sites that have relatively short T_2 values, a positive-contrast MR signal is preferred for better visualization of transplanted capsules. These microcapsules are stable for at least 1 year in physiologic saline, without perceivable changes in capsule size and morphology and/or leakage of nanoparticles. Human islets encapsulated inside GadoGold microcapsules are functional for at least 2 weeks in culture, as indicated by the human C-peptide levels secreted by the islets (24–896 pg/islet over a total period of 3 days) (36). Blood glucose levels in STZ-induced diabetic mice transplanted with GadoGold-encapsulated mouse βTC6 insulinoma cells returned to normal values within 1 week after transplantation, and normoglycemia was sustained for at least 6 weeks without the use of immunosuppressive drugs.

When comparing the sensitivity of detection for the three types of microcapsules, i.e. magnetocapsules, fluorocapsules and GadoGold capsules, it has been shown that, in all three cases, single capsules can be detected *in vitro* in gel phantoms. In animals, however, single capsules can only be detected if they are labeled with SPIO (40,43). When comparing GadoGold capsules with fluorocapsules, the sensitivity is about equal, with a detection limit *in vivo* of between 20 and 100 capsules.

FURTHER DEVELOPMENT OF TRACKABLE MICROCAPSULES: CAPSULE-IN-CAPSULES

Capsule-in-capsules (CICs) consist of two separate capsules, the inner capsule containing iron oxide and gold particles, and the outer capsule containing cells (Fig. 8) (39). Conceptually, the inner capsule shields the nanoparticles from the islet cells, preventing direct contact (and potential toxicity) of the contrast agents with the cells. Indeed, a small, but significant, improvement in cell function has been noted *in vitro* (39). For the synthesis of CICs, first, primary capsules are prepared using 2% w/v LVG alginate containing iron oxide and gold nanoparticles (0.156 μg of iron and 1.38 μg of gold per capsule). The primary capsules are fabricated using a bead generator flow rate of 0.01 mL/min, and gelated in 20 mM BaCl_2 solution. The cells and primary capsules are then mixed in 1.8% w/v LVG alginate, followed by the second capsule synthesis using a flow rate of 0.1 mL/min and 20 mM BaCl_2 solution. By precisely adjusting the flow rates, one is able to control the size of the overall capsules.

In STZ-induced diabetic mice transplanted intra-abdominally with CICs, blood glucose levels returned to normal (<200 mg/dL) immediately after transplantation and persisted for at least 75 days. CICs could be visualized *in vivo* using MRI, micro-CT and US imaging, even at 3 months post-transplantation, indicating the stability of the dual capsule layers.

When CICs were recovered from the animals at 4 months after transplantation, cell viability was still at 55%, and the recovered CICs had an intact appearance without any breakage.

CONCLUDING REMARKS

Over the last few decades, microencapsulation has been widely used to immunoprotect therapeutic cells after transplantation. Clinical trials have been initiated, but much still needs to be learned with respect to the ideal capsule formulation, engraftment site, and delivery method. Recently, a series of trackable microcapsules has been developed, which can be used to image cells with multiple clinically applicable imaging modalities. Similar to 'naked' cell tracking (50), microencapsulated cell tracking may become an important adjunct for further development of cellular therapies based on microencapsulation. It should be realized, however, that the use of contrast agents to label naked or microencapsulated cells does not allow the assessment of cell viability or function. To accomplish this, reporter genes need to be used, as exemplified by the combined use of magnetoencapsulated cells transfected with the reporter gene firefly luciferase (43).

Acknowledgments

The authors are supported by the National Institutes of Health (NIH) (RO1 EB007825, RO1 EB012590 and R21/R33 HL89029), the Maryland Nanotechnology Fund and the Maryland Stem Cell Research Foundation (MSCRFII-0161, MSCRFII-0399, MSCRFII-0043 and MSCRFF-0035).

Abbreviations used

APLLA	alginate–poly-L-lysine–alginate
APSA	alginate–protamine sulfate–alginate
CD	cluster designation
CIC	capsule-in-capsule
CT	computed tomography
DSA	digital subtraction angiogram
DTDTPA	dithiolated diethylenetriaminepentaacetic acid
FDA	Food and Drug Administration
GadoGold	gadolinium–gold
IRON	inverse recovery with on resonance
LVG	low-viscosity guluronate
LVM	low-viscosity mannuronate
MSC	mesenchymal stem cell
PFC	perfluorocarbon
PLL	poly-L-lysine
PS	protamine sulfate
SPIO	superparamagnetic iron oxide
STZ	streptozotocin
US	ultrasound

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Figure 1.

Schematic illustration of general microcapsule synthesis. Cells (a) and contrast agents (b) are suspended in low-viscosity guluronate (LVG) alginate solution inside a syringe (c) in step 1. The syringe is mounted on a syringe pump (d) and connected to a high-voltage power supply (e). The gelation bath (f) is connected to the ground. Microcapsules are produced by an electrostatic droplet generator and gelated in the cationic bath in step 2 (g). (h) Schematic drawing showing microencapsulation of cells and contrast agents. The first alginate (LVG) layer of the microcapsule is cross-linked with protamine sulfate (PS) or poly-L-lysine (PLL) as polycationic polymer (i, red) in step 3, and is further cross-linked with low-viscosity mannuronate (LVM) alginate (j, blue) in step 4.

Figure 2. Macroscopic (a, c) and fluoroscopic (b, d) images of bismuth X-caps (a, b) and barium X-caps (c, d). Single capsules can be clearly identified. Reproduced, with permission, from ref. (38).

Figure 3.

Post-treatment angiographic analysis of collateral vessel development. (a) Bar graph of the average modified thrombolysis in myocardial infarction (TIMI) frame count (a measure of collateral vessel development) for the mesenchymal stem cell (MSC)-X-cap, empty X-cap, unencapsulated MSC and sham injection-treated animals demonstrates a significant improvement in distal filling only in rabbits with peripheral arterial disease that received microencapsulated cells. (b-g) Representative digital subtraction angiograms (DSA, red) obtained during peak contrast opacification performed at 2 weeks post-injection of encapsulated MSC-X-caps (b) and empty microcapsules (c) with an overlay of microcapsule injections (green) obtained from the mask image of DSA. An increased collateralization can be appreciated in the MSC-X-cap-treated animal DSA (d) relative to the X-cap-treated animal DSA (e). Native mask digital radiographs demonstrate the location of the MSC-X-

caps (f) and empty X-caps (g) in the same animals. (h) Box-whisker plot showing the difference between left and right distal deep femoral artery diameters at baseline and 2 weeks after superficial femoral artery occlusion in treated (MSC-X-caps) and untreated (empty X-caps) animals. There was no statistically significant difference in vessel diameter between the treatment groups. Reproduced, with permission, from ref. (23).

Figure 4.

Micro-computed tomography (CT) images of 100 phantom barium microcapsules in saline 1 day (a) and 15 months (b) post-synthesis. These capsules are intrinsically radio-opaque, whereas alginate–protamine sulfate–alginate (APSA) capsules gelated with calcium ions (c) and alginate–poly-L-lysine–alginate (APLLA) capsules (d, each with 100 capsules per tube) are not visible under CT imaging. Axial (e), sagittal (f) and three-dimensional volume-rendered (g) micro-CT images of 3000–4000 barium capsules transplanted subcutaneously in mouse abdomen. Yellow arrows, bladder; white arrows, capsules. Reproduced, with permission, from ref. (37).

Figure 5.

(a) Using conventional T_2^* -weighted images, individual magnetocapsules can be easily identified as hypointensities. (b) Using the inverse recovery with on resonance (IRON) sequence to generate positive contrast, individual magnetocapsules appear as a bright signal with depiction of the capsule surface. (c, d) Magnetocapsules before (c) and after (d) rupture using glass bead treatment. After rupture, a significant loss of hypointensity occurs and the Feridex[®]-induced contrast decreases to a pinpoint double-dipole T_2^* susceptibility effect. (e) MR image of a mouse following injection of 500 magnetocapsules in the peritoneal cavity. Single capsules can be readily identified (arrows). Reproduced, with permission, from ref. (40).

Figure 6.

(a) *In vitro* proton T_2 -weighted MRI (left) and corresponding ^{19}F MRI (right) scans at 11.7 T of perfluoropolyether fluorocapsules in a standard 5-mm glass tube (the capsules were introduced in a 500- μm capillary tube). (b) *In vivo* ^{19}F MR images at 9.4 T of a mouse following intraperitoneal transplantation of 2000 perfluoropolyether fluorocapsules. Shown is the overlay of the ^{19}F image (pseudo-color) on the ^1H image (gray scale). (c) ^{19}F MR image of perfluoropolyether fluorocapsules obtained using a 3-T clinical system. Left to right: images of gel phantoms containing 1, 2, 5, 10, 15, 25 and 50 capsules. Reproduced, with permission, from ref. (44).

Figure 7. Positive-contrast 9.4-TMR (a) and micro-computed tomography (CT) (b) images of gadolinium-gold (GadoGold) microcapsules (arrow) engrafted subcutaneously into the mouse abdomen. (c, d) Ultrasound (US) images at 40 MHz of the cavity between the abdominal skin and parietal peritoneum of a mouse with (c) and without (d) injected GadoGold microcapsules. Reproduced, with permission, from ref. (36).

Figure 8.

Dual shielding of pancreatic islets and multimodal imaging using capsule-in-capsules (CICs). The semi-permeable outer alginate membrane blocks the penetration of immune cells and antibodies, yet allows unhindered diffusion of nutrients, glucose, oxygen and insulin produced by islets. The inner capsule, containing iron oxide and gold nanoparticle contrast agents, which enable concurrent MRI, computed tomography (CT) and ultrasound (US) imaging, prevents direct exposure of nanoparticles to cells. Islets within CIC exhibit improved insulin secretion compared with single capsules that contain the two types of nanoparticles and islets altogether. NP, nanoparticle. Reproduced, with permission, from ref. (39).