Hot spot imaging of microcapsules: An initial assessment of detection with fluorine and magnetization transfer imaging

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Introduction: Immuno-isolation of cells in semi-permeable membranes has been proposed as a means to prevent their immune destruction following transplantation. Immuno-isolation techniques such as microencapsulation are particularly attractive as they both abrogate the need for chronic immunosuppressive therapy and open the possibility of immuno-isolating xenogenic grafts. To date, microencapsulation has shown great clinical potential for a wide range of diseases requiring enzyme or endocrine replacement therapy. Nevertheless, several fundamental issues remain to be addressed before considering widespread clinical applications of this method. If microcapsules could be non-invasively monitored through techniques such as MRI, questions such as ideal transplantation site, best means of delivery and long-term survival of such grafts could be better addressed. Here we present two MRI techniques that may be utilized for the imaging of microcapsules. The first technique requires loading of the standard alginate-poly-L-lysine (PLL) microcapsule with a perfluoropolyether (PFPE) agent thereby creating fluorocapsules (fig. 1). Because tissues have a negligible background ^{19}F signal, fluorocapsules may be selectively imaged using "hot spot" MRI[1]. By overlaying conventional ^{1}H imaging with ^{19}F imaging, the underlying anatomic location of fluorocapsules can be determined. The second method, unlike fluorine imaging, does not require the addition of an MR contrast agent but instead utilizes magnetization transfer (MT) to detect the PLL inherent in alginate-PLL-alginate (APA) microcapsules. Contrast based on PLL-water proton interactions can be exploited by using a single off-resonance irradiation of the relatively small PLL associated proton pool[2]. In contrast to metal-based agent imaging, fluorine and MT imaging allow for the detection of capsules without perturbation of the signal from surrounding tissue. For certain anatomic locations such as the peritoneal cavity in which inherent T_2* effects are strong, such techniques may prove superior to metal-based contrast imaging.

Methods: Fluorocapsules and traditional APA microcapsules were both formed with FDA approved Protonal HD alginate. In the case of fluorocapsule synthesis, 20% vol/vol PFPE was added to a stock solution of 2% alginate before extrusion through an electrostatic droplet generator. The PFPE solution was composed of perfluro-15-crown-5 ether that was emulsified (40%vol/vol) in a mixture of H_20 and 2% lecithin. Standard APA capsules were also formed with an electrostatic droplet generator with a solution of 1.5% alginate. In both cases, the alginate droplets were transformed to alginate beads by gelling in 100 mM $CaCl_2$. Microcapsules were then transferred to a .1% solution of PLL thereby forming an electrostatic complexation between the negatively charged polyanion alginate and positively charged PLL. Capsules were washed and a final layer of .5% alginate was applied. MR experiments were performed using a vertical 11.7T Bruker spectrometer equipped with a 5 mm imaging probe. For anatomical imaging a RARE TR/TE=2000/15 ms sequence and for MT imaging a spin echo sequence of TR/TE= 9000/6.3 ms was used[3]. Eight images were acquired alternately with a saturation power of 4.6μT for 4000ms at Δω=+/-3.75 ppm from the water proton's frequency. The total FOV was 5x 5 mm with slice thickness=1mm and matrix=64x64 pixels.

Results: Permeability of standard APA capsules and fluorocapsules was determined by incubation for 48 hours with fluorescently labeled lectins of varying molecular weight. APA capsules and fluorocapsules were both found to be permeable to fluorescent lectins <75kD but were found to be impermeable to lectins >150kD, thus capable of blocking antibody penetration (immuno-isolation) while allowing inflow of nutrients and secretion of therapeutic factors by encapsulated cells. Using a microfluorometric assay to selectively label live cells with Newport Green and dead cells with propidium iodide, the difference in viability of βTC-6 murine insulinoma cells in fluorocapsules and APA capsules was not found to be statistically significant 48 hours post-encapsulation. *In vitro* imaging of 350 μm fluorocapsules at 11.7T demonstrated the ability to detect single capsules (fig. 2). MT imaging could similarly be used to detect individual APA micocapsules at 11.7T (fig. 3). The sensitivity of MT imaging to PLL was confirmed by the absence of signal from alginate microcapsules lacking PLL. Of note, MT imaging was more sensitive to detecting alginate-PLL capsules lacking an outer layer of alginate than complete APA microcapsules.

Discussion: Here we present the initial characterization of fluorocapsules and a new application of MT imaging to detect the PLL in APA microcapsules. Although further *in vitro* characterization of fluorocapsules is necessary, initial results appear promising. Such a technique should prove particularly robust for *in vivo* applications since PFPE is clinically used as an artificial blood substitute. Further, as PFPE agents are encapsulated and therefore have no direct contact with the surrounding tissue, standard parameters such as vapor pressure and tissue clearance half-life that have traditionally affected fluorine imaging are not applicable. As APA microcapsules are well-characterized and have repeatedly shown the ability to effectively immuno-isolate cellular grafts, further exploration of MT imaging of APA capsules is warranted.

References: 1) Bulte JWM.. Nature Biotech. 23:945-946 (2005). 2) Geoffeny N, JACS 123: 8228-8629 (2001). 3)Henkelman R, NMR Biomed: 14:57-64 (2001).

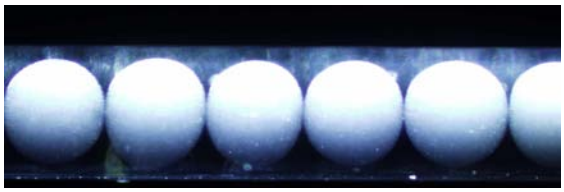

Figure 1: Microscopic image of fluorocapsules in a capillary tube

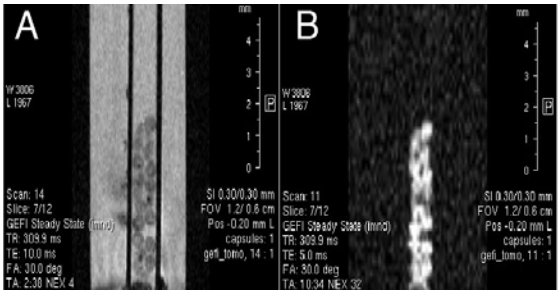

Figure 2: Fluorocapsules at 11.7T. A) Gradient echo ^{1}H image. B) Gradient echo ^{19}F image.

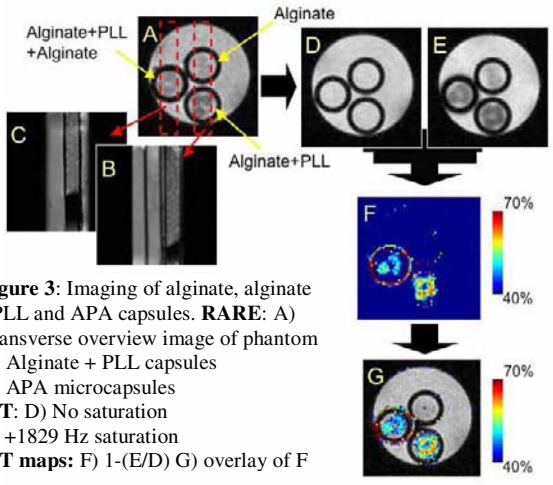

Figure 3: Imaging of alginate, alginate +PLL and APA capsules. **RARE**: A) Transverse overview image of phantom
B) Alginate + PLL capsules
C) APA microcapsules
MT: D) No saturation
E) +1829 Hz saturation
MT maps: F) 1-(E/D) G) overlay of F