Multifunctional Perfluorinated Microcapsules for Mesenchymal Stem Cell Delivery and Engraftment Tracking using ^{19}F MRI, X-ray, and Ultrasound

Y. Fu[1], D. A. Kedziorek[1], R. Ouwerkerk[1], S. M. Shea[2], N. Azene[1], A. Arepally[1], J. W. Bulte[1,3], R. Krieg[4], F. Wacker[1], and D. L. Kraitchman[1]

[1]Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University, Baltimore, MD, United States, [2]Imaging and Visualization, Siemens Research Corporate, Inc., Baltimore, MD, United States, [3]Institute of Cell Engineering, Johns Hopkins University, Baltimore, MD, United States, [4]Siemens AG Healthcare Sector, Erlangen, Germany

Introduction:

Peripheral arterial disease (PAD) affects millions Americans [1], and due to the extent or severity of their atherosclerotic disease, many PAD patients cannot undergo conventional medical or surgical therapy. Stem cell therapy, which has shown promise for treating PAD [2, 3], has problems associated with poor survival of transplanted cells and the inability to noninvasively monitor and track cell fate. We present here a novel mesenchymal stem cell (MSC) delivery and engraftment tracking method utilizing perfluorooctylbromide (PFOB) incorporated alginate-poly-L-lysine-alginate microcapsules (PFOB Caps). We hypothesized that PFOB Caps would not only maintain high viability of MSCs , but also enable noninvasive MSC tracking *in vivo* with clinical X-ray, c-arm CT, ultrasound (US), and MR imaging systems.

Methods:

Microencapsulation of bone marrow-derived rabbit or human MSCs ($1.5x10^6$cells/ml) was performed by extruding a PFOB-impregnated 2% (w/v) alginate solution from a syringe pump in conjunction with an electrostatic droplet generator, followed by cross linking with poly-L-lysine to form X-ray-, US- and MRI-visible microcapsules. Unlabeled capsules lacked PFOB. MSCs viability was determined using a fluorometric assay and compared between unlabeled capsules and PFOB Caps. New Zealand White (NZW) rabbits were randomized to receive 6 injections (~5000 capsules/injection) of either unlabeled microcapsules or PFOB Caps in the medial thigh. X-ray angiograms, c-arm CT, US, and ^{19}F MR images (3D-TrueFISP, BW=1000 Hz/px, TR/TE=3.2/1.6 ms, 2.0x2.0x5.0 mm^3, 40 partitions, 32 avgs, 7 min acquisition) were taken within 1-7 days after injection in a rabbit PAD model. X-ray angiography was repeated up to 5 weeks post-injection. *In vitro* phantom studies using ^{19}F MRI (3D-TrueFISP, BW=1500 Hz/px, TR/TE=3.0/1.5 ms, 2.0x2.0x5.0 mm^3, 24 partitions, 4 avgs, 62 s acquisition) and c-arm CT imaging were performed to determine the minimum detectable number of PFOB Caps using standard clinical imaging systems (Tim-Trio and Axiom Artis dFA, Siemens AG).

Results:

The viability of rabbit MSCs encapsulated with PFOB was 90±3% immediately after encapsulation and remained high (88±5% at 4 weeks post-encapsulation). PFOB Caps containing human MSCs had enhanced cell viability relative to unlabeled capsules (83±3% for PFOB vs. 50±1% for control at 65 days post-encapsulation, P<0.001). Viability of human MSCs in PFOB Caps was maintained up to 100 days, while it decreased sharply to <10% in unlabeled capsules at 80 days post-encapsulation. *In vitro* c-arm CT and ^{19}F MR imaging of PFOB Caps demonstrated the ability to detect as few as 2 and 25 capsules (Figure 1A, B), respectively. *In vivo* PFOB visibility on c-arm CT images was demonstrated relative to unlabeled capsules (Figure 1C) with persistence of intact microcapsules up to 5 weeks post delivery in PAD rabbits. Using ^{19}F MRI, transplanted PFOB Caps in rabbit medial thigh were clearly identifiable (Figure 1D). PFOB Cap injection sites were also readily seen with clinical US (Figure 1E).

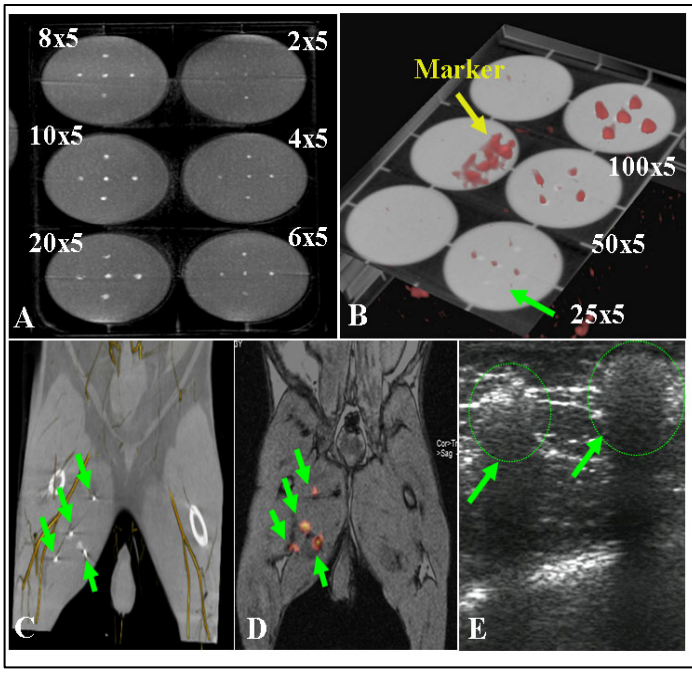

Figure 1:Multimodality- visible PFOB Caps. (A) Maximum intensity projection (MIP) of a c-arm CT image of a PFOB Cap 6-well plate phantom containing different numbers of PFOB Caps arranged in five locations per well. (B) ^{19}F MRI of PFOB Cap phantom fused with CT image demonstrating the delectability of 25 capsules. (C) A c-arm CT angiogram demonstrating the detection of four PFOB Cap injections in a rabbit right thigh, while unlabled capsule injections in left thigh not detectable. (D) *In vivo* ^{19}F MRI of PFOB Caps (arrow) overlaid on anatomical ^{1}H MRI (3D GRE, BW=320 Hz/px, TR/TE=15/3.7 ms, 1.0x0.9x1.5 mm^3, 64 partitions, 1avg, 2 min acquisition) showing the detection of four PFOB cap injection sites of the same rabbit as shown in C. (E) *In vivo* visibility of two PFOB Cap injections with clinical ultrasound (note the acoustic shadow caused by PFOB Caps).

Conclusion:

By adding PFOB, a contrast agent and oxygen carrier, to the alginate microcapsules, we have developed novel perfluorocapsules that provide a means to enhance the viability of MSCs and ensure the ability to monitor stem cell delivery and track engraftment *in vivo* using multiple clinical imaging modalities.

References:

1. Heart Disease and Stroke Statistics — 2008 Update, *American Heart Association*
2. Tateishi-Yuyama et al. *Lancet* 2002.
3. Bartsch et al. *Clin Res Cardiol* 2007.