Novel Perfluorooctylbromide Alginate Microcapsules for Enhanced Mesenchymal Stem Cells Survival and Noninvasive Imaging using Clinical CT and ^{19}F MRI

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Introduction: Stem cell therapies have shown great promise for peripheral arterial disease (PAD) patients who are not amenable to conventional revascularization therapy. However, even autologous cellular therapies have exceedingly low engraftment rates-often with the inability to confirm the delivery success and track cell fate.

Stem cell microencapsulation in conjunction with contrast agents may provide a means to enhance cell survival and enable cell tracking. The goal of this study was to evaluate a novel MRI- and X-ray-visible, immunoprotectable alginate microcapsule containing perfluorooctylbromide (PFOB), an imaging contrast agent and oxygen carrier, for allogeneic mesenchymal stem cell (MSC) delivery and tracking in a PAD rabbit model.

Methods: Microencapsulation of bone marrow-derived allogeneic rabbit or human MSCs was performed using a modified alginate-poly-L-lysine-alginate method[1] with the addition of 12% (v/v) PFOB. Unlabeled microcapsules lacked PFOB.

In vitro MSCs viability was determined using a fluorometric assay. PFOB microencapsulated MSCs were transplanted into a rabbit and viability was assessed at 3 days post transplantation using TUNEL staining.

The minimum number of PFOB microcapsules that could be detected using clinical c-arm CT and 3T ^{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) was determined in phantoms. New Zealand White (NZW) rabbits with (n=13) and without (n=16) left superficial femoral artery occlusion were randomized to receive 6 injections of unlabeled microcapsules, PFOB microcapsules, or naked MSCs in the medial thigh. X-ray angiograms, c-arm CT (Axiom Artis, Siemens, DynaCT®, 8 s rotation, scan angle 240° with an increment of 0.5°, dose per pulse of 0.36 μGy), and ^{19}F MR images (Tim Trio, Siemens, 3D-TrueFISP, BW=1000 Hz/px, TR/TE=3.9/2.0 ms, 1.25x1.25x2.0 mm^3, 40 partitions, 4-32 avgs) were acquired within 1-14 days after injection in a rabbit using standard clinical imaging systems. Radiopacities on c-arm CT and hotspots on ^{19}F MRI were registered using a custom 3D visualization software (Dextroscope). Postmortem gross anatomical visualization of the microcapsules was used to validate c-arm CT and ^{19}F MRI.

Results: *In vitro* MSC viability within PFOB microcapsules was high immediately after encapsulation (rabbit MSCs: 90±3%; human MSCs: 82±7%). TUNEL staining revealed 10 times higher cell survival in PFOB microcapsules than survival of naked MSCs (~5%) 3 days after transplantation into rabbit medial thigh.

In vivo visualization of PFOB microcapsules with c-arm CT images was confirmed (Figure 1A) in 95% of the injections whereas unlabeled capsules could not be detected. Despite high resolution of c-arm CT, detection of PFOB microcapsule injection sites in PAD rabbits was difficult because of the appearance of other radiopacities caused by platinum coils, bones, etc (Figure 1A). Using ^{19}F MRI, transplanted PFOB microcapsules in rabbit medial thigh were clearly identified as "hotspots" without background (Figure 1B) and showed a one-to one correspondence to the radiopacities on c-arm CT (Figure C). PFOB microcapsule injections that were not seen on CT and ^{19}F MRI were failed injections which were confirmed by postmortem histology

Conclusion: This study demonstrated for the first time a novel perfluorinated microcapsule for allogeneic MSC delivery and tracking using clinical c-arm CT and ^{19}F MRI. PFOB microencapsulation of MSCs maintains cell viability *in vitro* and *in vivo* and provides a means to monitor cellular delivery and tracking using clinical noninvasive imaging.

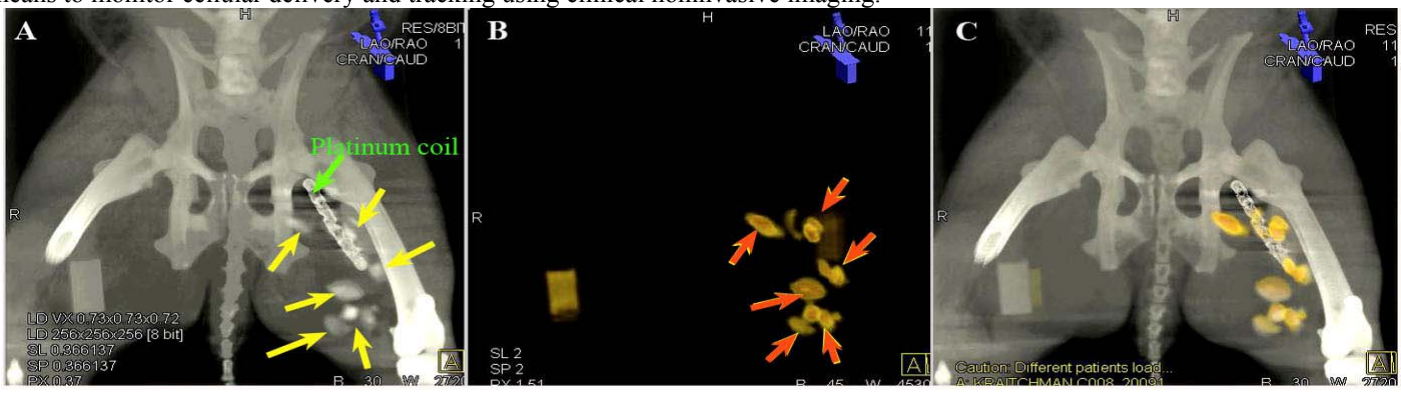

Figure 1. (A) Anterior-posterior view of a c-arm CT of a rabbit with left superficial femoral artery occlusion demonstrating the detection of PFOB microcapsules (yellow arrow) in left medial thigh 2 weeks after injection. (B) ^{19}F MRI of the same rabbit as (A) showing the clear identification of PFOB microcapsule injection sites (red arrow) without any other interfering caused by platinum coils or bones. (C) Fusion of C-arm CT image with ^{19}FMRI of PFOB microcapsules revealed one-to-one correspondence of PFOB microcapsule injection sites.

References:

1. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science.* 1980; 210:908-910.