# 19F MRI and C-arm CT Guiding of Reporter Probe Injection to Microencapsulated Mesenchymal Stem Cells for in vivo Cell Viability Assessment with Bioluminescence Imaging

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## Introduction:

Stem cell transplantation is an experimental treatment for peripheral arterial disease (PAD) that has the potential to restore a sufficient blood supply to the ischemic tissue by enhancing arteriogenesis. The major problems with stem cell therapies are the poor cell survival in vivo and difficulties with visualization of cell delivery and engraftment. These issues have provided a motivation for the modification of the alginate-poly-L-lysine (PLL)-alginate (APA) microencapsulation method(*1*), which can protect cells from early destruction and avoid immunosuppressive regimes, for non-invasive imaging. The addition of a contrast agent to the APA microcapsules enables cell tracking by MR, ultrasound, and X-ray imaging. In combination with reporter gene methods, bioluminescence imaging (BLI) provides a means for *in vivo* longitudinal monitoring of cell survival(*2*).

In the present study, mesenchymal stem cells (MSCs) were transfected with triple fusion reporter (TF) gene (TF-MSCs) containing red fluorescent protein, truncated thymidine kinase (SPECT/PET reporter) and firefly luciferase (BLI reporter) and microencapsulated in either unlabeled APA or perfluorooctylbromide (PFOB) impregnated APA capsules and transplanted into a rabbit model of PAD. Unlike small murine species, injection of the reporter gene probe, i.e., luciferin, on follow-up imaging studies would require large systemic doses. Thus, we sought to use C-arm CT localization of the radiopaque PFOB capsules for accurate injections of the BLI probe directly into the prior injection sites. $^{19}$F MRI was used to demonstrate the PFOB capsules locations.

## Methods:

MSCs were isolated from bone marrow of male New Zealand White rabbits and expanded for 3 passages, than transfected with triple fusion reporter gene with lipofectamine 2000 (Invitrogen). For microencapsulation, the classical alginate method(*1*) was used with the addition of 12% PFOB to create MR- and X-ray-visible capsules.

Prior to the transplantation in a PAD rabbit model the microcapsules were incubated with D-luciferin (150 µg/ml, Caliper). Rabbits (n=5) were randomized to received two to six intramuscular injections of PFOB and APA capsules containing TF-MSCs in the medial thigh. BLI (Xenogen IVIS 200) was performed immediately after capsules transplantation as well as 1 and 2 days post injection. $^{19}$F MRI (Tim-Trio, Siemens AG) was performed following BLI on day 0 using a custom 4-channel phased array tuned to fluorine. $^{19}$F MR imaging parameters were: TR/TE= 3.2/1.6ms; pixel resolution = 2.0 x 2.0 mm$^2$; slice thickness = 2.5 mm; 32 signal averages; 1000 Hz/pixel bandwidth; and total scan time = 426s (7:06 min).

C-arm CT (Siemens Axiom Artis dFA) was performed using the 8sDR preset (DynaCT®, Siemens Medical Solutions, Forchheim, Germany) with an acquisition time of 8 s, a total projection angle of 240°, projection increment 0.5°, 1k-matrix, and a resolution of 0.4x0.4x0.4 mm on day 1 and 2 to target luciferin (15 mg per injection site) to the PFOB capsules using a custom needle targeting software (X-Loc). APA capsules were injected blindly on day 1 and 2.

## Results:

In vitro bioluminescence of encapsulated MSCs was not blocked by encapsulation itself both with APA and PFOB capsules. The BLI signal from PFOB encapsulated MSCs was reduced for about 5% in comparison to the same number of nonencapsuleted MSCs (Fig. 1).

Using $^{19}$F MRI and X-ray imaging for rabbit model of PAD, 87.5% of the injection sites of PFOB microcapsules were co-detectable (Fig. 2). The undetected PFOB injections were associated with problems with delivery where only vehicle was injected rather than a failure in sensitivity by the imaging methods.

Successful targeting of the needles to the PFOB injections using C-arm CT was obtained in all locations (Fig. 3). BLI revealed viable MSCs encapsulated in PFOB microcapsules 1 and 2 days post transplantation (Fig. 4). The blind luciferin injections to the thigh muscles containing APA injections resulted in successful BLI signal detection only ~15% of the injection locations.

## Conclusion:

We present here the first demonstration of MR- and X-ray-visible microcapsules that can also measure cell viability with non-invasive BLI. Importantly, $^{19}$F MRI provides a method to determine capsule engraftment without ionizing radiation. The failure to visualize 85% of blind injections of the TF reporter probe to unlabeled APA capsules with TF-MSCs demonstrated the utility of multi-modality targeting. While targeting of the TF-MSCs within PFOB microcapsules was performed using C-arm CT, $^{19}$F MRI could potentially be used as well.

## References:

1. F. Lim, A. M. Sun, *Science* **210**, 908 (Nov 21, 1980).
2. R. Mayerhofer, K. Araki, A. A. Szalay, *J Biolumin Chemilumin* **10**, 271 (Sep-Oct, 1995).

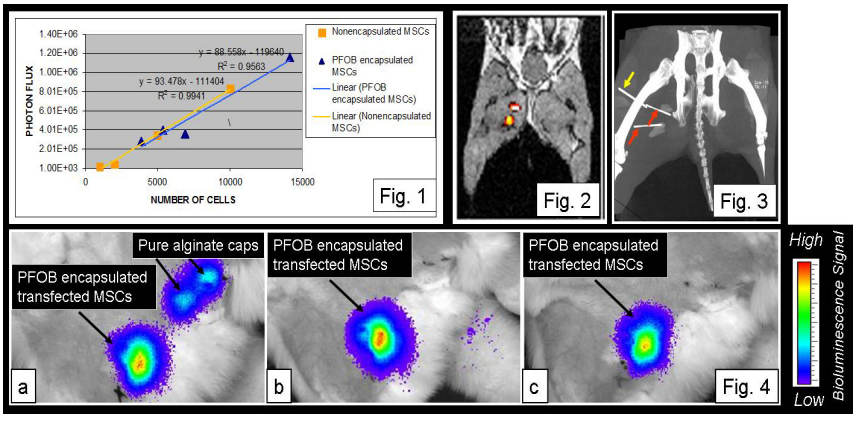


*Fig. 1. The BLI signal from PFOB encapsulated MSCs was reduced for about 5% in comparison to the same number of nonencapsuleted MSCs.*
*Fig. 2. $^{19}$F MRI all injection sites of PFOB microcapsules containing ~ 800 of capsules were detectable.*
*Fig. 3. Needles targeting to the PFOB injection sites using C-arm CT. Red arrows indicate needles guided to the PFOB transplants locations, yellow points to the orientation needle lying on the rabbit skin.*
*Fig. 4. BLI revealed viable MSCs PFOB encapsulated immediately (a), 1 (b) and 2 (c) days post transplantation.*

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