^{19}F MRI for non-invasive imaging of treatment failure in microencapsulated pancreatic cell therapy

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Target audience: Scientists and medical professionals in the field of type I diabetes, islet transplantation, and ^{19}F MRI. **Purpose:** To assess the feasibility of ^{19}F MRI to non-invasively visualize the degradation of fluoroencapsulated cell grafts. As fluorocapsules protect cells against immune-mediated destruction, we hypothesized that this technique can detect an impending failure of islet therapy. **Methods:** Fluorine emulsions were synthesized by sonicating an equal-volume mixture of 2.5% w/v lecithin and perfluoro-15-crown-5-ether (PFPE). Mouse insulinoma ßTC6 cells and PFPE emulsions were encapsulated inside alginate/protamine sulfate/alginate microcapsules (12% v/v PFPE emulsions/ml of alginate). To simulate capsule rupture *in vivo*, we designed weak fluorocapsules that can be rapidly degraded by alginate lyase. Weak fluorocapsules encapsulating *luciferase*-transduced ßTC6 cells were engrafted s.c. into diabetic NOD/Shiltj mice (n=3). The mice were imaged by ^{19}F MRI and bioluminescence imaging (BLI) pre- and post-injection of 1.2 mg lyase into the graft (or one and four days after transplantation). We cultured ßTC6 cells with the same concentration of injected lyase and followed cell viability with an MTS assay to prove that lyase did not affect cell viability. Unencapsulated ßTC6 cells with the same cell number as fluoroencapsulated grafts were s.c. transplanted in diabetic NOD/Shiltj mice (n=4) to demonstrate the pattern of graft rejection. MRI was performed with a Bruker 11.7T scanner using a dual-tuned ^{19}F /^{1}H surface coil. The parameters for ^{1}H/^{19}F MRI were: RARE, TR=4/3s, E=58/58ms, SI=1/1mm, matrix=256x160/256x160, NA=2/8, FOV=3.2x2/3.2x2cm. A reference (PFPE inside a capillary tube) was included for quantification of ^{19}F nuclei inside the implants. The total ^{19}F nuclei in the implants and the reference were calculated using Voxel Tracker software (Celsense, Pittsburgh, PA). For statistical significance, a Student's t-test with $p<0.05$ was used. **Results and Discussion:** *In vitro*, the MTS assay showed that cell death was not caused by lyase (A). *In vivo*, the graft viability decreased at three days after lyase injection as measured by BLI (B, D). In the same timeline, the release and dissipation of PFPE agents upon capsule rupture could be detected by ^{19}F MRI as a drop in signal intensity (C, D). The time course and magnitude of graft death by immunorejection were statistically similar in unencapsulated cell grafts and ruptured fluorocapsule grafts (E). Our data demonstrated that the degradation of immunoprotective fluorocapsules with subsequent graft rejection can be non-invasively detected by ^{19}F MRI. **Conclusion:** This strategy may be used to monitor the integrity of immunoprotection provided by fluorocapsules and the pending graft rejection when the capsules are compromised.

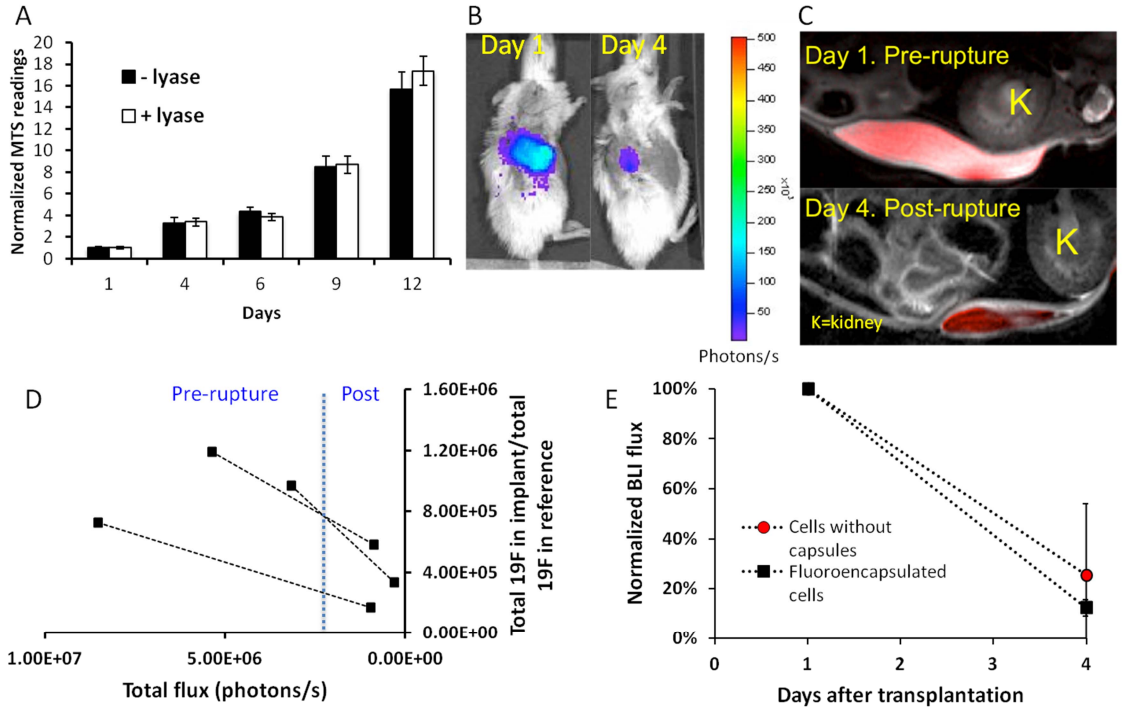

Figure 1: (A) MTS assay of mouse insulinoma cells cultured with and without alginate lyase. The readings were normalized to the values at day 1. BLI (B) and overlaid ^{19}F/^{1}H MR (C) images of a NOD/Shiltj mouse s.c. transplanted with fluoroencapsulated *luciferase*-insulinoma cells pre- and post-capsule rupture. (D) A decrease in graft viability (as indicated by BLI) upon capsule rupture in three mice was simultaneously detected with a decrease in the ratio of the total ^{19}F nuclei in the implants/reference. (E) Graft survival (BLI) of unencapsulated and encapsulated insulinoma cells at day 1 (pre-rupture) and 4 (post-rupture) after transplantation.