MR-Visible and Immunoprotective Alginate Microcapsules for Treatment of Fulminant Liver Failure

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Introduction: Liver transplantation is the primary treatment for fulminant liver failure. However, , approximately 20-30% of patients die due to a shortage of good quality , immune-matched livers from human organ donors. Recently, transplantation of isolated xenogenic hepatocytes has been explored as an alternative or intermediate bridge to full organ liver transplant. While studies have reported some success transplanting microencapsulated hepatocytes into mice with fulminant liver failure, an accurate and reproducible method for serial noninvasive tracking transplanted hepatocytes is needed. To this end, we have incorporated Feridex, an FDA approved superparamagnetic iron oxide (SPIO) nanoparticle formulation, into novel alginate-protamine sulfate-alginate microcapsules to create MR-visible, immunoprotected magnetocapsules. Furthermore, transgenic luciferase-hepatocytes were used to enable optical imaging as a means of assessing cell viability *in vivo*. We present here the functional and MRI properties of encapsulated human hepatocytes following intra-abdominal transplantation in mice.

Methods: Human (Luc-transduced) hepatocytes (HepG2) were encapsulated in 2% semi-permeable alginate microcapsules containing 20% Feridex and cross-linked with clinical grade protamine sulfate (.05%). In vitro viability and cell functionality was assessed with bioluminescent imaging (Xenogen) and an ELISA kit (AssayPro) specific for human albumin on days 1, 7, 14, 21, 28 and 35. 3,000 and 6,000 microcapsules (7.5E6 and 15E6 cells) were injected into the peritoneal cavity of 8 mice. Bioluminescence imaging was done (10ul luciferin/g) and blood samples were taken from the tail vein and assayed for human albumin using ELISA on days 0, 1, 7, 14, 21 and 28. Animals were imaged at 9.4T using a Bruker horizontal bore magnet after 28 days.

Results: As determined by ATP-dependent bioluminescent imaging , the viability of encapsulated human hepatocytes decreased by an order of magnitude after 3 weeks and then remained stable after 1 week *in vitro* Human albumin secretion was stable at about 0.02 ug/ml per week through one month. Upon delivery within the intraperitoneal cavity, magnetocapsules were readily detected by T2-weighted MRI as dispersed hypointensities throughout the peritoneal space (Fig. 1). *In vivo,* the viability of encapsulated human hepatocytes decreased gradually over a 4 weeks (Fig. 2) and plasma human albumin was detectable for up to 2 weeks, with negligible levels at later time points (Fig. 3).

Conclusion: Magneto-encapsulated human hepatocytes are functional and remain viable in the peritoneal cavity of mice for at least 2 weeks post transplantation. Taken together, these results support the ultimate application of using encapsulated hepatocytes as a intermediary therapeutic supplement to support liver function in patients with fulminant liver who are waiting for a suitable organ match. Furthermore, by making these capsules MR-visible, we have developed a method of tracking and monitoring immunoprotected hepatocyte transplants *in vivo*, including MR-guided real time targeted delivery. Funded by NIH RO1 EB007825.

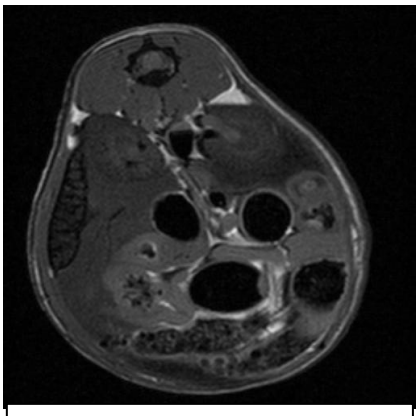

Figure 1: T2-weighted spin echo image of transplanted magnetocapsules dispersed in the peritoneal cavity (white arrows). TR=1500ms, TE=15ms, FOV=3x3cm.

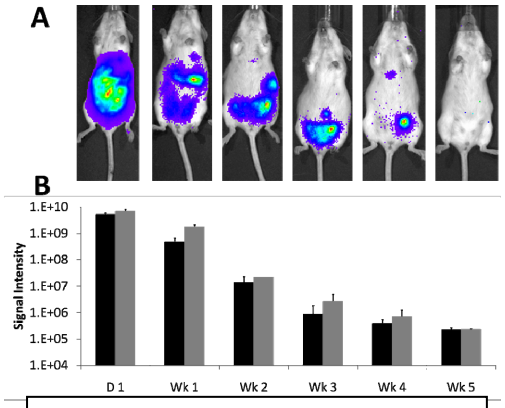

Figure 2: **A.** Bioluminescence image of 2 mice injected with 6,000 magnetocapsules. Total intensity is used to evaluate overall viability of the transplants. **B.** Average bioluminescence signal intensity in mice with 3,000 (black) and 6,000 (gray) magnetocapsules. Cell viability decreases initially and subsequently levels off around 4-5 weeks.

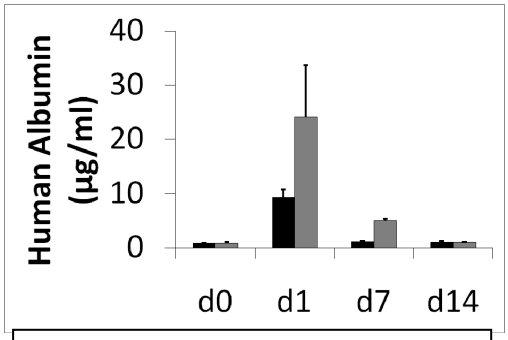

Figure 3: Human albumin (ug/ml) in plasma from mice transplanted with 3,000 (black) and 6,000 (gray) magnetocapsules. Detectable human albumin secretion occurs for up to 2 weeks.