Novel Single Layer MR-Visible Alginate Microcapsules for Visualization and Immunoprotection of Hepatocytes

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Introduction: To date, liver transplantation is the primary treatment for chronic liver failure and autoimmune hepatitis. However, due to shortages of human organ donors, approximately 20-30% of patients die before a liver becomes available[1]. Recently, transplantation of isolated xenogeneic hepatocytes has been explored as an alternative to full organ liver transplant[2]. While studies have reported success transplanting microencapsulated hepatocytes into mice with fulminant liver failure, an accurate and reproducible method of tracking transplanted hepatocytes *in vivo* is needed. To this end we have incorporated Feridex®, an FDA approved superparamagnetic iron oxide (SPIO) formulation, into novel single layer barium cross-linked alginate microcapsules to create MR-visible magnetocapsules for immunoprotection of hepatocytes. Conventional alginate-poly-L-lysine-alginate (APA) magnetocapsules[3] were included as control. We have assessed the permeability of the magnetocapsules and cell viability *in vitro*, and MRI properties in gelatin phantoms.

Methods: Both APA and barium cross-linked magnetocapsules were formed using a solution of human hepatocytes suspended in 2% w/v Protonal HD Alginate with 20% v/v Feridex in conjugation with an electrostatic droplet generator. Alginate beads were transformed into alginate capsules by gelling in a 100mM solution of either $BaCl_2$ or $CaCl_2$. For $BaCl_2$, single layer alginate capsules were prepared. For $CaCl_2$, cross-linked microcapsules were subsequently suspended in a solution of 0.1% PLL allowing positively charged PLL to bind to the negatively charged alginate. A final layer of 0.5% alginate was then added. Microcapsule permeability was determined via incubation with fluorescent lectins of varying molecular weight. Viability of encapsulated cells was determined by a microfluorometric assay using fluorescein diacetate and propidium iodide. Microcapsules were suspended in gelatin phantoms and imaged on on a clinical 3T XMR scanner (Philips Medical Systems) using a 6-element cardiac phased-array receiver coil. For T_2*-w GRE, imaging, the parameters were: TR=7.0 ms; TE=2.3 ms; flip angle =15°; FOV=22 cm; matrix=512x512; slice thickness= 1 mm. A 3D IRON sequence[4] was run with the same parameters with the exception of TR = 1300ms, TE = 12.0ms, IRON pulse bandwidth = 170Hz and turbofactor = 18.

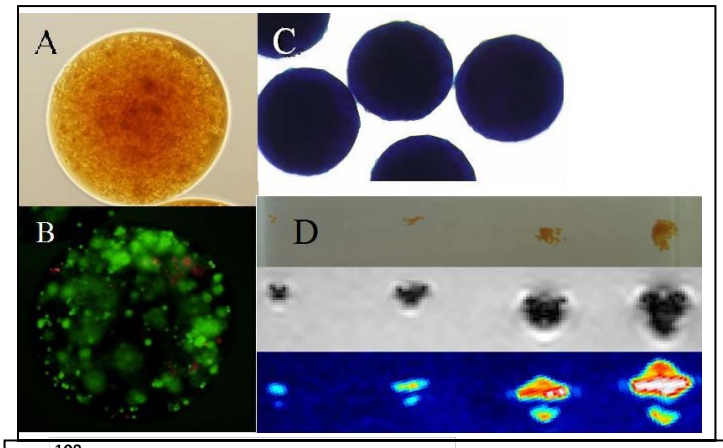

Figure 1: Microscopic imaging of a 400µm single-layer microcapsule: Bright field microscopy (A) and live/dead cell viability assay (B, green = alive, red = dead). (C) Prussian Blue staining of single layer barium cross-linked magnetocapsules. (D) MR imaging of single-layer magnetocapsules in a gelatin phantom. Top: Picture of gelatin phantom. Middle: T2*-w GRE scan, Bottom: IRON scan. From left to right, results are shown for 3, 10, 50, and 100 capsules, respectively

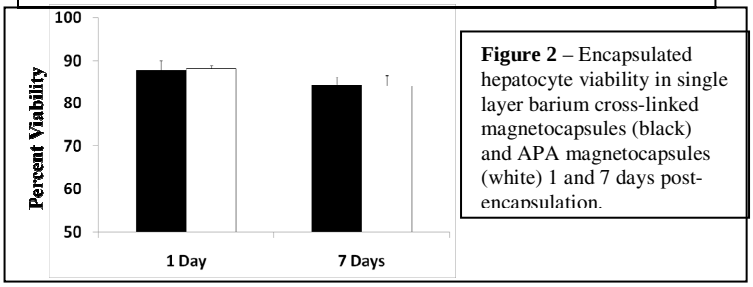

Figure 2 – Encapsulated hepatocyte viability in single layer barium cross-linked magnetocapsules (black) and APA magnetocapsules (white) 1 and 7 days post-encapsulation.

Results: Permeability assays with fluorescent lectins demonstrated that single layer barium cross-linked magnetocapsules have a permeability that lies between 75kD and 120kD, providing adequate immunoisolation. Viability assays conducted *in vitro* indicate that single-layer magnetocapsules display similar viability to standard APA magnetocapsules (Figs. 1B, 2). Prussian Blue staining illustrated that the Feridex was spread diffusely throughout the single layer magnetocapsules (Fig. 1C). Imaging of single layer magnetocapsules on a clinical 3T scanner demonstrated the ability to image individual capsules (Fig. 1D).

Conclusions: By encapsulating hepatocytes into microcapsules containing Feridex, we have developed a way to visualize encapsulated cells with MRI. By incorporating Feridex into barium cross-linked microcapsules, we have created smaller, single-layer MR-visible microcapsules. This modification reduces manufacturing time and has no effect on capsule permeability or encapsulated cell viability. Moreover, single layer magnetocapsules have MRI porperties that are comparable to their APA counterparts.

References

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