Microcapsules used in cell based therapy were designed for post implantation tracking

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Introduction: Cell based therapy has shown promise in restoring insulin independence in diabetic mice, and encapsulated cells have been locally delivered for gene therapy. As compared with injections, oral drugs or other exogenous delivery of therapeutics, transplanted cells can respond physiologically to the microenvironment and secrete hormones, neurotransmitter, and other useful biotherapeutic products that can restore, retain or alter host function. Transplanted cell xenografts can also overcome acute human organ shortage. However, it is necessary to protect xenografts from the host's immune system. To that end, we are developing an immunoprotective and biocompatible cell encapsulation device with controlled porosity (Fig. 1a) to immunoprotect the transplanted cells while allowing the free diffusion of nutrients and hormones (1,2). The device must be tracked *in vivo* so as to ascertain post-transplant location and to evaluate the physiological and biochemical environment proximal to the implant. Here we show *in vivo* device tracking with MRI for both diamagnetic and ferromagnetic cell encapsulation devices.

Theory: Diamagnetic (copper) encapsulation devices were designed with conductor thickness several times larger than the skin depth of copper at the 500 MHz operating frequency of our MR scanner; the devices were gold-coated for bioinertness. The thick conductor prevents the passage of electromagnetic waves to the device interior and results in a shielding effect where signal loss is localized to the device interior. Finite element simulations of the electro-magnetic field were performed to confirm the same. Additionally, we designed ferromagnetic (nickel) containers so as to provide a region of negative contrast enhancement that is several times larger than the microcontainer dimensions. In the presence of an external magnetic field, nickel becomes permanently magnetized with a saturation magnetization slightly larger than 1 T. Nickel may therefore be considered fully saturated at our scanning field strength of 11.7 T, creating a local field distortion that results in a symptomatic arrow-shaped signal void that is considerably larger than the microcontainer and oriented in the direction of the readout gradient.

Methods: Cells were dispersed in an extracellular matrix gel and encapsulated within a cubic microcontainer (copper or nickel). The microcontainer was then implanted within a human prostate tumor PC-3 xenograft or a human breast cancer MDA-MB-231 xenograft in SCID mice. For the copper microcontainer, MRI was performed with a spin echo sequence with a slice thickness = 3 mm, field-of-view = 1.4 cm x 1.4 cm, 55 μm x 55 μm in-plane resolution, TE = 6.3 ms, TR = 803 ms, number of signal averages = 2. Images for the nickel microcontainer were similarly acquired with a slice thickness = 0.5 mm, field-of-view = 2 cm x 2 cm, acquisition matrix = 512 x 256, TE = 10 ms, TR = 600 ms, and one signal average. All MRI was performed on a 500 MHz (11.7 T) scanner with a Bruker Avanace spectrometer and triple-axis imaging gradients (300 G/cm maximum). The microcontainers were retrieved 48 h post implantation and cells within and proximal to the device were stained with the viability stain calcein-AM (Sigma, www.Sigma,com), as well as with ethidium homodimer-1 (Sigma, www.Sigma,com) which stains for dead cells.

Results and Discussion: MRI of diamagnetic, copper microcontainers using the Faraday shielding effect show the signal void localized within the microcontainer (Fig. 1b). The technique allows precise *in vivo* identification of a microcontainer of any arbitrary shape, and the ability to discern the device from other susceptibility based artifacts in MRI. MRI of ferromagnetic, nickel microcontainers (Fig. 1c) shows a region of negative contrast enhancement that is much larger than the microcontainer dimensions. This provides an avenue for further miniaturization of the microcontainers, potentially to encapsulate single cells as opposed to cellular clusters, while still permitting non-invasive device detection and tracking of microcontainers that are much smaller than the image voxel resolution in MRI. For devices retrieved 48 h post implantation, staining with calcein-AM showed viable cells within and proximal to the device; there was no uptake of ethidium homodimer-1, indicating the absence of dead cells. These data suggest the bioinertness of the device and suggest that there was no adverse consequence of imaging with the pulse sequences used in this study.

Conclusions: MRI of microcontainers for cell based therapy was successfully demonstrated with two strategies for *in vivo* tracking. This tracking can be used in conjunction with vascular and metabolic MRI to periodically and longitudinally evaluate the physiological and biochemical environment proximal to the implant and to longitudinally and periodically assess the implant's effect on its microenviroment. We are currently exploring the encapsulation of cells that secrete therapeutic molecules for various tumor models.

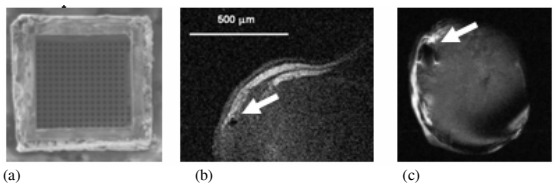

Fig 1: (a) SEM image of cell encapsulation microcontainer (2). MRI of a copper microcontainer with Faraday shielding of the device interior (b) and MRI of a nickel microcontainer with an enhanced region of negative contrast (c) provide the ability to uniquely distinguish and track the microcontainers post implantation.

References:

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