Monitoring re-cellularization of acellularized organs with iron oxide nanoparticles and T2W-MRI

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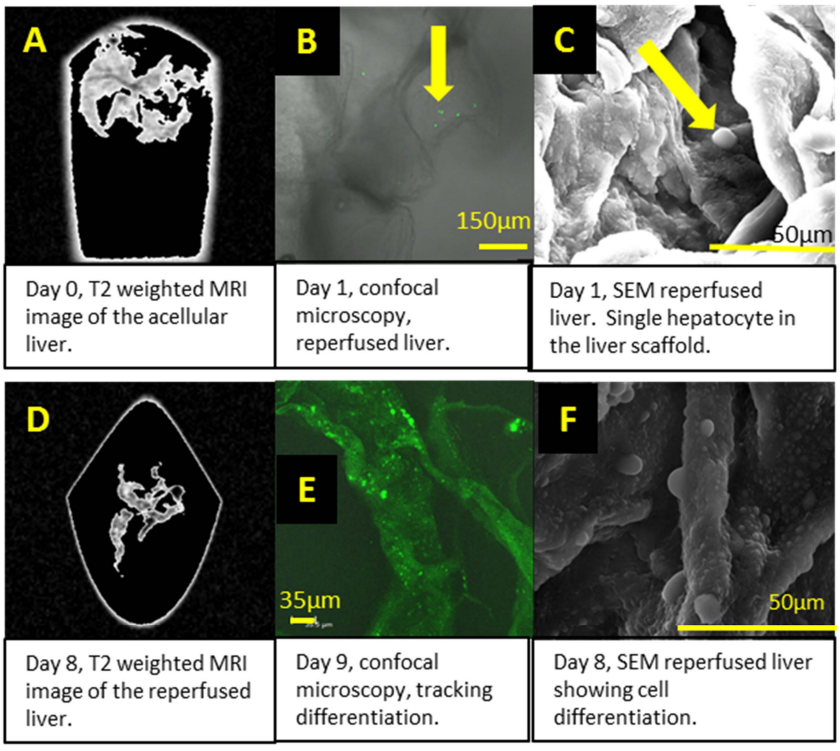
Introduction: Re-seeding organ scaffolds with a patient's own cells is a promising way to eliminate graft-versus-host disease and improve organ transplant procedures. However, to properly re-seed the extracellular matrix with cells, non-invasive tracking of the cells is required to determine proper re-seeding and scaffold engineering pattern. Cells labeled with iron oxide nanoparticles have been used to track cells within in vitro cell cultures and in vivo animal models. This study investigated whether T2-weighted MRI could track the reseeding of murine liver scaffolds with iron oxide-labeled liver cells.

Methods: Iron oxide nanoparticles with 1.63 µm average diameter that were coated with polystyrene and dragon green fluorophore were purchased from Bangs Labs, conjugated with protamine sulfate and glutamine, and sterilized. Liver cells were isolated from donor mice and loaded with the iron oxide particles. The donor liver was acellularized using a previously reported protocol. The MRI of the donor liver was performed on Day 0, then the liver was reperfused with the iron oxide-loaded liver cells. MR images were obtained on Days 1, 3, 6, and 8 to monitor cellular re-seeding of the liver. To validate re-seeding, liver biopsies were imaged with confocal microscopy on Day 1 and 9, and with SEM on Days 1 and 8. The T2 weighted images were acquired using a Bruker 7T MRI scanner using a MSME pulse sequence, with a 12000 ms TR, 20 ms - 960 ms TE, 48 echos, 3x3 cm FOV, 234x234 µm in-plane resolution, 1.0 mm SL, 12 slices, and 1 average. T2 parametric maps were calculated using Paravision Software Version 5.1. The SEM images were acquired with a Hitachi S-3400N at 30 Kv, 35 degree x-ray angle, 2,222x-2,784x magnification, under high vacuum, and 2,560 x 1920 pixel resolution. The confocal images were captured with a Leica SP5-II spectral confocal microscope and processed using Leica LAS-AF software version 2.7. A 40x/1.25NA PL Apo oil emersion lens and 488 nm wavelength Ar laser was used to capture the images, with excitation at 520 nm, 387.5x387.5 µm FOV and 2691x2691 pixels per slice while Z-stacking every 2 µm over 70 µm.

Results: The iron oxide particles were successfully modified. The zeta analysis demonstrated a 5 mV change in surface potential to a final charge of -20.2 mV. AOPI staining of the uploaded liver cells showed that the cells were viable. Liver cell upload of particles was verified with confocal microscopy demonstrating nearly 95% upload of 2.6 million cells. The acellular liver (Fig. A) was reperfused with liver cells containing the iron oxide particles and verified via MRI. Initial sparsely populated cell loading was confirmed with confocal microscopy (Fig. B) and SEM (Fig. C) of liver biopsy one day after reperfusion. More heavily populated cell load caused darker contrast in T2-weighted MR images (Fig. D) and was confirmed with confocal microscopy (Fig. E) and SEM (Fig. F).



Discussion: These findings demonstrate that MRI can track re-cellularization of organs. Therefore MRI can be used as a non-invasive tool to determine appropriate techniques for human organ re-seeding to eliminate graft-versus-host issues with transplants.

References:

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