MRI characterization of agarose 'cocoons'

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Introduction: Early and severe cell attrition in vivo may limit the efficacy of stem cell therapy. Strategies which promote cell survival include tissue angiogenic pre-treatment, cell transformation, and cell encapsulation. Agarose encapsulation strategies, in which single cells are 'cocooned' ex vivo in agarose matrices, have strong pro-survival properties because of matrix supplementation with provisional matrix proteins. MRI should be effective for tracking capsule fate in vivo, because agarose gels have long served as tissue-mimicking phantoms. Furthermore, capsule imaging may be facilitated by transient loading with paramagnetic contrast. This paper provides a characterization using MRI of pelleted agarose 'cocoons' ex vivo, and in vivo following direct injections into the lumbar muscles of rabbits, targeted towards the optimization of capsule design for MRI capsule tracking in animal stem cell therapy models.

Methods: Capsules (mean diameter 50μm) were constructed according to the methodology of Courtman.[1] T1 and T2 were modulated by varying agarose content (2.5% and 1.8%). Some capsules were also incubated for 20 minutes ex vivo in 5mM Gd-DTPA. Post-aspiration, capsule pellet relaxation times (~150μl in eppendorf tubes) were quantified using the head coil of a 1.5 Tesla GE Signa, with validated magnetization-prepared spiral imaging sequences.[2]

Capsule pellets were injected directly (up to 100μl) using a precision Hamilton syringe (250μl volume) or standard insulin syringe (1ml volume) and at discrete locations into the lumbar muscles of rabbits (n=6, New Zealand White, 2-5kg). The spatial distributions of T2 were evaluated using stacks of spiral images (TE of 11 and 54ms, TR=3000ms, 0.8x0.8x1.7mm^3, 24 nex) while T1 scanning was performed in select slices using a Look-Locker method (train of 9 small-tip angle pulses separated by 160ms, same resolution and TR). The persistencies of contrasts from agarose and Gd-DTPA were tracked via repetitive imaging over the first five hours post-injection, using heavily T1-weighted spgr imaging (TR of 50ms), which should highlight Gd-DTPA capsules only, and T2-weighted spiral imaging (TR of 3000ms), which should highlight all capsules. Distribution volumes (DV) and relaxation times of regions of significantly-elevated T2-weighted signal intensity were evaluated using custom software (xcinema, Stanford).

Results: Reducing agarose content increased T2 (2.5%: 88±13ms; 1.8: 104±13ms; p=0.006, n=7), while T1 remains constant (2.5%: 2741±705ms; 1.8%: 2671±494ms; p=0.63, n=6). Capsule immersion in Gd-DTPA reduced T2 moderately (2.5%: 61±7ms, p<0.0001; 1.8%: 34±5ms, p=0.0005, n=6) and T1 considerably (2.5%: 129±12ms, p=0.0018; 1.8%: 122±9ms, p=0.0005, n=5). Preliminary studies demonstrated very small increases in capsule T1 and T2 following cell encapsulation. In vivo, 1.8% capsules presented with DV of 44±15mm^3, T2 elevation of 53±26% from remote T2 of 29ms, and T1 elevation of 50±25% from remote T1 of 1147ms (n=13). 2.5% capsules presented with DV of 32±17mm^3, T2 elevation of 41±10% (n=9), and T1 elevation of 41±26% (n=11). Assuming a two-compartment model of slow water exchange, DV and T2 data are consistent with fractional DV filling with agarose by 48±15% at 1.8% and 44±7% at 2.5%. Only sites of Gd-DTPA enhancement were visible in early short-TR images (<2 hours), while no sites were visible in late short-TR images (>4 hours). Persistence of agarose contrast was retained across early and late time points.

Conclusions: MRI can effectively visualize agarose 'cocoons' following direct muscular injection in vivo. Capsule visualization in short TR images is facilitated tby immersion in paramagnetic contrast agents. Persistent labeling may prove essential in applications targeting tissue states with pathologically-elevated T2 (ie. edema). Gel 'stickiness' (ie. agarose biotinylation) or agent size (agarose pore size ~ 60-100nm) are design targets.[3] Mild dilution of the discrete pelleted capsules should reduce pellet viscosity, and facilitate needle delivery. Multiple contrast mechanisms may facilitate more comprehensively tracking of encapsulated cell fate, including cell emergence.

References: 1) Courtman D. et al. Canadian patent #2450650; 2) Foltz WD. et al, JCMR, 2006; 3) Serwer, P. Anal Biochem, 1985.

Figure: (a) coronal T2 image including 6 discrete lumbar injection sites (no Gd-DTPA samples); (b, c) early and late spgr (+1 and 4 hours) depicting 4 Gd-DTPA sites with white arrows; and (d) corresponding late fse (+4 hours).