Monitoring acellular matrix-based soft tissue regeneration: multiexponential diffusion and T2* for improved specificity

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INTRODUCTION

Cell-seeded natural scaffolds derived from acellular matrices (ACMs) hold great promise for regenerating large soft-tissue organs. The ACM contains site-preferred structural and functional molecules, and ACM-derived degradation products play important roles in tissue remodeling. MRI assessment has demonstrated sensitivity to tissue microstructure and composition, but specificity remains limited. In this study, multiexponential diffusion and effective transverse relaxation time T2* were investigated for their ability to assess cell growth and tissue composition, respectively.

METHODS

ACMs were prepared from fresh porcine urinary bladders according to a published protocol [1]. Cell cultures were obtained from passage 2 smooth muscle cells (SMCs) isolated from porcine bladder. Cells were seeded at 10^6 cells/cm^2 of ACM.

Cell-seeded (N=20) and unseeded (Control) (N=15) ACMs were prepared. Six of the unseeded ACMs were further processed for hyaluronic acid (HA) incorporation to arrest matrix porosity. MR imaging was performed on day 1, 3, and 7; seeded ACMs were imaged additionally at 21 days. Samples were placed in 5mL round Falcon tubes and imaged on a 1.5T scanner (Signa EXCITE Twinspeed, GE Healthcare) using a 3-inch surface coil. Quantitative T1, T2, T2*, and diffusion imaging was performed. T1 was measured with a spoiled gradient echo sequence (FA=2,3,10,20°, NEX=4) [2]. T2 was measured with a 2D spin echo sequence (TE=9-300ms, TR=3s, NEX=1). T2* was measured with a 2D multi-echo gradient-echo sequence (16 equally spaced TEs=[2.6-34.4] ms, TR=118 ms, FA=20°, NEX=4). Diffusion imaging was performed with a spin echo diffusion-weighted sequence [b-value=0-3000s/mm^2 in steps of 200s/mm^2, TR=4s, NEX=16). In-plane resolutions were 0.4mm (T1,T2,T2*) and 0.8mm (diffusion), and slice thickness was 3mm. Data analysis was performed using in-house developed software in Matlab (v.7.0). Differences were assessed using a two-tailed Student's t-test.

Samples were assessed on histology (H&E and Masson's trichome). Cell density was assessed qualitatively and 4 classes were defined: I (very few cells), II (few cells), III (patches of cells), and IV (good cellularity).

Fig. 1. a) Diffusion-weighted and **b)** T2*-weighted MR images of an ACM in a 12-mm diameter tube. Diffusion-weighted images are shown for b-value=200 s/mm^2 (left) and 1600 s/mm^2 (right). T2*-weighted images are shown for TE=2.6 ms (left) and 34.4 ms (right).

RESULTS

Representative diffusion-weighted and T2*-weighted MR images of the ACM are shown in Fig.1. The diffusion decay is different between unseeded and cell-seeded ACMs, in which a second slow diffusion component emerges in association with the intra-cellular compartment (Fig.2). Fig.3 shows that the slow diffusion fraction has the highest correlation with cellularity (r=0.954, $P<10^{-5}$) compared to single diffusion coefficient (r=-0.153, P=0.654), T1 (r=-0.614, $P<0.05$), and T2 (r=-0.309, P=0.355). T2* measurements demonstrated the greatest relative change for both cell-seeding and incorporation of HA (Fig.4). The most interesting observation on T2* was that in the presence of increased tissue hydration due to the hydrophilic nature of HA, T2 increased but T2* decreased as expected for the addition of a large macromolecule.
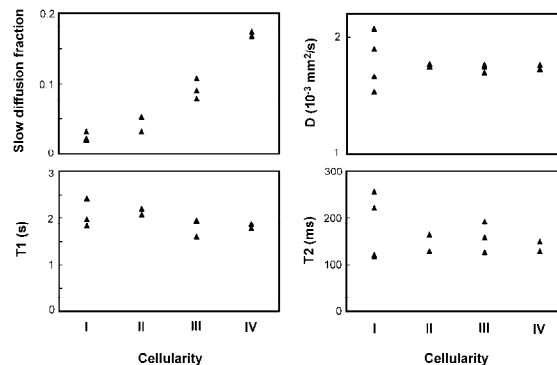
CONCLUSIONS

This study has demonstrated the potential role of T2* and multiexponential diffusion for more specific assessment of cell growth and tissue development. The results are relevant in applications where concurrent biological changes exist, for example, where cellularity needs to be determined independent of biochemical changes or where tissue hydration is a confounder and needs to be removed.
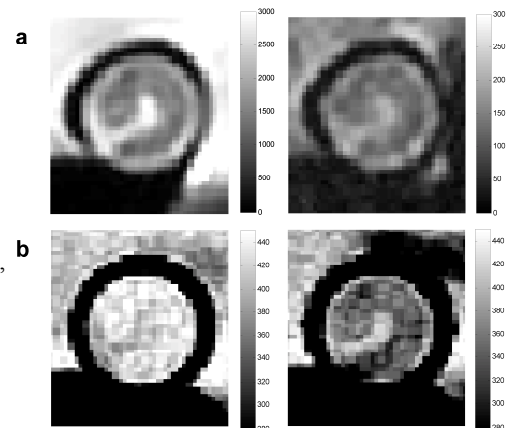
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[1] Brown AL. Biomaterials 2002; 23:2179.
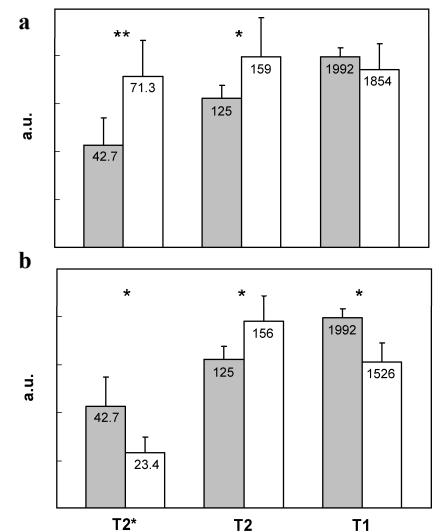[2] Cheng HL. MRM 2006; 55:566.
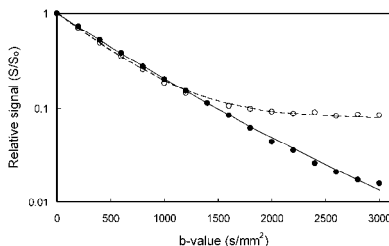
Fig. 2. Diffusion decay curve for unseeded (filled circles) and cell-seeded ACM (open circles). Latter displays biexponential decay.

Fig. 3. Quantitative MRI for distinguishing various levels of cellularity. The slow diffusion fraction is the most significantly correlated with cellularity.

Fig. 4. Effect on MR parameters from **a)** cell-seeding and **b)** incorporating HA. Note: T2* changes in opposing directions (**$P<10^{-4}$, *$P<0.05$).