Quantitative MRI assessment of matrix development in cell-seeded natural urinary bladder smooth muscle tissue-engineered constructs

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INTRODUCTION

Cell-seeded natural scaffolds derived from acellular matrices (ACMs) hold great promise for tissue-engineering large soft-tissue organs such as the urinary bladder and heart. The ACM has advantages of biological recognition and structural and biochemical similarities with the target tissue that are not easily reproduced using conventional synthetic or tissue-mimicking materials [1]. However, experience with cell-seeded natural scaffolds is relatively young, and little is known about cell-matrix interaction or its influence on MRI characterization. This study investigates a battery of quantitative MRI methods to monitor cell-matrix interaction and matrix development in a model of smooth muscle cell-seeded urinary bladder ACM.

METHODS

ACMs were prepared from fresh porcine urinary bladders according to a published protocol [2]. 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. Unseeded ACMs were prepared as controls.

Cell-seeded (N=24) and unseeded (N=15) ACMs were prepared 1, 3, and 7 days prior to MRI. 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, and diffusion imaging was performed. T1 was measured with a spoiled gradient echo sequence (FA=2,3,10,20°, NEX=4) [3]. Both single T2 (spin echo: TE=9-300ms, TR=3s, NEX=1) and multicomponent T2 (96-echo CPMG: TE=11.4-1094ms, TR=2.5s, NEX=2 [4]) were measured. Diffusion coefficients (D) were measured using 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) and 0.8mm (D), and slice thickness was 5mm for CPMG and 3mm for all other sequences. Data analysis was performed using in-house developed software in Matlab (v.7.0).

Samples were also assessed on histology, immunofluorescence, and biochemistry. H&E and Masson's trichome staining was performed to provide qualitative assessment of cellularity and collagen fibers in the matrix. Immunofluorescence staining for matrix metallopeptidase 1, or interstitial collagenase, was performed in a separate set of SMCs cultured on sterilized plastic cover slips in medium. Finally, biochemical assay (Chondrex Inc, USA) was performed to quantify the amount of type I collagen in the matrix of both cell-seeded and unseeded ACMs.

RESULTS

Cell numbers were relatively stable in the first week post cell-seeding. However, higher T2 and increasing D in seeded matrices were incompatible with cell presence (Fig.1). Multicomponent T2 was more specific, revealing time-course changes in the short T2 component that were suggestive of collagen degradation in the extracellular matrix (Fig.2). This hypothesis is confirmed on immunofluorescence showing the release of collagenase from seeded cells (Fig.3), and on biochemical detection of the resultant breakdown of collagen I in the extracellular matrix (Fig.4).

CONCLUSIONS

This initial quantitative MRI study of SMC-seeded bladder ACMs reveals matrix collagen degradation from SMC-released collagenase, noted for the first time and perhaps unique to natural matrices. These changes are revealed on MRI, with greatest specificity provided on multicomponent T2. Quantitative MRI is useful for monitoring cell-matrix interaction, but our results also underscore the potential challenge of distinguishing cell- and matrix-related events.

REFERENCES: [1] Zhang Y et al. Biomaterials 2009; 30:4021. [2] Brown AL et al. Biomaterials 2002; 23:2179. [3] Cheng HL et al. MRM 2006; 55:566. [4] Poon CS et al. JMRI 1992; 2:541.

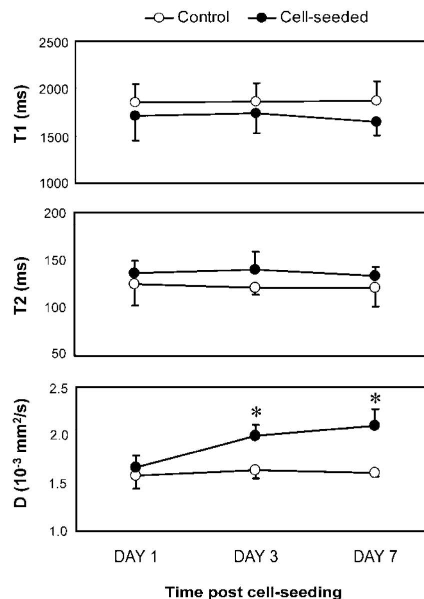

Fig. 1. Lowered parameter values expected from cell-seeding was not seen for T2 and diffusion coefficient D (*P<0.05)

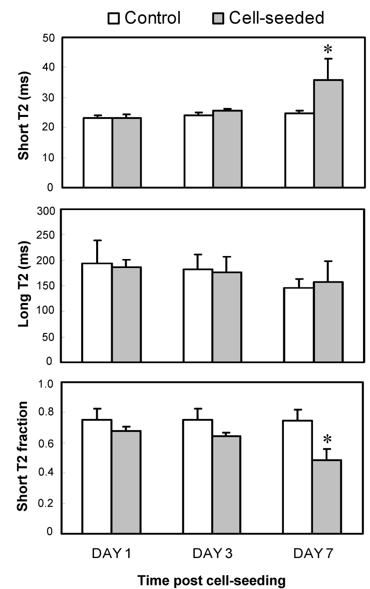

Fig. 2. Multicomponent T2 shows significant changes in the short component T2 and fraction (*P<0.05)

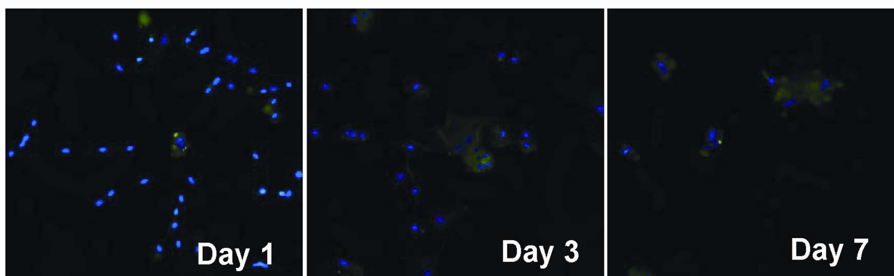

Fig. 3. Immunofluorescence staining for collagenase (green) confirms its release from SMCs (blue, DAPI) at all time-points (magnification, 10×)

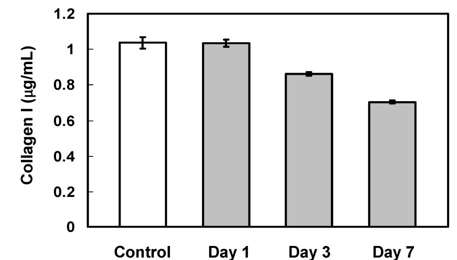

Fig. 4. Collagen I content progressively declines in cell-seeded matrices (grey), whereas no changes with time were seen in unseeded matrices (Control).