The acellular matrix for bladder tissue-engineering: a quantitative MRI study

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INTRODUCTION

Tissue scaffolds are an integral part of many tissue-engineering and regenerative therapies, as they provide a 3D support to guide cell growth and facilitate the delivery of biochemicals, nutrients, and cells. Recently, there is growing interest in using naturally derived acellular matrices (ACM) as scaffolds [1]. Unlike synthetic materials, ACMs possess native biomechanical and many acquired biological properties, but many questions remain in efforts to optimize scaffold design. To date, imaging of scaffolds has been achieved mainly with microCT, electron and confocal microscopy [2]. In this study, we explore the role of quantitative MRI in scaffold characterization. Specifically, we investigate the bladder ACM and effects of incorporating hyaluronic acid (HA), a natural biomaterial that has shown considerable potential in the development of engineered tissues [3-5].

METHODS

ACMs were prepared from fresh porcine urinary bladders according to a published protocol [6]. To incorporate HA, ACMs were cut into 2×2cm^2 squares, dehydrated in ethanol and lyophilized for 24 hours, and rehydrated in increasing concentrations of HA (0.05, 0.1, 0.2, 0.5 mg/100mL) (Sigma). Alcian blue staining was performed to confirm HA uptake. Both ACM with HA (HA-ACM) and without HA (non-HA-ACM) were prepared (N=17 total) for MRI. A separate group of ACMs (N=17) was prepared for hydration and biochemical assays.

MRI was performed on a 1.5T scanner (Signa EXCITE Twinspeed, GE Healthcare) using a 3-inch surface coil on ACM samples placed in 5mL round Falcon tubes. Quantitative T1, T2, and diffusion coefficients were measured. T1 was measured with a spoiled gradient echo sequence (FA=2,3,10,20°, NEX=4) [7]. 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 [8]) 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).

Water content in ACMs was determined by measuring its wet weight and dry weight prior to and after, respectively, dehydration with ethanol and lyophilization. The total water uptake was calculated as the difference between wet and dry weights.

Biochemical assays were performed to measure the glycosaminoglycan (GAG) content. Since HA is a non-sulfated GAG and is distinct from sulfated GAG (sGAG), two assays were performed. The Dimethyl-Methylene Blue (DMMB) method was used to determine total GAG content [9]. For this, dried ACM pieces were digested using 250μg/mL papain from papaya latex, mixed with 1,9-DMMB, and measured for absorbance at 530 nm, using chondroitin sulfate sodium salt as the standard. The second assay used Stains-all® at 480 nm to determine only sGAG content, according to a previous protocol [10]. All products were from Sigma.

RESULTS

Biochemical and physical changes were observed from incorporating HA in the ACM. The hydration assay (Table 1) showed significantly higher absolute water uptake and retention, by nearly two-folds (P<0.05). Biochemical assays (Table 2) showed a nearly three-fold increase in total GAG (P<0.01) attributed to HA only, since sGAG content was unchanged. On MRI, single T2 and diffusion measurements (Fig.1) were significantly higher in HA-ACM (P<0.01), consistent with the overall effect of greater hydration, increased space in the extracellular matrix, and higher GAG content. Multicomponent T2 appeared more specific and able to separate effects of increased GAG and hydration (Table 3, *P<0.05). The fast T2 increased likely due to improved mobility of water now associated with HA. The slow T2 increase reflects greater hydration.

CONCLUSIONS

This study has provided baseline quantitative MRI measurements of the bladder ACM, which is necessary for understanding MRI changes with further manipulation such as cell-seeding. The effect of HA incorporation was also assessed. Both increased GAG content and two-fold water uptake in HA-ACM were detected on T1, T2, and diffusion measurements, with multicomponent T2 being most specific. These results are valuable in guiding further regeneration development using ACMs and tissue-engineering strategies involving HA.

Table 1. Hydration assay

Units (g)	non-HA-ACM	HA-ACM
Wet weight	0.133 ± 0.025	0.248 ± 0.054
Dry weight	0.011 ± 0.003	0.039 ± 0.005
Water uptake	0.123 ± 0.022	0.210 ± 0.053

Table 2. Biochemical assays

Units (μg/mL)	non-HA-ACM	HA-ACM
sGAG	14.9 ± 1.7	12.7 ± 1.1
HA + sGAG	12.8 ± 6.9	33.5 ± 6.6

Table 3. Multicomponent T2 results

	Fast component		Slow component	
	$T2_{fast}$ (ms)	Fraction (%)	$T2_{slow}$ (ms)	Fraction (%)
non-HA-ACM	26.3 ± 3.7	71 ± 11	* 146.8 ± 11.5	26 ± 10
HA-ACM	31.4 ± 3.4	64 ± 11	* 237.7 ± 57.5	25 ± 8

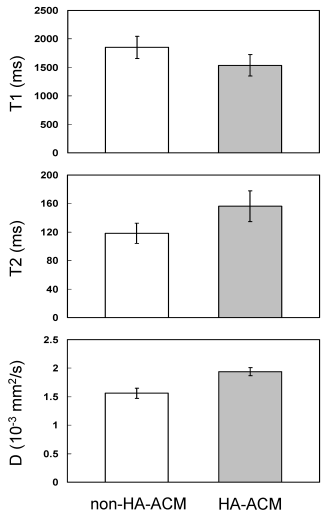

Fig. 1. All quantitative MRI parameters are significantly different between HA-ACM and non-HA-ACM (T1: P<0.05; T2,D: P<0.01)

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