Quantitative 11.7 T MRI and EPIC-µCT Assessment of Cartilage Repair in a Rabbit Glenohumeral Joint Model following Microfracture and Autologous Matrix Induced Chondrogenesis

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Target Audience: Clinicians and researchers investigating cartilage regeneration will find this information useful as it correlates cartilage repair of tissue following MFX and AMIC treatments with $T_{1\rho}$ and T_2 measurements.

Introduction: Microfracture (MFX) tissue stimulation is a first-line surgical treatment for cartilage defects [1]. Autologous matrix induced chondrogenesis (AMIC) is also under investigation for the repair of cartilage defects which combines MFX with a collagen membrane [2]. Here we present a comparison of X-ray and MR based imaging methods to compare the effectiveness of the MFX and AMIC treatments. MRI maps of $T_{1\rho}$ and T_2 relaxation times are highly correlated with the proteoglycan (PG) and collagenous changes that occur in cartilage regeneration [3-4], while EPIC (Equilibrium Partitioning of an Ionic Contrast-agent)-µCT provides complementary data for cartilage morphology and PG content distribution [5-6]. To our knowledge, MRI and EPIC-µCT have not been used together to assess cartilage regeneration following MFX and AMIC. The purpose of the current study is to examine the regenerative potential of AMIC treatment in comparison to MFX in an *in vivo* rabbit glenohumeral joint repair model using $T_{1\rho}$ and T_2, and EPIC-µCT.

Methods: Fifteen adult New Zealand white rabbits (4-5 kg) randomized into 3 treatment groups, underwent unilateral shoulder surgery under IACUC approval. Full-thickness cartilage defects (6 mm diameter) were created on the left shoulder and treated with MFX alone, MFX augmented with a collagen I/III scaffold (AMIC), or left untreated (surgical control (SC)). Contralateral shoulders (intact) served as uninjured controls. Rabbits were sacrificed at 8-9 months after surgery. ***MRI***: Immediately after sacrifice, cartilage plugs (6 mm diameter) were harvested from the surgical and intact joints, and imaged using a vertical 54 mm diameter clear bore Bruker DRX-11.7 T AVANCE micro-imaging system. The data were acquired with an in-plane resolution of 62.5 µm and a slice thickness of 0.5 mm. The imaging plane was perpendicular to the B_0 field in all cases. The $T_{1\rho}$ data were acquired using a preparatory self-compensation pulse cluster followed by a FSE sequence (TE/TR: 8 ms/ 3 s, spin-lock duration: 10 - 160 ms, spin-lock strength: 100 µT) [7]. The T_2 data were acquired using a modified CPMG sequence (TR: 10 s, TE: 6.2 - 100 ms, 16 echoes) [8]. Custom written MATLAB programs were used to calculate $T_{1\rho}$ and T_2 maps on a pixel-by-pixel basis. $T_{1\rho}$ and T_2 indices (defined as the ratio: repair tissue / contralateral normal cartilage) were calculated for each rabbit to standardize the absolute $T_{1\rho}$ and T_2 changes. The Mann-Whitney statistical test was used to compare the $T_{1\rho}$ and T_2 indices between the MFX and the AMIC groups. The significance level was set at $p < 0.05$. ***EPIC-µCT***: After MRI, cartilage plugs were fixed in formalin, incubated in Hexabrix contrast agent, and then scanned using a SCANCO µCT-40 scanner with a 12 µm resolution in all three spatial planes [6]. The scanner software was used to segment the cartilage, calculate its thickness and volume, and to create cartilage thickness maps. Repair site filling was quantified as the ratio of repair tissue volume to contralateral normal cartilage volume.

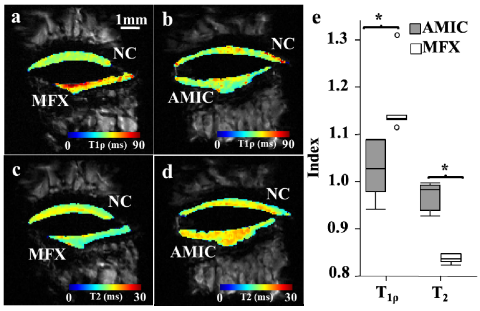

Fig.1. $T_{1\rho}$ (a-b) and T_2 (c-d) maps of native cartilage (NC) and repaired tissue (RT) after MFX and AMIC. Boxplot of the $T_{1\rho}$ and T_2 indices (ratio of RT/NC) of cartilage after MFX and AMIC are shown in (e). * $p < 0.01$.

Results: Representative $T_{1\rho}$ and T_2 maps of normal cartilage (NC) and repaired tissue (RT) after MFX and AMIC are shown in Figs. 1a-d. No repaired tissue was observed in the SC group using MRI (images not shown). The $T_{1\rho}$ index was 1.17 ± 0.08 and 1.03 ± 0.07 for MFX and AMIC groups, respectively. The T_2 index was 0.84 ± 0.01 in the MFX group and 0.97 ± 0.03 in the AMIC group (Fig. 1e). Significant differences between the MFX and AMIC groups were observed for both $T_{1\rho}$ and T_2 indices ($p < 0.01$). Typical thickness maps for NC and RT after MFX and AMIC treatments are shown in Fig. 2. The RT volume fill grade results are provided in Table 1.

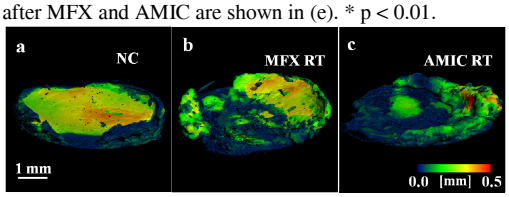

Fig.2. Representative thickness maps of NC (a), RT after MFX (b) and AMIC (c). The colors represent thickness as described by the key.

Volume Fill (%)	MFX (N = 5)	AMIC (N = 5)
0-50	1	1
50-100	2	3
>100	2	1

Tab.1. Defect volume filling grade

Discussion and conclusions: To the best of our knowledge, this work provides the first long-term MRI and EPIC-µCT data comparing *in vivo* glenohumeral joint cartilage repair following MFX and AMIC. After 9 months of healing there was a significant difference in both the $T_{1\rho}$ and T_2 indices when comparing MFX and AMIC treatments. $T_{1\rho}$ is known to be inversely correlated with the PG content in cartilage [9]. Therefore, the lower $T_{1\rho}$ index observed in the AMIC group relative to the MFX group indicates a higher PG content in RT following AMIC surgery. On the other hand, T_2 has been demonstrated to be dependent on the integrity and concentration of collagen in cartilage [10]. The lower T_2 index in the MFX group compared to the AMIC group indicates a higher fibrous tissue composition in RT after MFX treatment. Hence, our $T_{1\rho}$ and T_2 results suggest that the tissue growth observed in RT following AMIC is more biochemically similar to native hyaline cartilage. The morphological outcomes derived from EPIC-µCT show that an RT volume fill grade above 50% was seen in 4 out of 5 animals for both treatment groups. RT hypertrophy (above 100%) occurred in both groups. Thus, no superiority of AMIC over MFX could be determined on the basis of the volume filling grade results. In addition, attenuation values and spatial distribution maps derived from EPIC-µCT are currently under analysis. In conclusion, our results suggest that MR relaxation times ($T_{1\rho}$ and T_2) combined with EPIC-µCT can be used to monitor and assess cartilage regeneration in regenerating tissue following MFX and AMIC treatments.

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