In Vivo Quantification of Cartilage Regeneration in an Equine Model at 3T Following Gene Therapy

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Introduction

Currently, there is no established human sized model for cartilage regeneration. Previous studies with artificially created defects in rabbits [1] and horses [2], have shown the effectiveness of certain gene therapies in healing full-thickness cartilage injuries via *ex vivo* CT and histological examination. This study is the first to assess the time course of healing *in vivo* using quantitative MRI in live ponies with cartilage thicknesses comparable to humans in a 3T clinical scanner. We use several innovative, quantitative methods including delayed contrast-enhanced MRI of cartilage (dGEMRIC), dynamic contrast-enhanced MRI (DCE-MRI), and T_2 mapping in order to assess cartilage health and tissue regeneration. These methods have been shown to probe the structure of healthy hyaline cartilage [3,4], which is composed of a fibrous collagen matrix, glycosaminoglycans (GAG), a sparse population of chondrocytes, and an aqueous electrolytic fluid.

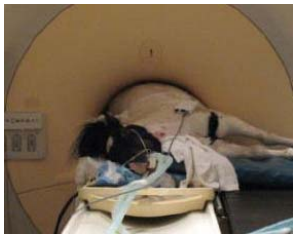

Figure 1 Pony inside a clinical 3T MRI.

Materials and Methods

In each of four ponies, full thickness articular cartilage and sub-chondral bone defects were created by drilling two large cylinders in the condyles of each stifle joint. During the procedure, four different gene therapies were randomly injected into each defect (16 total). The ponies were imaged in a 3T MR system (Achieva, Philips) and data was blindly and randomly analyzed.

dGEMRIC: A sagittal slice through each defect was imaged via a multi-inversion recovery turbo spin echo (IR-TSE) sequence (TR/TE=3740/28 ms; TSE factor=10; FOV=165 x 165 mm^2; matrix=332x328; slice thickness=3mm). Six acquisitions were taken of each slice with varied inversion times (0, 60, 150, 350, 1100, 1680 ms). Post-contrast imaging was performed after a 30 minute passive exercise following injection. T_1 values were calculated by performing Levenberg-Marquardt least-squares fit.

DCE-MRI was performed by administering a bolus injection of double dose (0.2mmol/kg Gd-DTPA) contrast agent while acquiring a 3D T_1 weighted turbo field echo (T_1-TFE) sequence (TR/TE=3.15/1.60 ms; flip angle=12°; TFE factor=50; FOV=64x180x180mm^3; matrix=32x120x120; slice thickness=4mm; 30 dynamic scans, 15.14s per scan). Pharmacokinetic parameters were calculated by fitting to a modified Brix model using Levenberg-Marquardt.

T_2 mapping: A multi-echo TSE sequence (TR/TE=3000/10, 20, 30, 40, 50, 60, 70, 80ms; FOV=165x165mm^2; matrix =164x165; slice thickness=3mm) was performed on each defect. T_2 values were calculated via least-squares fit.

Calculations were performed using in-house software written in the IDL environment (ITT Visual Information Systems). The ponies were imaged 6, 12, and 24 weeks post surgery. Exams at 6 weeks used a transmit/receive quadrature body coil. As a result of SNR limitations, 12 and 24 week data were obtained using one 4-channel array of 10 cm loop coils per stifle joint.

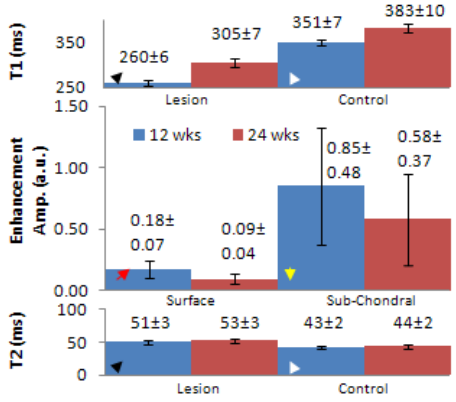

Figure 3 12 week (blue) and 24 week (red) average data for all 16 lesions. The arrow indicators match regions of interest from figure 2.

Results

Lower relative GAG concentrations are measured in new-growth cartilage compared to distal regions of the femoral cartilage (Figure 3). Relative GAG increases in both regions from 12 to 24 weeks. Higher enhancement amplitude is observed in the sub-chondral region compared to slight enhancement on the surface. There is no significant change from 12 to 24 weeks. No enhancement is observed in control cartilage. Higher T_2 is measured in new-growth cartilage compared to control and no change is observed from 12 to 24 weeks.

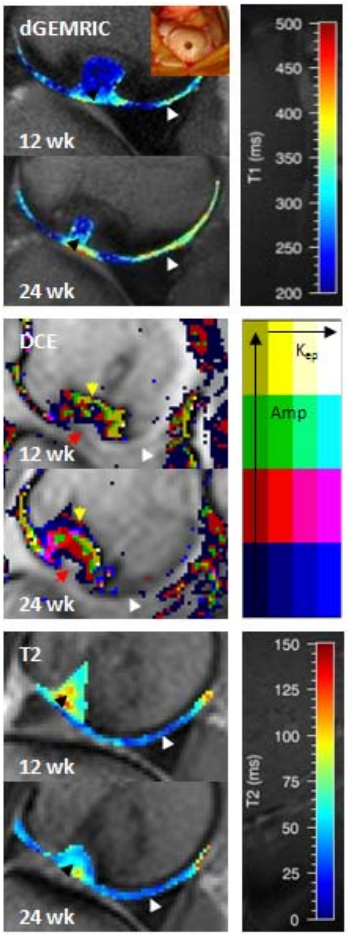

Figure 2 Representative images of dGEMRIC, DCE, and T_2 mapping at 12 and 24 weeks post surgery. Black arrow head: lesion, white: control, yellow: sub-chondral enhancing region, red: slightly enhancing surface of lesion. A photo of the surgical defect is shown in the upper right.

Discussion and Conclusion

The dGEMRIC measurements indicate that new-growth cartilage is GAG depleted, though concentrations increase as regenerated cartilage matures. Increasing GAG concentration observed in control cartilage is an indication of recovery from trauma-induced osteoarthritis. Elevated enhancement amplitudes in the sub-chondral bone indicate an increase in microvasculature near the injury site, while mild enhancement near the regenerated cartilage surface implies that new tissue is fibrous as opposed to hyaline like, slowly sequestering contrast agent. As indicated by higher T_2 measurements within the defects, the collagen fibers are likely to lack organization and therefore mimic the transitional zone as opposed to the radial zone of hyaline cartilage. This study strongly suggests that *in vivo* quantitative MRI can be used to monitor cartilage healing and characterize the physiological state of repaired tissue.

References

1. Bertone A L., et al. *J Orthop Res*, 2004. **2.** Ishihara A., et al. *J Orthop Res*, 2008. **3.** Trattnig S., et al. *Eur Radiol*, 2008. **4.** Lee J H., et al. *Osteoarthr Cartilage,* 2009.