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 T2 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.

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 mm2; 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. T1 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 T1 weighted turbo field echo (T1-TFE) sequence (TR/TE=3.15/1.60 ms; flip angle=12⁰; TFE factor=50; FOV=64x180x180mm3; 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.

T2 mapping: A multi-echo TSE sequence (TR/TE=3000/10, 20, 30, 40, 50, 60, 70, 80ms; FOV=165x165mm2; matrix =164x165; slice thickness=3mm) was performed on each defect. T2 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.

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 T2 is measured in new-growth cartilage compared to control and no change is observed from 12 to 24 weeks.

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 T2 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