VALIDATION OF MR IMAGE BASED MEASURES OF OSTEOARTHRITIC CARTILAGE BIOMARKERS BY DIRECT COMPARISONS TO HISTOLOGICAL SECTIONS

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Introduction

MR imaging techniques have become more widely accepted to assess the progression of osteoarthritis (OA) changes in the knee joint. Images may be used to characterize the size of osteochondral defects, for grading of OA changes, or for more quantitative measurements of cartilage volume, thickness and/or surface areas. Semi-automated image segmentation methods have been developed to calculate these biomarkers, however only limited validations of these measurements have been possible. In previous studies, cartilage volumes have been quantified through direct removal of cartilage from the subchondral surfaces, or creation of artificial defects [1]. These techniques may be prone to error due to incomplete removal of the cartilage, or potential damage to the subchondral bone junction. In addition, the irregularities in naturally occurring defects or cartilage degradation may cause further challenges to segmentation algorithms.

The goal of this study was to directly compare measures of cartilage volume, thickness and surface area made from MR image segmentation algorithms and from histological sections of naturally occurring OA in the human patella.

Materials and Methods

- Seven human cadaveric knees from five donors (2 males/3 females, average age 80) were transversely sectioned mid femur and mid tibia/fibula. The skin and subcutaneous tissues were then removed and the knees were stored frozen.
- The knees were allowed to thaw for 36 hours before they underwent MR imaging.

MAGNETIC RESONANCE IMAGING

- The knees were slightly flexed and imaged using a custom-designed four element phased array surface coil.
- Four MR sagittal image sequences were acquired in the sagittal plane:
  1. 3D gradient recall echo (GRE), no fat suppression (TR:29.0, TE: 15.0, flip angle: 40°, slice thickness 1.5mm)
  2. 3D gradient recall echo with fat suppression (TR: 47.0, TE: 15.0, flip angle: 40°, slice thickness 1.5mm)
  3. 3D spoiled gradient recall (SPGR) with fat suppression (TR: 39.0, TE: 7.0, flip angle: 20°, slice thickness 1.5mm)
  4. Dual echo fast spin echo with fat suppression (TR: 4000, TE: 14.2/85.1, flip angle: 90°, slice thickness 2mm)
- All sequences used a 256 x 256 matrix with a 12 cm field of view, resulting in an in-plane resolution of 0.469 mm/pixel
- Following imaging, a complete arthrotomy was performed to allow full inspection, grading and photography of all cartilage surfaces.
- To allow direct comparison of MR images with histological sections, patellae were then positioned with nylon screws in an MR-compatible rectangular holding device. Following fixation in 10% formalin, the patellae were rescanned in isolation, with imaging parameters and resolutions set to be identical to those used for the intact knee.

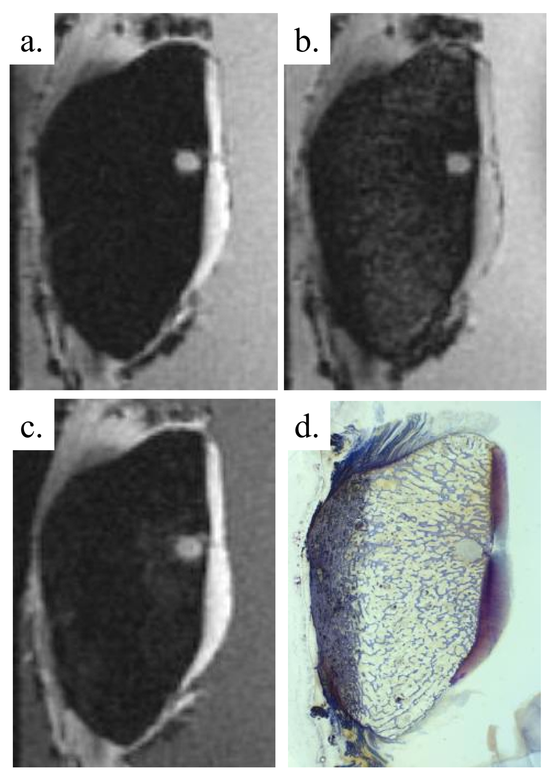
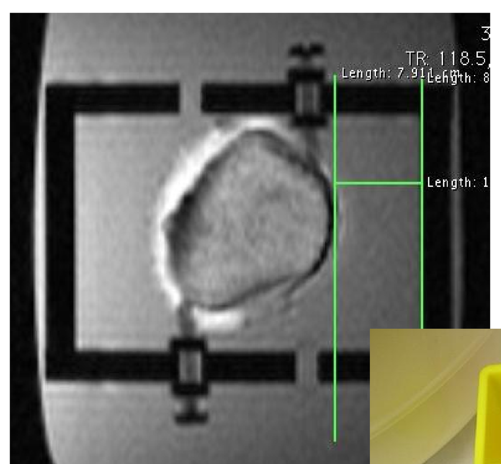
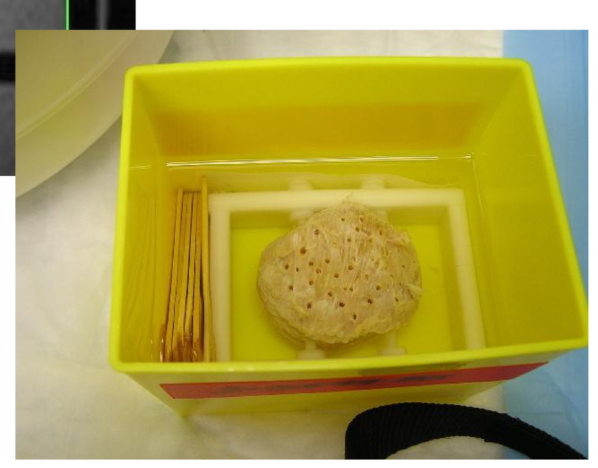


Figure 1: MR images and corresponding histological section: (a) 3D GRE with fat suppression, (b) 3D GRE without fat suppression, (c) 3D SPGR with fat suppression, (d) histological section stained with toluidene blue.





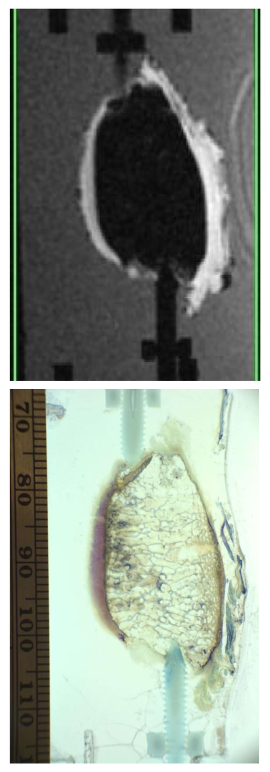


Figure 2: Patella in holding device for alignment of histology with MR imaging. Specimens were embedded in PMMA, sectioned and surface stained with toluidene blue.

HISTOLOGICAL SECTIONING

- Each patella and its holding device were embedded in polymethylmethacrylate and sectioned with a low speed diamond saw parallel to the imaging plane. With careful alignment, a low speed diamond saw was used to cut sections corresponding to every MR image, including approximately 25 sections for each patella. Sections were then mounted on translucent plastic and surfaces stained with toluidene blue. Color photographs were taken, and ImageJ software used to define the cartilage area, and length of the subchondral bone junction. Cartilage areas and subchondral lengths were multiplied by the nominal slice thickness of 1.5 mm to obtain measures of cartilage volume and subchondral bone surface area, respectively. Osteophytes were excluded from these measurements. To test for repeatability, a single investigator experienced in cartilage histomorphometry measured each histological section twice.

MR IMAGE ANALYSIS

- MR images were evaluated using a semi-automated segmentation algorithm to obtain cartilage volume, subchondral bone surface area and overall cartilage thickness maps for each patella. An experienced musculoskeletal radiologist inspected each segmentation map to exclude osteophytes, and confirm definitions of cartilage boundaries. Once voxels containing cartilage were identified, a 3D surface reconstruction algorithm was used to compute volumes and surface areas for the entire patella [2]. For individual slices, volumes were also calculated based on a voxel integration algorithm.

STATISTICAL ANALYSIS

- Statistical comparisons were made between histology and MR for overall volumes and surface areas for each patella, and for volumes from 177 individual slices from these patellae. Absolute and paired t-tests were used to identify significance of systematic bias. Pearson's correlations and standard errors of the estimate were also calculated.

Results

The selected knees exhibited wide ranges of naturally occurring osteoarthritis, with numerous osteochondral defects, diffuse thinning of cartilage, surface fibrillation, regions of denuded bone, subchondral bone cysts, and osteophytes as well as regions of intact cartilage (Fig.3). All patellae included at least one focal defect warranting an Outerbridge grade of either 3 or 4 as identified by MR imaging and confirmed after arthrotomy. Results of histology repeatability and statistical analysis are shown in Tables 1 and 2.

Table 1. Overall Patellar Cartilage Measurements	Histology Repeatability	MRI Analysis & Comparison
Volume mm^3 (n = 7)	1608 ± 426	1634 ± 444
Correlation	0.998	0.981
Standard Error of Estimate	16.5 mm^3, 1.03%	58.7 mm^3, 3.6%
Systematic difference	0.8%	1.6% (N.S.)
Surface Area mm^2 (n = 7)	991 ± 107	1008 ± 144
Correlation	0.972	0.938
Standard Error of Estimate	17.7 mm^2, 1.78%	30.7 mm^2, 3.1%
Systematic difference	0.3%	1.8% (N.S.)
Calculated Mean Thickness (n=7)	1.60 (0.34)	1.59 (0.27)
Correlation	0.988	0.909
Standard Error of Estimate	0.04 mm, 2.3%	0.09 mm, 5.6%
Systematic difference	0.7%	0.5% (N.S.)

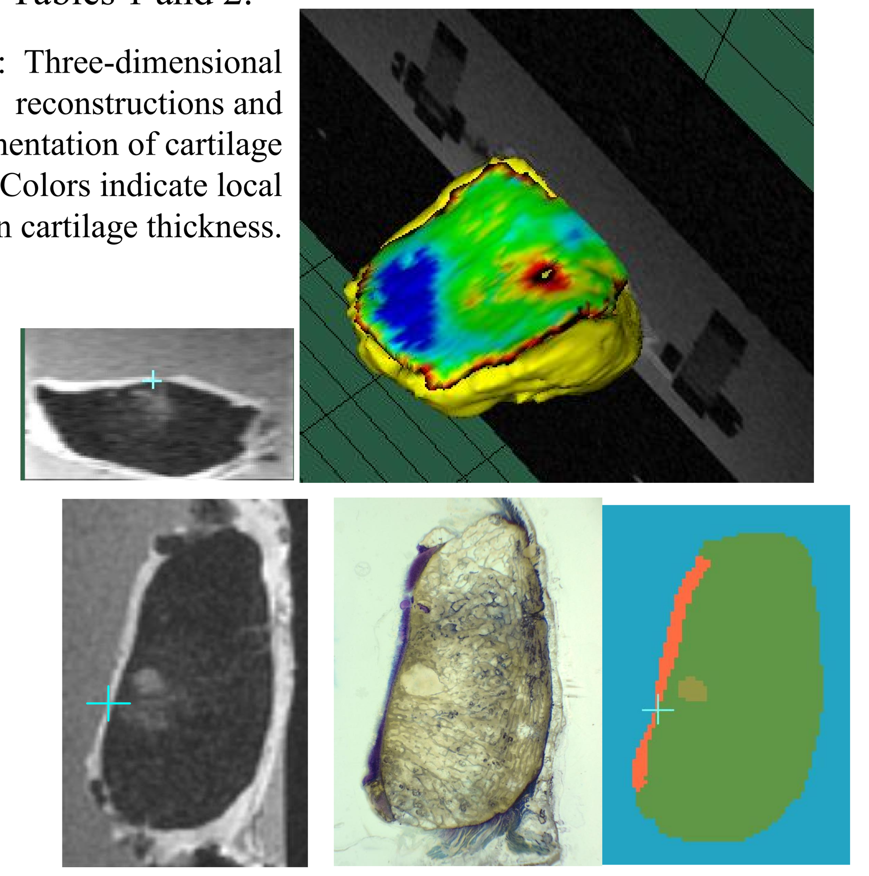


Figure 3: Three-dimensional reconstructions and segmentation of cartilage surface. Colors indicate local variations in cartilage thickness.

Overall Patellar Cartilage Biomarkers

- No significant differences between histological and MRI based measurements of volume, surface area and mean thickness
- Precision of MRI biomarkers 3-5%

Slice by Slice Cartilage Volumes

- High correlations between MRI and histological measures
- Systematic overestimation of cartilage volume due to voxel based calculations rather than surface reconstruction methods used in clinical biomarkers

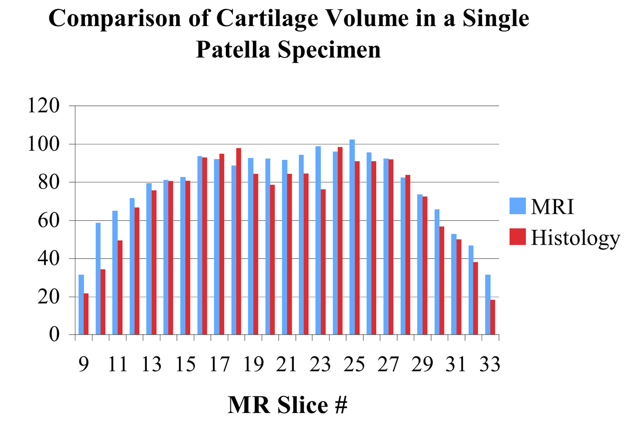


Figure 4: Comparison of MR to histologic measurements of cartilage volumes for 24 slices within a single patella specimen. Both methods clearly identify focal defect.

Table 2. Slice by Slice Cartilage Volume Measurements	Histology Repeatability	MRI Analysis & Comparison
Slice volume mm^3 (n = 177)	62.06 ± 29.7	68.4 ± 31.8
Correlation	0.984	0.954
Standard Error of Estimate	3.75 mm^3, 6.0%	6.61 mm^3, 10%
Systematic difference	0.3%	10% ($p < 0.05$)

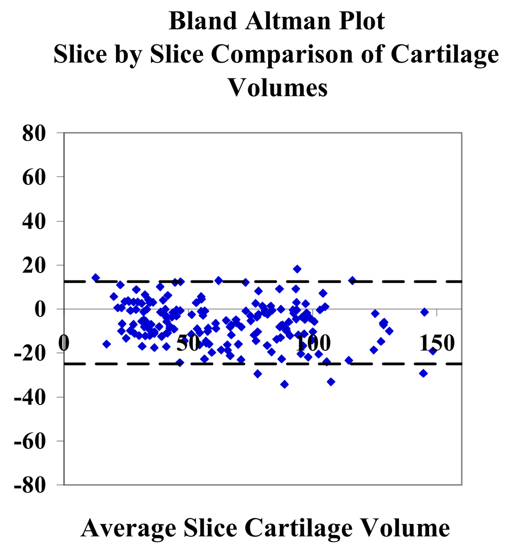
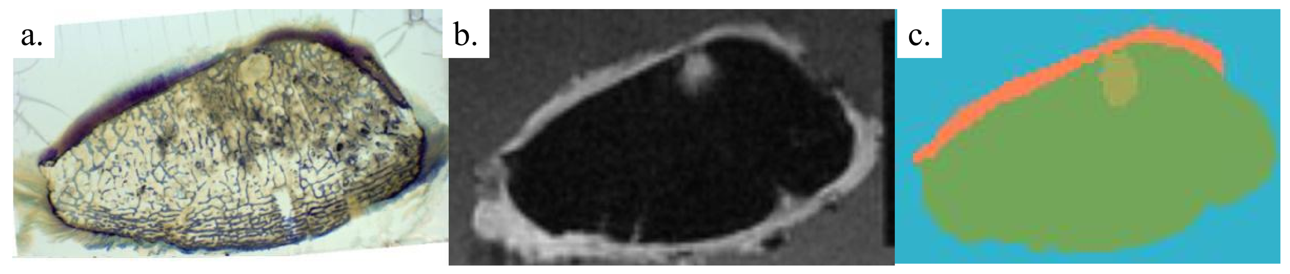


Figure 5: MRI measurements of cartilage volumes on each slice systematically overestimate histological measurements

Discussion

This study provided both quantitative and qualitative direct comparisons between MR images and histological analysis of naturally occurring osteoarthritis. Osteoarthritic features such as fibrillation, osteophytes, subchondral bone cysts and osteochondral defects, were detectable on both histology and in MR imaging. Toluidine blue staining clearly defined the subchondral bone junction, as well as variations in proteoglycan content. Excellent agreement was found for overall comparisons between the volumes, surface areas and mean thickness, with no significant systematic differences found in any pairwise comparisons of these overall measures which are typically used in clinical studies. Our study suggests accuracy comparable to the findings of Graichen et al., although the knees investigated in our study exhibited more severe OA which may be more challenging to segment (1). Consistent matches between MR and histological sections also allowed slice-by-slice comparisons of volumes and localized thickness measurements to provide more rigorous evaluation of the MR image analysis. Such measurements help to assess the potential for MR imaging to track focal defects. Individualized measures of slice volumes showed very high correlations, but MR measurements using the voxel integration algorithm slightly, but systematically, overestimated volume. Errors in histological repeatability were also increased for this more rigorous comparison, and both were affected by irregularities seen in these severely osteoarthritic specimens. Slice and thickness measure location correspondence errors also may have affected histology to MR agreement. In fact, the "gold standard" of histomorphometry includes many subjective decisions that present challenges and sources of error.



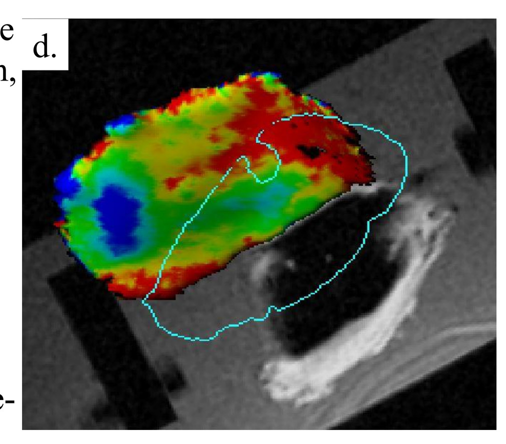


Figure 6: Typical comparison of a.) histological section to b.) MR image demonstrates the surface fibrillation, loss of protoeglycan, osteophytes and focal defects that make both histomorphometry and c.) segmentation challenging. Surface reconstruction and d.) rendering of the cartilage layer allows more accurate quantification and facilitates interpretation of the three-dimensional cartilage thickness.

It is important to note that both MR and histological measurements included any cartilage identified, regardless of the "quality" of this tissue. Therefore, measures of volume and thickness should be supplemented with other indications of cartilage quality such as evaluation of signal changes within the cartilage layer, T2 mapping, or evaluations of weightbearing MR imaging sequences to evaluate functional behavior of the cartilage.

Acknowledgements

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