Journal of Biomechanics xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Zonal variation of MRI-measurable parameters classifies cartilage degradation

Allison K.M. Fleck [a], Uwe Kruger [a], Kyrsten Carlson [a], Caitlin Waltz [a], Scott A. McCallum [b], X. Lucas Lu [d], Leo Q. Wan [a,b,c,*]

[a] Department of Biomedical Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, United States
[b] Center for Biotechnology & Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, United States
[c] Center for Modeling, Simulation and Imaging in Medicine, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, United States
[d] Department of Mechanical Engineering, University of Delaware, 130 Academy Street, Newark, DE 19716, United States

ARTICLE INFO

Article history:
Accepted 15 October 2017
Available online xxxx

Keywords:
Articular cartilage
Magnetic resonance imaging
Cartilage degradation
Osteoarthritis

ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease resulting in the deterioration of articular cartilage, a tissue with minimal ability to self-repair. Early diagnosis of OA with non-invasive imaging techniques such as magnetic resonance imaging (MRI) could provide an opportunity to intervene and slow or reverse this degeneration process. This study examines the classification of degradation states using MRI measurements.

Enzymatic degradation was used to specifically target proteoglycans alone, collagen alone and both cartilage components sequentially. The resulting degradation was evaluated using MRI imaging techniques (T_1, T_2, diffusion tensor imaging, and gadolinium enhanced T_1) and derived measures of water, glycosaminoglycan and collagen content. We compared the classification ability of full thickness averages of these parameters with zonal averages (superficial, medial, and deep). Finally, we determined minimum variables sets to identify the smallest number of variables that allowed for complete separation of all degradation groups and ranked them by impact on the separation.

Zonal analysis was much more sensitive than full thickness averages and allowed perfect separation of all four groups. Superficial zone cartilage was more sensitive to enzymatic degradation than the medial or deep zone, or the full thickness average. Variable ranking consistently identified collagen content and organization as the most impactful variables in the classification algorithm.

The aim of this study is to classify cartilage degradation using only non-invasive MRI parameters that could be applied to OA diagnosis. Our results highlight the importance of zonal variation in the diagnosis of cartilage degeneration. Our novel, non-invasive collagen content measurement was crucial for complete separation of degraded groups from control cartilage. These findings have significant implications for clinical cartilage MRI for disease diagnosis.

1. Introduction

Osteoarthritis (OA) is a degenerative disease affecting the synovial joints of an estimated 27 million people in the United States (Murphy and Helmick, 2012). Current treatments are unable to regenerate the damaged cartilage and early diagnosis of OA is critical for utilization of treatments slowing or reversing the damage (Aigner and McKenna, 2002; Eckstein et al., 2001). Early stages of OA are associated with damage or loss of both collagen and glycosaminoglycans (GAGs) in the tissue, as well as an increase in water content (Xia et al., 2014). Current imaging techniques used to diagnose OA include X-ray imaging and morphological MRI. X-ray images only hard tissues, and so, without contrast agents can only diagnose OA when significant damage, such as joint space narrowing and osteophyte growth, has already occurred. Morphological MRI is sensitive to soft tissues including articular cartilage and is used to image gross morphology including regions of hypo- or hyper-intensity or areas where cartilage has been eroded. Eroded cartilage occurs in mid- or late-stage OA, and no imaging techniques are currently used clinically to

* Corresponding author at: Director of the Laboratory for Tissue Engineering and Morphogenesis, Rensselaer Polytechnic Institute, Biotech 2147, 110 8th Street, Troy, NY 12180, United States.
E-mail address: wanq@rpi.edu (L.Q. Wan).
URL: http://www.rpi.edu/~wanq (L.Q. Wan).

https://doi.org/10.1016/j.jbiomech.2017.10.011
0021-9290/

diagnose early OA. Quantitative MRI is a promising technique for detecting early changes in cartilage composition and mechanical properties (Fleck and Wan, 2015).

Zonal variation in cartilage structure is integral to its mechanical function. The concentration of collagen, GAG, and water vary with cartilage depth, and the orientation of cartilage changes dramatically from surface to the osteochondral interface (Mow et al., 2005). OA damage is also zonal in nature, originating at the surface of the cartilage and moving progressively deeper into the tissue as the disease progresses (Aigner and McKenna, 2002; Guilak et al., 1994; Raya et al., 2013). While current analyses depend on full thickness averages of MR parameters to assess cartilage health, differentiating by zone may increase sensitivity to early stages of OA damage.

Quantitative MRI has been used in many cases to classify degraded cartilage using a variety of statistical methods. Enzymatic degradation using chondroitinase, trypsin, collagenase, and combinations of chemicals have been used to model degradation from OA (Lukas et al., 2015; Nieminen et al., 2000; Wang and Xia, 2012; Xia et al., 1995). Unlike previously studied classification methods, which include cluster analysis (Lin et al., 2009), Euclidean distance classification analysis (Lukas et al., 2015), and multivariate support vector machine analysis (Irrechukwu et al., 2011; Reiter et al., 2012), we employed Fisher discriminant analysis (FDA). FDA operates on one-dimensional projections with linear combinations of selected input variables (Fisher, 1936). This approach has several advantages for quantitative MRI: taking into account all samples instead of focusing on those at the border of clusters in hyperdimensional space, allowing for the analysis of input variables that may be inherently correlated (such as water content, T1, and diffusivity of articular cartilage in MRI), permitting easy visualization of group separability, and finally providing statistics or scores for clinical translation in the future.

The aim of this study is to classify cartilage degradation using only non-invasive MRI parameters that could be applied to the diagnosis of early OA. This discriminant analysis relies on routine MRI measurements and MR-derived chemical contents (water, GAG and a novel collagen content measurement). We hypothesize that zonal variation in MR parameters is more sensitive to degradation and a more powerful classifier of degradation groups than full thickness averages, and that MRI determination of tissue biochemical contents is more important to classification than other classic MRI variables.

2. Materials and methods

2.1. Sample preparation

Bovine stifle joints were obtained from a local abattoir (NY Custom Processing), and the patella was removed for further processing. Cartilage-on-bone samples of approximately 1 cm × 2 cm × 1 cm were cut with a band saw (control group had n = 6). Surface degradation models were created by incubating samples at 37 °C in serum-free DMEM (Thermo Fisher Scientific) supplemented with 1% penicillin-streptomycin (Sigma-Aldrich) with 0.2 units/mL chondroitinase ABC (cABC, Sigma-Aldrich) for 22 h (n = 4), 30 units/mL collagenase type II (Worthington Biochemical) for 14 h (n = 4), or sequential incubation first in cABC then collagenase (Pratta et al., 2003) (n = 5). Samples were then washed in saline and transferred to sample tubes for MRI.

2.2. MR imaging

MRI was performed with a Bruker AVIII 13 cm 7T Horizontal Bore Scanner using a 23 mm quadrature TR volume coil. Samples were immersed in 0.15 M saline before T1 (T1 RARE; TE = 12.5 ms; TR = 100, 300, 500, 700, 900, 1500, 3000 ms; 1 repetition, RARE factor 1) and T2 (MSME; TR = 15 s; TE = 10.5, 21, 35.5, 42, 52.5, 63 ms; 1 repetition) imaging. Samples were then incubated overnight at 4 °C in 1 mM gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA, Sigma-Aldrich) in 0.15 M saline, then brought to room temperature before another T1 scan and diffusion tensor imaging (DTIStandard; TE = 12.5 ms, TR = 700 ms; b = 100, 300, 550; 6 directions, 1 repetition). Resolution was 0.126 × 0.126 mm with a slice thickness of 2 mm.

2.3. MRI analysis

Images were analyzed using a combination of Paravision 5.1 (Bruker) and custom-written MATLAB (MathWorks) programs. T1 (before and after Gd-DTPA incubation (T1 and T1(Gd))), T2, apparent diffusion coefficient (ADC), and fractional anisotropy (FA) were calculated. Water fraction (ϕ^w) was calculated from the T2 fitting equation:

$$S(TE) = S_0 e^{-TE/T2} + C$$

where TE is the echo time, C is an offset term (Milford et al., 2015), and S_0 is the signal intensity at time zero, which is a measurement of the total proton density. ϕ^w is found by taking the S_0 term in the T2 fitting equation and dividing it by the average S_0 of a region of interest (ROI) in the saline bath solution (Xia et al., 1994). GAG content ([GAG]) was calculated using ROI analysis as described previously, using R = 4.4 (Bashir et al., 1996). Briefly, the concentration of fixed charges inside the cartilage can be calculated from the concentration of the negatively charged Gd-DTPA using the Donnan equilibrium model. The concentration of Gd-DTPA was found using:

$$[GdDTPA^{2-}] = \frac{1}{R}\left(\frac{1}{T1(Gd)} - \frac{1}{T1}\right)$$

where R is the relaxivity of the Gd-DTPA in $(\mathrm{mM/sec})^{-1}$. Fixed charge density (FCD) of the tissue was then calculated with:

$$FCD = \frac{[Na^+]_b\sqrt{[GdDTPA^{2-}]_t}}{\sqrt{[GdDTPA^{2-}]_b}} - \frac{[Na^+]_b\sqrt{[GdDTPA^{2-}]_b}}{\sqrt{[GdDTPA^{2-}]_t}}$$

where the subscript b indicates the concentration of the solute in the bath and the subscript t indicates the concentration in the tissue.

Collagen content was calculated by assuming that the solid matrix of bovine articular cartilage (ρ_{solid}) has a density of 1.323 g/mL (Gu et al., 1996). The mass fraction (in mg/mL) of the solid fraction is found by subtracting the water fraction (ϕ^w) from the tissue volume and multiplying by solid fraction density. Collagen content (in mg/mL) is then found by subtracting GAG content (in mg/mL) from the total solid matrix. The equation is:

$$[Collagen] = (1 - \phi^w) * \rho_{solid} - [GAG]$$

The density of the solid matrix does not change significantly with enzymatic degradation and so we assume that any zonal differences are also not significant (Gu et al., 1997).

Zonal variation in parameters was calculated using a custom MATLAB script. An ROI from the center of the sample containing the full cartilage thickness was specified. The uppermost 20% was designated as superficial zone, the next 30% as medial zone, and the lower 50% as deep zone (Mow et al., 2005). ROI averages were taken by averaging all the voxels within the zones.

2.4. Statistical analysis

Pairwise comparisons were done using Minitab 17. Data is reported as mean ± standard deviation. Assumptions of normality

Please cite this article in press as: Fleck, A.K.M., et al. Zonal variation of MRI-measurable parameters classifies cartilage degradation. J. Biomech. (2017), https://doi.org/10.1016/j.jbiomech.2017.10.011