Osteoarthritis and Cartilage 22 (2014) 275–283

Osteoarthritis and Cartilage

Deleterious effects of osteoarthritis on the structure and function of the meniscal enthesis

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ARTICLE INFO

Article history:
Received 20 June 2013
Accepted 26 November 2013

Keywords:
Graded tissue interface
Mechanical properties
Histomorphometry
Tidemark
Root Insertions

SUMMARY

Objective: The ability of menisci to prevent osteoarthritis (OA) is dependent on the integrity of the complex meniscal entheses, the attachments of the menisci to the underlying subchondral bone (SB). The goal of this study was to determine mechanical and structural changes in meniscal entheses after the onset of OA.

Design: Healthy and osteoarthritic meniscal entheses were evaluated for changes in histomorphological characteristics, mineralization, and mechanical properties. Glycosaminoglycans (GAG) and calcium in the insertion were evaluated with histological staining techniques. The extent of calcium deposition was assessed and tidemark (TM) integrity was quantified. Changes in the mineralized zone of the insertion were examined using micro-computed tomography (μCT) to determine bone mineral density, cortical zone thickness, and mineralization gradient. Mechanical properties of the entheses were measured using nano-indentation techniques to obtain material properties based on viscoelastic analysis.

Results: GAG thickness in the calcified fibrocartilage (CFC) zone and calcium content were significantly greater in osteoarthritic anterior meniscal entheses. TM integrity was significantly decreased in OA tissue, particularly in the medial anterior (MA) enthesis. The mineralized zone of osteoarthritic meniscal entheses was significantly thicker than in healthy entheses and showed decreased bone mineral density. Fitting of mineralization data to a sigmoidal Gompertz function revealed a lower rate of increase in mineralization in osteoarthritic tissue. Analysis of viscoelastic mechanical properties revealed increased compliance in osteoarthritic tissue.

Conclusions: These data suggest that significant changes occur at meniscal enthesis sites with the onset of OA. Mechanical and structural changes in meniscal entheses may contribute to meniscal extrusion, which has been shown to increase the progression of OA.

Introduction

The menisci of the knee joint aid in stability, lubrication, and load distribution, and are comprised of inhomogeneous collagen fiber layers[1]. When the knee is loaded the menisci experience high levels of compressive and shear forces at the articularing surface of the joint. The hierarchical morphology of collagen fibers in the meniscus mainbody allows for the transduction of applied compressive and shear forces into tensile hoop forces. Meniscal attachments, or entheses are graded tissue interfaces that anchor the mainbody of the meniscus to the underlying subchondral bone (SB). Each of the four meniscal enthesis in the knee joint function to diffuse tensile loads which are transmitted, *via* collagen fibrils, from the mainbody of the meniscus[1–7]. In order to effectively attenuate joint loads each enthesis must remain firmly rooted to the tibial plateau[8–10]. Identified enthesopathies at other tissue interfaces have revealed a variety of structural degenerations that may jeopardize enthesis functionality[7,11,12]. Clinically, if a meniscal enthesis is torn or avulsed, excessive transverse meniscal extrusion results[9]. Meniscal extrusion has been shown to be a precursor of secondary osteoarthritis (OA)[13,14]. Individuals with primary OA have also presented with meniscal extrusion, indicating a progressive degeneration of the meniscal enthesis[14–16]. To date, there have been no investigations on the integrity of meniscal entheses in the arthritic knee.

Similar to other fibrocartilaginous entheses, the meniscal entheses are compositionally graded to withstand a myriad of interfacial loading mechanisms. Primarily type I collagen fibrils, extending from the mainbody of the meniscus, form a ligamentous

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http://dx.doi.org/10.1016/j.joca.2013.11.013

(LI) zone which sustains longitudinal tensile forces manifested by compression on the meniscus[5,17]. These fibers then join with type II collagen fibers forming interwoven uncalcified and calcified fibrocartilage zones (UFC and CFC, respectively), separated by a tidemark (TM)[2,6,18]. These proteoglycan rich zones withstand compression and shear generated by dynamic changes in fiber angle and avulsion stress shielding[7]. Lastly, the CFC zone joins the SB at an interdigitated cement line[2,6,18]. These four zones can vary in size at each meniscal enthesis site, presumably structurally adapting to their unique functional environment[2,6,18]. Coupling mechanical and magnetic resonance imaging studies typifies this as the posterior sites, known to translate more during flexion, are significantly more compliant than the anterior sites[19–21].

Examination of joint and enthesis degeneration identifies various stimuli that may influence insertion mechanics. Regulation of inflammatory and anabolic cytokines can have detrimental effects on ECM integrity. In the OA joint increased production of aggrecanase, resulting in proteoglycan cleavage, and matrix metalloproteinase-13, causing irreversible degeneration of type II collagen, erodes the structural and mechanical efficacy of articular cartilage. The osteochondral interface also exhibits demonstrable changes in mineralization state and integrity, dependent upon disease progression[22,23]. Similarly, ligament and tendon pathophysiology at various insertion sites in the body exhibit ECM disruption, TM breakdown, micro-fissures, and osteophyte formation which impact structural organization and functionality[7]. Assimilating these findings gives rise to the supposition that the meniscus-to-bone interface is a potential disease-forming pathway, possibly preceding or catalyzing other harbingers of degradation.

In this study we examined meniscal entheses from normal and osteoarthritic knees for changes in histomorphometry, mineralization, and mechanical properties. Our hypothesis was that osteoarthritic entheses would exhibit deleterious effects similar to those observed at degenerative tissue interfaces in other articulating joints. These pathologies include breakdown of the TM; clefts and micro-fissures; osteophyte formation; calcium deposition and increased GAG content in the soft-tissue; and changes in mineralization. These changes would then result in degeneration of the viscoelastic properties of the entheses, thereby contributing to meniscal extrusion.

Methods

Sample preparation

Tissue samples were collected over 1 year based on several exclusion criteria. End-stage osteoarthritic tissue was obtained from patients undergoing total knee arthroplasty who signed an institutional review board approved waiver. Samples were included only if all four meniscal entheses could be identified by gross inspection. This resulted in seven total samples ($n = 7$). Healthy tissue was obtained from the Mayo Clinic tissue donor program. Selection was based on fluoroscopic evaluation and visual inspection by an orthopedic surgeon for no apparent signs of degeneration, and a donor age of <65 years. This resulted in eight total samples ($n = 8$, ages 41–61, average: 55). All samples were obtained with institutional review board approval.

All meniscal entheses (medial anterior (MA), lateral anterior (LA), medial posterior (MP), and lateral posterior (LP)) were excised and bisected along the main fiber axis. Adaxial (AD) sections, used for histomorphometry, were fixed in 10% neutral buffered formalin, serial dehydrated in ethanol, defatted in xylene and then embedded in methyl methacrylate (Technovit 9100 New, Heraeus Kulzer GmbH, Wehrheim, Germany). Abaxial (AB) sections, used for micro-computed tomography (μCT) and indentation, were embedded in a high viscosity acrylic resin (ClaroCit, Struers Inc., Cleveland, OH) that did not penetrate the specimen, wrapped in phosphate buffered saline soaked gauze, and stored at −20°C.

Histomorphometry

Histological sections (30 μm thick) were made using standard cutting and grinding techniques. Samples were stained with toluidine blue (TB) to identify GAGs and then counterstained using the Von Kossa (VK) technique for calcium. Changes in GAG presence in the insertion zones were quantified using Bioquant software (Bioquant Osteo 12.5, Bioquant Image Analysis Corporation, Nashville, TN) to identify the thickness of the TB stained and the TB + VK stained regions. Each region was outlined and measurements were performed at 20 μm intervals. TM integrity was assessed quantitatively. First the TM was isolated using image processing software (Image J), upper and lower color thresholds were determined using intensity histograms and visual inspection of a subset of images. Threshold values were then kept fixed and all images were converted to a binary image, and pixel locations were stored. A custom written Matlab script (version 7.14 (R2012a), Natick MA) then determined the variance of the first derivative and the mean amplitude of the peaks/valleys, estimating TM smoothness. Calcium deposition was scored using a modified grading scale as described by Sun *et al.* 2010[24]. Briefly, deposits were scored on a scale of 0–4 with 0 being no calcium and 4 being large deposits within and around the fibrous insertion.

μCT

The bisected insertions were scanned in saline *via* μCT (Scanco μCT 80, Scanco Medical AG, Brüttisellen, Switzerland) using the following parameters: voltage: 55 kVp, current: 114 μA, integration time: 500 ms. A resolution of 18 μm was obtained, yielding approximately 250 axial slices per specimen. The area of insertion was visually determined using the original specimen and multiple scanned views as reference. Tissue mineral density measurements (mgHA/cm^3) across the TM were obtained using Image Processing Language (Scanco Medical AG, Brüttisellen, Switzerland). For each specimen density measurements were taken at the center of the insertion and 54 μm above and below the center line (Fig. 1). Three additional measurements were taken 54 μm deeper into the initial reference plane, analogous to the tissue depth penetrated by the indenter tip (Section 2.4). A custom Matlab script was used to identify the first peak value of density measurements, which was considered to be indicative of mineralization at the TM, and the

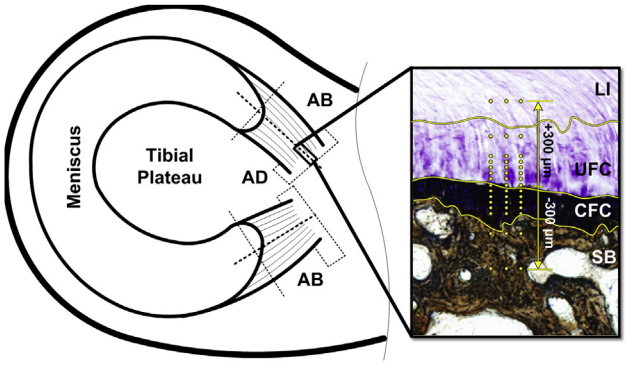

Fig. 1. Schematic of sample location. AD sections used for histomorphometry. AB sections used for indentation and μCT. Inset – Meniscal enthesis stained with TB/VK to highlight the four unique regions: LI, UFC, CFC, and SB. Yellow lines highlight the demarcations between zones. Yellow dots represent location of indentation test points.