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Osteoarthritic changes in the biphasic mechanical properties of the chondrocyte pericellular matrix in articular cartilage

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Abstract

The pericellular matrix (PCM) is a narrow region of cartilaginous tissue that surrounds chondrocytes in articular cartilage. Previous modeling studies indicate that the mechanical properties of the PCM relative to those of the extracellular matrix (ECM) can significantly affect the stress–strain, fluid flow, and physicochemical environments of the chondrocyte, suggesting that the PCM plays a biomechanical role in articular cartilage. The goals of this study were to measure the mechanical properties of the PCM using micropipette aspiration coupled with a linear biphasic finite element model, and to determine the alterations in the mechanical properties of the PCM with osteoarthritis (OA). Using a recently developed isolation technique, chondrons (the chondrocyte and its PCM) were mechanically extracted from non-degenerate and osteoarthritic human cartilage. The transient mechanical behavior of the PCM was well-described by a biphasic model, suggesting that the viscoelastic response of the PCM is attributable to flow-dependent effects, similar to that of the ECM. With OA, the mean Young's modulus of the PCM was significantly decreased (38.7 ± 16.2 kPa vs. 23.5 ± 12.9 kPa, $p < 0.001$), and the permeability was significantly elevated ($4.19 \pm 3.78 \times 10^{-17}$ m^4/N s vs. $10.2 \pm 9.38 \times 10^{-17}$ m^4/N s, $p < 0.001$). The Poisson's ratio was similar for both non-degenerate and OA PCM (0.044 ± 0.063 vs. 0.030 ± 0.068, $p > 0.6$). These findings suggest that the PCM may undergo degenerative processes with OA, similar to those occurring in the ECM. In combination with previous theoretical models of cell–matrix interactions in cartilage, our findings suggest that changes in the properties of the PCM with OA may have an important influence on the biomechanical environment of the chondrocyte.

Keywords: Chondron; Cell mechanics; Micropipette; Pericellular matrix; Osteoarthritis; Articular cartilage; Modulus; Permeability; Biphasic; Finite element model

1. Introduction

Articular cartilage lines the surfaces of diarthrodial joints and serves as the resilient, low-friction, load-bearing material for joint motion. A sparse single population of cells, termed chondrocytes, is responsible for maintaining the extracellular matrix (ECM) of this tissue through a balance of anabolic and catabolic activities. During normal joint activity, chondrocytes are exposed to a complex mechanical environment characterized by time-varying stresses and strains, hydrostatic pressure, interstitial fluid flow, and physicochemical phenomena, such as streaming and diffusion potentials and osmotic pressure changes (Frank and Grodzinsky, 1987; Lai et al., 1991; Maroudas et al., 1991; Mow and Guo, 2002; Mow et al., 1999). The mechanical environment of chondrocytes, in conjunction with biochemical factors (e.g., growth factors, cytokines), plays an important role in cartilage homeostasis and, as a consequence, the health of the joint (Guilak et al., 1994, 1997; Setton et al., 1994; Stockwell, 1987; van Campen and van de Stadt, 1987).

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Chondrocytes in articular cartilage are enclosed in a narrow region of tissue, the pericellular matrix (PCM), which together with the chondrocyte has been termed the "chondron". This region was first described by Benninghoff (1925) as a "fluid filled bladder", but was not investigated until Smirzai observed the presence of intact chondrons as a byproduct of homogenization of cartilage (Smirzai, 1974). The PCM is primarily characterized by the presence of type VI collagen but also possesses a high concentration of proteoglycans, including aggrecan, hyaluronan, and decorin, as well as fibronectin, and types II and IX collagen (Poole, 1997). More recently, chondrons have been isolated either mechanically or enzymatically and their morphology, composition, and metabolic activity have been the focus of many studies (Alexopoulos et al., 2003; Knight et al., 2001; Lee et al., 1997; Poole, 1992; Poole et al., 1987, 1988a, b). However, the precise biomechanical function of the PCM is not fully understood. It has been postulated that the PCM may provide a protective effect for the chondrocytes during loading through an "adaptive water loss from PCM proteoglycans" (Poole et al., 1988a, b). Other studies have suggested that the chondron serves as a mechanical transducer (Greco et al., 1992; Knight et al., 1998; Poole, 1992; Smirzai, 1974), potentially through an interaction of type VI collagen with cell surface integrins or hyaluronan (Knudson and Loeser, 2002; Lee et al., 2000c; Loeser et al., 2000). These hypotheses are supported by a computational study of cell–matrix interactions in cartilage that suggests that the ratio of mechanical properties of the PCM and the ECM may significantly influence the mechanical environment of the chondrocyte (Guilak and Mow, 2000). In that study, however, the biphasic properties of the chondron were not known and were examined parametrically over a range of values.

Several studies have used either theoretical or experimental models to characterize the micromechanical environment of the chondrocytes to better understand the sequence of events through which these signals are converted to an intracellular response (Baer et al., 2003; Baer and Setton, 2000; Guilak, 1995; Guilak et al., 1995; Haider, 2004; Lee et al., 2000a; Mobasheri et al., 2002; Mow et al., 1994, 1999; Wang et al., 2003; Wu et al., 1999). The predictions of these models rely on physiologically relevant measures of the material properties of the ECM, PCM, and chondrocyte. The mechanical properties of the cartilage ECM have been well-characterized in tension, compression, and shear with substantial evidence of both flow-dependent and flow-independent viscoelastic behaviors (Ateshian et al., 1997; Athanasiou et al., 1991; Chen et al., 2001; Hori and Mockros, 1976; Mow et al., 1980; Schinagl et al., 1997; Zhu et al., 1993). The elastic and viscoelastic properties of chondrocytes have also been determined using gel compression (Freeman et al., 1994; Knight et al., 2002), micropipette aspiration (Guilak, 2000; Haider, 2004; Jones et al., 1999; Trickey et al., 2000), and cytoindentation (Koay et al., 2003). More recently, we developed a new technique to isolate chondrons from their ECM and an analytical solution to an elastic model of micropipette aspiration to determine the Young's modulus of the PCM (Alexopoulos et al., 2003). This model accounted for the different mechanical properties of the cell and the PCM, and revealed the effect of several parameters on PCM aspiration experiments, including the thickness of the PCM, the micropipette radius, and the micropipette wall thickness. However, in these experiments we observed that the chondron exhibits significant transient behavior that an elastic model cannot predict.

The goal of this study was to determine the Young's modulus, hydraulic permeability, and Poisson's ratio of the PCM by applying a linear biphasic model to represent the flow-dependent transient behavior of the chondron during a micropipette aspiration experiment. Furthermore, we tested the hypothesis that these material properties of the PCM are altered with osteoarthritis (OA) in a manner that depends on their zone of origin from the cartilage ECM.

2. Materials and methods

Chondrons were mechanically isolated from full-thickness articular cartilage of human femoral heads obtained at the time of joint replacement surgery under a protocol approved by the Duke University Institutional Review Board ($n = 81$ chondrons from 13 donors, ages: 19–75 yr). Small slices, about 50% of the cartilage thickness, were removed from the femoral head, washed with Dulbecco's Phosphate Buffered Saline (Gibco, Grand Island, NY), and placed in a petri dish. Chondrons were extracted with a custom-built "micro-aspirator" that applies suction pressure to the cartilage surface with a modified syringe (Alexopoulos et al., 2003). To isolate chondrons from the surface or the middle/deep zone of the cartilage, the microaspirator was applied to the top or bottom side of the tissue slice, respectively. It is important to note that the "surface" chondrons isolated from OA cartilage may not have originated from the true superficial zone because this zone is often lost with degeneration.

Chondrons were classified as osteoarthritic ('OA') or non-osteoarthritic ('non-OA') based on a semi-quantitative histologic grading of the cartilage adjacent to the site from which chondrons were extracted (Carlson et al., 2002). The histological grading scale was based on assessment of the following parameters: surface fibrillation (0–10), toluidine blue staining (0–4), gross appearance of the femoral head (0–3), and chondrocyte