BIPHASIC PROPERTIES OF NORMAL AND OSTEOARTHRITIC HUMAN CHONDRONS

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INTRODUCTION

Chondrocytes in articular cartilage are surrounded by a pericellular matrix (PCM), which together with the chondrocyte have been termed the chondron [1]. This region is characterized by the presence of type VI collagen and increased proteoglycan concentration relative to the extracellular matrix (ECM). While the specific function of the PCM is not fully understood, it has been hypothesized to play important roles in regulating the biomechanical, biophysical, and biochemical signals that the chondrocyte perceives during normal joint activity [1,2,3]. Furthermore, modeling studies have suggested that the mechanical properties of the PCM, and their relation to the cell and ECM, may be important regulators of biophysical and biomechanical signals in the pericellular regions [2]. Recently, we used an analytical solution for the chondron as an elastic shell to analyze the equilibrium stress-strain behavior of chondrons in a micropipette aspiration experiment [3]. We found the mean Young's modulus of the PCM to be ~40% lower in osteoarthritic (OA) specimens as compared to non-OA controls [3]. The chondron exhibits significant viscoelastic behaviors in this experiment, which are not predicted by the elastic model.

The goal of this study was to model the flow-dependent viscoelastic behaviors of the chondron in the micropipette experiment, and to determine a hydraulic permeability for the PCM from matching the model to experimental data and to test the hypothesis that the permeability of the PCM is altered with OA. Using a newly developed isolation technique, chondrons were mechanically extracted from non-degenerate and OA human cartilage. The Young's modulus and permeability of the PCM were determined using a linear biphasic finite element model [4].

MATERIALS AND METHODS

Chondrons were mechanically isolated from full-thickness articular cartilage of human femoral heads obtained at the time of joint replacement surgery (n=73 chondrons from 13 donors, ages: 19-75 yr). Cartilage regions were classified as OA or non-OA based on a semi-quantitative histologic grading scale from 0 (normal) to 20 (OA). Chondrons were extracted with a custom-built "microaspirator"; briefly, suction pressure was applied to the cartilage surface with a modified syringe (Fig. 1A). Chondrons were classified as OA or non-OA based on a semi-quantitative histologic grading scale from 0 (normal) to 20 (OA) of the local site of chondron extraction. The specimens were classified as OA or non-OA based on a cut-off grade of 7, and the mean grades were 4.5±3.1 for non-OA and 15.8±2.1 for OA specimens. Two types of experiments were performed using micropipette aspiration [3]. To determine the elastic properties of the PCM, a series of step pressures (3 kPa) was applied to the chondron using a small glass pipette (~12 μm diam.), and the equilibrated aspiration length was recorded. To determine hydraulic permeability, a larger single step pressure (~18 kPa) was applied and the ensuing creep deformation was recorded for 4 min (Fig. 1B).

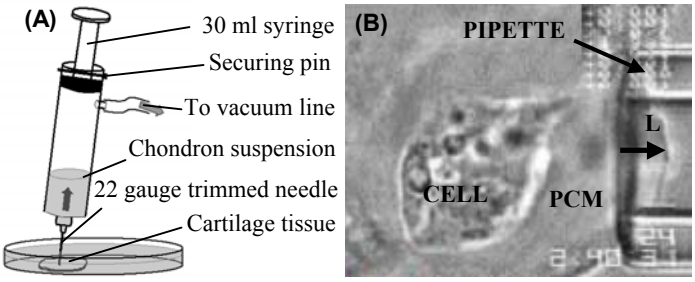

Figure 1A: The "microaspirator" used for chondron isolation: A constant suction pressure (~50 kPa) was applied to the surface of a cartilage slice to extract chondrons. Figure 1B: Micropipette aspiration of mechanically isolated chondron.

The Young's modulus and the hydraulic permeability of the chondrons were determined using an inverse method based on a custom-written axisymmetric, linear, biphasic finite element model. This finite element model uses the Galerkin weighted residual method applied to a *u-p* formulation of the biphasic theory and treats solid

displacement, u, and pressure, p, as continuous variables computed at element nodes [4]. In this formulation, the solid matrix is assumed to be an isotropic, linearly elastic material. The PCM was modeled as a biphasic half-space using bilinear quadrilateral elements (R=30μm, H=40μm). The mesh was refined in areas close to the pipette wall where large solid velocities and displacements were expected to occur. An aspiration pressure of 18 kPa was applied to nodes interior to the pipette wall in a single time-step, and all surfaces but the annular contact region were modeled as traction-free and free-draining. The solid axial displacement at the annular region was set to zero. The Poisson's ratio of the PCM was set equal to that of articular cartilage (ν=0.04) [5].

RESULTS

The FEM solution was convergent and stable for the tested configurations. The FEM predicted an initial jump in displacement following application of the step pressure, followed by a period of creep to equilibrium, consistent with behaviors observed experimentally. The Young's modulus of the PCM of chondrons from non-OA cartilage was $E_{non\text{-}OA}$= 34.7±14.5kPa. With OA, the Young's modulus of the PCM was reduced to E_{OA}=24.2±13.8kPa ($p<0.005$). The hydraulic permeability of the PCM of chondrons from non-OA cartilage was $k_{non\text{-}OA}=0.76\pm0.68\times10^{-16}$ $m^4/N\bullet s$. With OA, the permeability of the PCM was significantly increased to $k_{OA}=2.17\pm2.2\times10^{-16}$ $m^4/N\bullet s$ ($p<0.005$) (Fig. 2).

DISCUSSION

This study presents the first reported values for the hydraulic permeability of the PCM in articular cartilage, determined from a biphasic FEM model and micropipette aspiration of a mechanically-isolated chondron. The permeability of the PCM from non-OA chondrons was found to be nearly one order of magnitude less than that of non-degenerate ECM ($k_{ECM}\cong9x10^{-16}$ $m^4/N\bullet s$, [5]). In addition, the Young's modulus of the PCM from non-OA chondrons was found to be approximately one order of magnitude lower than that of the ECM ($E_{ECM}\cong1.3$ MPa, [5]). Differences in the biphasic properties of the PCM relative to ECM can have a dramatic influence on the local stress-strain and fluid-flow environments of the chondrocyte *in situ* [2]. Our previous FEM studies have shown the local strain in the vicinity of the chondrocyte to be amplified because of a mismatch of the Young's modulus between PCM and ECM (E_{PCM}/E_{ECM} <1). Because the Young's modulus of cartilage increases with depth [6], the ratio E_{PCM}/E_{ECM} becomes significantly smaller and the strain amplification is more pronounced. This phenomenon may represent a mechanism for amplifying mechanical signals under conditions where tissue-level strain magnitudes may be relatively small. Additional FEM studies will be required to reveal the effects of the significantly lower permeability of PCM on regulation of cellular signals in articular cartilage, as observed in this study.

A unique aspect of this study was the development of a new chondron isolation technique, based on mechanical extraction of chondrons by applying suction pressure via a custom-built device. This technique requires minimal tissue preparation, and can be used to extract chondrons from precise sites (i.e., zones) of articular cartilage. Compared with the homogenization technique [1], this new method is fast and easy and yields a large number of chondrons with a smaller amount of debris. Furthermore, the Young's modulus of the mechanically isolated chondrons studied here is nearly 25 times larger than that of enzymatically isolated chondrons [7], suggesting a significant effect of enzymatic isolation [8] on the properties of the PCM.

An important result of the current study was the finding of decreased stiffness and elevated permeability for the PCM in chondrons from OA cartilage. The mean Young's modulus of the PCM was ~30% lower in OA specimens, and the hydraulic permeability ~ 300% higher, as compared to the non-OA controls. These changes follow the same trends as those reported for the ECM regions of OA cartilage, and are believed to reflect a degradation of the collagen-proteoglycan matrix with disease. The consistency of trends for biphasic properties between PCM and ECM regions suggests that the mechanisms for degeneration may impact the local mechanical signals in the cellular environment, as well as the load-support and weight-bearing functions of articular cartilage.

In a previous study, we modeled the micropipette aspiration experiment with a linearly elastic, layered half-space model for the chondron and cell, and found the Young's moduli of non-OA and OA chondrons to be 67 kPa and 41 kPa, respectively [3]. These values are approximately twice the values calculated with the biphasic FEM [4]. This difference may be attributable to our modeling of the free-draining boundary conditions in the current model, which gives rise to constant fluid-flow during micropipette aspiration that cannot be predicted with a solid model. Furthermore, the current model does not account for the cell as a substrate in the chondron, which may additionally impact the resulting biphasic properties. Both models give rise to Young's moduli that are consistent with previous studies examining the deformation behavior of mechanically isolated chondrons embedded in an agarose matrix (E_{PCM}>25kPa) [9].

Increasing evidence suggests that the chondron is a distinct functional compartment in articular cartilage and serves to regulate the mechanical environment of the chondrocytes [3,9]. The mechanical properties of the PCM determined in this study will be important for examining the role of the PCM in regulating cellular signals, as well as changes associated with degeneration, injury and repair.

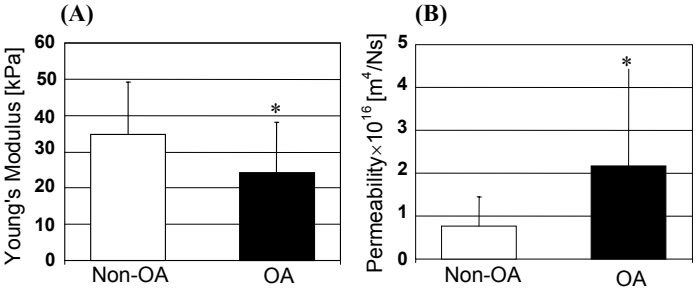

Figure 2A: The Young's modulus of the PCM is significantly decreased with OA. Figure 2B: The permeability of the PCM is significantly increased with OA (*p<0.005 vs. non-OA PCM).

ACKNOWLEDGMENTS

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