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An Axisymmetric Boundary Element Model for Determination of Articular Cartilage Pericellular Matrix Properties In situ via Inverse Analysis of Chondron Deformation

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Abstract

The pericellular matrix (PCM) is the narrow tissue region surrounding all chondrocytes in articular cartilage and, together, the chondrocyte(s) and surrounding PCM have been termed the chondron. Previous theoretical and experimental studies suggest that the structure and properties of the PCM significantly influence the biomechanical environment at the microscopic scale of the chondrocytes within cartilage. In the present study, an axisymmetric boundary element method (BEM) was developed for linear elastic domains with internal interfaces. The new BEM was employed in a multiscale continuum model to determine linear elastic properties of the PCM in situ, via inverse analysis of previously reported experimental data for the three-dimensional morphological changes of chondrons within a cartilage explant in equilibrium unconfined compression (Choi et al., *J Biomech*, 40:2596–603, 2007). The microscale geometry of the chondron (cell and PCM) within the cartilage extracellular matrix (ECM) was represented as a three-zone equilibrated biphasic region comprised of an ellipsoidal chondrocyte with encapsulating PCM that was embedded within a spherical ECM subjected to boundary conditions for unconfined compression at its outer boundary. Accuracy of the three-zone BEM model was evaluated and compared to analytical finite element solutions. The model was then integrated with a nonlinear optimization technique (Nelder-Mead) to determine PCM elastic properties within the cartilage explant by solving an inverse problem associated with the in situ experimental data for chondron deformation. Depending on the assumed material properties of the ECM and the choice of cost function in the optimization, estimates of the PCM Young's modulus ranged from ~24 to 59 kPa, consistent with previous measurements of PCM properties on extracted chondrons using micropipette aspiration. Taken together with previous experimental and theoretical studies of cell-matrix interactions in cartilage, these findings suggest an important role for the PCM in modulating the mechanical environment of the chondrocyte.

Keywords

osteoarthritis; collagen; proteoglycan; aggrecan; numerical model; inverse problem

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1. Introduction

Articular cartilage is a hydrated connective tissue which lines bone surfaces in diarthrodial joints (e.g. hips, shoulders, knees), and serves as a low-friction, load-bearing material that facilitates joint motion (Mow et al. 1992). Articular cartilage is comprised primarily of a fluid phase of interstitial water (~75–80% by volume) that saturates a solid extracellular matrix (ECM). The biomechanical properties of articular cartilage are attributed to the unique structure and composition of its ECM, which is comprised of a cross-linked network of type-II collagen fibers and proteoglycan macromolecules that have a net negative charge (Urban et al. 1979). Structural degradation of cartilage ECM is associated with degenerative joint diseases such as osteoarthritis (OA), a painful condition that affects over 20 million people in the United States. The physiology of articular cartilage is regulated by a population of specialized cells called chondrocytes. Chondrocytes are sparsely dispersed within the ECM, occupying roughly 1–10% of the tissue volume (Stockwell 1979), and are responsible for maintenance and repair of the ECM. Since cartilage is avascular and aneural, the metabolic activities of the chondrocytes are highly dependent on their local biophysical and biochemical environments. Under joint loading, chondrocytes are exposed to a complex and diverse combination of biophysical phenomena, arising from coupling between matrix deformation, fluid pressurization, diffusive fluid-solid drag and ionic effects within the charged and hydrated ECM. Thus, theoretical or computational models that quantitatively characterize the local environment of the chondrocyte can contribute to understanding relationships between local biophysical variables and ensuing alterations in the physiological response of the tissue.

Within the ECM chondrocytes are surrounded, individually or in small groups, by a distinct region of tissue called the pericellular matrix (PCM) (Hunziker et al. 1997; Poole et al. 1987). Together, the unit consisting of the chondrocyte(s) and the surrounding PCM has been termed a chondron (Benninghoff 1925; Poole et al. 1992), and the PCM is known to have a distinct structure and composition as compared to cartilage ECM. Specifically, in normal cartilage, the presence of type VI collagen in cartilage is exclusive to the PCM (Poole et al. 1988) and deletion of the *col6a1* gene, which prevents formation of type VI collagen, results in a significant loss of PCM stiffness (Alexopoulos et al. 2009). In the presence of OA, chondron size is enlarged and is associated with an increase in cell proliferation (Lee et al. 2000).

While the role of this tissue region is not fully understood, there has been considerable speculation that the PCM plays a critical biomechanical role in regulating cell-matrix interactions in articular cartilage (Poole et al. 1987; Szirmai 1974). With its high stiffness relative to the chondrocyte, the PCM is believed to serve as a protective layer while, simultaneously, facilitating transmission of mechanical signals induced by macroscopic external loading to the microscopic scale of the chondrocytes (Guilak et al. 2006). Previous biomechanical modeling studies that represent the ECM and PCM as linear biphasic materials have provided further support for this hypothesized dual role of the PCM (Alexopoulos et al. 2005a; Guilak and Mow 2000; Haider 2004; Kim et al. 2008). For example, multiscale computational finite element models of biphasic cell-matrix interactions suggest that the inclusion of a PCM region strongly influenced the transient micromechanical environment of the chondrocyte (Guilak and Mow 2000). Subsequent multiscale biphasic modeling studies of cell-matrix interactions further demonstrated that the inclusion of a PCM region can lead to concurrent effects of strain amplification and stress shielding in configurations of static unconfined compression (Alexopoulos et al. 2005a) and dynamic confined compression (Kim et al. 2008), depending on parameters such as the zone within the cartilage and the assumed mechanical properties of the ECM, the PCM and the chondrocyte.

The mechanical properties of cartilage ECM are well established, based on in vitro mechanical tests performed on cartilage explants (Ateshian et al. 1997; Athanasiou et al. 1991; Mow et al. 1980), with a compressive Young's modulus typically on the order of 1MPa. Furthermore, a number of previous studies have reported the mechanical properties of enzymatically isolated chondrocytes using micromechanical testing methods such as micropipette aspiration (Trickey et al. 2000) or microindentation (Darling et al. 2008; Darling et al. 2006; Koay et al. 2003; Shieh and Athanasiou 2006), with measurements of the Young's modulus roughly on the order of 1kPa.

Micropipette aspiration, in combination with a theoretical layered elastic halfspace solution (Alexopoulos et al. 2003; Guilak et al. 2005) or a layered biphasic finite element model (Alexopoulos et al. 2005b), has also been used to determine mechanical properties of the PCM in mechanically isolated chondrons. These studies have shown that the PCM Young's modulus is several orders of magnitude larger than that of the chondrocyte, but an order of magnitude lower than that of the ECM in the middle and deep zones (Alexopoulos et al. 2003; Alexopoulos et al. 2005a; Guilak et al. 2005), with mean values ranging from 25–70 kPa. Furthermore, the elastic and biphasic mechanical properties of the PCM exhibit significant alterations in the presence of osteoarthritis (Alexopoulos et al. 2003; Alexopoulos et al. 2005b). In other studies, inverse finite element approaches have been used to estimate PCM properties based on the deformation of isolated chondrons compressed in agarose gel (Michalek and Iatridis 2007). While these studies provide quantitative measurements of PCM mechanical properties, one potential limitation of these measurement techniques is that they require extraction of the chondron from the native ECM. Thus, the extent to which the chondron isolation procedure may alter PCM mechanical properties, potentially due to damage or swelling during extraction, is not yet known. For example, previous studies have shown that enzymatic extraction of chondrons significantly alters the mechanical properties of the PCM as compared to mechanical extraction methods (Guilak et al. 1999; Hing et al. 2002).

In situ studies of deformation in cartilage explants can facilitate the determination of chondron mechanical properties within native cartilage ECM. Previously, either confocal microscopy (Guilak et al. 1995) or histologic fixation and physical sectioning (Buschmann et al. 1995; Quinn et al. 1998) were employed to study the three-dimensional (3D) deformation behavior of individual chondrocytes within the native ECM or in artificial agarose constructs (Freeman et al. 1994; Knight et al. 2001). These studies demonstrated that cell shape and volume are highly dependent on local ECM deformation. Recently, in situ changes in three-dimensional morphology of the chondron under equilibrium unconfined compression were measured within the ECM of cylindrical porcine cartilage explants subjected to 10–50% compressive strain (Choi et al. 2007). This study employed a novel 3D fluorescence imaging technique based on confocal microscopy and immunolabeling of type VI collagen, which is exclusive to the PCM, to obtain 3D ellipsoidal approximations of undeformed and deformed chondron shape and chondrocyte volume in three zones of the ECM (superficial, middle, deep).

The primary objective of the current study was to develop an efficient computational model for estimation of cartilage PCM linear elastic properties based on previously reported experimental data for in situ chondron deformation (Choi et al. 2007) (Fig. 1). The boundary element method (BEM), which employs analytical fundamental solutions of the governing partial differential equations, is an alternative to the finite element method in the case of linear models and reduces the geometric complexity by one spatial dimension. BEMs are particularly efficient when quantities of interest reside, exclusively, on the domain boundary or internal interfaces. A new axisymmetric and linear elastic BEM was developed by extending a previous elastic BEM formulation for boundary value problems (Haider and

Guilak 2002) to the case of a three-zone (cell-PCM-ECM) domain, i.e. with internal interfaces. The ability of the BEM to represent axisymmetric ellipsoidal chondron geometry using elements along a set of one dimensional (parametric) curves leads to a highly efficient method that was directly integrated into an optimization algorithm for parameter estimation of PCM elastic properties. The resulting BEM code, written in C, was employed in a multiscale model of equilibrated chondron deformation under 10% compressive strain in unconfined compression to analyze the process of estimating in situ PCM elastic material properties via least squares optimization in the context of previously reported experimental data (Choi et al. 2007).

2. Computational model and methods

2.1 Multiscale continuum model

Within articular cartilage, chondrocytes are sparsely dispersed throughout the ECM and the cellular volume fraction is relatively low (~1–10%) (Stockwell 1979). A common simplification in modeling mechanical cell-matrix interactions is to employ a two-scale continuum approach. Specifically, chondrons are modeled as soft inclusions within a stiff ECM. First, a macroscopic problem that neglects presence of the chondrons is solved in a homogeneous region that represents the ECM. Subsequently, a microscopic problem is formulated to model deformation at the microscopic scale in the local environment a single chondrocyte, accounting for inhomogeneities due to the presence of the chondron. To date, several studies have used this multiscale continuum modeling approach to analyze biphasic cell-matrix interactions in articular cartilage (Alexopoulos et al. 2005a; Benedict 2008; Guilak 2000; Haider and Guilak 2007; Kim et al. 2008; Korhonen and Herzog 2008; Wu and Herzog 2006).

In modeling cartilage biomechanics using the biphasic theory, equilibrium is associated with a state in which motion of interstitial fluid ceases and the governing equations reduce to those of elastostatics. In the linear and isotropic case, the governing equations reduce to:

$$\nabla \cdot \sigma = \mathbf{f}, \quad \sigma = \lambda \text{tr}(\mathbf{e})\mathbf{I} + 2\mu\mathbf{e}, \quad 2\mathbf{e} = \nabla\mathbf{u} + (\nabla\mathbf{u})^T, \quad (1a-c)$$

where $\mathbf{u}$ is the displacement, $\mathbf{e}$ is the infinitesimal strain tensor, σ is the stress tensor, $\mathbf{I}$ is the identity tensor, $\mathbf{f}$ is the body force and λ, μ are solid phase Lamé coefficients associated with the state of “drained” biphasic equilibrium. In the case of a cylindrical domain ($0 \leq r \leq a, 0 \leq z \leq h$) subjected to axisymmetric unconfined compressive loading with engineering strain ε applied along $z = h$, the analytical solution of Eq. 1 (with $\mathbf{f} = \mathbf{0}$) along the domain boundary is (Armstrong et al. 1984):

$$u_r = \nu \varepsilon r, \quad u_z = -\varepsilon z, \quad t_r = 0, \quad t_z = -\varepsilon E n_z. \quad (2a-d)$$

In Eq. 2, E, ν are the solid phase Young's modulus and Poisson's ratio, respectively, u_r, u_z are the radial and axial displacements and t_r, t_z are the radial and axial tractions, respectively, where n_r, n_z are components of the unit outward normal along the domain boundary. The analytical solution in Eq. 2 is based on assumptions that the surfaces $z = 0, h$ are in frictionless contact with rigid and porous platens, and that the surface $r = a$ is traction-free. Eq. 2 was employed in a two-scale axisymmetric continuum model of equilibrium chondron deformation, in situ, within cartilage ECM (Fig. 2). This theoretical solution was used to formulate far-field boundary conditions in a three-zone (cell-PCM-ECM) linear elastic model of microscopic deformation in the local environment of a single cell located along the central axis of the cylindrical explant (Fig. 2b). The cell-PCM and PCM-ECM boundaries were idealized as internal elastic interfaces. Choi et al. (2007) quantified the

three-dimensional in situ equilibrium deformation of porcine chondrons subjected to unconfined compression at 10–50% engineering strain, reporting changes in height, medial-lateral (ML) width and anterior-posterior (AP) of chondrons idealized as ellipsoids (Choi et al. 2007). Given the assumption of small strains and isotropy in our model, only data for mid-zone chondron deformation at 10% engineering strain was considered, and mean values are summarized in Table 1. Based on these findings, in our multiscale continuum model the chondrocyte and the chondron were idealized as axisymmetric ellipsoids (Fig. 3) with undeformed heights ($2H_C$, $2H_P$) and widths (W_C , W_P) based on Table 1. The boundary of the microscopic domain was taken as a spherical curve with a radius of $R_E (= 30 \mu\text{m})$. The center of the microscopic domain was taken at a depth of $z/h = 0.5$, where $h = 1 \text{ mm}$, and boundary conditions along the outer spherical curve were formulated using Eq. 2 evaluated with ECM material properties.

2.2 Computational methods

2.2.1 Boundary integral formulation—An axisymmetric elastic boundary integral formulation for boundary value problems (Bakr 1986), that has been previously used by our group to model the micropipette aspiration contact problem (Haider and Guilak 2002), was extended to the case of domains with internal interfaces. Motivated by the in situ chondron deformation experimental data, a three-zone domain is considered with one external boundary (Γ_E) and two internal interfaces representing the cell-PCM (I_{CP}) and PCM-ECM (I_{PE}) internal boundaries (Fig. 3). Extension to the interface problem employs the same integral operators and fundamental solutions as for the boundary value problem, with the distinction that the fundamental solutions are evaluated separately in each subdomain. The resulting boundary integral operators are assembled into a system of boundary integral equations that, on the internal interfaces (I_{CP} , I_{PE}), have twice as many unknowns as on the external boundary (Γ_E). Introducing cylindrical polar coordinates $\mathbf{x} = (x_1, x_2, x_3) = (r, \theta, z)$, the following system of boundary integral equations (for $i = 1, 3$) is formulated by taking limits as an interior point approaches a boundary or interface point ($\mathbf{y}$) in each of the three subdomains:

$$\begin{aligned} \sum_{j=1,3} C_{ij}(\mathbf{y})u_j(\mathbf{y}) &= \sum_{j=1,3} \int_{\Gamma_E \cup I_{PE}} \left[U_{ij}^{ECM}(\mathbf{y}, \mathbf{x})t_j(\mathbf{x}) - T_{ij}^{ECM}(\mathbf{y}, \mathbf{x})u_j(\mathbf{x}) \right] d\Gamma(\mathbf{x}) \text{ for } \mathbf{y} \in \Gamma_E \\ \sum_{j=1,3} C_{ij}(\mathbf{y})u_j(\mathbf{y}) &= \sum_{j=1,3} \int_{\Gamma_E \cup I_{PE}} \left[U_{ij}^{ECM}(\mathbf{y}, \mathbf{x})t_j(\mathbf{x}) - T_{ij}^{ECM}(\mathbf{y}, \mathbf{x})u_j(\mathbf{x}) \right] d\Gamma(\mathbf{x}) \text{ for } \mathbf{y} \in I_{PE}^+ \\ \sum_{j=1,3} C_{ij}(\mathbf{y})u_j(\mathbf{y}) &= \sum_{j=1,3} \int_{I_{PE} \cup I_{CP}} \left[U_{ij}^{PCM}(\mathbf{y}, \mathbf{x})t_j(\mathbf{x}) - T_{ij}^{PCM}(\mathbf{y}, \mathbf{x})u_j(\mathbf{x}) \right] d\Gamma(\mathbf{x}) \text{ for } \mathbf{y} \in I_{PE}^- \\ \sum_{j=1,3} C_{ij}(\mathbf{y})u_j(\mathbf{y}) &= \sum_{j=1,3} \int_{I_{PE} \cup I_{CP}} \left[U_{ij}^{PCM}(\mathbf{y}, \mathbf{x})t_j(\mathbf{x}) - T_{ij}^{PCM}(\mathbf{y}, \mathbf{x})u_j(\mathbf{x}) \right] d\Gamma(\mathbf{x}) \text{ for } \mathbf{y} \in I_{CP}^+ \\ \sum_{j=1,3} C_{ij}(\mathbf{y})u_j(\mathbf{y}) &= \sum_{j=1,3} \int_{I_{CP}} \left[U_{ij}^{Cell}(\mathbf{y}, \mathbf{x})t_j(\mathbf{x}) - T_{ij}^{Cell}(\mathbf{y}, \mathbf{x})u_j(\mathbf{x}) \right] d\Gamma(\mathbf{x}) \text{ for } \mathbf{y} \in I_{CP}^- \end{aligned} \quad (3a-e)$$

In Eqs. 3, u_j and t_j are displacement and traction variables, respectively, along the boundary and internal interfaces. $U_{ij}^K(\mathbf{y}, \mathbf{x})$ and $T_{ij}^K(\mathbf{y}, \mathbf{x})$ are axisymmetric displacement and stress fundamental solutions in each of the three subdomains, i.e. $K = ECM, PCM, Cell$, and can be explicitly represented in terms of elliptic integrals (Bakr 1986). The displacement fundamental solutions $U_{ij}^K(\mathbf{y}, \mathbf{x})$ and series approximations for evaluation of the elliptic integrals are summarized in Appendix A. The stress fundamental solutions $T_{ij}^K(\mathbf{y}, \mathbf{x})$ can be obtained by substituting $U_{ij}^K(\mathbf{y}, \mathbf{x})$ into the stress-strain relations, i.e. Eqs. 1b, but are omitted for brevity and found in Appendix D of Bakr (1986). Note that $I_{PE}^+ \equiv I_{PE}^- \equiv I_{PE}$ and $I_{CP}^+ \equiv I_{CP}^- \equiv I_{CP}$ in Eqs. 3, and the superscripts are included to delineate the direction in which the limit is taken to the interface since it affects the choice of fundamental solution that is

utilized, e.g. in Eq. 3d I_{CP}^+ denotes taking a limit to I_{CP} from the PCM subdomain, and hence the fundamental solutions $U_{ij}^{PCM}(\mathbf{y}, \mathbf{x})$ and $T_{ij}^{PCM}(\mathbf{y}, \mathbf{x})$ are employed. The second-order tensor $C_{ij}(\mathbf{y})$ is determined by the interior solid angle at the point $\mathbf{y}$, e.g. $2C_{ij} = \delta_{ij}$ when the boundary or interface is smooth at $\mathbf{y}$. Eqs. 3 are in the category of direct boundary integral formulations, in that there is a direct correspondence between the mathematical unknowns and the physically relevant variables, i.e. displacement and traction components.

2.2.2 Boundary element method—To discretize the boundary integral Eqs. 3, a boundary element method (BEM) is employed with 3-node isoparametric quadratic elements, as in Haider and Guilak (2002). A computational mesh is required on the curve

$x_1 = \sqrt{R_E^2 - (x_3 - 0.5)^2}$ for the ECM boundary Γ_E , on the curve $x_1 = W_p \sqrt{1 - (x_3 - 0.5)^2 / H_p^2}$ for

the PCM-ECM interface I_{PE} , and on the curve $x_1 = W_c \sqrt{1 - (x_3 - 0.5)^2 / H_c^2}$ for the cell-PCM interface I_{CP} (Fig. 3). M_E , M_P and M_C denote the number of boundary elements employed on Γ_E , I_{PE} and I_{CP} , respectively, and element interpolations of the coordinates, displacements and tractions on the m^{th} element γ_m are denoted by $\mathbf{x}^m(\xi)$, $\mathbf{u}^m(\xi)$ and $\mathbf{t}^m(\xi)$ ($m = 1, \dots, M_E + M_P + M_C$), respectively, where $\xi(-1 \leq \xi \leq 1)$ is the local element coordinate. These interpolations can be represented in terms of their 3 nodal values within the m^{th} boundary element γ_m via the relations:

$$\mathbf{x}^m(\xi) = \sum_{\tau=1}^3 \mathbf{x}_{\tau}^m N_{\tau}(\xi), \quad \mathbf{u}^m(\xi) = \sum_{\tau=1}^3 \mathbf{u}_{\tau}^m N_{\tau}(\xi), \quad \mathbf{t}^m(\xi) = \sum_{\tau=1}^3 \mathbf{t}_{\tau}^m N_{\tau}(\xi). \quad (4a-c)$$

In Eqs. 4, the nodal values are given by $(\bullet)_{\tau}^m = (\bullet)^m(\tau-2)$, where $\tau = 1, 2, 3$ is the local nodenummer, and the quadratic basis consists of the three shape functions $N_1(\xi) = -\xi(1-\xi)/2$, $N_2(\xi) = (1-\xi)(1+\xi)/2$ and $N_3(\xi) = \xi(1+\xi)/2$. Eqs. 4 are substituted into Eqs. 3 and global mesh coordinates are introduced by labeling each mesh node with a global index $p = p(m, \tau)$ where $p = 1, \dots, n$ and $n = 2(M_E + M_C + M_P) + 3$.

The following system of discrete boundary integral equations is obtained at each mesh node p ($p = 1, 3$):

$$\sum_{j=1,3} C_{ij}^{(p)} u_j^{(p)} = 2\pi \sum_{\gamma_m \in \Gamma_E \cup I_{PE}} \sum_{\tau=1}^3 \sum_{j=1,3} \left[(t_j)_{\tau}^m \int_{-1}^1 U_{ij}^{ECM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi - (u_j)_{\tau}^m \int_{-1}^1 T_{ij}^{ECM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi \right] \quad \text{for } \mathbf{y}^{(p)} \in \Gamma_E \quad (5a)$$

$$\sum_{j=1,3} C_{ij}^{(p)} u_j^{(p)} = 2\pi \sum_{\gamma_m \in \Gamma_E \cup I_{PE}} \sum_{\tau=1}^3 \sum_{j=1,3} \left[(t_j)_{\tau}^m \int_{-1}^1 U_{ij}^{ECM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi - (u_j)_{\tau}^m \int_{-1}^1 T_{ij}^{ECM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi \right] \quad \text{for } \mathbf{y}^{(p)} \in I_{PE}^+ \quad (5b)$$

$$\sum_{j=1,3} C_{ij}^{(p)} u_j^{(p)} = 2\pi \sum_{\gamma_m \in I_{CP} \cup I_{PE}} \sum_{\tau=1}^3 \sum_{j=1,3} \left[(t_j)_{\tau}^m \int_{-1}^1 U_{ij}^{PCM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi - (u_j)_{\tau}^m \int_{-1}^1 T_{ij}^{PCM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi \right] \quad \text{for } \mathbf{y}^{(p)} \in I_{PE}^- \quad (5c)$$

$$\sum_{j=1,3} C_{ij}^{(p)} u_j^{(p)} = 2\pi \sum_{\gamma_m \in I_{CP} \cup I_{PE}} \sum_{\tau=1,3}^3 \left[(t_j)_\tau^m \int_{-1}^1 U_{ij}^{PCM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi - (u_j)_\tau^m \int_{-1}^1 T_{ij}^{PCM}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi \right] \quad \text{for } \mathbf{y}^{(p)} \in I_{CP}^+ \quad (5d)$$

$$\sum_{j=1,3} C_{ij}^{(p)} u_j^{(p)} = 2\pi \sum_{\gamma_m \in I_{CP}} \sum_{\tau=1,3}^3 \left[(t_j)_\tau^m \int_{-1}^1 U_{ij}^{Cell}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi - (u_j)_\tau^m \int_{-1}^1 T_{ij}^{Cell}(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi)) K_{\tau,m}(\xi) d\xi \right] \quad \text{for } \mathbf{y}^{(p)} \in I_{CP}^- \quad (5e)$$

In Eqs. 5, $K_{\tau,m}(\xi) = N_\tau(\xi) x_1(\xi) J_m(\xi)$, where $J_m(\xi)$ is the Jacobian of the transformation from global to local coordinates in element γ_m . By mapping the element level nodal variables $(u_j)_\tau^m$ and $(t_j)_\tau^m$ to their global node numbers, and grouping together all element integrals associated with the same nodal variables, the assembled system of linear algebraic equations can be represented in block matrix form as:

$$\begin{pmatrix} \mathbf{A}_{11}^{ECM} & \mathbf{A}_{11}^{PCM} & \mathbf{0} & \mathbf{A}_{13}^{ECM} & \mathbf{A}_{13}^{PCM} & \mathbf{0} \\ \mathbf{0} & \mathbf{A}_{11}^{PCM} & \mathbf{A}_{11}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{A}_{13}^{Cell} \\ \mathbf{0} & \mathbf{0} & \mathbf{A}_{11}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{A}_{13}^{Cell} \\ \mathbf{A}_{31}^{ECM} & \mathbf{A}_{31}^{PCM} & \mathbf{0} & \mathbf{A}_{33}^{ECM} & \mathbf{A}_{33}^{PCM} & \mathbf{0} \\ \mathbf{0} & \mathbf{A}_{31}^{PCM} & \mathbf{A}_{31}^{Cell} & \mathbf{0} & \mathbf{A}_{33}^{PCM} & \mathbf{A}_{33}^{Cell} \\ \mathbf{0} & \mathbf{0} & \mathbf{A}_{31}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{A}_{33}^{Cell} \end{pmatrix} \begin{pmatrix} \mathbf{u}_1^E \\ \mathbf{u}_1^{PE} \\ \mathbf{u}_1^{CP} \\ \mathbf{u}_3^E \\ \mathbf{u}_3^{PE} \\ \mathbf{u}_3^{CP} \end{pmatrix} = \begin{pmatrix} \mathbf{B}_{11}^{ECM} & \mathbf{B}_{11}^{PCM} & \mathbf{0} & \mathbf{B}_{13}^{ECM} & \mathbf{B}_{13}^{PCM} & \mathbf{0} \\ \mathbf{0} & \mathbf{B}_{11}^{PCM} & \mathbf{B}_{11}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{B}_{13}^{Cell} \\ \mathbf{0} & \mathbf{0} & \mathbf{B}_{11}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{B}_{13}^{Cell} \\ \mathbf{B}_{31}^{ECM} & \mathbf{B}_{31}^{PCM} & \mathbf{0} & \mathbf{B}_{33}^{ECM} & \mathbf{B}_{33}^{PCM} & \mathbf{0} \\ \mathbf{0} & \mathbf{B}_{31}^{PCM} & \mathbf{B}_{31}^{Cell} & \mathbf{0} & \mathbf{B}_{33}^{PCM} & \mathbf{B}_{33}^{Cell} \\ \mathbf{0} & \mathbf{0} & \mathbf{B}_{31}^{Cell} & \mathbf{0} & \mathbf{0} & \mathbf{B}_{33}^{Cell} \end{pmatrix} \begin{pmatrix} \mathbf{t}_1^E \\ \mathbf{t}_1^{PE} \\ \mathbf{t}_1^{CP} \\ \mathbf{t}_3^E \\ \mathbf{t}_3^{PE} \\ \mathbf{t}_3^{CP} \end{pmatrix} \quad (6)$$

In Eq. 6, the nodal variables are grouped by the boundary or interface on which they reside, illustrating coupling between displacements and tractions on the three surfaces bounding the cell, PCM and ECM regions. The overwhelming majority of non-zero entries in Eq. 6, are element integrals that can be evaluated using standard regular or logarithmic Gaussian quadrature formulas (Stroud and Secrest 1966). Specifically, all but the diagonal entries of the submatrices $\mathbf{A}_{ij}^{ECM}$, $\mathbf{A}_{ij}^{PCM}$, $\mathbf{A}_{ij}^{Cell}$ are integrals along the boundary elements that can be evaluated using regular or logarithmic Gaussian quadrature. The exceptions are the diagonal entries of $\mathbf{A}_{ij}^{ECM}$, $\mathbf{A}_{ij}^{PCM}$ and $\mathbf{A}_{ij}^{Cell}$, which contain strongly singular integrals that require special treatment. In the BEM literature, there are primarily two techniques for treatment of the strongly singular integrals: an indirect approach and a direct approach. In the indirect approach, see e.g. (Bakr 1986), all non-singular integrals are first computed. Subsequently, boundary displacements and tractions based on known analytical solutions of linear elasticity (e.g. rigid translation, plane stress, plane strain), are substituted into Eq. 6 to solve for the remaining, strongly singular, matrix entries in terms of the non-singular entries (already computed). However, this approach relies on the availability of analytical solutions, which is practical for boundary value problems on a homogeneous domain, yet impractical for problems with internal interfaces. Consequently, in contrast to our previous BEM

computational models of cell mechanics (Haider and Guilak 2000, 2007; Haider and Guilak 2002), this study employs the direct approach to evaluate the strongly singular integrals. In particular the method of Guiggiani and Casalini (1987) was used and is summarized in the following section.

2.2.3 Evaluation of strongly singular integrals—Computation of strongly singular integrals is similar to evaluation of Cauchy Principal Value integrals in the sense that two integrals, each with equivalent singularities of opposite signs, are summed together. For example, when $\mathbf{x}^m(\xi)$ and $\mathbf{y}^{(p)}$ in Eqs. 5 lie in the same element, the pair of two adjacent boundary elements can be denoted as γ^m and γ^{m+1} . The singularity at the node common to both elements corresponds to $\xi = 1$ in element γ^m and $\xi = -1$ in element γ^{m+1} . The strongly singular integrands in Eqs. 5 are re-written as:

$$\begin{aligned} T_{ij}^K(\mathbf{y}^{(p)}, \mathbf{x}^m(\xi))K_{\tau,m}(\xi) &= J_m(\xi) \frac{f_m(\xi)}{\xi-1}, \quad \mathbf{x}^m \in \gamma^m, \\ T_{ij}^K(\mathbf{y}^{(p)}, \mathbf{x}^{m+1}(\xi))K_{\tau,m+1}(\xi) &= J_{m+1}(\xi) \frac{f_{m+1}(\xi)}{\xi+1}, \quad \mathbf{x}^{m+1} \in \gamma^{m+1}, \end{aligned} \quad (7a,b)$$

where $K = \text{Cell}, \text{PCM}, \text{ECM}$, and $f_m(\xi)$, $f_{m+1}(\xi)$ are regular functions. The sum of the two singular integrals is then defined in the Cauchy Principal Value sense as:

$$I = \lim_{\varepsilon \rightarrow 0} \left(\int_{-1}^{1-\varepsilon/J_m(1)} \frac{f_m(\xi)J_m(\xi)}{\xi-1} d\xi + \int_{-1+\varepsilon/J_{m+1}(-1)}^1 \frac{f_{m+1}(\xi)J_{m+1}(\xi)}{\xi+1} d\xi \right). \quad (8)$$

By letting $h_a(\xi) = f_a(\xi)J_a(\xi)$ where $a = m, m+1$, and noting that existence of the integral sum I in Eq. 8 necessitates that $h_m(1) = h_{m+1}(-1)$, Eq. 8 can be rewritten as:

$$\begin{aligned} I &= \lim_{\varepsilon \rightarrow 0} \left(\int_{-1}^{1-\varepsilon/J_m(1)} \frac{h_m(\xi)-h_m(1)}{\xi-1} d\xi + \int_{-1+\varepsilon/J_{m+1}(-1)}^1 \frac{h_{m+1}(\xi)-h_{m+1}(-1)}{\xi+1} d\xi \right) + \lim_{\varepsilon \rightarrow 0} \left(\int_{-1}^{1-\varepsilon/J_m(1)} \frac{h_m(1)}{\xi-1} d\xi + \int_{-1+\varepsilon/J_{m+1}(-1)}^1 \frac{h_{m+1}(-1)}{\xi+1} d\xi \right) \\ &= \int_{-1}^1 \frac{h_m(\xi)-h_m(1)}{\xi-1} d\xi + \int_{-1}^1 \frac{h_{m+1}(\xi)-h_{m+1}(-1)}{\xi+1} d\xi - h_m(1)\ln|J_m(1)| + h_{m+1}(-1)\ln|J_{m+1}(-1)|. \end{aligned} \quad (9a,b)$$

The integrals in Eq. 9b are now regularized, in the sense that they contain removable singularities, and can be accurately evaluated using standard Gaussian quadrature. When the point $\mathbf{y}^{(p)}$ lies at the middle node of the element, the element is divided into two sub-elements on the intervals $[-1,0]$ and $[0,1]$, and a similar procedure (not described) is employed.

2.2.4 Boundary conditions and final assembly—Once all components of the matrices $\mathbf{A}_{ij}^K$ and $\mathbf{B}_{ij}^K$ ($K = \text{ECM}, \text{PCM}, \text{Cell}$) in Eq. 6 have been determined, the boundary conditions can be applied. At each node of Γ_E , exactly two quantities must be specified: either u_1 or t_1 and either u_3 or t_3 . Eq. 6 is then rearranged such that matrix entries that multiply unknown quantities are assembled on the left hand side of the linear system, while matrix-vector products corresponding to prescribed nodal values are moved to the right hand side. A new linear system $\mathbf{K}\mathbf{x} = \mathbf{b}$ is obtained where the matrix $\mathbf{K}$ has the following block form:

$$\mathbf{K} = \begin{bmatrix} \mathbf{K}_{11} & \mathbf{K}_{13} \\ \mathbf{K}_{31} & \mathbf{K}_{33} \end{bmatrix},$$

$$\text{where: } \mathbf{K}_{ij} = \begin{bmatrix} \mathbf{X}_{ij}^{ECM} & \mathbf{A}_{ij}^{ECM} & -\mathbf{B}_{ij}^{ECM} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{A}_{ij}^{PCM} & \mathbf{B}_{ij}^{PCM} & \mathbf{A}_{ij}^{PCM} & -\mathbf{B}_{ij}^{PCM} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{A}_{ij}^{Cell} & \mathbf{B}_{ij}^{Cell} \end{bmatrix}, \text{ for } i, j=1, 3, \quad (10a-c)$$

$$\text{and } \mathbf{x} = [\mathbf{x}_1, \mathbf{u}_1, \mathbf{t}_1, \mathbf{x}_3, \mathbf{u}_3, \mathbf{t}_3]^T$$

In Eqs. 10, individual components of $\mathbf{X}_{ij}^{ECM}$ are the corresponding components of either $\mathbf{A}_{ij}^{ECM}$ or $\mathbf{B}_{ij}^{ECM}$ depending on whether a traction component or a displacement component, respectively, is prescribed at the associated node of Γ_E . Similarly, the entries of the vectors of unknowns $\mathbf{x}_1$ and $\mathbf{x}_3$ are taken as the complementary components of traction or displacement on Γ_E . The remaining displacement and traction components, i.e. on Γ_{PE} and Γ_{CP} , are all unknown and assembled in the vectors $\mathbf{u}_1, \mathbf{u}_3$ and $\mathbf{t}_1, \mathbf{t}_3$, respectively. Due to the assumption of axisymmetric geometry, six rows of the linear system $\mathbf{K}\mathbf{x} = \mathbf{b}$ are modified to directly enforce conditions that the radial displacement and radial traction are zero at nodes along the symmetry axis $x_1 = 0$. For the application under consideration, the fully assembled linear system is small but dense and can be accurately solved using Gaussian elimination with partial pivoting.

2.3 Parameter estimation

The computational BEM model developed in the previous section reduces the in situ chondron deformation problem to consideration of a mesh on three parametric curves. This geometric simplification, combined with efficiency and accuracy of BEMs, allows the forward solver to be directly integrated into an optimization algorithm for parameter estimation based on the experimental study of Choi et al. (2007). In their study, all chondrons used to quantify morphological changes were selected from radially central regions of the tissue to avoid inhomogeneous strain fields due to friction between the compressing platen and the tissue. This choice is also consistent with use of an axisymmetric model for analysis of the experimental data, and mean values of mid-zone morphological data are summarized in Table 1.

Based on Table 1, different parameter estimation problems can be formulated. In this study, undeformed morphological data were used to set up boundary element meshes for axisymmetric ellipsoids approximating the undeformed shapes of the chondrocyte and the chondron. BEM forward simulations were then used to solve an inverse problem that minimized a least squares cost function comparing model predictions to deformed morphological data. The formulation of robust parameter estimation problems requires careful selection of a subset of chondron material properties for estimation, and choice of experimental data components to employ in formulating an appropriate cost function for minimization. Due to the statistical variability in chondrocyte morphology and depth within the middle zone of the explant, the cost functions considered in this study employed only mean values of undeformed and deformed chondron lengths along principal directions (Table 1). These mean values were then compared to BEM simulations with the chondron centered at $z/h = 0.5$, i.e. the middle depth within the explant. In addition, since the PCM is the primary focus of this study, emphasis was placed on robust estimation of PCM Young's modulus (E_{PCM}) and PCM Poisson's ratio (ν_{PCM}), assuming fixed values of these parameters for the chondrocyte and the ECM.

Two different cost functions were considered. First, a function based on deformed coordinates of the chondron, i.e. at the PCM-ECM interface, was formulated. To increase

resolution in the cost function, $N = 19$ radial lines emanating from the center of the undeformed chondron were generated. This central point was then aligned to the axial center of deformed ellipsoid, as simulated by the BEM, and a local coordinate system was introduced. Distances between the undeformed and deformed coordinates were then used to construct the following cost function for PCM parameter estimation:

$$f_1 = \sum_{i=1}^N \left(|r_i^m - r_i^d|^2 + |z_i^m - z_i^d|^2 \right) \quad (r_i, z_i) \in I_{PE}, \quad (11)$$

where the superscript d denotes experimental values and m denotes BEM model predictions. To examine the effect of incorporating cell volumetric deformation data, cell volume data V_C^m was also added into the cost function of Eq. 11 via a scalar term with scaling coefficient $\omega = 0.01$:

$$f_2 = \omega |V_{cell}^m - V_{cell}^d|^2 + \sum_{i=1}^N \left(|r_i^m - r_i^d|^2 + |z_i^m - z_i^d|^2 \right) \quad (r_i, z_i) \in I_{PE}. \quad (12)$$

Both cost functions were minimized using a custom implementation of a Nelder-Mead direct search method (Nelder and Mead 1965), based on the specific algorithm presented by Kelley (1999). This algorithm employs and manipulates a simplex for each parameter to be optimized. In particular, one-parameter estimation of E_{PCM} and two-parameter estimation of E_{PCM} and ν_{PCM} were considered and, in the optimization method, the associated simplexes had two and three components per parameter, respectively. Initial parameter ranges in the simplex were varied to test for sensitivity of the optimal parameter values to their initialization and, hence, evaluate robustness of the inverse BEM method.

3. Results

The boundary element method described in Sec. 2.2 was implemented in a custom C-code on a MacBookPro laptop computer (2.0 GHz Intel Core Duo processor), and was first tested for accuracy. All results shown employed $M_E = M_P = M_C = 13$ quadratic boundary elements on one-dimensional curves for the ECM, the PCM and the chondrocyte. Numerical integration with 10-point Gaussian quadrature was sufficient for demonstrating accuracy between numerical and analytical solutions. Accuracy of the BEM model was first verified by comparison to analytical linear elasticity solutions (Appendix B) for plane strain, plane stress, confined compression and unconfined compression in a homogeneous domain. Reduction to a homogeneous domain was achieved in the BEM simulation by assigning material properties in all three zones (cell, PCM, ECM) to be equal. All comparisons of BEM simulations to analytical solutions demonstrated excellent agreement and a typical comparison is shown in Figure 4. Numerical convergence was also assessed via a least squares error comparing analytical and numerical displacements along all boundary elements of I_{CP} . When the same number of elements was chosen on Γ_E , I_{PE} and I_{CP} the effect of including more elements is demonstrated in Figure 5. For the non-homogeneous case, BEM solutions were also verified against finite element solutions obtained using the software package *COMSOL Multiphysics* via the axisymmetric stress-strain application model in the *Structural Mechanics Module* (COMSOL, Inc., Burlington MA). Chondrocyte, PCM and ECM material properties were based on the in vitro micropipette aspiration study of Alexopoulos et al. (2005b), having the fixed values $E_{Cell} = 0.35\text{kPa}$, $E_{PCM} = 38.7\text{kPa}$, $E_{ECM} = 1000\text{kPa}$ and $\nu_{Cell} = 0.43$, $\nu_{PCM} = \nu_{ECM} = 0.04$, and were used in multiscale simulations of macroscopic plane stress, plane strain, confined compression, and unconfined compression of a linear elastic cylinder with a chondron located at 50% depth. Comparisons

of BEM simulations of deformed chondron shape were in excellent agreement with the independent finite element computations (Fig. 6).

Once accuracy of the new elastic BEM was verified, the associated code was integrated as a function call into a custom C-code for inverse BEM analysis using the Nelder-Mead optimization algorithm. Typical CPU times were on the order of a few seconds for the forward BEM computation, and less than three minutes for solution of the optimization problem. The resulting inverse BEM code was then used to analyze the inverse problem associated with the in situ mid-zone small strain (10% compression) data for chondron deformation of Choi et al. (2007) (Table 1). Note that, in their study, cell deformation was reported only as changes in chondrocyte volume and, hence, the original data measurements for the mean undeformed and deformed cell height and ML width were obtained and have been included in Table 1. Material properties for the chondrocyte were chosen based on Alexopoulos et al. (2005b) as $E_{Cell} = 0.35\text{kPa}$ and $\nu_{Cell} = 0.43$. The ECM Young's modulus was fixed at $E_{ECM} = 0.5\text{MPa}$ and two values for the ECM Poisson's ratio (ν_{ECM}) were considered. The first value, $\nu_{ECM} = 0.04$ was based on the value used in the previous in vitro study of isolated chondrons (Alexopoulos et al. 2005b). As part of their experimental study of in situ chondron deformation, Choi et al. (2007) also reported site-specific ECM Poisson's ratio in their cartilage explants based on a ratio of compressive to lateral engineering strain determined via texture correlation. Hence, their reported mid-zone value of $\nu_{ECM} = 0.12$ was also assumed in this study.

First, the inverse BEM code was employed using the cost function f_1 of Eq. 11 in a one parameter optimization to estimate E_{PCM} assuming fixed values for ν_{PCM} and ν_{ECM} . Results are shown in Table 2 for the three cases considered. Estimated values of E_{PCM} obtained via the inverse analysis demonstrate a high degree of consistency, with values of 24.5kPa (Case I), 58.6–58.7kPa (Case II) and 58.0–58.1kPa (Case III) as the initial simplex for E_{PCM} was varied. Increasing the assumed value of ν_{ECM} from 0.04 to 0.12 had a significant effect on the estimated value of E_{PCM} for both assumed values of ν_{PCM} (Case I vs. Cases II&III). Although the costs in Cases II&III were significantly higher than for Case I ($f_1 = 0.08 - 0.09$ vs. 0.01), comparisons of simulated and experimental chondron shapes demonstrated excellent agreement (Fig. 7).

Next, two parameter optimization was conducted in an attempt to, simultaneously, estimate both E_{PCM} and ν_{PCM} using the cost function f_1 in Eq. 11. More robust estimates, i.e., with low sensitivity to choice of the initial simplex, were obtained when the initial simplexes for E_{PCM} and ν_{PCM} were increased together. Results are shown in Table 3 for the two values of ν_{ECM} under consideration (Cases IV&V). In Case IV, estimated values of E_{PCM} and ν_{PCM} varied between 25.5–26.7kPa and 0.16–0.18, respectively, with the cost f_1 on the order of 0.01. Case V exhibited less variation in estimates of E_{PCM} , ranging between 57.2–57.3kPa. Estimates of ν_{PCM} were very close to zero, and the cost f_1 was significantly higher than in Case IV, and on the order of 0.07. Overall, comparisons of simulated and experimental chondron shapes demonstrated excellent agreement (Fig. 8).

Lastly, the inverse BEM was applied to the cost function in Eq. 12 to consider the effects of incorporating a measure of deformed chondrocyte volume into the optimization (Table 4). It is noted that the form of Eq. 12 was chosen for the cost function after an attempt was made to add an additional least squares term accounting for multiple points along the cell-PCM interface. However, in these cases, a 19-point least squares cost function on the cell-PCM interface yielded cost values that were between 25–100 times greater than optimal values of f_1 achieved in cases I–V. Generally, this resulted in non-convergence of the optimization algorithm or high sensitivity to choice of the initial simplex, indicating limitations in the range of applicability of the inverse BEM mode to the in situ data. Using Eq. 12, when

$\nu_{ECM}=0.04$ (Case VI), estimated values of E_{PCM} increased in magnitude but exhibited less variation, when compared to Case IV, to 45.6–45.8kPa. Estimated values of ν_{PCM} also increased in magnitude to 0.36, but exhibited little variation. For Case VI, the new cost function f_2 was on the order of 0.02. When $\nu_{ECM}=0.12$ (Case VII), the magnitude of E_{PCM} decreased, when compared to Case V, to 48.5–48.6kPa, and the magnitude of ν_{PCM} increased to 0.38–0.39, but the new cost f_2 was higher than for Case V ($f_2 = 0.18$ vs. 0.02). However, typical comparisons of simulated and experimental chondron shapes demonstrated excellent agreement between the model and the experimental data (Fig. 9). In the case of the cost function f_2 , the estimates of E_{PCM} were less sensitive to the choice of ν_{ECM} , for the two cases under consideration.

4. Discussion

In this study, a new axisymmetric elastic BEM was developed for domains with internal interfaces, and was used in a multiscale model to simulate deformation in the microscopic environment of an articular cartilage explant. Efficiency of the BEM approach, combined with its geometric simplicity, enabled direct integration into an efficient inverse optimization algorithm. The resulting inverse BEM computational model was applied, using an optimization algorithm, to confocal microscopy data for chondron deformation in articular cartilage to determine the mechanical properties of the PCM in situ. Consistent with previous studies on isolated chondrons, the results of this study showed that the elastic modulus of the PCM is approximately an order of magnitude lower than that of the ECM, and several orders of magnitude higher than that of the chondrocyte. To our knowledge, this study represents the first determination of the in situ material properties of the PCM, based on experimental data for in situ deformation within the native ECM of an articular cartilage explant.

The measures of PCM stiffness presented in this study are in excellent agreement with magnitudes reported in previous studies on isolated chondrons. Specifically, our overall ranges for E_{PCM} are 24.5–45.8kPa when $\nu_{ECM}=0.04$ and 48.5–58.7kPa when $\nu_{ECM}=0.12$. These ranges are highly consistent with previous in vitro micropipette aspiration studies in normal human chondrons that reported values of $E_{PCM}=66.5\pm 23.3$ kPa using a layered elastic halfspace theoretical solution (Alexopoulos et al. 2003) and $E_{PCM}=38.7\pm 16.2$ kPa using a transient biphasic finite element model accounting for cell geometry (Alexopoulos et al. 2005b). Furthermore, a previous inverse FEM study of isolated chondrons compressed in agarose (Michalek and Iatridis 2007) estimated E_{PCM} to lie in the range 43–240kPa, but was also based on the analysis of isolated chondrons, in this case compressed within agarose gel.

While the estimated PCM properties were consistent with the range of properties previously reported on extracted chondrons, the choice of ECM properties had some influence on the results of the inverse optimization. The analysis of one parameter optimization indicates that estimations of E_{PCM} are significantly lower (24.5kPa vs. 58.0–58.7kPa, Table 2) when ν_{ECM} is chosen based on typical literature values associated with mechanical testing of cartilage explants versus the texture correlation method used to estimate ν_{ECM} in Choi et al. (2007). This difference persists in the case of two parameter optimization (25.5–26.7kPa vs. 57.2–57.3kPa, Table 3), but decreases significantly in magnitude when the cost function, i.e. Eq. 12, is modified to account for chondrocyte volumetric deformation (45.6–45.8kPa vs. 48.5–48.6kPa, Table 4). Estimations of ν_{PCM} based on two parameter optimization indicate that analysis of chondron shape alone may be inadequate to accurately estimate this parameter. Predictions of deformation at the PCM-ECM interface consistently exhibited higher accuracy than those at the cell-PCM interface (Figs. 7–9), indicating potential limitations of a linear elastic model of the chondrocyte within the in situ chondron. Overall, incorporation of cell volumetric deformation into the cost function via Eq. 12 significantly

increased the estimated values of ν_{PCM} (0.36–0.39 vs. 0.00–0.18) as well as decreasing the variation in these estimations.

Estimations of ν_{PCM} presented in this study indicate sensitivity to the choice of cost function. In particular, the cost function accounting for cell volumetric deformation estimated ν_{PCM} in a more consistent fashion, to lie in the range 0.36–0.39, when $\nu_{ECM} = 0.04$ or $\nu_{ECM} = 0.12$. However, these estimates are significantly larger than previous estimates based on FEM analysis of in vitro micropipette aspiration of isolated human chondron that reported a range of $\nu_{PCM} = 0.044 \pm 0.063$. Further studies are required to investigate whether these discrepancies are inherent differences between the testing environments (in vitro vs. in situ) and/or species, or are due to challenges in solving the inverse problem related to high sensitivity of chondron deformation to small changes in Poisson's ratio. Focusing exclusively on the case $\nu_{ECM} = 0.12$, which used measurements in the same explants as the chondron data (Table 1), the inverse BEM analysis using Eq. 12 suggests that articular cartilage PCM may be less compressible within the ECM as compared to chondrons isolated in vitro. These differences may be due to constraint of the PCM by the surrounding native ECM, the complex nature of attachments between the chondrocytes and ECM, and their cumulative effects on PCM elastic properties.

It is important to note that there may be inherent differences between the in situ measurements of PCM elastic properties determined in this study and previous ex situ measurements of the same properties. Within the ECM, the detailed structure of attachment between the PCM and the ECM is not well understood. Thus, chondrons from the same site or zone of a cartilage explant may exhibit differences in PCM stiffness and Poisson's ratio between the ex situ and in situ environments. For example, mechanical extraction, particularly by tissue homogenization, may result in damage or swelling of the PCM (Hing et al. 2002). In one previous study, micro-indentation using an atomic force microscope was used to measure the in situ properties of the growth plate PCM, reporting a Young's modulus $E_{PCM} = 265 \pm 53 \text{ kPa}$ (Allen and Mao 2004). While this value is significantly higher than the estimates reported in the present study, it is important to note that there may be significant differences in the properties of growth plate and articular cartilage. Species and age are also other factors that may significantly influence a comparison between PCM properties measured in the in situ and ex situ settings.

Overall, this study demonstrates the potential utility of integrating (forward) mechanical models for cartilage biomechanics with (inverse) optimization methods for estimation of microscopic material properties in the context of novel experimental techniques based on confocal microscopy. Since an axisymmetric BEM approach was employed, this study relies on assumptions of small strain and isotropy and, as such, was limited to analysis of mid-zone data at small levels of macroscopic strain for chondrons near the central axis of the explant. A consideration of the full set of data reported in Choi et al. (2007) would require the use of alternate computational models that account for cartilage anisotropy and nonlinear finite strain. As demonstrated in this study with a BEM approach, extended models that can be readily and efficiently integrated into an optimization algorithm will have a greater potential for successful application to more comprehensive analysis of in situ data. Future studies will also need to consider the optimal selection and combination of experimental data subsets, (forward) computational mechanical models, and inverse optimization algorithms to achieve more detailed and accurate estimates of microscopic biomechanical properties in the local cellular environment of articular cartilage.

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Appendices

A. Axisymmetric displacement fundamental solutions and evaluation of elliptic integrals

The axisymmetric displacement fundamental solutions can be represented, explicitly, in terms of elliptic integrals as (Bakr 1986) (Appendix E):

$$U_{11}(\mathbf{y}, \mathbf{x}) = \frac{1}{16\pi^2(1-\nu)} \frac{1}{y_1 x_1 C} \left\{ [(3-4\nu)(y_1^2 + x_1^2) + 4(1-\nu)(y_3 - x_3)^2] K(m) + [-C^2(3-4\nu) - (y_3 - x_3)^2(y_1^2 + x_1^2 + (y_3 - x_3)^2) D^{-1}] E(m) \right\} \quad (13a)$$

$$U_{13}(\mathbf{y}, \mathbf{x}) = \frac{1}{16\pi^2(1-\nu)} \frac{y_3 - x_3}{y_1 C} \left\{ K(m) - [x_1^2 - y_1^2 + (y_3 - x_3)^2] D^{-1} E(m) \right\} \quad (13b)$$

$$U_{31}(\mathbf{y}, \mathbf{x}) = \frac{1}{16\pi^2(1-\nu)} \frac{y_3 - x_3}{y_1 C} \left\{ -K(m) + [y_1^2 - x_1^2 + (y_3 - x_3)^2] D^{-1} E(m) \right\} \quad (13c)$$

$$U_{33}(\mathbf{y}, \mathbf{x}) = \frac{1}{16\pi^2(1-\nu)} \frac{2}{C} \left\{ (3-4\nu)K(m) + (y_3 - x_3)^2 D^{-1} E(m) \right\} \quad (13d)$$

where

$$C = \sqrt{(y_1 + x_1)^2 + (y_3 - x_3)^2}, \quad D = (y_1 - x_1)^2 + (y_3 - x_3)^2, \quad m = \frac{2\sqrt{y_1 x_1}}{C} \quad (14a-c)$$

and $K(m)$, $E(m)$ are the elliptic integrals:

$$K(m) = \int_0^{\pi/2} \frac{ds}{\sqrt{1-m^2 \sin^2 s}}, \quad E(m) = \int_0^{\pi/2} \sqrt{1-m^2 \sin^2 s} ds. \quad (15a,b)$$

When $m \rightarrow 1$, the elliptic integrals in Eqs. 15 are nonsingular and can be evaluated accurately using a small number of terms ($n = 5$) of the series (Bakr 1986):

$$K(m) = \log(4) + \sum_{i=1}^n a_i (1-m^2)^i - \log(1-m^2) \left[\frac{1}{2} + \sum_{i=1}^n b_i (1-m^2)^i \right] \quad (16a)$$

$$E(m) = 1 + \sum_{i=1}^n c_i (1-m^2)^i - \log(1-m^2) \left[\sum_{i=1}^n b_i (1-m^2)^i \right] \quad (16b)$$

where:

$$a_1 = 0.09657862, a_2 = 0.03155943, a_3 = 0.02376122, \\ a_4 = 0.02596289, a_5 = 0.00663980 \quad (17a)$$

$$b_1 = 0.12499930, b_2 = 0.07014876, b_3 = 0.04498388 \\ b_4 = 0.01875166, b_5 = 0.00184723 \quad (17b)$$

$$c_1 = 0.44315287, c_2 = 0.05756700, c_3 = 0.03176115 \\ c_4 = 0.03066235, c_5 = 0.00765296 \quad (17c)$$

$$d_1 = 0.24999920, d_2 = 0.09356491, d_3 = 0.05426052 \\ d_4 = 0.02183602, d_5 = 0.00212479. \quad (17d)$$

As $m \rightarrow 1$, the elliptic integral $E(m)$ remains nonsingular and can be evaluated as described above. The elliptic integral $K(m)$ contains a logarithmic singularity and must be decomposed into singular and nonsingular parts. This decomposition is made after the quadratic elements in Eq. 4 are introduced and $K(m)$ in Eq. 16a can be written in the form $K(t) = f(t)\log(t) + g(t)$ where t is a local coordinate along the element ($t \in [0,1]$) and $f(t)$, $g(t)$ are nonsingular functions.

B. Elementary linear elasticity solutions

Plane stress

In a state of plane stress, $\sigma_{33} = \sigma_{13} = \sigma_{23} = 0$ and the displacement and traction components are:

$$u_1 = (1-\nu)x_1 = (1-\nu)r, \quad u_3 = -2\nu x_3 = -2\nu z \\ t_1 = En_1 = En_r, \quad t_3 = 0. \quad (18)$$

Plane strain

In a state of plane strain, $e_{33} = e_{13} = e_{23} = 0$ and the displacement and traction components are:

$$\begin{aligned} u_1 &= (1+\nu)(1-2\nu)x_1 = (1+\nu)(1-2\nu)r, & u_3 &= 0, \\ t_1 &= En_1 = En_r, & t_3 &= 2\nu En_1 = 2\nu En_z. \end{aligned} \quad (19)$$

Uniaxial confined compression

The confined compression test is commonly used for in vitro analysis of deformation in cylindrical articular cartilage explants. A compressive normal displacement or normal traction is applied over the top surface of the sample that is laterally confined, thus preventing radial displacement. At static equilibrium, the displacement and traction components are:

$$\begin{aligned} u_1 &= 0, & u_3 &= -\frac{\varepsilon_z}{h}, \\ t_1 &= -\frac{\lambda\varepsilon_z}{h}n_1 = -\frac{\lambda\varepsilon_z}{h}n_r, & t_3 &= \frac{(\lambda+2\mu)\varepsilon_z}{h}n_1 = \frac{(\lambda+2\mu)\varepsilon_z}{h}n_z. \end{aligned} \quad (20)$$

Figure 1.

Confocal microscopy images of in situ chondron deformation within the ECM of a cylindrical porcine articular cartilage explant under static unconfined compression, adapted from Choi et al. (2007) [with permission]. (left) cartilage explants were fixed at 0% and 10% compression and immunolabeled for type VI collagen to quantify the boundaries of the chondrocyte and PCM, (right) 3D reconstructions were made of chondrons in control and compressed tissue explants.

Figure 2.

Illustration of two-scale axisymmetric continuum model of in situ deformation of chondrons in the middle zone of a cylindrical cartilage explant subjected to static unconfined compression. (a) A single chondron is located at 50% depth (middle zone), along the symmetry axis of a cylindrical explant. (b) The unconfined compression analytical solution for a homogeneous cylinder of ECM is used to formulate boundary conditions for a three-zone (cell-PCM-ECM) interface problem at the microscopic scale of the chondrocyte.

Figure 3.

The local environment of a single chondron is represented as a three-zone domain in an axisymmetric BEM model of coupled cell-PCM-ECM deformation in articular cartilage.

Figure 4.

Verification of the three-zone BEM model in the case $\nu_{ECM} = \nu_{PCM} = \nu_{Cell} = 0.2$ and $E_{ECM} = E_{PCM} = E_{Cell} = 2.0\text{kPa}$ by comparison to known analytical solutions from linear elasticity theory. Deformed displacement profiles for the BEM model (circles) are compared to each analytical solution (solid lines) on the cell-PCM and PCM-ECM interfaces for: (a) plane stress, (b) plane strain, (c) confined compression, and (d) unconfined compression.

Figure 5.

Illustration of convergence for the BEM model when an identical number of elements are used on I_{CP} , I_{PE} and Γ_E . Errors were calculated as a least squares sum comparing radial (a) and axial (b) deformed coordinates of the BEM model at all nodes of I_{CP} to corresponding values for the unconfined compression analytical solution.

Figure 6.

Verification of the three-zone BEM model against an independent finite element solution. Deformed profiles for the BEM simulations (solid circles) and finite element simulations (open circles) are compared along the cell-PCM and PCM-ECM interfaces for the cases of macroscopic: (a) plane stress, (b) plane strain, (c) confined compression, and (d) unconfined compression. Note that the undeformed chondron was centered at $z/h = 0.5$ and its deformed shape is a combination of an axial translation and deformation.

Figure 7.

BEM simulations of deformed chondron shape for estimated parameter values in the case of one parameter optimization using the cost function of Eq. 11. Computational predictions (dots) are compared to deformed (black line) and undeformed (grey line) shapes of the chondron based on Table 1 for the cases of Table 2: (a) Case I, (b) Case II, and (c) Case III.

Figure 8.

BEM simulations of deformed chondron shape for estimated parameter values in the case of two parameter optimization using the cost function of Eq. 11. Computational predictions (dots) are compared to deformed (black line) and undeformed (grey line) shapes of the chondron based on Table 1 for the cases of Table 3: (a) Case IV, and (b) Case V.

Figure 9.

BEM simulations of deformed chondron shape for estimated parameter values in the case of two parameter optimization using the cost function of Eq. 12. Computational predictions (dots) are compared to deformed (black line) and undeformed (grey line) shapes of the chondron based on Table 1 for the cases of Table 4: (a) Case VI, and (b) Case VII.

Table 1

Mean values (n=12) of morphological parameters for in situ chondron deformation in the middle zone of a cylindrical porcine cartilage explant subject to 10% surface-to-surface compression, based on Choi et al. (2007).

	Chondron		Chondrocyte	
	Undeformed	Deformed	Undeformed	Deformed
ML width [μm]	18.10	18.38	11.49 *	11.66 *
Height [μm]	18.93	15.58	11.26 *	8.89 *
AP width [μm]	18.13	18.58	Not recorded	Not recorded

* Chondrocyte values were not reported in the original study and were obtained from the authors.

Table 2

Results for one parameter estimation of E_{PCM} using the cost function of Eq. 11.

Case	$E_{PCM}^{Initial}$ [kPa]	$E_{PCM}^{Estimated}$ [kPa]	$f_1(E_{PCM}^{Estimated})$
I) $\nu_{ECM} = \nu_{PCM} = 0.04$	(10,15)	24.5	0.01000013
	(20,25)	24.5	0.01000002
	(30,35)	24.5	0.01000001
	(40,45)	24.5	0.01000002
II) $\nu_{ECM} = \nu_{PCM} = 0.12$	(10,15)	58.7	0.08763329
	(20,25)	58.7	0.08763329
	(30,35)	58.6	0.08763329
	(40,45)	58.7	0.08763329
III) $\nu_{ECM} = 0.12, \nu_{PCM} = 0.04$	(10,15)	58.1	0.07654386
	(20,25)	58.0	0.07654386
	(30,35)	58.1	0.07654387
	(40,45)	58.0	0.07654387

Table 3
Results for two parameter estimation of E_{PCM} and ν_{PCM} using the cost function of Eq. 11.

Case	$E_{PCM}^{Initial}$ [kPa]	$E_{PCM}^{Estimated}$ [kPa]	$\nu_{PCM}^{Initial}$	$\nu_{PCM}^{Estimated}$	$f_1(E_{PCM}^{Estimated}, \nu_{PCM}^{Estimated})$
IV) $\nu_{ECM}=0.04$	(10,15,20)	25.5	(0.0,0.05,0.1)	0.16	0.01000001
	(30,35,40)	26.7	(0.2,0.25,0.3)	0.18	0.01000090
	(40,45,50)	26.3	(0.3,0.35,0.4)	0.16	0.01000030
V) $\nu_{ECM}=0.12$	(10,15,20)	57.3	(0.0,0.15,0.1)	0.00	0.07202031
	(20,25,30)	57.2	(0.1,0.15,0.2)	0.00	0.07202032
	(30,35,40)	57.3	(0.2,0.25,0.3)	0.00	0.07202031
	(40,45,50)	57.3	(0.3,0.35,0.4)	0.00	0.07202031

Table 4

Results for two parameter estimation of E_{PCM} and ν_{PCM} using the cost function of Eq. 12.

Case	$E_{PCM}^{Initial}$ [kPa]	$E_{PCM}^{Estimated}$ [kPa]	$\nu_{PCM}^{Initial}$	$\nu_{PCM}^{Estimated}$	$f_2(E_{PCM}^{Estimated}, \nu_{PCM}^{Estimated})$
VI) $\nu_{ECM} = 0.04$	(10,15,20)	45.6	(0.0,0.05,0.1)	0.36	0.02103019
	(20,25,30)	45.6	(0.1,0.15,0.2)	0.36	0.02103019
	(30,35,40)	45.6	(0.2,0.25,0.3)	0.36	0.02103019
	(40,45,50)	45.8	(0.3,0.35,0.4)	0.36	0.02103019
VII) $\nu_{ECM} = 0.12$	(10,15,20)	48.6	(0.0,0.05,0.1)	0.39	0.17654582
	(20,25,30)	48.5	(0.1,0.15,0.2)	0.38	0.17654582
	(30,35,40)	48.5	(0.2,0.25,0.3)	0.39	0.17654582
	(40,45,50)	48.6	(0.3,0.35,0.4)	0.38	0.17654582