Multiscale cartilage biomechanics: technical challenges in realizing a high-throughput modelling and simulation workflow

Ahmet Erdemir1,2, Craig Bennetts1,2, Sean Davis1,2,3, Akhil Reddy1,2,4 and Scott Sibole1,2,5

1Computational Biomodeling (CoBi) Core, and 2Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA
3Department of Mechanical Engineering, University of Akron, Akron, OH 44325, USA
4Weill Cornell Medical College, New York, NY 10065, USA
5Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Understanding the mechanical environment of articular cartilage and chondrocytes is of the utmost importance in evaluating tissue damage which is often related to failure of the fibre architecture and mechanical injury to the cells. This knowledge also has significant implications for understanding the mechanobiological response in healthy and diseased cartilage and can drive the development of intervention strategies, ranging from the design of tissue-engineered constructs to the establishment of rehabilitation protocols. Spanning multiple spatial scales, a wide range of biomechanical factors dictate this mechanical environment. Computational modelling and simulation provide descriptive and predictive tools to identify multiscale interactions, and can lead towards a greater comprehension of healthy and diseased cartilage function, possibly in an individualized manner. Cartilage and chondrocyte mechanics can be examined in silico, through post-processing or feed-forward approaches. First, joint–tissue level simulations, typically using the finite-element method, solve boundary value problems representing the joint articulation and underlying tissue, which can differentiate the role of compartmental joint loading in cartilage contact mechanics and macroscale cartilage field mechanics. Subsequently, tissue–cell scale simulations, driven by the macroscale cartilage mechanical field information, can predict chondrocyte deformation metrics along with the mechanics of the surrounding pericellular and extracellular matrices. A high-throughput modelling and simulation framework is necessary to develop models representative of regional and population-wide variations in cartilage and chondrocyte anatomy and mechanical properties, and to conduct large-scale analysis accommodating a multitude of loading scenarios. However, realization of such a framework is a daunting task, with technical difficulties hindering the processes of model development, scale coupling, simulation and interpretation of the results. This study aims to summarize various strategies to address the technical challenges of post-processing-based simulations of cartilage and chondrocyte mechanics with the ultimate goal of establishing the foundations of a high-throughput multiscale analysis framework. At the joint–tissue scale, rapid development of regional models of articular contact is possible by automating the process of generating parametric representations of cartilage boundaries and depth-dependent zonal delineation with associated constitutive relationships. At the tissue–cell scale, models descriptive of multicellular and fibrillar architecture of cartilage zones can also be generated in an automated fashion. Through post-processing, scripts can extract biphasic mechanical metrics at a desired point in the cartilage to assign loading and boundary conditions to models at the lower spatial scale of cells. Cell deformation metrics can be extracted from simulation results to provide a simplified description of individual chondrocyte responses. Simulations at the tissue–cell scale can
be parallelized owing to the loosely coupled nature of the feed-forward approach. Verification studies illustrated the necessity of a second-order data passing scheme between scales and evaluated the role that the microscale representative volume size plays in appropriately predicting the mechanical response of the chondrocytes. The tools summarized in this study collectively provide a framework for high-throughput exploration of cartilage biomechanics, which includes minimally supervised model generation, and prediction of multiscale biomechanical metrics across a range of spatial scales, from joint regions and cartilage zones, down to that of the chondrocytes.

1. Background and motivation

The mechanics of cartilage and chondrocytes is a function of complicated load sharing between musculoskeletal joints of the body, individual tissue structures within the joint, and the organization and mechanical properties of cells and microstructure within the tissue (figure 1). The external loads applied to the body are translated to musculoskeletal joints, which may also endure large applied muscular forces. To achieve equilibrium, the passive structures of the joints often must sustain high stresses. Contact is the modality through which cartilage sustains load, where different regions of the musculoskeletal joint may exhibit varying contact force transmission. As one moves from the intermediate scale of joint compartments to the lower spatial scale resolution of the tissue, the load transmission at the articular surface is now resolved as a contact stress field on the cartilage surface. This load distribution is not only dependent on the total contact force, but it is also likely to be influenced by the cartilage geometry and properties, i.e. its thickness, radius of curvature and macroscopic material properties. Different zones along the depth of the cartilage (superficial, transitional and deep) exhibit varying stresses, strains, fluid pressures, etc., depending on the zonal thickness and underlying material properties, which are dictated by variations in collagen matrix organization and proteoglycan content [2]. These zonal loads influence the deformation and loading of chondrocytes, which are dependent on the relative mechanical properties of chondrocytes, and pericellular and extracellular matrices [3]. Chondrocyte shape, size and possibly their organization affect the loads transmitted to these cells and to their nuclei, which may cause cell damage and death [4] or may trigger mechanobiological responses for the regulation of extracellular matrix integrity [5].

A comprehensive understanding of the mechanical environment of cartilage and chondrocytes potentially has significant implications to evaluate the function of this tissue in health and disease. Such an understanding is necessary as cartilage bears loads exceeding multiple body weights during the activities of daily life and the tissue’s resulting mechanical environment can lead to regulation of its structural integrity. Mechanical insults not only result in matrix damage, but may also decrease chondrocyte viability [6]. Chondrocytes also respond to their mechanical environment, where mechanical transduction results in biological responses to maintain the structure of the extracellular environment [5]. Changes in the multiscale load sharing pathway alter mechanical signals transmitted to the cells, possibly compromising the mechanobiological function of chondrocytes. When quantified for healthy populations, the joint–tissue and tissue–cell scale mechanical environments of cartilage can better elucidate the forcing functions of chondrocyte mechanobiology, i.e. cellular tractions and osmotic pressure gradients [7]. Such information can be used as a design specification for tissue engineering of cartilage, i.e. how construct loading protocols should be implemented to promote matrix growth and adaptation in cell-seeded constructs. Mechanics also has a significant role in chronic and acute disorders of the cartilage. For example, the mechanical capacity and function of the cartilage and chondrocytes are affected during the onset and progression of osteoarthritis [8]. This debilitating musculoskeletal disease is the most common form of arthritis. Osteoarthritis influences more than 30% of adults above the age of 65 [9] and can manifest itself in any synovial joint. The knowledge of cartilage and chondrocyte loading can also indicate when and how trauma or tears may occur and it may offer an explanation for the formation of chondral defects. In any of these diseased or injured populations, quantification of cartilage and chondrocyte mechanics may have additional value for mechanical risk assessment and for diagnostic purposes, and may assist in the evaluation of disease progression or efficacy of prevention and intervention strategies.

Computational modelling and simulation provides a means of quantifying the mechanical environment of the cartilage and chondrocytes [1]. In vivo measurement of cartilage and chondrocyte mechanics in human subjects, where the joints are exposed to loadings of daily activities, is not necessarily within the reach of the current state of the art. Recent experimentation illustrated the possibility to quantify cartilage and chondrocyte deformations of fresh cadaveric samples in situ [10] and of animals in vivo [11], through invasive approaches. Modelling and simulation provide a means for in-depth exploration of such data through descriptive analysis [12]. In addition, through predictive simulations, it may be possible to establish multiscale mechanical interactions and to evaluate the influence of disease- or intervention-related perturbations on the mechanics of cartilage and chondrocytes [1].

Musculoskeletal joints exhibit large variations, anatomically and mechanically, at multiple scales, within an individual and across populations. Regional cartilage anatomy (curvature, thickness), stiffness, zonal properties, i.e. delineation of superficial, transitional and deep layers, and fibrillar architecture [13–16], and chondrocyte organization, shape, size [17,18] and mechanical properties [19] all vary largely among species and between individuals and can be altered by injury or disease. A high-throughput modelling and simulation framework seeks to provide the ability to represent such variations and conduct individualized analysis to assess this multiscale mechanical system under various loading conditions, which can be used for understanding trauma risk, for evaluating the protective performance of interventions, and for diagnosis and prognosis through the use of biomechanical markers. Likewise, parametric analysis supported by large-scale unsupervised simulations can allow probabilistic studies (for population analysis or for uncertainty estimation) [20], inverse analysis (for estimation of patient-specific mechanical properties) [21] and virtual prototyping (to design new therapeutic, surgical or rehabilitative management strategies) [22]. While the use of modelling and simulation for explorations of cartilage and chondrocyte mechanics is appealing, routine investigations are hampered by many technical challenges associated with the need to accommodate the nonlinear, multiscale and multiphasic biomechanical nature of this tissue [1].
The goal of this paper is to summarize various strategies to address the technical challenges of realizing a modelling and simulation workflow to characterize the mechanical environment of cartilage and chondrocytes. The tissue of interest is articular cartilage, but the outlined strategies are equally applicable to fibrocartilage and other tissues. The modelling and simulation strategy is based on a post-processing-type analysis approach (from higher spatial scales to lower spatial scales), where mechanical load sharing across joint–tissue and tissue–cell scales can be differentiated in a feed-forward manner. Individual components of the workflow are aimed to address difficulties in model development and its automation, scale coupling, computational burden and extraction of relevant biomechanical metrics from simulation results. Immediate utilization of a streamlined in silico framework for understanding the regional and population-wide mechanical responses of cartilage and chondrocytes may still not be computationally feasible, particularly when the physical phenomena need to be represented at a higher fidelity. Nonetheless, approaches outlined here lay the foundation of a high-throughput framework for large-scale analysis of cartilage mechanics at multiple scales.

2. High-throughput framework

The modelling and simulation work presented herein provides a feed-forward workflow where body-level musculoskeletal loading is differentiated to tissue-level and cell-scale deformations using multiple models in sequence (figure 2). If one is interested in a certain contact region of the joint, e.g. cartilage-to-cartilage contact area of the knee’s medial compartment, a model at the joint–tissue scale (spatial lengths of 0.001–0.01 m) can be constructed for prediction of detailed contact mechanics and, more importantly, the internal mechanics of the cartilage. Such models should incorporate the articulation between the cartilage surfaces for evaluation of the distribution of load transmission through the contact interface. These models will ideally include zonal thickness and material properties of the cartilage as well to appropriately predict the internal mechanical response of the tissue. Driven by the regional contact force (or articulation kinematics), simulations can predict contact pressure distributions and the detailed mechanics of the superficial, transitional and deep zones. In the case of solid phase analysis, tissue-level mechanical metrics include strains and stresses. With the addition of the fluid phase, as in biphasic simulations, they also include fluid flux and pressure. Such information can be used to drive cell scale models to predict chondrocyte deformation metrics. For example, one can pick up any point in the joint–tissue scale model, e.g. mid-point of the transitional zone, extract the deformation gradient, fluid pressure and fluid pressure gradient from the solution of the joint–tissue scale model, and apply these as loading and boundary conditions to a tissue–cell scale model (spatial lengths of 10⁻⁷ to 0.0001 m) representative of the cartilage and chondrocyte characteristics of that specific region [23]. This process is equivalent to using a one term Taylor series to approximate the field of displacements and fluid pressures in the neighborhood of the point of interest. From the results of the cell scale models, chondrocyte mechanical metrics can be calculated including the cell aspect ratio change, average effective (von Mises) stress and strain, and mass exchange rate. Through appropriate parametrization, streamlined data flow in between scales, automation of post-processing and effective computing strategies, a high-throughput modelling and simulation framework can be realized for prediction of cartilage and chondrocyte mechanics.

At the spatial scale of the musculoskeletal joint, simulations using a finite-element (FE) representation of the complete joint anatomy [24], e.g. the tibiofemoral joint, including its substructures such as the ligaments and meniscus, can provide the necessary information to define loading and boundary conditions of the aforementioned joint–tissue scale model. It should be noted that a comprehensive model, where all structures of the joint are represented, may be used to bypass the use of the compartmental joint–tissue scale model [25]. Yet, a detailed representation of zonal anatomy, requiring a finer level of discretization to accommodate zonal thicknesses, may be computationally prohibitive to include in the representation of the entire musculoskeletal joint. Contact information, e.g. force or articulation kinematics, to drive the joint–tissue scale model may be estimated from experimentation. Such measurements are possible, either directly on cadaver specimens by using contact pressure measurement systems [26], or indirectly on live subjects through the use of biplanar fluoroscopy by estimating proximity of articulating...
surfaces [27]. In other words, an elaborate model of the joint can be foregone, and a regional model can be employed instead, with boundary conditions and loads taken directly from regional measurements in the experiment.

Extensibility is paramount for a modelling and simulation framework as the desired utility of the in silico workflow in future may necessitate replacing models and analysis types with higher fidelity versions. The feed-forward approach lends itself to expansion towards higher and lower spatial scales. At the higher spatial scale, body-level musculoskeletal models can be used to determine muscular joint loading [28], which can be processed to drive joint-level FE analysis, e.g. for muscle force-driven patellofemoral joint contact analysis [29]. At the lower spatial scale, cell deformation metrics can be used to drive cellular–subcellular scale models, i.e. incorporating the cytoskeleton (actin and tubulin) [30], to understand the mechanical load transmission to intracellular components, in particular to the nucleus. Similar submodelling can be performed for the extracellular matrix, for example, to evaluate microstructural-level loading of collagen fibres, which may provide the relationship between macroscopic tissue damage accumulation and microscale fibre failure [31]. At all spatial scales, it is also possible to increase the fidelity of mechanical representation; in terms of physics (nonlinear elasticity, multiphasic response) [32], with additional anatomical components, and by using more advanced constitutive relationships [24,33]. Utilization of open source and freely accessible software, e.g. Python (for scripting to automate tasks; https://www.python.org/), Salome (for geometry and mesh generation; http://www.salome-platform.org/) and FEbio (for FE analysis; http://www.febio.org/) [34], facilitates future development by any interested parties.

3. Tackling challenges

This section summarizes our recent published and unpublished work to overcome various technical challenges associated with high-throughput modelling and simulation in multiscale biomechanics of the cartilage and chondrocytes. The term throughput is used in a general sense, referring to the rate at which something, in this case modelling and simulation, can be conducted. The challenges (and the proposed solutions) span the life cycle of the modelling and simulation workflow. To enable high-throughput modelling and simulation, the ‘challenge of automated model development’ should be met. Both joint–tissue and tissue-cell scale models need to be developed on-the-fly and in an unsupervised manner to facilitate large-scale analysis. Irrespective of being high-throughput or not, the ‘challenge of appropriate scale coupling’ exists. If the information exchange between scales is erroneous, multiscale analysis of cartilage may become a useless effort. High-throughput multiscale simulations of cartilage and chondrocytes also require expedited solutions of large number of models, i.e. the ‘challenge of computing’, for different joint regions, tissue zones and loading conditions. Interactive post-processing of simulation results, in order to understand biomechanics of cartilage and chondrocytes, is not suitable for high-throughput analysis. Therefore, the ‘challenge of streamlined extraction of information’ needs to be addressed. The ‘challenge of accessible modelling and simulation framework’ is readily addressed by using free and open source software and publicly sharing models and tools developed as part of the summarized studies (see §5).

3.1. Automation of modelling

3.1.1. Joint–tissue scale model development

For a given contact region at the joint–tissue scale, the articulating cartilage tissue exhibits variations in its gross geometry, e.g. thickness [13]. These parameters dictate how contact pressures are distributed during load transmission between interfacing cartilage, e.g. for the hip [35]. Within the tissue, superficial, transitional and deep zones of the cartilage may have varying thicknesses and their mechanical responses depend on the fibrous network of collagen (primarily type
II), water content (75–80% wet weight), dissolved ions and aggregating proteoglycans [2]. The material properties are depth-dependent [36], and as a result of varying fibre alignment in each zone, they also exhibit anisotropy [37]. All these influence how the mechanical deformations and loads distribute within the tissue.

A streamlined workflow for model generation at the joint–tissue scale should exploit the parametrization of anatomical and mechanical properties such that any desirable variations can be represented expeditiously in a minimally supervised manner, e.g. within an input text file. Our team has been working on the development of an automated model generation procedure to represent regional anatomy and depth-dependent material properties for articulating regions of cartilage [38,39] (figure 3). Python scripting was used along with SALOME to automate geometry and mesh generation and to prepare a model ready to be analysed using FEBio, including definitions of material properties and prescription of loading and boundary conditions. The user can specify anatomical parameters in a human-readable configuration file, which includes overall dimensions of the articulating region of interest, cartilage boundaries and zonal delineation with ellipsoidal surface approximations (for all zones, essentially allowing representation of non-uniform thickness), and mesh density. The execution of Python scripts generates the model geometry and discretizes the whole region (into a hexahedral mesh) with appropriate element, node and surface sets. The user can also prescribe depth-dependent material properties, e.g. elastic modulus, Poisson’s ratio, hydraulic permeability, fibre properties [37], all as a function of FE centroid location. The regional contact force (to drive the model) and boundary conditions for the margins of the model, e.g. confined or unconfined, are other user-specified inputs. In an additional step, execution of Python scripts allows the generation of a human-readable model input file that can be used with FEBio to conduct FE analysis. Results of such analyses quantify the mechanical response of the cartilage at a given anatomical and mechanical state and for preferred loading and boundary conditions, which can also be fed-forward to tissue–cell scale models for further simulations. This scripted environment allows generation of a plethora of models with different characteristics (figure 3), with minimal user interaction through the automation of geometry, mesh and model definition processes.

![Figure 3. Parametrization can simplify the modelling process at the spatial scale incorporating joint- and tissue-level features. Articular cartilage exhibits large variations at this spatial level. The curvature of contact surfaces and boundaries of the superficial, transitional and deep zones can be different based on the individual, the musculoskeletal joint and the region of interest within the joint. Cartilage also has depth-dependent mechanical properties, dictated by collagen and proteoglycan content, and collagen fibre architecture. Through an automated pipeline, the generation of numerous models and the execution of simulations under different loading conditions may be conducted with ease. (Online version in colour.)](https://rsfs.royalsocietypublishing.org/content/5/20140081)

### 3.1.2. Tissue–cell scale model development

Models at the tissue–cell scales, when and if generated in an expedited fashion, can allow large-scale estimation of chondrocyte mechanics at any desired location within a given zone of the cartilage. Diverse anatomical variations exist for chondrocyte organization, shape and size across the layers of cartilage and sometimes within the layers [17]. A pericellular matrix surrounds the chondrocyte(s) to form a unit called a chondron. In the superficial zone, the chondrocytes exhibit flatter, ellipsoidal shapes, with relatively denser cellular distribution [17]. Depending on the body region, e.g. for the ankle [40], several cells may cluster to form a multicellular chondron. In the transitional zone, chondrocyte distribution is sparser [17]. Chondrocytes are nearly spherical and typically form chondrons with a single cell. In the deep zone, stacking of chondrocytes into columnar chondrons is observed [17]. Shape, size and organization (and variations in each) can influence the way mechanical loading is transmitted to individual cells, and therefore the cell’s mechanotransduction. The relative material properties of chondrocytes, their pericellular environment and the extracellular matrix are also important to resolve the mechanical load sharing problem. The pericellular matrix has a significant role in the regulation of chondrocytes’ mechanical environment and their mechanobiological response [41]. The region is primarily of type VI collagen, which is exclusive to this zone [41]. The extracellular matrix includes a zone-dependent fibrous network of collagen of primarily type II [2] (as described above).

A robust workflow for model development at the tissue–cell scale can include parametrization of anatomical and mechanical properties such that rapid and appropriate representations specific to specimen, and tissue location and depth can be constructed for prediction of chondrocyte mechanics in a
high-throughput manner. In a recent study, our team developed an automated model generation procedure to represent cellular organization, shape and size for zone-specific tissue–cell scale models [42] (figure 4). PYTHON scripting was used to interact with SALOMÉ to automate geometry and mesh generation for a rectangular volume with a distribution of spherical/ellipsoidal inclusions. This geometrical model is a representative volume for cellular-level description of the tissue. In a human-readable file, the user can specify the tissue arrangement (a variable rectangular volume, with single or multiple stacked layers), cell shape and size (spherical or ellipsoidal with variable radii), pericellular layer shape and size (spherical or ellipsoidal with variable radii), cell arrangement (location and orientation, clustering of multiple cells within a chondron) and mesh density. The geometrical properties can be specified explicitly, or by providing cellular geometry and distribution statistics, e.g. mean and standard deviations for cell radii, number of cells per unit volume, and pericellular thickness. The execution of PYTHON scripts provides discretized (tetrahedral) model geometry in a human-readable form, including appropriate element, node and surface sets to subsequently define loading and boundary conditions. Through additional configuration parameters, users can assign material properties for the extra-cellular matrix in a similar fashion as was done for the cartilage layer properties described for the joint–tissue scale models. In addition, direction-dependent material properties of the pericellular matrix can be prescribed, i.e. the region’s fibre organization aligned tangentially to the chondrocyte surface [43] as a function of element centroid location relative to the chondrocyte. Finally, the loading and boundary conditions can be prescribed using deformation gradient and fluid pressure information (for biphasic case), e.g. from the results of joint–tissue scale simulations. PYTHON scripting manages the assembly of this heterogeneous information to generate a human-readable model input file that can be used with FEBIO to conduct FE analysis. Simulations can quantify the mechanical response of individual or clusters of chondrocytes for a given anatomical and mechanical property set and for preferred loading and boundary conditions. Similar to the pipeline provided for the joint–tissue scale modelling, this scripted environment allows generation of a plethora of models with varying characteristics individualized for the cartilage layer of interest (figure 4), with minimal user interaction and through the automation of the geometry, mesh and model definition processes.

3.2. Scale coupling
Multiscale analysis requires the transfer of mechanical information from joint–tissue scale simulations (where cartilage mechanics is quantified) to tissue–cell scale models (where chondrocyte mechanics can be predicted). This data exchange requires a multitude of assumptions. A fundamental assumption is the required consistency of the tissue mechanical behaviour between the joint–tissue and tissue–cell scales. At the higher spatial scale, the cartilage may be represented by homogenized material properties [24]. This constitutive response should be equivalent to the average response of the tissue–cell scale model, which represents the cartilage in more detail, including the delineation of chondrocytes, pericellular matrix and the extracellular matrix. Multiple strategies exist to enforce this constraint. For example, the homogenized tissue properties (of the joint–tissue scale model) and the properties

![Diagram of cartilage structure and tissue zones](image)

**Figure 4.** Based on statistical descriptors of cellular anatomy and organization, model generation can be automated at the spatial scale of chondrocytes. Zonal cartilage features and chondrocyte properties influence the mechanics of cells. Chondrocyte size, shape and distribution vary largely between individual layers of the cartilage. These properties are also dependent on the location of the cartilage within the joint and across joints. Cell size, shape and density can be prescribed in a statistical manner to generate a representative volume for a desired zone of interest. Definitions of parameters representing pericellular matrix anatomy and material properties for chondrocytes, and pericellular and extracellular matrices complete the computational representation of the cartilage at the tissue–cell scale. Tissue-level mechanics information can be mapped on the boundaries of the representative volume as loading and boundary conditions to carry out simulations. This streamlined model generation approach provides the opportunity to build models in an expedited fashion. (Online version in colour.)
of the extracellular matrix (of the tissue–cell scale model) can be treated as the same. When the volume fraction of the extracellular matrix is large, the contribution of the pericellular matrix and chondrocytes to the tissue scale response can be neglected. We have used this assumption in our previous studies where 94.2% of the tissue–cell scale model was the extracellular matrix (for an 11 cell model of the transitional zone of cartilage) [23,25]. Alternatively, homogenization based on spherical inclusions [44] can be adapted to estimate joint–tissue scale macroscopic properties of the cartilage as an analytical function of tissue–cell scale constituent properties and their volume fraction. Finally, computational homogenization strategies can be employed [45], where average mechanical response of a representative volume element (in this case, the tissue–cell scale model) can be calculated from simulations of uniform loading cases, and a surrogate macroscopic constitutive law can be fitted to determine homogenized material properties for the joint–tissue scale model. This approach may be beneficial for complicated and dense cellular arrangements [17] and for explicit fibre-based modelling of the tissue matrix microstructurally [31]. The gross impact of tissue heterogeneity and the contributions of cells and fibres on tissue behaviour can be incorporated within the continuum mechanics framework.

Another assumption when transferring information from the higher spatial scale to the lower one is related to the level of approximation of loading and deformation metrics that need to be provided as boundary conditions for the tissue–cell scale model. One option is to overlay the boundary of the tissue–cell scale model within the joint–tissue scale model geometry and linearly interpolate the solution of the macroscopic scale as displacement (and fluid pressure if necessary) boundary conditions [46,47]. While this approach does not require any further assumptions for the required detail of data exchange, it may be cumbersome to do so, particularly for large numbers of cellular-level analyses. Alternatively, one can use loading and deformation information from a given point within the cartilage of the joint–tissue scale model to quantify chondrocyte deformations [25]. If the size of the tissue–cell scale model is negligible when compared with the characteristic size of the corresponding geometry in the joint–tissue scale model, a first-order approximation may be sufficient, i.e. using the deformation gradient at a given macro-scale point to set boundary conditions of the cell scale model. Unfortunately, for cartilage and chondrocytes, decoupling of length scales can be problematic, potentially requiring a second-order approximation for data exchange [45], i.e. using the deformation gradient, which is a measure of local deformations (shape change) at a given point, and deformation Laplacian, which provides the spatial derivatives of the deformation gradient. In a recent study, our team evaluated the role of data passing assumptions in the characterization of biphasic chondrocyte mechanics [23]. A 1 x 1 mm homogeneous and isotropic cartilage tissue underwent non-uniform compression (imitating a curved indenter, 0–10% nominal strain across the boundary; figure 5). Along the loading axis, asymmetric permeability conditions were prescribed (permeable at the indentation site, impermeable at the fixed bottom); lateral walls of the model were confined and impermeable. Full loading was applied in 0.1 s with a relaxation duration up to 100 s. The biphasic response of the model was obtained using FEBio. Loading and deformation information at the centre point of this model was extracted to drive a
tissue–cell scale model. The lower scale model was a 100 μm × 100 μm × 100 μm representative volume with spherical cellular inclusions representative of the transitional layer of the cartilage [48] (11 cells within the volume; chondrocyte radius of 5 μm and pericellular thickness of 2.5 μm; figure 5). Chondrocyte, pericellular matrix and extracellular matrix material properties were homogeneous, isotropic and biphasic. Two types of data passing schemes were employed to test the required fidelity when prescribing tissue–cell scale boundary conditions. First, a first-order Taylor series expansion was used to map macroscale deformation gradient, and fluid pressure and its gradient to the boundaries of the tissue–cell scale model. Then, a second-order Taylor series expansion, with additional terms containing the deformation and fluid pressure Laplacians, was used. Results of both these cases were compared against the predictions of a control model, where the joint–tissue scale model had a region at its centre explicitly incorporating the chondrocyte and pericellular matrix representation exactly as in the separate tissue–cell scale model. While the results were not necessarily compared against the behaviour of actual cells, this investigation revealed that data passing assumptions may lead to additional misrepresentation of the chondrocytes’ mechanical environment, at least for the case presented here (figure 5). It is advised to use a higher-order data passing scheme or one that relies on interpolation (albeit with the increased difficulty for high-throughput analysis). It should also be noted that for each additional term included in a Taylor series approach, the order of the required tensor increases by one. In three dimensions, this will contribute $3^{n+1}$ additional coefficients to be calculated for both the solid and fluid phases, where $n$ is the order of the series.

Another challenge for cell scale analysis has been the selection of an appropriate volume for the tissue–cell scale model. Ideally, the representative volume should be small to allow the scale separation assumption in that the characteristic lengths of higher and lower scales differ by orders of magnitude. The representative volume should also be large enough to include characteristic geometrical features of the region’s cellular organization. Traditionally, computational simulations of chondrocytes used models with an edge length of 100 μm [49], originated from the study by Guilak & Mow [46]. This model and its successors placed a single chondron at the centre of the modelling volume, commonly overlooking the actual cell density of the cartilage zone. One has multiple options when building models which keep the volume ratio of the chondrocytes and extracellular matrix the same. Cartilage can be represented at the tissue–cell scale using a single cell model with a small overall model volume or by placing multiple cells in larger volumes. Ideally, all these models should result in similar chondrocyte mechanics predictions. Yet, with the increased size of the representative volume, mechanical interactions between the cells can be investigated and the influence of a cell’s location within the neighbourhood of the point of interest can be explored. Our ongoing work explored the influence of representative volume size on the prediction of chondrocyte loading and deformation [50]. Tissue–cell scale models representative of chondrocyte density of the cartilage’s transitional zone were developed, starting with representative volumes containing a single cell, through 20 cells (figure 6). The model edge length varied between 30 and 120 μm to keep cell density constant. The cells were randomly distributed within the representative volume maintaining a prescribed minimum distance between cells and the volume boundaries. For each level of cellular representation, multiple models were generated and solved providing mechanics information for at least 130 chondrocytes in total. Chondrocytes were spherical, each with a radius of 5 μm and a pericellular thickness of 2.5 μm. Biphasic simulations were conducted with loading and boundary conditions reflecting the deformation gradient and fluid pressure (and their derivatives based on a second-order data passing scheme) obtained from macro-level simulations. The macroscale joint–tissue model was loaded by a non-uniform surface displacement with asymmetric fluid behaviour (described above) [23]. The average predicted cell metrics (and their variability) became more consistent as the representative volume size included larger number of cells, e.g. for chondrocyte shape factor (defined in [51]; figure 6). Differences can be attributed to the variability of cell location within the representative volume and/or cell-to-cell mechanical interactions. Simulations were completed approximately 10 times faster at smaller representative volumes with lower cell numbers. However, for this specific illustrative case, distribution of cell mechanical metrics was better captured as the volume of interest increased—possibly providing location-dependent variability of cell mechanics and cell-to-cell mechanical interactions.

### 3.3. Computing

Simulations both at the joint–tissue scale and the tissue–cell scale can be inherently computationally intensive. Geometrical and material nonlinearity, consideration of multiple...
material phases, and large mesh sizes may all contribute to the added cost of conducting simulations at the single spatial scale. Multiscale analysis further complicates this issue as multiples of models need to be solved for quantification of cartilage and chondrocyte mechanics. Nonetheless, the post-processing-based feed-forward approach to multiscale simulations provides various opportunities to tackle the computing burden. From a user’s perspective, multiple simulation strategies may exist (figure 7): parametric studies at the joint–tissue scale, parametric studies at the tissue–cell scale, a combination of both, and lastly large-scale simulations at the tissue–cell scale for characterization of chondrocyte mechanical environment for a given solution of a joint–tissue scale model. In the first use case, one may be interested in the cartilage mechanics for a set of loading and boundary conditions or in the sensitivity of the cartilage mechanical environment to variations in anatomical and mechanical properties. Similarly, as an example of the second use case, one may be interested in chondrocyte mechanics as a function of macroscopic deformations or owing to variations in anatomical and mechanical properties of the chondrocytes, pericellular matrix and extracellular matrix. These analyses can be conducted in conjunction to evaluate the changes in multiscale load sharing path as model parameters at all scales are perturbed simultaneously (third use case). All these use cases can lead into probabilistic analysis [20], i.e. for assessment of population variations, sensitivity studies and uncertainty quantification. The final use case illustrates the multiscale feed-forward analysis approach. First, a joint–tissue scale model is solved. At each point of interest in that model, e.g. all locations across all zones of the cartilage, tissue–cell scale models are simulated to map chondrocyte mechanical metrics on the topology of the macroscale tissue anatomy. This may require the solution of hundreds to thousands of tissue–cell scale models, all driven by the results of a single joint–tissue scale model. In all the aforementioned cases, simulations are loosely coupled, i.e. minimal or no communication is required between models of the same scale. Massively scalable parallel computing strategies can be employed by distributing simulation workload to independent computing nodes in order to expedite the whole solution process. Albeit various computational strategies, case-specific model simplifications may still be necessary to balance the requirements of a high-throughput simulation workflow and scientific accuracy.

In a previous study, an approach to tackle large-scale simulations through the use of loosely coupled computing strategies was presented for the prediction of chondrocyte deformations in tibial and femoral cartilage [25]. First, a tibiofemoral joint model was solved to obtain tissue-level deformation metrics for the cartilage during the application of a compressive load with a magnitude of one body weight. This model incorporated the anatomical detail of the joint articulation and the passive tissue structures, e.g. ligaments, and a homogenized representation of tissue material properties, i.e. apparent properties at the macroscale. Nonlinearly elastic FE analysis provided deformation gradients at each element centroid within the transitional layers of femoral and tibial cartilage. For each element centroid, a tissue–cell scale model was solved to obtain a joint-level distribution of chondrocyte deformations within femoral and tibial cartilage. The tissue–cell scale model was a $100 \times 100 \times 100 \, \mu m$ representative volume with spherical cellular inclusions and homogeneous and isotropic elastic properties for chondrocytes, pericellular matrix and extracellular matrix. The cellular organization represented the transitional zone [48]: 11 cells within the volume;
chondrocyte radius of 5 μm and pericellular thickness of 2.5 μm. The boundary conditions of the model reflected deformations based on a first-order data passing scheme. This analysis required the solution of 7822 tissue–cell scale models (same model simulated with different boundary conditions extracted from 7822 locations in tibial and femoral cartilage). Exploiting the parallel nature of feed-forward analysis, these models were solved in a high-performance computing environment (multiple compute nodes using 101 threads on Glenn Cluster of the Ohio Supercomputer Centre), using FEBio for FE analysis. At the time, Glenn Cluster provided up to 9572 compute cores, ranging in frequencies of 2.4–2.6 GHz, offering a peak performance of more than 75 teraflops. Through parallel processing, solution of 7822 models resulted in a wall-clock time of 19 h. If using a single CPU, estimated wall-clock time would be 1735.1 h (72.3 days), indicating a prohibitive computing burden. A study of scalability was not performed, but because each tissue-scale model is entirely independent, the limiting factor should only be the initial distribution of problems to computer nodes, and likewise, the pooling of results after simulations have completed. Some network overhead is expected but it is ideally low, and thus scaling to many thousands of nodes is highly feasible. The feed-forward analysis provided the possibility for this loosely coupled parallel processing. In turn, timely prediction of chondrocyte mechanics for large geometrical regions of femoral and tibial cartilage was attainable (figure 7).

For cartilage and chondrocyte mechanics, nonlinear simulations incorporating the biphasic or multiphasic nature of the tissue and cell behaviour may also increase the computational cost. It should be noted that FE analysis software provides capabilities for multithreading (on a single computing node) [52–54] and parallel processing (on a number of distributed computing nodes) [53,54], during the generation of system stiffness matrices and/or the solution of linearized system equations. The use of these built-in computing strategies may expedite single simulation of a given joint–tissue or tissue–cell scale model. FEBio, the FE analysis package used in our modelling and simulation workflow, provides multithreading on shared memory computing architectures [52]. By relying on PARDISO, a shared-memory multiprocesssing parallel direct sparse solver for linear systems of equations [55], solution times can be reduced on a given compute node equipped with multiple processors.

3.4. Post-processing
FE analyses, both at the joint–tissue and tissue–cell scales, result in a large set of variables to evaluate the mechanical environment of the cartilage and chondrocytes. However, it may be challenging to interpret the raw simulation results without post-processing, where desirable biomechanical metrics within the context of research question or clinical purpose can be calculated. Similarly, movement of information from joint–tissue simulations down to tissue–cell scale models requires preparation of the raw simulation results of the higher spatial scale in a form amenable to use in the models of the lower spatial scale. In this section, post-processing solutions relying on Python scripting are provided for simulations conducted using freely accessible FEBio software.

At the spatial scale of joint–tissue, interpretation of FE analysis results, e.g. for individual zones of the cartilage, is possible by using principal stress and strain (solid phase) and fluid flux and pressure (fluid phase) distributions, which are common for the evaluation of biphasic mechanics. For multiscale analysis, more detailed information about the mechanical loading and deformation is needed. One may want to solve a tissue–cell scale model associated with a point in the macroscale model, e.g. at an element centroid. The complete mechanical state of the point of interest needs to be mapped to the tissue–cell scale model as boundary conditions. For biphasic simulations for example, the time histories of the deformation gradient (and possibly the deformation Laplacian) and the fluid pressure (and its derivatives) will be necessary for data passing (also see discussion on scale coupling above). Such information is not readily available in FE analysis results and it needs to be derived from nodal displacement and fluid pressure information. To facilitate this procedure, Python scripts were developed to parse through FEBio output files to extract raw nodal and element results for desired sets, e.g. elements for which multiscale analysis would be performed [23]. Using the FE isoparametric formulation, the nodal displacements and fluid pressures can be used to compute time histories of deformation gradient, fluid pressure and their spatial derivatives for desired points of the joint–tissue scale models. This information can subsequently be provided to tissue–cell scale models in a streamlined human-readable form. For illustration of joint–tissue scale post-processing, the solution of a 1 x 1 x 1 mm homogeneous and isotropic cartilage tissue was explored (figure 8). A summary of the model and simulation conditions is provided here, and further details are available elsewhere [23]. The cartilage model underwent non-uniform compression, essentially representing the influence of a curved indenter (0–10% nominal strain across the boundary). The biphasic response was sought, with asymmetric permeability conditions along the loading axis (permeable at the indentation site, impermeable at the fixed bottom), and with confined and impermeable walls. Full loading was applied in 0.1 s with a relaxation duration up to 100 s. The time histories of compressive strain and fluid pressure for the centroid of the element at the centre of the tissue are shown in figure 8.

At the spatial scale of tissue–cell, interpretation of FE analysis results, e.g. for the chondrocytes, pericellular matrix and extracellular matrix, is also possible through the evaluation of principal stress and strain (solid phase) and fluid flux and pressure (fluid phase) distributions. This information can provide an understanding of nearby focal stresses and strains on chondrocyte surfaces and within, which may indicate potential failure and mechanical signalling mechanisms. Yet, an understanding of multi-modal loading and overall deformations of individual cells and/or populations of cells may be necessary. Metrics such as cell height and width, aspect ratio and volume (undeformed and deformed) have commonly been used for describing experimental data [11] and simulation results [56], with the intention to associate cell mechanics with cell damage and mechanical stimuli. Such descriptors, however, are not readily available in raw simulation results, and automated procedures are necessary if these variables need to be evaluated for hundreds of cells and for a large number of simulation conditions. Motivated by this need, Python scripts were developed to parse FEBio output files to extract raw results for nodes, elements and surfaces representing each chondrocyte [23,51]. The analysis scripts are capable of calculating the volume of each cell,
and using volume-weighted averaging, the effective stress and strain, and maximum shear strain. The net mass exchange rate for each chondrocyte is also calculated by the surface integral of fluid flux. This metric, obtained from biphasic analysis, can be an indicator of cell-to-cell signalling.

More common cellular deformation metrics are calculated by first finding the mass moment inertia of each cell and then finding the eigenvalues of this tensor, which represent the principal moments of inertia \[57\]. These eigenvalues are processed to calculate axis lengths of the inertial ellipsoid, which in turn provide a shape factor from their ratio \[51\]. Further processing provides chondrocyte height, width and depth, and therefore aspect ratio \[51\]. From the results of biphasic FE analysis, time histories of all these cell scale metrics are available in a human-readable form. Post-processing capabilities at the tissue–cell scale were illustrated using the results of a \(100 \times 100 \times 100 \) \(\mu m\) representative volume with spherical cellular inclusions and homogeneous and isotropic biphasic properties for chondrocytes, pericellular matrices and extracellular matrix (figure 9). The cellular organization represented the transitional zone \[48\]: 11 cells within the volume; chondrocyte radius of 5 \(\mu m\) and pericellular thickness of 2.5 \(\mu m\). The boundary conditions of the model reflected deformation gradient and fluid pressure (and their derivatives based on a second-order data passing scheme) at the centre of a joint–tissue scale model described previously in this section. Extracellular matrix properties were kept the same as the macroscopic properties of the joint–tissue scale. Time histories of various biomechanical loading and deformation metrics for each chondrocyte are provided in figure 9.

4. Concluding remarks

The vision and initial realization of a high-throughput modelling and simulation workflow for large-scale analysis of cartilage at the joint–tissue scale, post-processing of simulation results can provide common descriptors of the tissue's mechanical environment at any desired location. In this case, time histories of compressive strain and fluid pressure were extracted for a point at the centre of a cartilage region \(1 \times 1 \times 1 \) \(mm\). A non-uniform displacement profile was prescribed at the top. Impenetrable walls confined the tissue. (Online version in colour.)
cartilage and chondrocyte mechanics was provided in this document. Various strategies to realize individual components of the modelling framework were implemented with a specific focus on the automation of developing models at the joint–tissue and tissue–cell scales that are capable of incorporating subject-specific and regional variations in anatomical and mechanical parameters. Some of the technical challenges associated with multiscale analysis were addressed. In separate studies, the requirement for higher-order data passing from the joint–tissue scale down to the tissue–cell scale was emphasized, and the appropriateness of the selection of the tissue–cell scale model size was confirmed. From a usability perspective, the need for streamlined extraction of important biomechanical metrics, for the evaluation of cartilage and chondrocyte mechanics and for feed-forward movement of the data through the spatial scales was met. Computing strategies, in particular the use of parallel processing, were presented as a means to exploit the loosely coupled nature of post-processing-based multi-scale analysis to conduct thousands of simulations in an expedited fashion.

The outlined modelling and simulation approach focused on a feed-forward analysis strategy relying on the sequential solution and post-processing of computational models at the joint–tissue scale and then at the tissue–cell scale. This approach was found to be suitable for solving the load sharing problem starting from the higher spatial scales, and determining the loads and deformations at various cartilage layers and at the level of individual chondrocytes. A significant advantage of this feed-forward approach is related to the reduced demand on computing as macro- and microscale models need to be solved only once for a desired loading condition at preferred locations. This advantage fundamentally relies on the assumption that tissue representations at multiple scales are mechanically consistent, i.e. they provide the same overall constitutive behaviour. It may not provide the structure–function relationships, which can be accomplished through a feedback loop, i.e. by computational homogenization that relies on iterative solutions of lower scale models during the solution process of the model at the higher spatial scale [45]. It should be noted that such a feedback loop is not necessary to obtain the mechanical environment of cartilage and chondrocytes for a given tissue state. It will be necessary when constitutive properties change as a result of tissue growth and damage [31]. Nonetheless, the tools presented in this study can be adapted for such purposes. It is worth mentioning that this study did not provide an understanding of error propagation when one uses the proposed multiscale modelling workflow. Owing to the feed-forward nature of the simulations, it is anticipated that the errors will accumulate in an additive manner as one moves from the higher spatial scale of the joint down to the scale of chondrocytes.

The components of the modelling and simulation workflow were prototyped using Python scripting, for pre-processing of the models using user-specified parameters and for post-processing of the simulation results. The integrated and scriptable computer-aided design package SALOME was used in an unsupervised manner for generation of models. Likewise, FEBio, FE analysis software for biomechanics [34], was used to conduct simulations. These readily available, open source and freely accessible pieces of software provided the means for quick prototyping. Nonetheless, it is possible to integrate model development, simulation and post-processing tools into a turn-key modelling and simulation platform. Some desirable features for the expansion of this framework are noted as follows. Geometrical model generation procedures can use explicitly defined individual anatomy, e.g. from image segmentation, for tissue representation at the macroscale, and for cell representation at the microscale. For models at the joint–tissue scale, the representation of additional components, e.g. meniscus, will be necessary when the desired contact region of interest becomes wider. For models at the tissue–cell scale, incorporation of cell membrane may be needed to investigate the influence this structure’s permeability on the mechanical behaviour of the chondrocyte. Robust physics formulations provided in FEBio [32] can also allow the modelling and simulation workflow to include multiphasic behaviour of the cartilage and chondrocytes beyond biphasic analysis, to incorporate the mechanical effects of charged particles and proteoglycans, namely osmotic pressure gradients and swelling.

The capacity to explore cartilage mechanics for any joint of interest, at any region of contact within the joint, for any zone within the tissue and under different loading conditions when supported by high-performance computing can empower large-scale analysis of cartilage and chondrocyte function in health and disease. Population responses can be quantified based on probabilistic analysis and in-depth sensitivity studies can be performed to understand the role of biomechanical markers in cartilage function. It is anticipated that this framework will be used to understand cartilage and chondrocyte mechanics during ageing. Ageing manifests anatomical and mechanical changes at multiple scales [13,16,19,58], which in turn perturb the mechanical environment and therefore associated biological responses to mechanical stimuli. Similarly, this modelling and simulation workflow will likely be a significant toolset to evaluate the complicated mechanical state of the tissue and cells during the onset and progression of osteoarthritis.

5. Access to data, models and scripts
Various scripts, models, data and simulation results can be found at the project site, https://simtk.org/home/j2c, in the downloads section.

Acknowledgements. This study was conducted at the Cleveland Clinic and was previously presented at the Seventh World Congress of Biomechanics, 6–11 July 2014, Boston, MA, USA [59]. The document provides a synthesis of our group’s published and unpublished work on in silico mechanics of cartilage and chondrocytes. The authors acknowledge Simbios, NIH National Center for Biomedical Computing at Stanford University, http://simbios.stanford.edu/ (project hosting); Ohio Super Computer Center, https://www.osc.edu/ and Extreme Science and Engineering Discovery Environment, https://www.xsede.org/ (high-performance computing); FEBio, http://www.febio.org/ (FE analysis software) [34]; SALOME, http://www.salome-platform.org/ (integrated software for computer-aided design); Python, https://www.python.org/ (scripting). The authors are also grateful for in-depth discussions with Jason Halloran and Snehil Chokhandre (Cleveland Clinic), Cees Oomens and Rene van Donkelaar (Eindhoven University of Technology), Jeff Weiss and Steve Maas (University of Utah), and Farshid Guilak (Duke University).

Funding statement. The research activities were supported by the National Institutes of Health, in particular by the National Institute of Biomedical Imaging and Bioengineering (R01EB009643; Principal Investigator: A.E., https://simtk.org/home/j2c) and partially by the National Institute of General Medical Sciences (R01GM104139; Principal Investigator: A.E., https://simtk.org/home/openknee).