Modeling IL-1 induced degradation of articular cartilage

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Abstract

In this study, we develop a computational model to simulate the *in vitro* biochemical degradation of articular cartilage explants sourced from the femoropatellar grooves of bovine calves. Cartilage explants were incubated in culture medium with and without the inflammatory cytokine IL-1 α . The spatio-temporal evolution of the cartilage explant's extracellular matrix components is modelled. Key variables in the model include chondrocytes, aggrecan, collagen, aggrecanase, collagenase and IL-1 α . The model is first calibrated for aggrecan homeostasis of cartilage *in vivo*, then for data on (explant) controls, and finally for data on the IL-1 α driven proteolysis of aggrecan and collagen over a 4-week period. The model was found to fit the experimental data best when: (i) chondrocytes continue to synthesize aggrecan during the cytokine challenge, (ii) a one to two day delay is introduced between the addition of IL-1 α to the culture medium and subsequent aggrecanolysis, (iii) collagen degradation does not commence until the total concentration of aggrecan (i.e. both intact and degraded aggrecan) at any specific location within the explant becomes ≤ 1.5 mg/ml. and (iv) degraded aggrecan formed due to the IL-1 α induced proteolysis of intact aggrecan protects the collagen network while collagen degrades in a two-step process which, together, significantly modulate the collagen network degradation. Under simulated *in vivo* conditions, the model predicts increased aggrecan turnover rates in the presence of synovial IL-1 α , consistent with experimental observations. Such models may help to infer the course of events *in vivo* following traumatic joint injury, and may also prove useful in quantitatively evaluating the efficiency of various therapeutic molecules that could be employed to avoid or modify the course of cartilage disease states.

46 **Keywords:** Cartilage, Interleukin-1, Computational model, Proteolysis, Degradation,
47 Explant.

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Introduction

There are many signaling molecules that mediate the biochemical degradation of cartilage tissue, including inflammatory cytokines [1-3]. The most commonly reported inflammatory molecules mediating cartilage biochemical degradation include TNF- α , IL-1 β , IL-6, prostaglandins, nitric oxide (NO) and reactive oxygen species (ROS) [1, 2, 4-7]. Amongst these, the mechanisms of cartilage degradation mediated through the interleukin-1 (IL-1) family of inflammatory cytokines is an area of intense investigation [2, 4, 5, 8, 9]. In one such study [9], a detailed set of *in vitro* experiments were designed to investigate the response of bovine cartilage explants to interleukin-1 α (IL-1 α). As an important step towards a general model of cartilage homeostasis, the aim of the present paper is to develop an *in silico* analysis of these *in vitro* experiments [9]. In doing so, we hope to develop a suitable parameterized computational model that can simultaneously capture the key spatio-temporal biochemical interactions responsible for the experimentally observed degradation of cartilage explants upon exposure to IL-1 α . We expect that the general model may also be helpful in evaluating the therapeutic efficiency of various molecules that may be employed for the management of osteoarthritis (OA) following trauma.

Structurally, articular cartilage consists of a highly hydrated extracellular matrix (ECM) synthesized and maintained by a sparse population of chondrocytes [10]. The key biomechanically functional macromolecules of cartilage ECM are the proteoglycan, aggrecan, and a fibrillar network of collagens (mainly type II, with minor amounts of types IX and XI) [11, 12]. Aggrecan is comprised of a core protein, to which glycosaminoglycans (GAGs) are covalently attached [13]. In turn, aggrecan molecules bind non-covalently to hyaluronan to form aggregates [13, 14]. The aggrecan monomer attached to hyaluronan is

referred to here as ‘intact aggrecan’, while proteolytically degraded ‘intact aggrecan’ is referred to here as ‘degraded aggrecan’.

Together with interstitial fluid, these macromolecular components behave as a poroelastic biocomposite material, enabling cartilage to resist high static and dynamic compressive, tensile and shear loads [15, 16]. Under normal conditions, chondrocytes maintain tissue homeostasis via controlled synthesis of aggrecan, collagen and many other proteoglycans and glycoproteins that help to assemble and remodel the ECM in dynamic balance with cell-mediated matrix turnover [12, 17].

Chondrocyte distribution and activity vary with depth in cartilage tissue based on local structural and functional requirements [18, 19]. However, long-term homeostasis of cartilage is challenged when short-term rates of aggrecan and collagen damage exceeds rates of synthesis and repair [17]. Following traumatic injury to cartilage, the observed imbalance between production and degradation of ECM has been attributed to both direct mechanical damage and to upregulation of proteolytic enzymes by the chondrocytes, including aggrecanases and matrix metalloproteinases [17]. The change in chondrocyte gene and protein expression of these proteases is driven by elevated levels of inflammatory cytokines such as IL-1, IL-6 and TNF- α synthesized within cartilage and neighbouring synovial tissues [20-22].

The inflammatory cytokines bind to chondrocyte cell surface receptors, triggering signalling cascades that lead to an increase in the expression and activity of aggrecanases and collagenases in the ECM [23-25]. Aggrecanases (predominantly ADAMTS-4,5) [17, 26-28] are primarily involved in the degradation of aggrecan [17, 29], whereas the collagenases

(MMP-1,13) are primarily involved in degradation of collagen found in cartilage [30-32]. Chondrocytes also secrete TIMPs (tissue inhibitor of metalloproteinases) [33]. These proteins help to protect the cartilage ECM by inhibiting the activity of aggrecanases (TIMP-3) and other MMPs (mainly TIMP-1, 2) [17, 34]. TIMP activity and expression either remains unchanged or is down-regulated by the inflammatory cytokines [35, 36], which enables aggrecanases and collagenases to degrade ECM components more efficiently.

Importantly, we note previous reports suggesting that aggrecan protects collagen from degradation, probably by the association of aggrecan and other proteoglycans with the surface of Type II collagen fibrils [10, 37]. This association may obstruct the access of collagenases to the enzymatic clip sites along collagen fibrils [38]. This effect is taken into account in the model developed here.

The structural homeostasis of articular cartilage therefore depends on a complex set of biochemical interactions between chondrocytes, ECM and proteases as illustrated in Figure 1. We acknowledge that many other molecules play potentially important signalling and functional roles in the ECM. However when developing the model, we will implicitly assume that they are either constant or their effect is taken into account by change in the main model variables.

We seek a quantitative understanding of key interactions shown in Figure 1 to gain insight into the short-term role of inflammatory cytokines in altering ECM homeostasis following traumatic joint injury, with a view to better understanding short-term processes, and their role in promoting long-term cartilage degradation such as observed in OA. We do this by

122 simulating the *in vitro* experiments of Li, Wang et al. [9], which are designed to understand
123 the effects of the inflammatory cytokine IL-1 α on cartilage explant degradation. The model
124 simulates the transport and biochemical interactions of IL-1 α within cartilage explant tissue,
125 the production of proteases and the degradation of key structural molecules in the cartilage
126 explant over a 4 week period. Mathematically, these biochemical interactions can be
127 modelled using reactive transport partial differential equations [39, 40]. The model developed
128 here represents a step towards the development of a general cartilage model to help infer
129 likely *in vivo* events following traumatic injury to the cartilage.

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Materials and Methods

Model description: In this study we develop a computational model to simulate the *in vitro* experiments reported by Li, Wang et al. [9]. Our goal is to model the experimentally observed biochemical degradation of bovine calf cartilage tissue explants based on quantitative kinetic data related to the degradation and release of aggrecan and collagen to the culture medium over a period of 4 weeks as shown in Figure 2. Computational modelling of the experimental outcome would help elucidate the quantitative contributions of various processes occurring within the tissue. Additionally, histological studies indicating spatial variations in explant aggrecan and collagen concentrations (as shown in Figure 3) can be qualitatively compared with the corresponding model predictions.

The experiments by Li, Wang et al. [9] involved placing bovine cartilage explants in culture medium with or without the inflammatory cytokine IL-1 α (at a concentration of 1 ng/ml). The articular cartilage explants are cylindrical disks (3 mm diameter, 1mm thick) as shown in Figure 4A. The cylindrical disks of cartilage contain no subchondral bone, as the disks are separated from the bone during the harvesting and preparation procedure. Note that the superficial zone of each cartilage explant is left intact. The explants were incubated in 96-well plates at 37°C over a period of 4 weeks, resulting in transport of biomolecules between the explant and the culture medium for both control and treated groups. The culture medium surrounding the explant was changed every 2 days, and medium removed was analysed for aggrecan and collagen content.

Figure 2 shows the experimental time-dependent aggrecan and collagen loss profiles from controls and IL-1 α treated cartilage explants as reported by Li, Wang et al. [9]. In the treated group, IL-1 α interacts with the chondrocytes, inducing the expression of various proteases that lead to the accelerated degradation of aggrecan and collagen relative to that observed for controls. Figure 3 shows a typical histological section (stained with Toluidine blue) illustrating IL-1 α induced aggrecan depletion adjacent to a cartilage surface. A key experimental observation is that the aggrecan degradation occurs more rapidly than collagen degradation (see Figure 2). In other words, the fractional-loss of degraded collagen significantly lags the fractional loss of degraded aggrecan [9, 17]. Our key task here is to develop a computational model that can realistically simulate the experimental observations made during these experiments.

Based on the cylindrical explant geometry in 3D (Figure 4A), we adopted a simplified two-dimensional geometry for the computational analysis by assuming radial symmetry along the vertical axis of the explant as shown in Figure 4B. We expect this to be a good approximation. Hence the spatial domain can be represented in the computational model in cylindrical coordinates by a radius of $r = 1.5$ mm and thickness of $z = 1.0$ mm as shown in Figure 4B.

Governing equations: The model simulates the transport of IL-1 α , the interaction between IL-1 α and receptors (IL-1R) on the surface of the chondrocytes, and the subsequent generation of aggrecanases (ADAM-TS4 and ADAM-TS5) and matrix metalloproteinases (MMP-1 and MMP-13) by chondrocytes. For simplicity, we group together the two key cartilage based aggrecanases (ADAM-TS4 and ADAM-TS5) into a single chemical variable termed

aggrecanase (which we denote in the model as ‘aga’). Similarly, the matrix metalloproteinases (MMP) responsible for cartilage degradation (MMP-1 and MMP-13) are grouped together into a single chemical variable termed MMP (which we denote in the model as ‘mmp’). These proteases (aggrecanase and MMP) interact with components in the cartilage ECM, causing the sequential degradation of the aggrecan and collagen network. Note in our model, we have chosen to not explicitly include the presence or absence of a host of other modifiers of the protease activity (e.g. TIMPs). Additionally, MMP-1 and MMP-13 are initially generated in an inactive procollagenase form [41]. These procollagenases (proMMP-1 and proMMP-13) are eventually activated by other MMP’s (e.g. MMP-3 and MMP-10). Similarly, aggrecanases are generated as proenzymes that are activated by pro-protein convertases [42]. As such, the model should be thought of as describing the net protease activity, which implicitly includes the net consequence of all the other molecules mediating protease activity including TIMPs. Hence for simplicity, we assume that the terms ‘aga’ and ‘mmp’ refer to the net active form of the aggrecanases (ADAM-TS4 and ADAM-TS5) and collagenases (MMP-1 and MMP-13). However, it is not difficult to include the effect of additional processes involving protease activation in the model, should this become a focus in the future.

The transport and biochemical interactions of all key variables in the model satisfy mass conservation [39, 40]. Mass conservation, together with constitutive equations are represented mathematically by a set of transient 3D diffusion-reaction equations in cylindrical co-ordinates. A generic partial differential equation is shown below:

$$\frac{\partial C_i}{\partial t} = D_i \nabla^2 C_i \pm R_i \quad (1)$$

where the suffix ‘i’ represents the individual variables involved in the model. The nine variables include chondrocyte cell density, intact aggrecan (‘ag’) concentration, degraded aggrecan (‘agd’) concentration, intact collagen (‘col’) concentration, degraded collagen (‘cold’) concentration, net active aggrecanase (‘aga’) and collagenase (‘mmp’) concentration and IL-1 α (‘IL-1’) concentration.

D_i (m²/s) represents the effective diffusivity for the i^{th} chemical species and C_i (cells/m³ or moles/m³) the cell density or concentration of the i^{th} species. R_i (moles/m³/s or cells/m³/s) represents the source-sink terms i.e. the rates of generation or apoptosis/consumption of the individual species. Most chemical reactions are represented by classical equilibrium equations. However, upon exposure to inflammatory cytokines the response of a cell is to change its synthesis profile, but this change takes time. So we found it necessary to introduce two time dependent equations. These equations represent the time delays involved in (i) IL-1 α induced transcription, translation and secretion of proteases by chondrocytes and (ii) intracellular and extracellular post-translational processing. Michaelis-Menten kinetics is used to represent the net activity of the proteolytic enzymes in the ECM, including aggrecanase and MMP. The complete set of governing equations for each of the species involved in the cartilage degradation model is shown in Table 1.

Initial and Boundary conditions: Table 2 shows the initial conditions assigned for variables in the computational models. The concentration of intact aggrecan ($C_{\text{ag,b}}$), degraded aggrecan ($C_{\text{agd,b}}$) and MMP ($C_{\text{mmp,b}}$) are set to zero in the culture medium, as the medium is replenished every two days [9]. Further details related to their values are discussed in the subsequent section. Table 3 details the boundary conditions assigned to the different variables. Zero flux

boundary conditions are assigned for all species at the bottom surface of the well plate (denoted surface 1). Because the explant is in contact with the well plate, the flux along the interface between the explant and the well-plate is judged to be negligible. Support for this assumption is provided by histological images of cartilage tissue explants incubated in the presence of inflammatory cytokines including IL-1 α and TNF- α [5, 43]. Additionally, our own full-thickness histological images of IL-1 α treated bovine cartilage tissue explants (see Figure 3D) have shown negligible aggrecan loss from the surface of the explant resting on the bottom of a single well of a 96-well plate. These images indicate negligible access of IL-1 α and escape of any degraded aggrecan along this interface.

Zero flux boundary conditions are applied at the curved (denoted surface 2) and top (denoted surface 3) surfaces of the explant for chondrocytes (cell) and intact collagen (col), respectively. This is based on intact collagen being fixed in space [44, 45], together with the fact that chondrocyte movement through a stationary collagen network is slow relative to the time-scale of the experiments [46]. Robin boundary conditions are assigned for intact aggrecan (ag), degraded aggrecan (agd) and MMP (mmp) along these surfaces [45]. A Dirichlet type boundary condition is employed to represent the concentrations of aggrecanase, IL-1 α and degraded collagen (cold) at the interfaces between the explant and the culture medium along surfaces 2 and 3, respectively. It is convenient to present further details related to the boundary conditions for aggrecanase, IL-1 α and degraded collagen in the later result sections, as our ability to reproduce the experimental data are a result of the specific choices made. We note in passing that most previous *in vitro* experiments on cartilage explant degradation have reported a time dependent increase in the concentration of degraded ECM species in the surrounding culture medium [4, 47].

Modelling approach: To approximate the initial conditions for the explant accurately, we first model the *in vivo* steady state. To do this, we assumed the collagen network is formed and we calibrate the model by simulating the production and (transport) loss of intact aggrecan, so that the aggrecan profile in the cartilage resembles that reported in the literature for very young calves [18], and so the flux through the top surface accords with estimates for aggrecan loss to the synovial fluid (i.e. about 2% per week) [48]. We then simulated the removal of the explant from its steady-state *in vivo* condition in the tissue by changing the boundary conditions on the cylindrical model domain. This was done by allowing molecular transport through the sides of the explant as well as through its top surface. We first calibrated the model by simulating the *in vitro* ‘control experiment’ i.e. the evolution of the aggrecan and collagen released from the explant to the culture medium in the absence of IL-1 α . Having completed this second model calibration, it is then appropriate to finally simulate the effect of adding IL-1 α to the explant culture medium (including the exponential reduction of aggrecan synthesis due to the presence of IL-1 α) [9, 49, 50], and following a third model calibration, to discern the quantitative details of the effect that IL-1 α has on cartilage degradation.

Model parameters: Table 4 lists model parameters, their values and where they were sourced in the literature. R_1 (moles/cell/s) represents the amount of intact aggrecan formed by a single chondrocyte per unit time. H (m) represents the thickness of the cartilage tissue explant. The parameters k_1 and k_2 (s^{-1}) represent the rates of production and apoptosis of the chondrocytes. The activity of aggrecanase is represented by the catalytic rate constant, k_3 (s^{-1}) and a Michaelis constant, $K_{m,aga}$ (moles/ m^3) [51]. The binding affinity of IL-1 α to IL-1 receptors (IL-1R) present on the chondrocyte surface is represented by the binding affinity, k_6 (s^{-1}) and the dissociation rate constant, $K_{m,IL-1}$ (moles/ m^3).

It has been reported that binding of IL-1 α to IL-1 receptors (IL-1R) can result in up to a 60 fold increase in MMP expression and 300 fold increase in aggrecanase expression by chondrocytes [52]. Given that aggrecanase and MMP production are stimulated by formation of the IL-1-IL-1R complex, we can correlate the binding affinity of IL-1 to IL-1R (k_6) with the rates of production of aggrecanase (k_4) and MMP (k_{10}) as shown in Table 4. However, changes in MMP and aggrecanase secretion are delayed due to the finite time required for chondrocytes to change their expression profile. Following a sudden stimulus, chondrocytes ‘retool’, which involves time for mRNA expression, protein formation, post-translational processing, secretion of the final product by chondrocytes and the activation of proteases in the ECM [53].

While binding of IL-1 to IL-1R to form the IL-1-IL-1R complex (C^*) is modelled as an equilibrium reaction [23], the time delays in the expression of aggrecanase and MMP following a stimulus are represented by stimulus response variables S_1 and S_2 . The stimulus responses are driven by the concentration of the IL-1-IL-1R complex and are represented using first order reactions with rate constants α_1 and α_2 as shown in Table 1. The calibrated values for α_1 and α_2 are shown in Table 4 (note we have chosen $\alpha_1 = \alpha_2$, as there is currently little reason to suppose that the times required for the secretion of aggrecanase is significantly different from that required for MMP). Proteases in general are initially secreted in an inactive form [54] and are usually activated through complex biochemical interactions mediated by other activated proteases, TIMPs and the characteristics of the propeptide domain of the protease concerned [54, 55]. Here, we implicitly account for all these biochemical interactions through the rate equations of the stimulus responses S_1 and S_2 .

The parameters k_5 (s^{-1}) represent the rate of MMP induced degradation of aggrecanase and k_7 (s^{-1}) the rate of protease induced degradation of IL-1 α . The activity of MMP degrading collagen is represented by both its catalytic rate constant, k_8 (s^{-1}) and the Michaelis constant, $K_{m,mmp}$ (moles/ m^3). The parameters β_{max} and k_9 (moles/ m^3) relate to a function representing the dependence of MMP activity on aggrecan concentration (i.e. to the evidence that aggrecan is protective of collagen). The parameter k_{11} ($M^{-1}.s^{-1}$) represents the rate constant for binding of MMP to degraded collagen (cold). C_{IL-1R} (moles/ m^3) denotes the concentration of IL-1 receptors (IL-1R) within the cartilage explant. In our model the concentration of IL-1 receptors is related to chondrocyte density (see Table 4). The parameters $h_{i,r}$ and $h_{i,z}$ (m/s) represents the interfacial mass transfer coefficient of the i^{th} species from the curved and top surfaces (surface 2 and 3 respectively) of the explant to the surrounding culture medium.

Numerical solution: The governing equations for the individual species together with the appropriate initial and boundary conditions were solved numerically using the finite element software COMSOL Multiphysics (Version 4.2, Burlington, MA, USA). A mesh dependence study showed that increasing the number of mesh elements beyond 630 did not cause any significant changes in model predictions. We therefore selected 630 quadratic finite elements for all the simulations. For simulating the *in vitro* experiments, we selected a time span of 27 days based on the duration of the actual experiments [9]. The relative accuracy for all the simulations is set to 1×10^{-3} . Model simulation times on a high-end PC ranged from 11- 646 s.

Results

Calibration of model initial conditions

The explants for the *in vitro* experiments were sourced from the femoropatellar grooves of 1-2-week old calves [9]. Previous experiments [18, 56] and computational modelling studies [10, 45, 57] have reported depth based variation in intact aggrecan production and concentration for young bovine cartilage. To approximate the initial conditions for the explant accurately, we first model the *in vivo* steady-state for aggrecan homeostasis in young bovine articular cartilage. Specific details for this model are as follows:

(a) The aggrecan mass transfer coefficient through the curved surface of the future explant ($h_{r,ag}$) (surface 2 in Figure 4B) is set to zero, as this is a no-flux boundary condition *in vivo*.

(b) The chondrocyte density (C_{cell}) was assumed uniform over the entire explant domain, as chondrocyte densities are fairly uniform in young animals [18].

(c) The aggrecan concentration in the synovial fluid is taken as 0 moles/m³ based on reports of very low aggrecan levels in synovial fluid of healthy individuals [58].

(d) Aggrecanase mediated aggrecan degradation terms were neglected based on reports of minimal aggrecan degradation in very young animals [17, 59, 60]. Proteoglycan turnover is modelled via the aggrecan exiting through the superficial zone and cartilage surface [61]. As reported by Smith et al. [45], this approach resulted in an aggrecan profile that agrees closely with reported experimental observations on very young calves [18].

To capture the depth based variation in aggrecan synthesis [18, 19, 62], we assumed a linear relationship between the basal rate of intact aggrecan synthesis (R_1 , moles/cell/s) and the depth of the cartilage (z) as shown in Table 4. The aggrecan production rate term is also sensitive to the aggrecan concentration, such that local production ceases as the cartilage

aggrecan concentration builds to the target concentration (C_{tar}) of 0.024 moles/m³. The selection of C_{tar} is based on the maximum reported aggrecan concentration of 60 mg/ml in young bovine cartilage explants [18], assuming the molecular weight of aggrecan to be 2.5 MDa [17]. The model solution predicts a spatio-temporal variation for intact aggrecan concentration within the explant. We calibrated the model by adjusting the basal rate of aggrecan synthesis by a single chondrocyte (P_{ag}) and the mass transfer coefficient ($h_{z,ag}$) through the top surface of the cartilage (surface 3 in Figure 4B). The predicted rate of aggrecan loss from the explant was made consistent with the reported *in vivo* proteoglycan losses in cartilage tissue from young animals [48]. The calibrated $h_{z,ag}$ had a value of 0.8×10^{-10} m/s, while the calibrated basal rate of aggrecan synthesis by a single chondrocyte (P_{ag}) had a value of 2.40×10^{-22} moles/cell/s.

Figure 5A and 5B shows the results from the final calibrated model including the temporal variation in aggrecan loss from the explant *in vivo* at steady state and the temporal variation in the spatial average aggrecan concentration ($\hat{C}_{ag}$) of the explant, respectively. Figure 5C and 5D shows the predicted depth based variation in aggrecan concentration and production for the explant, respectively at 6, 12, 24, 120 and 180 days. The model is calibrated to have an average steady state spatial explant aggrecan concentration ($\hat{C}_{ag}$) of 55 mg/ml.

The steady state aggrecan concentration at the deepest region of the explant ($z=0$ mm) is approximately 60% higher than the aggrecan concentration at the articular surface ($z=1$ mm). The predicted average and depth based aggrecan concentration profiles are consistent with the experimentally measured aggrecan concentration profiles in young bovine cartilage explants [18]. After adjusting for a higher rate of aggrecan observed in younger age of

cartilage (i.e. turnover rates are 2 to 4 times higher in very young animals compared to mature adult animals), the rate of *in vivo* aggrecan loss through the top surface from the explant is in agreement with experimental observations [48]. The steady state *in vivo* aggrecan profile is employed as an initial condition in subsequent analyses given in Table 2. For the modelling assumptions employed here, at steady-state, aggrecan production is found to be concentrated towards the top of the cartilage explant.

Modeling of in vitro control experiments

Next we simulated the *in vitro* control experiments involving incubation of the cartilage explants in culture media without IL-1 α [9]. Degradation of aggrecan by aggrecanase and MMP's is negligible under such conditions [4, 8]. However experiments on explants [4, 5, 9] have found that aggrecan loss increases to about 6% of the initial explant aggrecan content per week, as shown in Figure 2. The rate of aggrecan release for control explants *in vitro* (i.e. 6%) exceeds the reported *in vivo* aggrecan loss rates (around 2%) due to additional release of aggrecan through the freshly cut explant surfaces (i.e. surface 2 in Figure 4B). The model was calibrated to account for the increased rate of aggrecan loss by adjusting the mass transfer coefficient ($h_{r,ag}$) from the freshly cut surface, until it agreed with the observations shown in Figure 2. The model was run to steady state (i.e. well beyond the duration of the experimental data). The calibrated $h_{r,ag}$ had a value of 1×10^{-10} m/s.

Figure 6 shows the results for the calibrated model. The predicted rate of aggrecan loss from the explant in the time range simulating the *in vitro* control experiment (Figure 6A) is consistent with proteoglycan loss measurements from young bovine cartilage explants

cultured in the absence of any inflammatory cytokines [9]. The spatial average aggrecan concentration (Figure 6B) of the explant reduced from its *in vivo* steady state value of 55 mg/ml to a new steady state value of 52 mg/ml. We also note that the rate of aggrecan production by chondrocytes in the deep and middle zones increases significantly, compared to the *in vivo* control, largely accounting for the increased rate of aggrecan loss.

Given that IL-1 α can significantly decrease the rate of aggrecan production, it is of interest to understand the effect of continuing aggrecan production by chondrocytes in explants. So we also simulated a limiting scenario for the *in vitro* control experiments, setting the aggrecan production rate to zero for the duration of the *in vitro* experiments ($t \geq 120$ days), while all other model parameters were unchanged (see Figure 7). The predicted rate of aggrecan loss from the explant in the time range simulating the *in vitro* experiments remained unaffected by the absence of aggrecan production in the early stages (i.e. first 2 or 3 days), but subsequently the rate of aggrecan loss is significantly lower relative to that observed in the presence of aggrecan synthesis (see Figure 7A). This suggests it is important to include the continued production of aggrecan by chondrocytes during *in vitro* experiments, and take into account any effect of IL-1 α on aggrecan production.

Modeling of cytokine mediated aggrecan degradation

The next step is to model the addition of 1 ng/ml of IL-1 α to the explant culture media. When simulating this process, we made the following model assumptions:

- (a) It is known that a variety of aggrecan degradation products are formed by aggrecanases [17]. However in our simulation all the degradation products of intact aggrecan are lumped

together into a single entity termed ‘degraded aggrecan’ (‘agd’)—similarly for collagen degradation products (‘cold’).

(b) Based on mass conservation principles, we assumed that intact aggrecan is proteolytically converted to degraded aggrecan according to a 1:1 stoichiometric ratio. The total aggrecan concentration at any spatial location within the explant includes concentration of intact and degraded aggrecan ($C_{ag}+C_{agd}$).

(c) We prescribed a Robin type boundary condition for degraded aggrecan along the curved (surface 2) and top (surface 3) surfaces of the explant. Because degraded aggrecan is smaller and more mobile than intact aggrecan, we expected that the mass transfer coefficient for degraded aggrecan through the cartilage surface would be higher than that for intact aggrecan.

(d) The effective diffusivity of degraded aggrecan, aggrecanase and IL-1 α are all assumed to be a function of the intact aggrecan concentration, as shown in Table 4. This is because the cartilage ECM containing intact aggrecan has a small effective pore size opening [10, 63], which can reduce the mobility of degraded aggrecan, aggrecanase and IL-1 α by about an order of magnitude [10, 14, 64].

(e) The concentration of aggrecanase at the curved (surface 2) and top (surface 3) surfaces of the explant are set at zero. This is because aggrecanase can rapidly diffuse from the cartilage-medium interface (surface 2 and 3) into the culture medium [10, 12].

The *in vitro* control experiments were simulated for 27 days—the duration of the actual *in vitro* experiments [9]. For these simulations, we set the initial intact aggrecan concentration of the explant as a function of the explant depth (z), as quantified by the expression shown in

Table 2, which matches the steady state profile under *in vivo* conditions. The initial concentrations of the remaining species are set to zero. The curved ($h_{r,ag}$) and top ($h_{z,ag}$) surface mass transfer coefficient for intact aggrecan are set at their calibrated values of 1×10^{-10} and 0.8×10^{-10} m/s, respectively. The curved ($h_{r,agd}$) and top ($h_{z,agd}$) surface mass transfer coefficient for degraded aggrecan is taken to be approximately two-orders of magnitude higher.

We simulated the aggrecanase mediated degradation of aggrecan in the absence (Case 1) and presence (Case 2) of aggrecan synthesis by the explant. Li, Wang et al. [9] also reported that aggrecan synthesis rates in young bovine cartilage explants are reduced to approximately 10% of their basal value when exposed to a culture medium containing 1ng/ml of IL-1 α . Additionally, *in vitro* experiments [49, 50] have shown that aggrecan synthesis from chondrocytes reduces exponentially in a dose-dependent manner with increase in IL-1 α concentration of the culture medium. Based on these findings, we simulated an additional case (Case 3) introducing the following functional relationship between the local rate of intact aggrecan synthesis (R_{ag} , moles/m³/s) and the local explant IL-1 α concentration:

$$R_{ag} = R_1 e^{-\lambda C_{IL-1}} \quad (2)$$

Equation (2) is based on the assumption that an increase in IL-1 α concentration from 0 to 0.1 ng/ml reduces the rate of intact aggrecan synthesis from chondrocytes by 90% [49], and an increase from 0 to 1.0 ng/ml reduces the aggrecan synthesis rate by chondrocytes to almost negligible levels (>95% reduction) [50]. The fitting constant in equation (2) (represented by the parameter λ) for this data has a value of 4.3×10^8 m³/moles. The Michaelis constant for aggrecanase ($K_{m,aga}$) is calibrated using Case 3. Calibration of $K_{m,aga}$ ensured that the predicted rate of aggrecan loss from the explant and the explant aggrecan concentration distribution were consistent with the experimental results of Li, Wang et al.[9]. The calibrated

Michaelis constant for aggrecanase ($K_{m,aga}$) is found to be 55 nM, which lies within the range reported in the literature for $K_{m,aga}$ (10.6 to 61.0 nM) [65, 66].

Figure 8A shows the time dependent variation in the spatial average concentration of intact aggrecan in the explant. The spatial average intact aggrecan concentration reduced by 95%, 86% and 94% over 27 days for cases 1, 2 and 3, respectively. The spatial distribution of intact aggrecan concentration within the cartilage explant for cases 1, 2 and 3 are shown in Figure 9A-D, E-H and I-L, respectively. In all cases, the intact aggrecan concentration within explants exposed to IL-1 α demonstrates a relatively sharp moving front of aggrecan degradation. It is observed that the presence of aggrecan synthesis slightly retards the speed of this moving aggrecan degradation front. Figure 8C shows the rate of aggrecan loss from the explant for all three cases. We note that the aggrecan loss and concentration profiles predicted by our model for cases 1 and 3 are most consistent with experimental measurements [9], compared to case 2.

The spatial average IL-1 α concentration for cases 1, 2 and 3 are shown in Figure 8B. For all cases, the spatial average IL-1 α concentration reached a steady state value of 0.4 ng/ml in 9 days. The spatial distribution of IL-1 α concentration within the cartilage explant for cases 1, 2 and 3 are shown in Figure 10A-D, E-H and I-L, respectively. The IL-1 α concentration profile within the explant also demonstrates a sharp moving front. The speed of the IL-1 α front progression is similar for all cases. The results show that aggrecan synthesis has minimal effect on IL-1 α transport within the explant. We speculate that the limited depth of transport of IL-1 α into the aggrecan depleted zones is attributed to its rapid protease induced degradation [67].

Modeling of cytokine mediated collagen degradation

Now we seek to include in the model the IL-1 α mediated degradation of collagen. It has been shown experimentally that IL-1 α mediated collagen degradation can only occur once the aggrecan concentration drops below a specific threshold [38] i.e. aggrecan in high enough concentrations protects the collagen network from enzymatic degradation. We therefore simultaneously solve the governing equations of aggrecan (intact and degraded), IL-1 α , aggrecanase, MMP and collagen (intact and degraded) to simulate the collagen degradation process. We made the following additional model assumptions:

(a) We prescribed a zero flux boundary condition for intact collagen along the curved (surface 2) and top (surface 3) surfaces of the explant because intact collagen is assumed to be immobile [10, 44].

(b) The concentration of degraded collagen at the curved (surface 2) and top (surface 3) surfaces of the explant are set to zero, as degraded collagen is assumed to diffuse rapidly [12] across the explant interfaces exposed to the culture medium.

(c) To estimate the total amount of collagen initially present, we first calculated the mass of aggrecan in the explant, which is the product of the steady-state *in vivo* average concentration of aggrecan and the volume of the explant. The mass of intact collagen was then estimated based on reported mass composition of cartilage tissue [68]. The collagen concentration is then calculated in terms of molar units (moles/m³), based on the reported molecular form and chain composition of cartilage based collagen [69]. We finally assumed that the initial concentration of intact collagen is uniform throughout the explant, based on reports from earlier studies [18, 70]. By this process, the initial concentration of intact collagen in the explant is estimated to be 79 mg/ml or 0.2 moles/m³.

(d) The effective diffusivity of degraded collagen and MMP are assumed to be a function of the local aggrecan concentration as shown in Table 4, based on reports that the cartilage ECM can significantly reduce their mobility [64, 71].

(e) We prescribed a Robin-type boundary condition for MMP along the curved (surface 2) and top (surface 3) surfaces of the explant. The curved ($h_{r,mmp}$) and top ($h_{z,mmp}$) surface mass transfer coefficient for MMP were adjusted so that the predicted collagen loss from the explant is consistent with experimental measurements [9]. Note that if the MMP concentration is set to zero on the boundary of the domain representing the cartilage explant, then the collagen network right on the boundary remains intact (this is not observed in explant experiments).

(f) By trial and error during the calibration process, we found that the time delay in collagen loss from the explant (Figure 2) [9] is accurately captured if MMP mediated catabolism of collagen fibres within the cartilage matrix commenced when the total aggrecan concentration (intact and degraded) is reduced below the threshold value of about 1.5 mg/ml. We employ a Hill-type function to mathematically represent the aggrecan concentration dependence on MMP activity ($k_{act,mmp}$), as shown in Table 4.

(g) Trial and error also revealed that we needed to employ a second order kinetic rate expression to represent the binding of MMP to degraded collagen. The kinetic rate data are based on reports that: (i) MMP's initially denature intact collagen into degraded collagen by binding to selective sites along its triple helical structure [72, 73], and (ii) the rate of degradation of denatured collagen triple helixes are independent of MMP type [73]. The rate constant for binding of MMP to degraded collagen is chosen from literature [17]. There is currently no quantitative information in literature related to the number of MMP binding sites on surface of degraded collagen molecules. We therefore calibrated this parameter ($n_{R,cold}$) by

trial and error. We assumed that MMPs bound to degraded collagen are eventually lost to the surrounding culture media.

The calibration process involved simultaneously accounting for the effect of intact collagen being protected by aggrecan, the rate of loss of MMP from the explant and the number of MMP binding sites on degraded collagen. It might thus appear to be a case of over-fitting the data. However, the time dependent shape of the collagen degradation curve is itself complex. So fitting these parameters in the model actually results in a reasonably constrained set of parameters. This calibration process is described further in the Discussion.

The collagen degradation process is simulated for different aggrecan synthesis rates as described by cases 1, 2 and 3 for the aggrecan degradation process. The final number of MMP binding sites chosen for the surface of degraded collagen ($n_{R,cold}$) is based on the calibration for the case where aggrecan synthesis in the explant is assumed to be a function of the local IL-1 α concentration, as defined in equation (2) (i.e. Case 3). The spatial distribution of intact collagen concentration within the cartilage explant for cases 1, 2 and 3 are shown in Figure 11A-D, E-H and I-L, respectively. The intact collagen concentration front moves at a much slower speed relative to those for intact aggrecan and IL-1 α concentration fronts, and the degradation front deepens over time. Figure 8D shows the rate of collagen loss from the explant for the three cases. The collagen losses predicted by our model are most consistent with experimental measurements [9] when aggrecan synthesis by the explant is either absent (Case 1) or when aggrecan synthesis is dependent on the local IL-1 α concentration as defined in equation (2) (Case 3).

Estimation of IL-1 α mediated aggrecan degradation rates under *in vivo* conditions

Our computational models have been shown to predict the *in vitro* losses of intact aggrecan and collagen from normal and IL-1 α treated cartilage explants with a reasonable degree of accuracy. However, the IL-1 α concentrations used for the *in vitro* experiments [9] are higher (1 ng/ml) in comparison to the reported synovial fluid IL-1 α concentrations for normal [74, 75] and osteoarthritis/rheumatoid arthritis [75-77] afflicted cartilage tissues (0.001-0.5 ng/ml). We hoped the results from this model may provide valuable quantitative information related to aggrecan turnover rates during osteoarthritis. Therefore, we trialled our computational model by simulating the *in vivo* degradation of aggrecan in cartilage tissue for a range of reported synovial fluid IL-1 α concentrations. The IL-1 α concentration range represents both normal and osteoarthritic cartilage tissues. The concentration of IL-1 α in synovial fluid is much lower than that used by essentially all investigators in explant culture systems [5, 28, 78]. Higher concentrations of IL-1 α are used *in vitro* to accelerate the cytokine mediated cartilage degradation process in post-traumatic knee osteoarthritis [9].

The geometry and mathematical form of the model is similar to the model replicating the *in vitro* aggrecan degradation stimulated by IL-1 α . However, in the case of an *in vivo* analysis, the curved surface (surface 2) is set as a zero flux boundary condition for all the species involved. This is because the cartilage explant/disc may now be considered as sitting within more cartilage tissue. We also assumed that the local rate of aggrecan synthesis within the *in vivo* cartilage is governed by the local IL-1 α concentration as shown in equation (2). We simulated the model for IL-1 α concentrations in the range of 0.001 ng/ml to 0.5 ng/ml. Figure 12A shows the predicted temporal variations in the spatial average concentration of intact

578 aggrecan in the cartilage tissue for a range of synovial fluid IL-1 α concentrations in the *in*
579 *vivo* geometry.

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Discussion

We have shown that we can model reasonably accurately the experimentally observed degradation of cartilage explants exposed to IL-1 α using a system of partial differential equations representing reaction-diffusion transport equations. Approximately two-thirds of the forty seven parameters employed to define the model are based on values reported in the literature, while the remaining one third of parameters are based on model calibrations to experimental observations. Three different sets of experimental observations are used to calibrate the model: (i) the cartilage condition *in vivo* (calibration of model to reflect aggrecan concentration profile and aggrecan turnover *in vivo*), (ii) the *in vitro* behaviour under control conditions (calibration of model to reflect the experimentally observed rate of aggrecan and collagen loss over time (see Figure 2)), and (iii) the IL-1 α treatment condition *in vitro* (calibration of the model to reflect the experimentally observed rate of aggrecan and collagen loss over time in the presence of IL-1 α (see Figure 2)).

This triad of calibrations applied sequentially to a time-independent curve (i.e. to find a steady-state profile *in vivo*), and to four time dependent curves (i.e. the aggrecan and collagen loss curves for both control and treatment conditions), serve to constrain the approximately 16 unknown model parameters reasonably well. For while calibrating the model, we were challenged to explain both curve timing and curve shapes. The calibrated model can accurately capture the regional variations in explant degradation rates by a single set of model parameters. This is reflected by the close agreement between the predicted and experimental aggrecan and collagen loss profiles from the explant (Figure 8C and D). In other words, our model has significant predictive power as we could fit the experimental data with a single set of model parameters.

Interestingly, we found that the best fit to the control data is obtained by including on-going aggrecan production in the model. This is consistent with observations that chondrocytes continue to produce aggrecan *in vitro*, providing their support medium is regularly replenished (as occurred for this experiment) [9]. However, we incorporated in the model the reported finding that aggrecan production for cartilage explants exposed to IL-1 α is greatly reduced [9, 49, 50, 79].

Furthermore, using only equilibrium reactions (i.e. reactions that are fast relative to time dependent transport processes) in our reaction-diffusion model, we found that we cannot represent the initial delay in the onset of aggrecan degradation that is observed experimentally [9]. Consequently, we found it necessary to include a first-order rate equation to represent the time delays involved in gene expression, protein synthesis, post-translational processing and secretion. Doing so gives a much better fit to the data. We note that this approximation is consistent with observations on the delay associated with gene expression in chondrocytes exposed to IL-1 α [53]. This is interesting as it suggests that following cartilage exposure to IL-1 α (e.g. following traumatic injury of a joint), there is a ‘window of opportunity’ of about one or two days before the effect of inflammatory cytokines on aggrecanase biosynthesis is realized as aggrecan degradation. This window of opportunity has been noted elsewhere [78]. Importantly, it has been shown that if IL-1 α is removed prior to collagen loss, chondrocytes within the explant can at least partially recover aggrecan content of the cartilage explant [78].

Li, Wang et al. [9] noted previously that the observed IL-1 α stimulated loss of aggrecan and collagen is consistent with the suggestion that aggrecan protects collagen network from

degradation (presumably by hindering access of MMPs to the collagen fibril surface cleavage sites) [38]. We have included this protective effect of aggrecan on collagen degradation in our model. However, our modelling suggests that: (i) it is not only intact aggrecan that is protective of the collagen network, but also that degraded aggrecan plays a significant role in protecting collagen network, and (ii) collagen is fully protected by total aggrecan concentrations as low as 1.5 mg/ml. For it was found that the inclusion of degraded aggrecan in the protective effect significantly modulates the timing of collagen network degradation observed in the explant experiment. For example according to our modelling results, by adding the protective effect of degraded aggrecan to the protective effect of intact aggrecan, the time at which collagen network degradation begins is ‘pushed out’ by about 4 days (compare case 4 in Figure 8D, which becomes case 5 in Figure 8D upon the inclusion of degraded aggrecan). Importantly, the shape of the collagen loss curve depended strongly on including the binding of MMP to degraded (denatured) collagen fragments (compare case 5 in Figure 8D, which becomes case 3 in Figure 8D upon the inclusion of MMP binding to degraded (denatured) collagen). Again as noted by Li, Wang et al. [9], these data suggest that there may be a comparatively long window of opportunity for drug intervention aimed at preventing collagen network degradation, at least in part because of the protective effect of degraded aggrecan.

The diffusion coefficient for very large molecules like aggrecan (approximately 2.5 MDa) and aggregate (up to 300 MDa) [14] is very small. Yet, the measured apparent diffusion coefficient along an aggrecan gradient is much larger due to the gradient in aggrecan osmotic pressure also varying with aggrecan concentration [63]. Despite this we found that a constant diffusion coefficient of about 10^{-14} m²/s coupled with an increased resistance at the boundary gave a reasonably accurate *in vivo* concentration profile. This is consistent with the reported

earlier findings of Smith et al. [45]. Degraded aggrecan was given a diffusion coefficient two orders of magnitude larger, which flattened the degraded aggrecan gradient within the cartilage explant. This flattening of the degraded aggrecan concentration profile proved significant by regulating the rate of collagen network degradation.

There is a significant challenge in representing the spatio-temporal degradation of aggrecan for the cartilage explant exposed to IL-1 α . Nevertheless, we have demonstrated that our system of partial differential equations is capable of representing a steep spatial gradient in intact aggrecan and IL-1 α concentration, and the time course of the degradation agrees reasonably well with experimental observations. The steep spatial gradient in intact aggrecan concentration during degradation is at least partly a result of making the diffusion coefficient of molecules through intact aggrecan dependent on the concentration of intact aggrecan. We note that this feature is consistent with photo-bleaching experiments, which show that diffusion coefficient for molecules depends on the aggrecan concentration [80]. However to finally obtain a relatively steep gradient in intact aggrecan requires that the diffusion coefficient for aggrecanase be smaller than that of IL-1 α (in our case, about 70 times smaller than aggrecanase). If diffusion coefficients for aggrecanase and IL-1 α are reversed, the spatial gradient of intact aggrecan is flattened. We are uncertain how realistic this modelling assumption is, as unfortunately there are no independent data in the literature to support or refute these findings.

Finally we applied our calibrated model to predict the *in vivo* aggrecan loss from cartilage tissue exposed to a range of synovial fluid IL-1 α concentrations, which approximate IL-1 α concentrations found in normal through to osteoarthritis afflicted cartilage tissue. Our results

showed a significant increase in *in vivo* aggrecan losses from cartilage tissue on transition from normal physiology to moderate osteoarthritis [75]. For example Figure 12A shows the spatial average intact aggrecan concentration reduced by 0.5%, 2%, 20% and 62% over 27 days for synovial fluid IL-1 α concentrations of 0.001, 0.01, 0.1 and 0.5 ng/ml, respectively. Figure 12B shows the *in vivo* turnover times of intact aggrecan at different synovial fluid IL-1 α concentrations. The model predicts that the turnover time for intact aggrecan under normal physiological conditions ($C_{IL-1}=0.001-0.01$ ng/ml) [75] ranges from 140 to 163 days. This helps demonstrate that the transition from normal physiology to mild ($C_{IL-1}=0.1$ ng/ml) and then moderate osteoarthritis ($C_{IL-1}=0.5$ ng/ml) [75] can result in a 70% to 90% reduction in the aggrecan turnover time. This is consistent with reports of increased aggrecan turnover rates following joint injury and osteoarthritis [81]. We note that both predicted *in vivo* intact aggrecan concentration and turnover rates under normal physiological conditions are consistent with experimentally measured aggrecan concentration and turnover rates in cartilage tissues [18, 48].

Overall, our models are successful in capturing the aggrecan and collagen losses from cartilage tissue when subjected to inflammatory stressors. However, there are limitations to the current modelling study including: (i) not explicitly taking into account the kinetics of the activation of various proteases (e.g. the activation of aggrecanases (ADAMTS-4,5) and collagenases (MMP-1 and MMP-13)), (ii) the model does not explicitly account for the feedback inhibition of aggrecanase and collagenase activity by TIMPs [17, 33, 34] and (iii) the model does not account for the effect of Donnan (charge based) partitioning of IL-1 α and other charged molecules [82, 83] between the culture medium and the cartilage tissue, which has negative fixed charge [84]. The parameters representing the catalytic activities of aggrecanase ($K_{m,aga}$) and collagenase ($k_{act,mmp}$, k_9 and k_{11}) need to be recalibrated to account

for protease activation and inhibition. The Donnan partitioning of IL-1 α between the culture medium and cartilage tissue can be modelled using the Nernst-Planck equation [85], but this is beyond the scope of the current study.

Conclusion

The fundamental benefit of computational modelling of physiological systems is that complex integrated physiological systems can be mathematically represented by starting with the principles and laws of chemistry and physics including mass conservation, known constitutive relationships and physiological data reported in the literature. However, the number of valid ways to arrange this information into an integrated system is significantly curtailed. We can then narrow down the possibilities by identifying the key mechanisms that regulate the experimental observations. Such integrated models even allow us to quantify certain system variables, which are not easy to measure. In this paper, we present an integrated system of partial differential equations that can reasonably accurately represent the IL-1 α stimulated degradation of cartilage explants *in vitro*. We have also shown that the calibrated model predicts increased aggrecan turnover rates *in vivo* for osteoarthritic cartilage compared to normal cartilage, which is consistent with experimental observations [75, 81]. Further calibration studies on independent data sets are required to build generality and robustness of the model. Hence model development requires increased experimental knowledge about specific interaction mechanisms of selected model variables. Nevertheless we are encouraged that further model development may in the future result in useful and reliable predictions about the response of cartilage to environmental stressors.

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Table 1: Governing equations of transport, generation and consumption of species involved in biochemical degradation of cartilage

Species	Equation
Chondrocyte (C_{cell})	$\frac{\partial C_{\text{cell}}}{\partial t} = D_{\text{cell}} \nabla^2 C_{\text{cell}} + k_1 C_{\text{cell}} - k_2 C_{\text{cell}}$
Intact Aggrecan (C_{ag})	$\frac{\partial C_{\text{ag}}}{\partial t} = D_{\text{ag}} \nabla^2 C_{\text{ag}} + R_1 C_{\text{cell}} \left(1 - \frac{C_{\text{ag}}}{C_{\text{tar}}} \right) - k_3 C_{\text{aga}} \left(\frac{C_{\text{ag}}}{C_{\text{ag}} + K_{\text{m,aga}}} \right)$
Degraded Aggrecan (C_{agd})	$\frac{\partial C_{\text{agd}}}{\partial t} = D_{\text{agd}} \nabla^2 C_{\text{agd}} + k_3 C_{\text{aga}} \left(\frac{C_{\text{ag}}}{C_{\text{ag}} + K_{\text{m,aga}}} \right)$
Stimulus aggrecanase (S_1)	$\frac{\partial S_1}{\partial t} = \alpha_1 (C^* - S_1)$
Aggrecanase (C_{aga})	$\frac{\partial C_{\text{aga}}}{\partial t} = D_{\text{aga}} \nabla^2 C_{\text{aga}} + k_4 S_1 - k_5 C_{\text{aga}}$
IL-1 ($C_{\text{IL-1}}$)	$\frac{\partial C_{\text{IL-1}}}{\partial t} = D_{\text{IL-1}} \nabla^2 C_{\text{IL-1}} - k_6 C_{\text{IL-1R}} \left(\frac{C_{\text{IL-1}\alpha}}{C_{\text{IL-1}\alpha} + K_{\text{m,IL-1}}} \right) - k_7 C_{\text{IL-1}}$
Intact Collagen (C_{col})	$\frac{\partial C_{\text{col}}}{\partial t} = D_{\text{col}} \nabla^2 C_{\text{col}} - k_{\text{act,mmp}} k_8 C_{\text{mmp}} \left(\frac{C_{\text{col}}}{C_{\text{col}} + K_{\text{m,mmp}}} \right)$
Degraded Collagen (C_{cold})	$\frac{\partial C_{\text{cold}}}{\partial t} = D_{\text{cold}} \nabla^2 C_{\text{cold}} + k_{\text{act,mmp}} k_8 C_{\text{mmp}} \left(\frac{C_{\text{col}}}{C_{\text{col}} + K_{\text{m,mmp}}} \right)$
Stimulus MMP (S_2)	$\frac{\partial S_2}{\partial t} = \alpha_2 (C^* - S_2)$
MMP (C_{mmp})	$\frac{\partial C_{\text{mmp}}}{\partial t} = D_{\text{mmp}} \nabla^2 C_{\text{mmp}} + k_{10} S_2 - k_{11} C_{\text{mmp}} C_{\text{cold}} n_{\text{R,cold}}$

893 **Table 2: Explant based model initial conditions**

Species	Values/ Expression (Units)	References
Chondrocyte (C_{cell})	1.5×10^{14} (cells/m ³)	[19]
Intact aggrecan (C_{ag}) (<i>in vivo</i>)	0 (moles/m ³)	Text, [45]
Intact aggrecan (C_{ag}) (<i>in vitro</i>)	$-0.0433\left(\frac{z}{H}\right)^4 + 0.0431\left(\frac{z}{H}\right)^3 - 0.0162\left(\frac{z}{H}\right)^2 + 0.002\left(\frac{z}{H}\right) + 0.0242$ (moles/m ³) ¹	Text ² , [18]
Degraded aggrecan (C_{agd})	0 (moles/m ³)	Text
Aggrecanase (C_{aga})	0 (moles/m ³)	Text
IL-1 α ($C_{\text{IL-1}}$)	0 (moles/m ³)	Text
Intact collagen (C_{col})	0.2 (moles/m ³)	[68]
Degraded collagen (C_{cold})	0 (moles/m ³)	Text
MMP (C_{mmp})	0 (moles/m ³)	Text

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¹ Based on reported depth dependent aggrecan concentration in young bovine cartilage explants [18]. The concentration units of aggrecan can be changed from mg/ml to moles/m³ using the reported [17] molecular weight of aggrecan (2.5 MDa).

² Text refers to the cartilage explant based model initial conditions, which were either assumed or evaluated during the model calibration procedure as described in the manuscript text.

896 **Table 3: Model boundary conditions**

Species	Surface	Mathematical Expression
Chondrocyte	1, 2, 3	$D_{\text{cell}} \nabla C_{\text{cell}} = 0$
Intact Aggrecan	1	$D_{\text{ag}} \nabla C_{\text{cag}} = 0$
Intact Aggrecan	2	$D_{\text{ag}} \nabla C_{\text{cag}} + h_{\text{r,ag}} (C_{\text{ag}} - C_{\text{ag,b}}) = 0$
Intact Aggrecan	3	$D_{\text{ag}} \nabla C_{\text{cag}} + h_{\text{z,ag}} (C_{\text{ag}} - C_{\text{ag,b}}) = 0$
Degraded Aggrecan	1	$D_{\text{agd}} \nabla C_{\text{agd}} = 0$
Degraded Aggrecan	2	$D_{\text{agd}} \nabla C_{\text{agd}} + h_{\text{r,agd}} (C_{\text{agd}} - C_{\text{agd,b}}) = 0$
Degraded Aggrecan	3	$D_{\text{agd}} \nabla C_{\text{agd}} + h_{\text{z,agd}} (C_{\text{agd}} - C_{\text{agd,b}}) = 0$
Aggrecanase	1	$D_{\text{aga}} \nabla C_{\text{aga}} = 0$
Aggrecanase	2, 3	$C_{\text{aga}} = 0$
IL-1 α	1	$D_{\text{IL-1}} \nabla C_{\text{IL-1}} = 0$
IL-1 α	2, 3	$C_{\text{IL-1}} = C_{\text{IL-1,b}}$
Intact Collagen	1, 2, 3	$D_{\text{col}} \nabla C_{\text{col}} = 0$
Degraded Collagen	1	$D_{\text{cold}} \nabla C_{\text{cold}} = 0$
Degraded Collagen	2, 3	$C_{\text{cold}} = 0$
MMP	1	$D_{\text{mmp}} \nabla C_{\text{mmp}} = 0$
MMP	2	$D_{\text{mmp}} \nabla C_{\text{mmp}} + h_{\text{r,mmp}} (C_{\text{mmp}} - C_{\text{mmp,b}}) = 0$
MMP	3	$D_{\text{mmp}} \nabla C_{\text{mmp}} + h_{\text{z,mmp}} (C_{\text{mmp}} - C_{\text{mmp,b}}) = 0$

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Parameters	Values/Expressions	References
Effective diffusivity (Chondrocyte) (D_{cell})	$0 \text{ m}^2 \cdot \text{s}^{-1}$	[46]
Effective diffusivity (Intact Aggrecan) (D_{ag})	$1 \times 10^{-14} \text{ m}^2/\text{s}$	[10, 44]
Effective diffusivity (Degraded Aggrecan) (D_{agd}) ³	$D_{\text{agd}} = D_{\text{agd}}^* e^{-95C_{\text{ag}}}$	Text, [64]
Diffusivity (Degraded Aggrecan) (D_{agd}^*)	$1 \times 10^{-10} \text{ m}^2/\text{s}$	[10, 44]
Effective diffusivity(Aggrecanase) (D_{aga})	$D_{\text{aga}} = D_{\text{aga}}^* e^{-120C_{\text{ag}}}$	Text, [64]
Diffusivity(Aggrecanase) (D_{aga}^*)	$1 \times 10^{-12} \text{ m}^2/\text{s}$	[86]
Effective diffusivity (IL-1 α) ($D_{\text{IL-1}}$)	$D_{\text{IL-1}} = D_{\text{IL-1}}^* e^{-95C_{\text{ag}}}$	Text ⁴ , [64]
Diffusivity (IL-1 α) ($D_{\text{IL-1}}^*$)	$7 \times 10^{-11} \text{ m}^2/\text{s}$	[87]
Effective diffusivity (Intact collagen) (D_{col})	$0 \text{ m}^2/\text{s}$	[44]
Effective diffusivity (Degraded collagen) (D_{cold})	$D_{\text{cold}} = D_{\text{cold}}^* e^{-95C_{\text{ag}}}$	Text, [64]
Diffusivity (Degraded collagen) (D_{cold}^*)	$1 \times 10^{-10} \text{ m}^2/\text{s}$	[10, 12]
Effective diffusivity (MMP) (D_{mmp})	$D_{\text{mmp}} = D_{\text{mmp}}^* e^{-95C_{\text{ag}}}$	Text, [64]
Diffusivity (MMP) (D_{mmp}^*)	$1 \times 10^{-12} \text{ m}^2/\text{s}$	[71, 86]
Basal aggrecan production rate (R_1)	$P_{\text{ag}} \left(1 - \frac{0.9z}{H} \right)$	Text
Target aggrecan (intact) concentration (C_{tar})	$0.024 \text{ moles}/\text{m}^3$	[17, 18, 45]
Chondrocyte based basal aggrecan production (P_{ag})	$2.4 \times 10^{-22} \text{ moles}/\text{cell}/\text{s}$	Text, [57, 88]
Thickness of cartilage tissue explant (H)	$1 \times 10^{-3} \text{ m}$	[9]
Chondrocyte production rate (k_1)	$0 \text{ cell}/\text{s}$	Text
Chondrocyte apoptosis rate (k_2)	$0 \text{ cell}/\text{s}$	Text
Catalytic rate constant (aggrecanase) (k_3)	0.9 s^{-1}	[66, 89]
Michaelis constant (aggrecanase) ($K_{\text{m,aga}}$)	$5.5 \times 10^{-5} \text{ moles}/\text{m}^3$	[42, 66]
Rate constant aggrecanase production (k_4)	$0.83k_6 \text{ s}^{-1}$	Text, [52]
Aggrecanase degradation rate (k_5)	$1 \times 10^{-4} \text{ s}^{-1}$	[90]
Binding affinity (IL-1 α binding to IL-1R) (k_6)	$4.32 \times 10^{-5} \text{ s}^{-1}$	[23]
Dissociation constant (IL-1 α binding to IL-1R) ($K_{\text{m,IL-1}}$)	$7.2 \times 10^{-8} \text{ moles}/\text{m}^3$	[23]
IL-1 α degradation rate (k_7)	$5.83 \times 10^{-4} \text{ s}^{-1}$	[67]
IL-1 α receptor concentration ($C_{\text{IL-1R}}$)	$\frac{n_{\text{R}} C_{\text{cell}}}{N_{\text{A}}}$	[91]
Number of IL-1 receptors in single chondrocyte (n_{R})	2700 cell^{-1}	[23]
Avogadro number (N_{A})	$6.023 \times 10^{23} \text{ \#}/\text{moles}$	[91]
IL-1 α concentration (culture medium) ($C_{\text{IL-1,b}}$)	$0, 5.7 \times 10^{-8} \text{ moles}/\text{m}^3$	Text, [9]
Catalytic activity (MMP) (k_8)	1.5 s^{-1}	[54]
Michaelis constant (MMP) ($K_{\text{m,mmp}}$)	$0.0021 \text{ moles}/\text{m}^3$	[54]
Rate constant MMP production (k_{10})	$0.17 k_6 \text{ s}^{-1}$	Text, [52]
Rate constant MMP binding to degraded collagen (k_{11})	$0.47 \text{ M}^{-1} \cdot \text{s}^{-1}$	[54]

³ The units for C_{ag} used in the expressions for calculation of effective diffusivity are in moles/m³.⁴ Refer to manuscript text for further details related to parameter value selection.

# of MMP binding sites on degraded collagen ($n_{R,cold}$)	320	Text
Aggrecan dependent MMP catalytic activity ($k_{act,mmp}$)	$\frac{\beta_{max}}{1 + \left(\frac{C_{ag} + C_{agd}}{k_9} \right)^n}$	Text
Maximum MMP activity (β_{max})	1	Text
C_{ag} at half-maximal MMP activity (k_9)	0.0003 moles/m ³	Text
Hill –coefficient MMP activity (n)	6	Text
IL-1-IL-1R complex equilibrium concentration (C^*)	$\left(\frac{C_{IL-1} C_{IL-1R}}{C_{IL-1} + K_{m,IL-1}} \right)$ moles/m ³	[92]
Rate constant aggrecanase stimulus (α_1)	$0.4 \times 10^{-5} \text{ s}^{-1}$	Text
Rate constant MMP stimulus (α_2)	$0.4 \times 10^{-5} \text{ s}^{-1}$	Text
Intact aggrecan radial mass transfer coefficient ($h_{r,ag}$)	$1 \times 10^{-10} \text{ m/s}$	Text
Intact aggrecan axial mass transfer coefficient ($h_{z,ag}$)	$0.8 \times 10^{-10} \text{ m/s}$	Text
Degraded aggrecan radial mass transfer coefficient ($h_{r,agd}$)	$1.5 \times 10^{-8} \text{ m/s}$	Text
Degraded aggrecan axial mass transfer coefficient ($h_{z,agd}$)	$1.2 \times 10^{-8} \text{ m/s}$	Text
MMP radial mass transfer coefficient ($h_{r,mmp}$)	$1 \times 10^{-9} \text{ m/s}$	Text
MMP axial mass transfer coefficient ($h_{z,mmp}$)	$1 \times 10^{-9} \text{ m/s}$	Text
Synovial fluid intact aggrecan concentration ($C_{ag,b}$)	0 moles/m ³	[45]
Culture medium intact aggrecan concentration ($C_{ag,b}$)	0 moles/m ³	Text
Culture medium degraded aggrecan concentration ($C_{agd,b}$)	0 moles/m ³	Text
Culture medium MMP concentration ($C_{mmp,b}$)	0 moles/m ³	Text

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Figure 1: Schematic of biochemical interactions regulating the structural homeostasis of cartilage extracellular matrix (ECM). The image boundary (outer blue lines) represents the entire cartilage tissue, made up of hydrated ECM and a sparse population of chondrocytes (gray). Chondrocytes produce the proteoglycan, aggrecan (Agg) and collagen fibrils (Col), which are the key matrix constituents of cartilage ECM (orange). Chondrocytes maintain ECM homeostasis through controlled production and degradation of aggrecan and collagen. The dotted green line separates the production and degradation pathways. Traumatic injury to the joint increases the concentration of inflammatory cytokines such as IL-1 α (green) in the cartilage tissue and synovial fluid. Relevant to the present explant studies, binding of IL-1 α to the chondrocyte IL-1 α receptors upregulates aggrecanases (Aga) and collagenases (MMP) (blue). Aggrecanases catalyze the degradation of aggrecan to degraded aggrecan (Agg-D)

915 while MMP-collagenases catalyzes the degradation of collagen to degraded collagen (Col-D).
916 Both degraded aggrecan and collagen are represented by orange compartments, as they are
917 originally a part of the ECM. The chondrocytes and synovium also secrete tissue inhibitor of
918 metalloproteinases (TIMP, yellow compartment), which inhibits the activity of aggrecanase
919 and collagenase. The net activity of aggrecanases (Aga) is mediated by TIMPs and a host of
920 other modifiers of the proteases activity. For modelling purposes, we have chosen not to
921 explicitly include the inhibition of aggrecanase and collagenase activity by TIMP. The model
922 captures the net protease activity, which implicitly includes the net consequence of all the
923 other molecules on protease activity including TIMPs. The production and inhibition
924 pathways are represented by solid black and red lines.

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928 **Figure 2:** Time-dependent *in vitro* loss profiles of aggrecan-sGAG (intact and degraded
 929 aggrecan) and collagen from young bovine cartilage tissue explants as reported in the
 930 experimental study by Li, Wang et al. [9]. The experimental data is statistically analysed
 931 using the linear mixed effects model followed by Tukey's Honestly Significant Difference
 932 (Tukey's HSD) test for pair-wise comparisons. $P < 0.05$ were considered statistically
 933 significant. The cartilage tissue explants were incubated in 96-well plates with appropriate
 934 culture medium, both in the presence (1 ng/ml concentration) and absence (Control) of the
 935 inflammatory cytokine IL-1 α .

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938 **Figure 3:** Micrographs (at $\times 20$ magnification) of toluidine blue stained young bovine
 939 cartilage explants incubated in single wells of a 96 well plate for two days with appropriate
 940 culture medium in the absence (**Panel A**) and presence (**Panel B**) of 10 ng/ml of IL-1 α . **Panel**
 941 **C** and **Panel D** shows micrographs (at $\times 4$ magnification) of toluidine blue stained full
 942 thickness young bovine cartilage explants incubated for two days in appropriate culture
 943 medium in the absence and presence of 1 ng/ml of IL-1 α , respectively. Exposure to IL-1 α
 944 leads to accelerated loss of aggrecan-sGAG (intact and degraded aggrecan) from the explant.
 945 Toluidine blue staining shows aggrecan depletion at regions of the explant surface exposed to
 946 IL-1 α (top edge of Panel B and top and side edge of Panel D). The bottom surface of the
 947 explant does not show any staining loss indicating that the bottom of the explant is
 948 maintained continuously flush against the plastic bottom of the well.

Figure 4: Schematic of cartilage explant geometry subjected to biochemical degradation *in vitro* in the absence and presence of IL-1 α . **Panel A** shows the actual cylindrical geometry of the explant (grey) cultured in a medium (orange) with/without the presence of the inflammatory cytokine IL-1 α . The locations of the bottom (surface 1), curved (surface 2) and top (surface 3) of the explant are shown by appropriate labels. **Panel B** shows the simplified two-dimensional (2D) cylindrical geometry adopted for the computational model. The dotted green line indicates the axis of symmetry as labelled. The red line corresponds to the explant surface in direct contact with the bottom surface of the well plate (surface 1). The black line corresponds to the curved (surface 2) and the brown line to the top (surface 3) surfaces of the

970 computational domain. In the computational model, only surfaces 2 and 3 are involved in
971 mass exchange between the explant and the surrounding culture medium. It is assumed that
972 direct contact of surface 1 of the explant with the well plate precludes significant mass
973 exchange. The symbols r and z represent the radial and axial co-ordinates of the cylindrical
974 geometry. The orange arrows adjacent to the co-ordinate symbols indicate the positive
975 directions for each co-ordinate axis. The symbol H represents the height of the cartilage
976 explant (1.0 mm) while the symbol R represents the radius of the cartilage explant (1.5 mm).

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Figure 5: Time dependent variation in aggrecan loss, aggrecan concentration and aggrecan production rates for *in vivo* model. **Panel A** shows the steady state *in vivo* rate of aggrecan loss from the explant. **Panel B** shows the temporal variation in the spatial average intact aggrecan concentration of the cartilage explant. **Panel C** and **Panel D** show the depth based variations in the concentration and production rate of intact aggrecan in the cartilage explant. Day 0 in Panel B, C and D corresponds to the start of the numerical simulation to estimate the steady state *in vivo* distribution of intact aggrecan in the cartilage explant. In Panel A, Day 0 corresponds to any time interval when the *in vivo* aggrecan concentration in the explant has reached steady state. The predicted depth based variation at steady state is compared with the measured depth dependent aggrecan concentration in young (2-3 week old) bovine articular cartilage by Klein et al. [18].

991

992 **Figure 6:** Time dependent variation in aggrecan loss, aggrecan concentration and aggrecan
 993 production rates in young bovine cartilage explants under *in vivo* ($0 \leq t < 120$ days) and *in*
 994 *vitro* ($t \geq 120$ days) conditions for controls. Under *in vitro* control conditions, the culture
 995 medium is devoid of the inflammatory cytokine, IL-1 α . **Panel A** shows the comparison
 996 between the predicted and reported [9] *in vitro* rate of intact aggrecan loss from the explant
 997 into the culture medium. **Panel B** shows the temporal variation in the spatial average intact
 998 aggrecan concentration of the cartilage explant. **Panel C** shows the depth based variation in
 999 intact aggrecan concentration in the cartilage explant. **Panel D** shows the depth based
 1000 variation in aggrecan production under steady state conditions in the cartilage explant. Note
 1001 that the basal production rate of aggrecan by chondrocytes is maintained at the calibrated

1002 value of 2.4×10^{-22} moles/cell/s throughout the entire duration of the simulation ($0 \leq t \leq 240$
1003 days).

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1007 **Figure 7:** Time dependent variation in aggrecan loss, aggrecan concentration and aggrecan
1008 production rates in young bovine cartilage explants under *in vivo* ($0 \leq t < 120$ days) and *in*
1009 *vitro* ($t \geq 120$ days) conditions. Under *in vitro* control conditions, the culture medium is
1010 devoid of the inflammatory cytokine, IL-1 α . **Panel A** shows the comparison between the
1011 predicted and reported [9] *in vitro* rate of intact aggrecan loss from the explant into the
1012 culture medium. **Panel B** shows the temporal variation in the spatial average intact aggrecan
1013 concentration of the cartilage explant. **Panel C** and **Panel D** show the depth based variation
1014 in intact aggrecan concentration and aggrecan production rate, respectively in the cartilage
1015 explant. The basal rate of aggrecan synthesis by the chondrocytes (P_{ag}) was maintained at the

1016 calibrated value of 2.4×10^{-22} moles/cell/s only during the *in vivo* state ($0 \leq t < 120$ days) and
1017 set to zero for the simulation of the *in vitro* control condition ($120 \leq t \leq 240$ days).

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Figure 8: Time dependent variation in intact aggrecan and IL-1 α concentration and the rates of loss of aggrecan and collagen from cartilage explants subjected to *in vitro* IL-1 α mediated biochemical degradation. The concentration of IL-1 α in the culture medium was maintained at 1 ng/ml. **Panel A** and **Panel B** shows the predicted temporal variation in the spatial average intact aggrecan and IL-1 α concentration of the cartilage explant. **Panel C** and **Panel D** shows the comparison between the predicted and reported [9] rates of losses of aggrecan (intact and degraded) and collagen, respectively from the cartilage explant into the culture medium. The basal rate of aggrecan synthesis by the chondrocytes (P_{ag}) was set to 0 and 2.4×10^{-22} moles/cell/s for case 1 and 2, respectively. For case 3, the rate of aggrecan synthesis was a function of the local IL-1 α concentration as shown in equation (2). Case 4 and Case 5

1030 shows the process of refinement for the collagen degradation model. For Case 4, the Hill-
1031 function representing the aggrecan dependence on MMP activity only accounts for intact
1032 aggrecan (C_{ag}). For Case 5, the hill-function representing the aggrecan dependence on MMP
1033 activity accounts for both intact and degraded aggrecan ($C_{ag}+C_{agd}$). For both the cases (Case 4
1034 and 5), the binding of MMP to degraded collagen is not taken into account.

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Figure 9: Spatial distribution of intact aggrecan concentration in young bovine cartilage explants subjected to *in vitro* IL-1 α mediated biochemical degradation as predicted by our computational model. The concentration of IL-1 α in the culture medium was maintained at 1 ng/ml. **Panel A-D, Panel E-H and Panel I-L** shows the spatial distribution of intact aggrecan concentration across the cartilage explant at 0, 6, 9 and 18 days corresponding to cases 1, 2 and 3. The basal rate of aggrecan synthesis by the chondrocytes (P_{ag}) was set to 0 and 2.4×10^{-22} moles/cell/s for case 1 and 2, respectively. For case 3, the rate of aggrecan synthesis was a function of the local explant IL-1 α concentration as shown in equation (2).

Figure 10: Spatial distribution of IL-1 α concentration in young bovine cartilage explants subjected to *in vitro* IL-1 α mediated biochemical degradation as predicted by our computational model. The concentration of IL-1 α in the culture medium was maintained at 1 ng/ml. **Panel A-D, Panel E-H and Panel I-L** shows the spatial distribution of IL-1 α concentration across the cartilage explant at 0, 6, 9 and 18 days corresponding to cases 1, 2 and 3. The basal rate of aggrecan synthesis by the chondrocytes (P_{ag}) was set to 0 and 2.4×10^{-22} moles/cell/s for case 1 and 2, respectively. For case 3, the rate of aggrecan synthesis by was a function of the local explant IL-1 α concentration as shown in equation (2).

Figure 11: Spatial distribution of intact collagen concentration in young bovine cartilage explants subjected to *in vitro* IL-1 α mediated biochemical degradation as predicted by our computational model. The concentration of IL-1 α in the culture medium was maintained at 1 ng/ml. **Panel A-D, Panel E-H and Panel I-L** shows the spatial distribution of intact collagen concentration across the cartilage explant at 0, 6, 9 and 18 days corresponding to cases 1, 2 and 3. The basal rate of aggrecan synthesis by the chondrocytes (P_{ag}) was set to 0 and 2.4×10^{-22} moles/cell/s for case 1 and 2, respectively. For case 3, the rate of aggrecan synthesis was a function of the local explant IL-1 α concentration as shown in equation (2).

Figure 12: Effect of variation in synovial fluid (SF) IL-1 α concentration on *in vivo* aggrecan temporal concentration profiles and turnover rates in cartilage tissue. **Panel A** shows the temporal variation in the *in vivo* spatial average intact aggrecan concentration of the cartilage tissue corresponding to synovial fluid IL-1 α concentrations of 0.001, 0.01, 0.1

1121 and 0.5 ng/ml, respectively. **Panel B** shows the variation in intact aggrecan turnover time
1122 with respect to synovial fluid IL-1 α concentration. The rate of aggrecan synthesis for these
1123 simulations was set as a function of the local tissue IL-1 α concentration as shown in equation
1124 (2).

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