Coupling cellular phenotype and mechanics to understand extracellular matrix formation and homeostasis in osteoarthritis. ⋆

Vikram Sunkara * **Max von Kleist** **

* *Zuse Institute & Freie Universität Berlin, D-14195 Germany (e-mail: sunkara@mi.fu-berlin.de).*
** *Freie Universität Berlin, D-14195 Germany (e-mail: max.kleist@fu-berlin.de)*

Abstract: Osteoarthritis of the knee is a common degenerative disease during aging. It is typically caused by articular cartilage degeneration. Cartilage, which is located between bone surfaces, is a viscoelastic material aiming to absorb, redirect and transmit mechanical forces during movement. Without the cartilages' buffering capacity, bones come into direct contact inducing severe pain up to the stage where affected individuals loose mobility. The mechanisms of cartilage remodeling are poorly understood, and there is currently no effective method to reconstitute damaged cartilage. Cartilage consists of extracellular matrix (ECM) and a low density of cells (chondrocytes), which generate matrix proteins. The composition of the matrix gives the cartilage specific viscoelastic properties, which are sensed by chondrocytes feeding back on ECM remodeling. The aim of this study is to build a mathematical model that couples mechanical ECM properties with chondrocyte phenotype in the upkeep of cartilage homeostasis. We model the viscoelastic properties of the cartilage in terms of a linear Kelvin-Voigt model, where the dampening ratio feeds back on the phenotypic switching behaviour in chondrocytes. The chondrocytes, depending on their phenotypic state, may either produce proteoglycans or collagens or both, which alters the viscoelastic properties of the cartilage. We formulate a coupled system of equations integrating mechano-sensitive phenotypic switching behaviour of chondrocytes with respect to ECM remodelling. We define cartilage homeostasis as the fixed point of the derived systems of equations. Using this framework we can reproduce the long term changes in cartilage composition during aging.

Keywords: Extra Cellular Matrix, Attractors, Mechanical Systems, Switching Networks, Mathematical Models, Osteoarthritis.

1. INTRODUCTION

Osteoarthritis is a degenerative disease that affects the majority of individuals in the later stages of their life Lawrence et al. (2008). Osteoarthritis of the knee, hip and spine are particularly common. They induce severe pain and reduce mobility up to the stage where individuals cannot pursue their day-to-day duties. Currently, there is no effective treatment available. Joint replacement is thus the method of last resort to ensure patients' autonomy and life-quality. However, besides the risks, it poses an enormous financial burden for the health care systems, particularly in aging societies Buckwalter et al. (2004); Kim (2008).
Ostearthritis of the knee is typically characterized by cartilage degeneration. The cartilage is located between bone surfaces (see Figure 1). It is a avascular, viscoelastic tissue typically constituting extracellular matrix (ECM) and a low cell (chondrocytes) density. The cells constantly build- or degrade components of the ECM. The extracellular matrix consists mainly of water, cells and polymers. The polymers are matrix proteins belonging to the family of

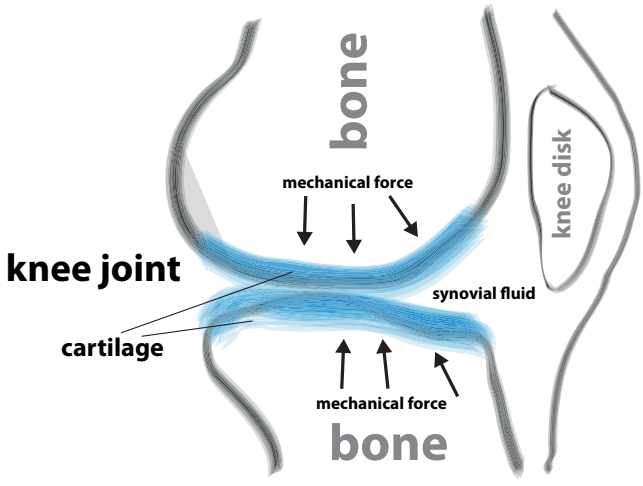

Fig. 1. Simplified depiction of the human knee with the cartilage highlighted in blue.

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collagens and proteoglycans, which determine both stiffness and elasticity of the tissue Humphrey et al. (2014); Mouw et al. (2014). The specific configuration and ratio of these constituents gives rise to mechanic properties, aiming to absorb, redirect and transmit mechanical forces that operate on the bone during movement. During osteoarthritis, these mechanical properties are lost and bones may come in direct contact provoking severe pain.

The molecular mechanisms of cartilage formation, homeostasis and functional loss are not well understood. Several biochemical factors have been identified in association with ECM formation and -loss (see Wescoe et al. (2008) for an overview), however there is currently no causative and integrative framework that puts all factors in context. There has been a great interest in understanding cartilage growth, which motivated a number of modelling approaches: Some authors have modelled a feedback between cartilage formation and nutrient gradients Lutianov et al. (2011), with the aim of modelling cartilage re-growth after cell implantation into a defect region, while others focus on the effects of insulin-like growth factors (IGF) Zhang et al. (2009) or nutrients plus IGF Asfour et al. (2015) in cartilage explants. Notably, Kar et al. (2016) modelled cartilage degradation subject to interleukin-1 stimuli. None of these models takes the bio-mechanical properties of the tissue into account and how this may affect cartilage re-modelling. However, it has been shown that the presence- or absence of biochemical factors does not suffice to build a functional cartilage and several authors LeBaron and Athanasiou (2000); Ikenoue et al. (2003); Lee and Bader (1997) have shown that mechanical stimuli are essential to generate functional cartilage tissue *in vitro*. Modelling approaches by Catt et al. (2011) and Klisch et al. (2003, 2008) take the mechanical properties of growing cartilage explants into account, however the mechanosensitive behavior of chondrocytes and its feedback on ECM composition is not regarded. Thus, although the role of mechanics in cartilage remodeling is widely appreciated Wescoe et al. (2008); Ramage et al. (2009), there is currently no functional model integrating tissue mechanics with chondrocyte phenotypes. The aim of this project is to provide a first prototypical framework that allows integrating mechanical and phenotypic switching that give rise to cartilage formation and homeostasis. We will hereby focus on long-term behaviour and homeostasis in healthy cartilage.

2. CORE MODEL

The core idea of the presented work is that there exists a strong feedback between mechanical characteristics of the ECM and the phenotypic state of the chondrocytes that regulate ECM composition (see Fig. 2), which in turn alters the mechanical characteristics of the ECM. The specific feedback between loading, ECM composition and the phenotypic state of chondrocytes has been shown to be regulated by mechanosensitive receptors (mainly integrins and mechano-sensitive ion channels Mobasheri et al. (2002)) and it has been reported that chondrocytes respond to mechanical events locally around the cells Bachrach et al. (1995); Valhmu et al. (1998). In the present work we model and investigate the mechano-sensitive phenotypic switch in chondrocytes and its feedback on the ECM surrounding the cells.

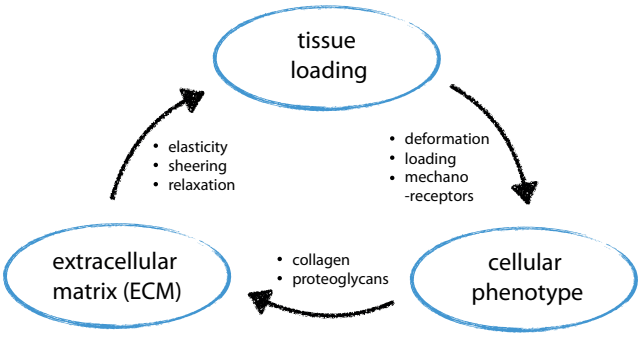

Fig. 2. Interplay between mechanics, metabolisms and tissue homeostasis.

2.1 Mechanical components

A viscoelastic material is modelled as a spring and a dashpot in parallel. The spring component provides the initial displacement as the cartilage experiences the impact, then after the impact is complete and the maximum deformation reached, the damper slowly reforms the cartilage back into its original shape using the stored energy of the initial impact. Biochemically, these properties can be explained by water displacement Mow (1989). Note that glycans can hold vast amounts of water due to their charged side chains. Viscoelastic materials can be modelled in different spring and damper configurations Li and Herzog (2004). We choose to model the cartilage as a linear Kelvin-Voigt model (the dashpot is a linear function of the velocity), since the non-linear dashpots considered elsewhere Edelsten et al. (2010) produce significant amounts of oscillations in the displacement of cartilage over time, which is biologically unrealistic. Furthermore, we omit the impact of velocities where the material will undergo an inelastic collision, which would be biologically unrealistic aswell.

Let $x(t)$ be the displacement in height of the cartilage at time t, then the equation of motion of the displacement through time is given by solving the following second order ordinary differential equation,

$$m\frac{d^2x(t)}{dt^2} + c_{damp}\frac{dx(t)}{dt} + c_{spring}x(t) = F_{external}, \quad (1)$$

where m is the mass of the cartilage, $c_{damp} > 0$ is the damping coefficient and $c_{spring} > 0$ is the elastic coefficient. In the absence of the damping term, the equation above would be the ODE of a simple harmonic oscillator. The damping controls how the tissue reforms back into its original shape. The term

$$\gamma := \frac{c_{damp}}{2 \times \sqrt{m \times c_{spring}}}, \quad (2)$$

is referred to as the *damping ratio*. If $\gamma < 1$, then the cartilage is *under damping*, that is, the damping is very small and the cartilage will keep oscillating. If $\gamma = 1$, then the cartilage is *critically damped*, meaning that the cartilage will come back to it original form in the shortest period of time. If $\gamma > 1$, then the cartilage is *over damped*, here the cartilage will take a larger period of time to get back to the original shape. In vivo studies of cartilage deformation have shown that articular cartilage is in the range of being critical to over damped Mauck et al. (2003b). In general loading scenarios, the cartilage reforms back into its original shape fairly quickly, being ready for the next loading

period. In equation (1) an increase in an external force increases the displacement of the cartilage, which is an oversimplification. In reality, there is a maximum amount of displacement a cartilage (or any material) can undergo after which the material responds as an inelastic. For the purpose of investigating displacements, we only consider external forces in the range that keep the displacement below the threshold of inelastic behavior.

3. COUPLING MECHANICS WITH CELLULAR PHENOTYPE

We now derive all the components of the mechano-regulatory feedback. The model is depicted in Figure 3. We assume that the ECM is made up of chondrocytes C, collagen K and proteoglycans G. Different concentration combinations of the C, K, G will give the ECM different damping ratios (as described in eq. (2)). The chondrocytes inside the ECM can be in one of three phenotypic states: Collagen producing cells KC, proteoglycan producing cells GC or idle cells IC. When a chondrocyte is idle, it is producing both collagen and proteoglycans at a slow rate for the regular up keep of the ECM. When the cell switches to KC or GC, then it is either producing collagen, or proteoglycans at a high production rate. Hence, chondrocytes then change the concentration of C, K and G, which then changes the damping of the ECM, feeding back on the chondrocyte's phenotype. We define the equilibrium state of all these dynamics as *homeostasis* of the cartilage.

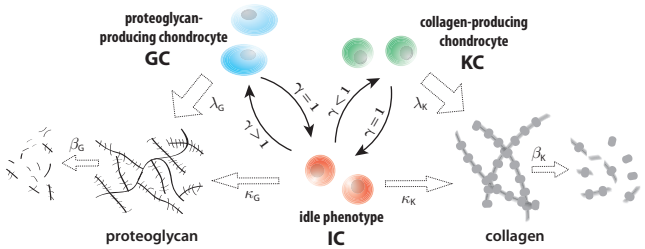

Fig. 3. **Mechanical and Phenotypic Coupling.** The cells can switch between different phenotypic states (indicated by blue, red and green) depending on the dampening ration of the ECM. Proteoglycan-producing (blue) and collagen producing cells (green) generate proteoglycan or, respectively collagen at a high rate λ_G, λ_C, whereas the 'idle' cell produces both proteoglycan and collagen, however at much lower rates κ_G, κ_C. Both proteoglycan and collagen are degraded at rates β_G, β_C respectively.

3.1 Mechanical coupling

We describe the mechanics of the ECM via a Kelvin–Vogit model (see section 2.1). In doing so, we have an analytic partitioning of dynamics into three categories, that is, the ECM is either under damped, critically damped and over damped. Now we wish to describe these damping ratios in terms of collagen, proteoglycans and chondrocytes, the most abundant elements found in the ECM. Let $G(t), C(t)$ and $K(t)$ be the concentrations (g/m^3) of proteoglycans, chondrocytes and collagen present in the ECM at time t. We model the spring and damping coefficients of the Kelvin–Vogit model eq. (1) as follows:

$$c_{damp} = \alpha_{damp} \times C(t) \times K(t), \tag{3}$$

$$c_{spring} = \alpha_{spring} \times G(t). \tag{4}$$

Here α_{damp} and α_{spring} are some scaling constants. Verbosely, the damping coefficient is proportional to the amount of chondrocytes and collagen present in the ECM, and the spring coefficient is proportional to the amount of proteoglycans. A link between mechanical coefficients and proteoglycan and collagen concentrations have been suggested in the literature Wu and Herzog (2002); Fox et al. (2009), we are extending on this by proposing a simple order one relationship as a first approximation. With the description of the damping and spring coefficients given in equations (3) and (4), we rewrite the damping ratio in eq. (2) as a function of C, K, G concentrations: for ease of notation we denote $\gamma(C(t), G(t), K(t) := \gamma(t)$,

$$\gamma(t) := \frac{\alpha_{damp} \times C(t) \times K(t)}{2 \times \sqrt{m \times \alpha_{spring} \times G(t)}}, \tag{5}$$

with m being the mass of the ECM. From the equation above we can rewrite the three different damping ratios as properties of C, K, G. Firstly, there exists concentrations of C, K, G such that the ECM is critically damped. We define the set of all configurations where $\gamma(\cdot, \cdot, \cdot) = 1.0$ as healthy cartilage. The three different damping ratio zones as a function of proteoglycan- and collagen concentrations are highlighted in Figure 4 [1]. Note that we assumed a fixed chondrocyte concentration at this point. We can see that as we increase the proteoglycans, the ECM becomes more elastic and is under damped. If we increase the collagen, then the ECM becomes more rigid and over damped.

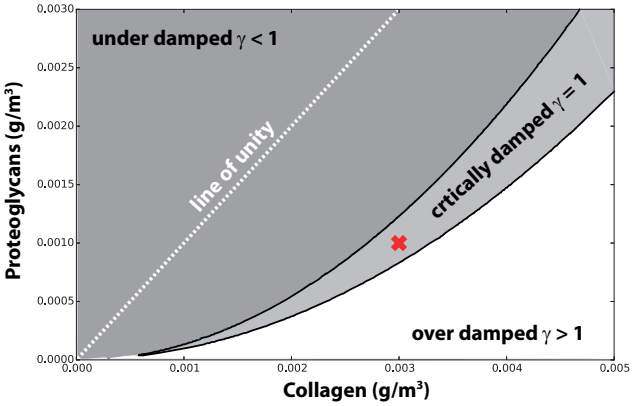

Fig. 4. The three damping ratio zones for a fixed chondrocyte concentration. The red cross highlights the fixed point.

3.2 Phenotype Switch

The proteoglycans and collagen are produced by the chondrocytes and released into the ECM Mauck et al. (2003a). A chondrocyte in an idle state will produce some proteoglycans and collagen for regular upkeep of the ECM Catt et al. (2011); Thibault et al. (2002). However, in a developmental stage we assume that the chondrocyte can

[1] See section 3.5 for parameters values used

be activated into a phenotypic state where it dedicates resources for solely producing either collagen or proteoglycans. We model this switching between phenotypes as a markov jump process. We recall the notation: Collagen-producing cell (KC), proteoglycan-producing cell (GC) or idle cell (IC). Since each cells' phenotype is an independent, identically distributed random variable, we consider the transition rate matrix for the switching of the single chondrocyte. Let $P_{IC}(t)$ be the probability of the chondrocyte being in an idle state at time t, similarly we denote $P_{GC}(t)$ and $P_{KC}(t)$ to be the probabilities of being in the state GC or KC respectively. The transition probabilities are given by the *Master Equation* :

$$\frac{dP_{IC}(t)}{dt} = \delta_{\gamma(t)=1}(P_{GC}(t) + P_{KC}(t)) - (\delta_{\gamma(t)>1} + \delta_{\gamma(t)<1})P_{IC}(t), \quad (6)$$

$$\frac{dP_{GC}(t)}{dt} = \delta_{\gamma(t)>1}P_{IC}(t) - \delta_{\gamma(t)=1}P_{GC}(t), \quad (7)$$

$$\frac{dP_{KC}(t)}{dt} = \delta_{\gamma(t)<1}P_{IC}(t) - \delta_{\gamma(t)=1}P_{KC}(t). \quad (8)$$

Where δ is a Kronecker delta. Verbosely, the system of equations above state that if the ECM is over damped, then the phenotype will switch to a GC and more proteoglycans will be produced. Conversely, if the ECM is underdamped, the phenotype of the chondrocytes will switch to KC and collagen production will be activated. Once the ECM becomes critical damped, the cell returns to the idle state IC. The probability distribution over multiple chondrocytes is simply a multinomial distribution with the solution to the master equations above as the sucess probabilities.

3.3 ECM Remodelling

We now derive the equations of motion for the proteoglycans and collagen. Currently there is no clear consensus in the community regarding the dynamics of the chondrocyte population in the cartilage. Hence for simplicity, we assume the chondrocyte population to be know *a priori* and furthermore, their dynamics on the time scale of interest to be constant or piecewise constant. We can now write down an ODE to describe the evolution of the collagen and proteoglycans over time given a fixed number of chondrocytes:

$$\frac{dK(t)}{dt} = \lambda_K P_{KC}(t)C(t) + \kappa_K C(t)P_{IC}(t) - \beta_K K(t), \quad (9)$$

$$\frac{dG(t)}{dt} = \lambda_G P_{GC}(t)C(t) + \kappa_G C(t)P_{IC}(t) - \beta_G G(t), \quad (10)$$

$$C(t) = C_0 \text{ fixed.}$$

Here λ_K and λ_G are the proteoglycan- and collagen production rate when a cell is in the corresponding phenotypic state. The coefficients κ_K and κ_G are the rates of production of collagen and proteoglycans when the cell is in the idle state. Lastly, β_K and β_G are the degradation rates of collagen and proteoglycans respectively. Note that we uses the expectation value, $P_{KC}(t)C(t)$ for the estimate of the number of chondrocytes which are in a particular phenotype. However, this term can easily be replaced with the random variable, as a consequence of which we would have multiple trajectories. For the purpose of demonstration we use the expected number of chondrocytes in the respective phenotype and hence all equations are deterministic.

3.4 Model Summary

Collating all different components we see that the ECM damping is given by the concentrations of C, K, G inside the ECM. The damping ratios then influence the phenotypes of the chondrocytes. The phenotype of the chondrocytes changes the concentrations of C, K, G, which in turn changes the ECM's damping ratio. We define the homeostasis of the ECM as the stationary state of coupling of all these dynamics. In the following sections we consider some parameters and simulate their corresponding stationary state.

3.5 Attractor analysis for fixed chondrocyte density

Let us now investigate the dynamics of this system for some fixed set of parameters. The parameters for the collagen and proteoglycan concentrations were extracted from the literature Catt et al. (2011); Thibault et al. (2002); Mauck et al. (2003b). The rest of the parameters are chosen such that a healthy piece of cartilage is the reference for homeostasis. That is, we chose the parameters such that when all component concentrations within a healthy cartilage are inputed, the derivatives for all equations above are zero. For a given chondrocyte concentration we have a fixed point, a stable point where the loading is critically damped and the production and degradation of the collagen and proteoglycans are balanced. We let $\alpha_{spring} = 89090.90$, $\alpha_{damp} = 1483163.95$, $m = 0.02$, $\lambda_K = 1e-5$, $\lambda_G = 1e-5$, $\kappa_K = 1e-6$, $\kappa_G = 1e-6$, $\beta_G = 0.2$, and $\beta_K = 0.066$. We also relax the condition for being critically damped to have a bigger region for visual aid. For numerical purposes we define $\gamma(t) \in (0.85, 1.2)$ to be the interval within which the ECM is critically damped.

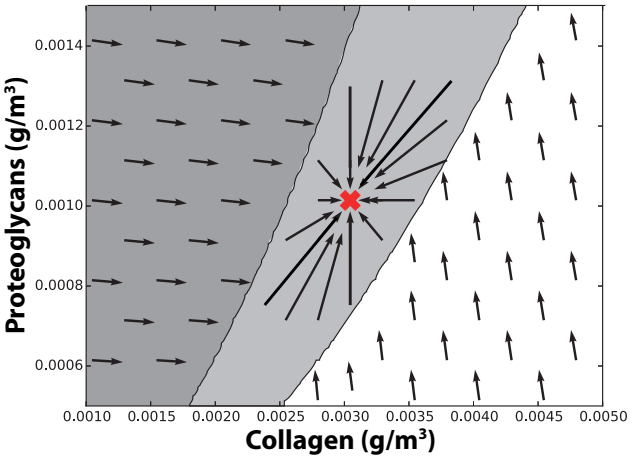

Fig. 5. The three damping ratio zones for a fixed chondrocyte concentration with the vector field indicated. The red cross highlights the attractor.

In Figure 5 we give a phase diagram of how the equations evolve on the space of proteoglycan and collagen

concentrations [2]. We can see that if we start in the over damped zone (lower right/white region in Fig. 5) more and more proteoglycans are being produced (near vertical arrows in Fig. 5), until the critically damped zone is reached, where the phenotype will switch to IC. Then from there the trajectory would slowly travel towards the fixed point at concentrations of 0.003 and 0.0015 [g/m^3] collagen and proteoglycan respectively. Analogously, for the under damped case, more and more collagen is being produced until the critically damped zone is reached.

3.6 Effects of aging

Previous studies have shown that chondrocyte proliferation decreases with aging Cancedda et al. (2003), which may result in their gradual depletion. In order to study articular cartilage homeostasis in aging, we analyse changes in the fixed point with regard to different collagen and proteoglycan concentrations for different chondrocyte densities.

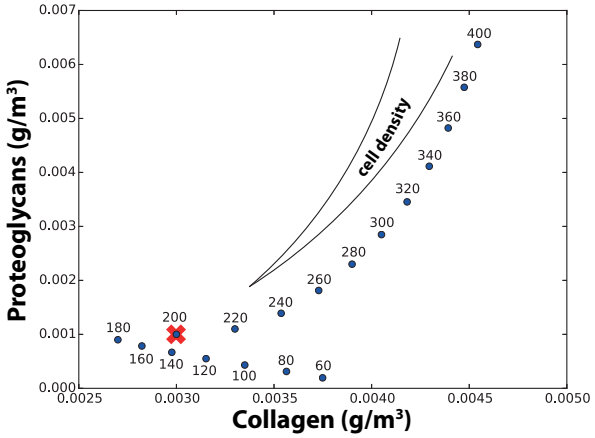

Fig. 6. Illustration of the respective fixed points for different chondrocyte densities. The cell concentration is given by $0.03 * 0.02 * ((cell\ density)/200)$ $[g/m^3]$.

In the previous section, we configured the parameters with the chondrocyte density to be 200. In Figure 6 we can see that as we decrease the number of chondrocytes from 400 to 180, the amount of proteoglycans and collagen in the fixed point configurations decrease. However, the proteoglycan levels seem to stabilise. After a chondrocyte density of 180 is reached these trends completely change, meaning that if chondrocyte densities are further decreased, collagen seems to increase at the respective stable fixed points. Biologically, the ECM is tending towards being a more over damped tissue (becoming more and more rigid). Also the ECM height is known to be given by the proteoglycans Guterl et al. (2010), hence, decrease in chondrocytes decreases the ECM height, exactly what is seen in aging individuals with functional cartilage Blazek et al. (2014); Duan et al. (2011).

[2] Note that the arrows in the critical damped zone have been amplified for visual aid to show the fixed point (the magnitude of the derivatives are smaller relative to the other two zones and hard to see).

3.7 Discussion

The aim of our study was to provide a mathematical framework that enables to couple mechano–regulatory feedbacks to model cartilage homeostasis. Note that most studies to date either focus solely on biochemical factors during *de novo* synthesis or cartilage remodeling Lutianov et al. (2011); Zhang et al. (2009); Asfour et al. (2015); Kar et al. (2016) or solely on bio-mechanical properties Catt et al. (2011); Klisch et al. (2003, 2008). To our knowledge, there is no model that regards the mechano–sensitive feedback regulating cartilage homeostasis.
In our framework, mechanical feedback is determined by a critical dampening ratio, which switches chondrocyte phenotypes from either producing proteoglycans, collagen or both. Note, that we assumed an instantaneous switching behaviour, which implicitly assumes a time-scale separation between cellular phenotypes and matrix production, as observed in related scenarios Yousef et al. (2015). We used the developed framework to describe the long–term dynamics of cartilage aging (Figure 6). It should be noted that the properties of aging cartilage are not only characterised by chondrocyte proliferation but also by metabolic factors. Hence, future research on cartlage in aging should incorporate metabolic factors. Future work will be focused on extending the presented framework towards a multi-layer structure, which takes external forces into account. This can be achieved by defining a layer-specific damping ratio function (see eq. (5)). Specifically, we would expect layer-specific dampening and spring coefficients for the superficial zone; middle zone and the deep zone. The latter is motivated by layer specific collagen networks and proteoglycan composition Ramage et al. (2009). The parameterisation of these layer specific coefficients will be extended to 2D and 3D models to fit experimental stress strain and shearing experiments.

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