Altered swelling and ion fluxes in articular cartilage as a biomarker in osteoarthritis and joint immobilization: a computational analysis

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In healthy cartilage, mechano-electrochemical phenomena act together to maintain tissue homeostasis. Osteoarthritis (OA) and degenerative diseases disrupt this biological equilibrium by causing structural deterioration and subsequent dysfunction of the tissue. Swelling and ion flux alteration as well as abnormal ion distribution are proposed as primary indicators of tissue degradation. In this paper, we present an extension of a previous three-dimensional computational model of the cartilage behaviour developed by the authors to simulate the contribution of the main tissue components in its behaviour. The model considers the mechano-electrochemical events as concurrent phenomena in a three-dimensional environment. This model has been extended here to include the effect of repulsion of negative charges attached to proteoglycans. Moreover, we have studied the fluctuation of these charges owning to proteoglycan variations in healthy and pathological articular cartilage. In this sense, standard patterns of healthy and degraded tissue behaviour can be obtained which could be a helpful diagnostic tool. By introducing measured properties of unhealthy cartilage into the computational model, the severity of tissue degeneration can be predicted avoiding complex tissue extraction and subsequent in vitro analysis. In this work, the model has been applied to monitor and analyse cartilage behaviour at different stages of OA and in both short (four, six and eight weeks) and long-term (11 weeks) fully immobilized joints. Simulation results showed marked differences in the corresponding swelling phenomena, in outgoing cation fluxes and in cation distributions. Furthermore, long-term immobilized patients display similar swelling as well as fluxes and distribution of cations to patients in the early stages of OA, thus, preventive treatments are highly recommended to avoid tissue deterioration.

1. Introduction

Articular cartilage is a highly specialized tissue covering bone epiphyses in synovial joints [1–3]. It serves as a load-bearing material of the joints, providing excellent lubrication and great wear characteristics [4]. Besides, it bears compressive and shear stresses owing to cyclic and intermittent loads [5]. The main feature of cartilage is the lack of blood vessels and nerves [6]. Thus, tissue maintenance is related to diffusive and convective-mediated processes triggered by mechano-electrochemical phenomena [7–9]. These are determined by the interaction of its predominant components: collagen fibres, proteoglycans and interstitial fluid [10].

Collagen fibres constitute 50–75% of the tissue volume; their specific orientation and distribution configure a dense network that resists compressive and
shear stresses [11]. Proteoglycan aggrecans (PGs), the major protein of cartilage, representing 15–30% of the tissue volume, are attached to the collagen fibres [12]. Proteoglycans consist of a protein core with one or more negatively charged covalently attached glycosaminoglycan chains (figure 1a). About 80–85% of the weight of healthy articular cartilage is an aqueous medium, interstitial fluid, in which inorganic salts, electrolytes sodium, calcium, potassium and chloride are dissolved [13].

The PGs are responsible for the turgid nature of the tissue and they provide the osmotic properties needed to resist compressive loads [14,15]. In this sense, cartilage swelling is considered a key parameter to characterize the functionality and integrity of the tissue. In addition, it is proposed as a helpful tool to quantify the severity of its degeneration [16,17]. Furthermore, the negative charges attached to PGs provide a repulsive force that enhances the compressive stiffness of the cartilage [18] (figure 1b).

It is well known that the PG content of cartilage is strongly correlated with the health of the tissue [19,20]. Of particular interest, initial stages of osteoarthritis (OA) as well as other cartilage degenerative diseases (costochondritis, achondroplasia and cartilage degeneration in immobilized patients) are marked by the progressive depletion of PGs in the extracellular matrix and the weakening of the network formed by collagen fibres and PGs [21]. In such cases, two phenomena are observed (i) severe hydration increment of ill cartilage compared with healthy tissue (swelling of the tissue) and (ii) irregular distribution of cations within the tissue [22].

To study this behaviour, emerging in vivo and in vitro experimental models have been developed [23–25]. Their main purpose is to clarify the contribution of structural components to the behaviour of healthy and/or pathological cartilage. MRI and histology are traditionally used to monitor the spatial distribution of molecules in cartilage but these are time-consuming techniques and highly influenced by the state of the tissue [26]. Therefore, they produce only semi-quantitative analysis (specific-molecule colour–density correlation) in two-dimensional sections that provide inaccurate three-dimensional representations. Additionally, longitudinal monitoring of changes with time is impossible because of the destructive nature of histology, and the high cost of MRI. The above-mentioned technical limitations mean that some basic aspects of cartilage remain obscure [27]. To overcome these limitations, computational models are proposed as a valuable tool to predict cartilage behaviour in vivo [28]. This technique can provide insights into correlation between intrinsic properties of cartilage and their effects in tissue behaviour which can help in clinical diagnosis and prognosis [28,29].

Despite the high number of existing material models to simulate cartilage behaviour [30–33], a marked tendency to consider multicomponent and multiphasic models has been established: from purely poromechanical models, where the tissue is considered as a mixture composed of a solid matrix with fluid inside the pores [34,35], to triphasic models, where the role of ions is also incorporated by a new ionic phase [36–38]. The interaction between the main tissue components generates specific electrochemical forces, these essential phenomena, which are neglected by most of the previous works, enable analysis of, for instance, the effects of cartilage disease on tissue behaviour. Altered ion distributions and outgoing fluxes in a three-dimensional environment are two key phenomena experimentally observed in degenerated cartilage [39]. Furthermore, modern medical imaging techniques such as MRI generate three-dimensional images containing valuable information [40]. These facts discourage the use of less accurate one- or two-dimensional computational models [41,42].

In our previous work [38], we developed a three-dimensional computational model of cartilage behaviour, based on the triphasic theory for hydrated soft tissue [10,42], which was able to accurately simulate the contribution of the main tissue properties to its behaviour. The model considers the mechano-electrochemical events as a concurrent phenomenon. Convective terms associated with coupled fixed charge density and solid phase velocity (FCD–PGs attachment) were also considered. However, the repulsion of negative charges attached to PGs and their fluctuation as a result of PGs variations was excluded. This phenomenon has been recently proposed as biomechanical marker to enable early detection of OA and monitor cartilage degenerative diseases [43,44]. Therefore, it is of interest to study this in healthy and pathological articular cartilage. In this work, we present an extended three-dimensional computational model of cartilage behaviour based on our previous work to include the effect of the PG repulsion (RPG) phenomenon. The new model has been validated by comparing its results with experimental swelling strain assays [10]. The model has been used to monitor and analyse cartilage behaviour at different stages of OA and in both short (four, six and eight weeks) and long-term (11 weeks) fully immobilized patients. Simulation results showed marked differences in swelling phenomena, in outgoing cation fluxes and in cation

![Figure 1. (a) Schematic of the cartilage network. It is composed of cross-linked type II collagen with proteoglycan aggrecans molecules bound. These proteoglycan aggregates consist of chondroitin-sulfate- and keratin-sulfate-rich regions (forming glycosaminoglycans molecules) linked to hyaluronic acid. (b) Proteoglycan repulsion phenomena owing to negative fixed charges attached in glycosaminoglycans.](http://rsif.royalsocietypublishing.org/)
distributions among healthy and pathological cartilage simulations. Interestingly, based on simulation results, patients who are immobilized for long periods present similar swelling behaviour to patients in the early stages of OA.

2. Material and methods

Based on our previous work [38], four phases are considered: negatively charged porous—elastic solid (s), fluid (f), cations (+) and anions (−). These phases dynamically interact with each other triggering essential mechano-electrochemical phenomena for cartilage maintenance (for more details, see [10,38]). Accordingly, the articular cartilage tissue is considered as a mixture of these four phases. The solid phase refers to the collagen network, negative-charged proteoglycans and intrafibrillar water. The fluid phase refers to extrafibrillar water. The following is the summary of the main model equations with special emphasis on RPG phenomena.

2.1. Mechano–electrochemical model

2.1.1. Governing equations

The four governing equations for the four basic unknowns (u* the displacement of the solid matrix, e* the chemical potential of water, e+ and e− the electrochemical potential for cations and anions, respectively) are given by,

\[ \nabla \cdot \mathbf{u} = 0. \]  

The momentum balance equation of the mixture

\[ \nabla \cdot \left( \sigma_{ref} \right) = 0. \]  

The mass balance equation of the mixture

\[ \nabla \cdot \mathbf{v} + \nabla \cdot \mathbf{J} = 0. \]  

The charge balance equations for ions

\[ \frac{\partial (\Phi^{c+} c^+)}{\partial t} + \nabla \cdot \left( j^+ \right) + \nabla \cdot \left( \frac{\Phi^{c+} \mathbf{v}^+}{c^+} \right) = 0 \quad (2.3) \]

\[ \text{and} \]

\[ \frac{\partial (\Phi^{c−} c−)}{\partial t} + \nabla \cdot \left( j^- \right) + \nabla \cdot \left( \frac{\Phi^{c−} \mathbf{v}−}{c−} \right) = 0 \quad (2.4) \]

where \( \sigma \) is the total mixture stress tensor and \( \sigma^+, \sigma^- \) and \( \sigma^* \) are the stress tensors related to the fluid, chemical and solid phase, respectively. \( \mathbf{v}^+ = \frac{\partial u^+}{\partial t} \) is the velocity of the solid matrix. \( c^+ \) and \( c^- \) are cation and anion concentrations and \( \Phi^* \) is the volume fraction of water (porosity) [10,25]. Here, the water flux, \( J^w \), cation flux, \( J^+ \) and anion flux, \( J^- \), are expressed as a combination of the electrochemical potentials,

\[ J^w = -\frac{RT \Phi^{c*}}{\alpha} \left( \nabla e^w + \frac{e^+}{e^w} \nabla e^+ + \frac{e^-}{e^w} \nabla e^- \right), \]  

\[ J^+ = -\frac{RT \Phi^{c+} c^+}{\alpha} \nabla e^+ - \frac{RT \Phi^{c+} D^+}{e^+} \nabla e^+ + \frac{RT \Phi^{c+} (c^+)^2}{\alpha e^+} \nabla e^+ \]  

\[ \text{and} \]

\[ J^- = -\frac{RT \Phi^{c−} c−}{\alpha} \nabla e^- - \frac{RT \Phi^{c−} D^−}{e^-} \nabla e^- + \frac{RT \Phi^{c−} (c−)^2}{\alpha e^-} \nabla e^−, \]

where \( \alpha \) is the drag coefficient between the solid and the water phase, \( D^+ \) and \( D^- \) the cation and anion diffusivities.

R is the universal gas constant and \( T \) is the absolute temperature [42].

Considering \( \Phi^* \) as the volume fraction of each component, thus we have the following saturation condition for the tissue

\[ \Phi^* + \Phi^{c+} + \Phi^+ + \Phi^− = 1, \]

because \( \Phi^* + \Phi^{c+} + \Phi^− \), the ion volume fractions can be neglected [10,42,45]. For infinitesimal deformation, the constitutive equation for the total stress of the tissue, considered as a mixture can be expressed as

\[ \sigma^* = -\Phi^* \rho I - T_{e} I + \sigma^*_e \]  

and

\[ \sigma^*_e = \lambda \varepsilon (\varepsilon) + 2 \mu \varepsilon, \]  

where \( T_e \), the chemical expansion, \( p \) is the fluid pressure, \( I \) is the identity tensor, \( \sigma^*_e \) the elastic stress related to collagen fibres, \( \lambda \) and \( \mu \) are the Lamé constants and \( \varepsilon \) is the infinitesimal strain tensor of the solid matrix [42]. Note that while \( p \) exists in both fluid and solid phases (because both phases are incompressible), \( T_e \) exists only in the solid phase and depends explicitly on the FCD–PGs (\( \varepsilon^* \)).

The constitutive equations for the rest state variables appearing in equations (2.3) and (2.4) in terms of the basic variables of the problem, may be also written as,

\[ e^w = \frac{p}{RT} - \Phi(c^+ + c^-) - \frac{B_v}{RT} \theta \]  

\[ e^+ = (\gamma^+ - c^+) \exp \left( \frac{F_c \psi}{RT} \right) \]  

\[ e^- = (\gamma^- - c^-) \exp \left( -\frac{F_c \psi}{RT} \right), \]

where \( F_C \) is the Faraday constant, \( \psi \) the electrical potential, \( B_v \) is the fluid–solid coupling coefficient, \( \Phi \) is the osmotic coefficient, \( \gamma^+ \) and \( \gamma^- \) are the activity coefficient of cation and anion, respectively. \( \theta = \text{div} u^* \) is the solid matrix dilatation related to the infinitesimal strain tensor of the solid matrix [42]. Small strains are considered in this model as well as linear-elastic isotropic and homogeneous material.

2.1.2. The chemical expansion stress (\( \sigma^* \))

To fully understand the effect of RPG phenomena, chemical expansion stress, \( \sigma^* \), was considered in the computational model through the constitutive equation (2.8). Specifically, this stress is due to the presence of charge-to-charge electrostatic repulsive forces exerting on the PG–collagen network (solid phase). It is of importance to note that the equilibrium of cartilage swelling is due to the combined action of \( \sigma^* \) and osmotic pressure. Both events depend on the internal concentration and distribution of ions and the interaction with each other. The dependence of the \( \sigma^* \) on the ion concentration is quantified as follows

\[ T_c = a_{s} e^w \exp \left( -k \frac{\sqrt{c^+ + c^-}}{\sqrt{c^+ + c^-}} \right), \]  

where \( a_{s} \) is a dimensional factor.
where $\alpha_0$ and $k$ are the RPG coefficients, $\gamma^+$ and $\gamma^-$ are the mean activity coefficient of ions along the process and in the reference state, respectively. Finally, $c$ refers to the external salt concentration [10].

### 2.1.3. Discretization

The multiphasic model described above is solved using the finite-element method. To obtain the fully coupled nonlinear system of equations describing the discretized model, we first establish the weak formulation of the governing equations

$$
\int \nabla \delta_\omega \cdot \sigma \, dV = \int \delta_\omega \sigma \cdot n \, d\Gamma, \quad (2.16)
$$

and

$$
\int \nabla \delta_{\omega -} \cdot J^+ \, dV + \int \nabla \delta_{\omega +} \cdot J^- \, dV = -\int \delta_{\omega +} J^- \cdot n \, d\Gamma, \quad (2.17)
$$

and

$$
\delta_\omega \frac{\partial (\Phi^+ \delta^+)}{\partial t} \, dV + \int \nabla \delta_\omega \cdot J^+ \, dV + \int \nabla \delta_\omega \cdot J^- \, dV = -\int \delta_\omega (J^+ - J^-) \cdot n \, d\Gamma, \quad (2.19)
$$

where $n$ is the unit vector normal to the boundary and $\delta_{\omega -}, \delta_{\omega +}, \delta^+, \delta^-$ are the so-called test functions fulfilling a set of mathematical requirements associated with continuity and integrability [46]. The superscript asterisk stands for the quantities in the bathing solution and $c^* = c^+ + c^-$.  

#### 2.1.4. Numerical implementation

The primary unknowns of the model $[\mathbf{u}, e^+, e^-]$ are interpolated from nodal values. Trilinear eight-noded hexahedral elements with $2 \times 2 \times 2$ Gaussian integration points are used. The selected average mesh size is of 2 mm, and the resulting total number of elements is 3528. The finite-element formulation has been implemented in a user-defined element subroutine of the commercial software package ABAQUS v. 6.11 [47]. Finally, the time derivatives are approximated with the Crank–Nicolson method that yields an implicit approximation to the solution of the initial value problem $y' = f(x, y)$ with $y(x_0) = y_0$ at $x$ for a given time step $h$ [48].

### 2.2. Experimental shrinking for proteoglycan aggrecans

#### 2.2.1. Initial conditions

Initially, the cartilage sample is equilibrated with a single salt (NaCl) solution with concentration $c^*$. The initial conditions for the computational model are

$$
t = 0; \quad u = 0; \quad e^* = e^+ + e^-;
$$

#### 2.2.2. Experimental shrinking for proteoglycan aggrecans

To validate the RPG phenomena incorporated in the computational model, the experimental design described in Lai et al. [10] is here computationally reproduced (figure 2b) to analyse the tissue response in confined conditions. Thus, a cartilage specimen of 1.5 mm diameter and 0.5 mm depth placed inside a circular confining ring is considered. No loads are applied to the sample in the $z$-direction. The properties of healthy cartilage collected in tables 1 and 2 are introduced into the computational model. Both simulations, with and without RPG, have the same input parameters. Additionally, in the case of considering RPG effects, those parameters collected in table 2 are also included to consider the repulsion between negatively charged proteoglycans.

#### 2.2.3. Interpretation of the data

The experimental shrinkage for proteoglycan aggrecans validation

Figure 2. (a) Schematic of the transient free swelling experiment and (b) boundary conditions of the cartilage sample applied in both, with and without PG repulsion phenomena, computational simulations. A cartilage sample of 0.5 mm thickness and 1.5 mm diameter is immersed in NaCl solution with an initial concentration of 0.15 M. The sample is confined in an impermeable chamber. (Online version in colour.)
Table 1. Model parameters used in both simulations, with and without PG repulsion effects, for healthy cartilage tissue.

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>value</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu$</td>
<td>Poisson's coefficient</td>
<td>0.3</td>
<td>[42]</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>drag coefficient between solid and water phase</td>
<td>$7 \times 10^{14}$</td>
<td>[42]</td>
</tr>
<tr>
<td>$c^0$ (mEq mL$^{-1}$)</td>
<td>initial FCD</td>
<td>0.1</td>
<td>[10]</td>
</tr>
<tr>
<td>$\phi^w$</td>
<td>initial amount of water in the tissue</td>
<td>0.75</td>
<td>[42]</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>osmotic coefficient</td>
<td>0.8</td>
<td>[10]</td>
</tr>
<tr>
<td>$D^+$ (m$^2$ s$^{-1}$)</td>
<td>diffusivity of the cations</td>
<td>$5 \times 10^{-10}$</td>
<td>[42]</td>
</tr>
<tr>
<td>$D^-$ (m$^2$ s$^{-1}$)</td>
<td>diffusivity of the anions</td>
<td>$8 \times 10^{-10}$</td>
<td>[42]</td>
</tr>
<tr>
<td>$\gamma^+$</td>
<td>activity coefficient of cations</td>
<td>0.86</td>
<td>[10]</td>
</tr>
<tr>
<td>$\gamma^-$</td>
<td>activity coefficient of anions</td>
<td>0.85</td>
<td>[10]</td>
</tr>
<tr>
<td>$\gamma^\text{int}$</td>
<td>main activity coefficient of the sample</td>
<td>1.0</td>
<td>[10]</td>
</tr>
<tr>
<td>$\gamma^\text{ext}$</td>
<td>main activity coefficient of the external solution</td>
<td>1.0</td>
<td>[10]</td>
</tr>
<tr>
<td>$R$ (J mol$^{-1}$ K$^{-1}$)</td>
<td>universal gas constant</td>
<td>8.314</td>
<td>[42]</td>
</tr>
<tr>
<td>$T$ (K)</td>
<td>absolute temperature</td>
<td>298</td>
<td>[42]</td>
</tr>
</tbody>
</table>

Table 2. Additional model parameters for PG repulsion phenomenon.

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>value</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$ (mol$^{-1}$)</td>
<td>chemical expansion coefficient</td>
<td>7.5</td>
<td>[10]</td>
</tr>
<tr>
<td>$a_c$ (Pa m$^2$ mol$^{-1}$)</td>
<td>PG repulsion coefficient</td>
<td>2500</td>
<td>[10]</td>
</tr>
</tbody>
</table>

Table 3. Experimental measurements of confined cartilage specimen in free swelling with $\nu = 0.3$ [10].

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>value</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c^*$ (mEq mL$^{-1}$)</td>
<td>0.0</td>
<td>0.015</td>
<td>0.15</td>
</tr>
<tr>
<td>$E$ (MPa)</td>
<td>—</td>
<td>1.48</td>
<td>0.74</td>
</tr>
</tbody>
</table>

The free-swelling state of tissue equilibrated with the bathing solution is chosen as the reference configuration for strain (time 0 s, undeformed configuration).

2.2.2. Boundary conditions

Similarly, the boundary conditions of the sample in confined conditions are (figure 2b)

free surface:

$$\sigma_z = 0; \quad e^w = e^w; \quad e^+ = e^+; \quad e^- = e^-.$$ 

lateral surface:

$$u_x = u_y = 0; \quad I^z_{xy} = I^z_{yx} = I^z_{yv} = 0.$$ 

lower surface:

$$u = 0; \quad I^z = I^z = I^z = 0.$$ 

At $t = 0$, the concentration of the external solution, $c^*$, is increased according to the values listed in table 3. The transient response of the solid displacement is here solved by using the new three-dimensional model considering RPG and compared with our previous results where the influence of this phenomenon was ignored (non-RPG). Furthermore, the obtained results were also compared with experimental ones reported by Lai et al. [10].

2.3. Numerical applications

Two different cartilage degenerative processes are studied herein; the progression of OA in adults and the degradation of the tissue after a certain period of full joint immobilization. With this aim, identical geometry and confined experimental conditions as in the previous validation case (§2.2), 1.5 mm diameter and 0.5 mm depth, are considered. However, in this case, the concentration of the external bath solution was decreased from 0.15 to 0.125 M to mimic cartilage swelling observed in the physiological situation. Both computational parameters for OA progression and cartilage after full joint immobilization were obtained from the literature [49–53]. The related results were compared with those obtained for healthy tissue. For each disease stage, different behavioural patterns for the tissue were obtained. Accurate quantification of cation fluxes and distributions within the samples and motorization of tissue changes along these degenerative processes were also performed.

2.3.1. Osteoarthritis progression

To establish a common criterion for OA tissue degeneration phases, the Osteoarthritis Research Society International (OARSI) has defined different grades which range from healthy cartilage (grade 0) to cartilage with advanced OA (grade 3). OARSI also correlates structural and behavioural changes with the symptoms observed (e.g. increment of the hydration of the cartilage) in each stage of the disease (table 4). Consistent with this classification, we introduce in our mechno-electrochemical computational model the experimentally obtained parameters, extracted from the literature for each OA stage (table 5). The results obtained are compared with those corresponding to the properties of healthy cartilage (grade 0).

2.3.2. Immobilized patients

In this second case, cartilage behaviour after different periods (four, six, eight and 11 weeks) of full joint immobilization is
also analysed. Geometry and confined conditions are the same as for the problem described in §2.2. The NaCl concentration of the external bath is again decreased from 0.15 to 0.125 M. In this situation, experimental observations of $E$ and $c_F$ are chosen as the two main parameters that suffer significant variations (table 6), whereas the rest remains similar to those exhibited by the healthy tissue (tables 1 and 2).

### 3. Results and discussion

The above-described three-dimensional mechano-electro-chemical model was used to simulate the behaviour of healthy articular cartilage after diluting the external bath solution in confined conditions. Computed axial strains of the cartilage samples with and without RPG were compared with those reported experimentally [10]. Finally, the model was applied to free swelling study and/or shrinking phenomena that occur in unhealthy cartilage processes as OA differentiation phases and degraded cartilage resulting from full immobilization. The results derived from abnormal cartilage free swelling patterns were also compared with those obtained for healthy tissue. Hence, correlation with the grade of progression of the disease is established.

#### 3.1. Validation of the extended model considering repulsion of proteoglycan aggrecans

Owing to the concentration increment of external bath solution, $c^*$, the cartilage samples exhibit two main phenomena: massive water outflow and ion entrance to balance the concentrations between both sides of the cartilage membrane. Simulation results with no RPG (figure 3, red line) show that after an increment of the external bath concentration up to 0.2 M of $c^*$, the cartilage begins to shrink sharply; from this threshold, shrinking becomes slower until maximal deformation is reached, $\varepsilon = -0.34$ at 2.5 M of $c^*$. When the RPG phenomenon is considered (figure 3, blue line), this tendency is sustained, whereas simulated shrinking is much closer to that obtained experimentally. Note that, a bath concentration of 0.3 M, without considering PG effects, resulted cartilage deformation

### Table 4. OARSI grade of cartilage degeneration [54].

<table>
<thead>
<tr>
<th>OARSI grade</th>
<th>tissue reaction: structure and biological changes</th>
<th>main associated physiological phenomena</th>
</tr>
</thead>
<tbody>
<tr>
<td>grade 0</td>
<td>normal articular cartilage; tissue keeps normal microstructure and properties</td>
<td>—</td>
</tr>
<tr>
<td>grade 1</td>
<td>early degradation of collagen fibres and abnormal cell proliferation</td>
<td>slight deterioration of mechanical properties ($\downarrow E$) increment of initial water content ($\uparrow d_{c_F}$)</td>
</tr>
<tr>
<td>grade 2</td>
<td>focal fibrillation and important degradation of the collagen network</td>
<td>significant deterioration of mechanical properties ($\downarrow \downarrow E$) FCD decrease ($\downarrow \downarrow c_F$)</td>
</tr>
<tr>
<td>grade 3</td>
<td>the matrix fibrillation extends vertically downward the mid zone</td>
<td>severe deterioration of mechanical properties ($\downarrow \downarrow \downarrow E$) sever FCD decrease ($\downarrow \downarrow \downarrow c_F$) increment of ions diffusivity ($\uparrow \uparrow \uparrow D^+ / D^-$)</td>
</tr>
</tbody>
</table>

### Table 5. Healthy and osteoarthritic (OA) human cartilage properties.

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>G0: healthy cartilage</th>
<th>G1: early OA</th>
<th>G2: intermediate OA</th>
<th>G3: advanced OA</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ (MPa)</td>
<td>Young’s modulus</td>
<td>0.74</td>
<td>0.50</td>
<td>0.37</td>
<td>0.28</td>
<td>[49]</td>
</tr>
<tr>
<td>$c_F^*$ (mEq ml$^{-1}$)</td>
<td>initial FCD</td>
<td>0.19</td>
<td>0.18</td>
<td>0.135</td>
<td>0.09</td>
<td>[51]</td>
</tr>
<tr>
<td>$d_{c_F}$</td>
<td>initial amount of water in the tissue</td>
<td>0.75</td>
<td>0.79</td>
<td>0.86</td>
<td>0.875</td>
<td>[50]</td>
</tr>
<tr>
<td>$D^+$ (m$^2$ s$^{-1}$)</td>
<td>diffusivity of the cations</td>
<td>$5 \times 10^{-10}$</td>
<td>$1.44 \times 10^{-9}$</td>
<td>$1.54 \times 10^{-9}$</td>
<td>$1.65 \times 10^{-9}$</td>
<td>[52]</td>
</tr>
<tr>
<td>$D^-$ (m$^2$ s$^{-1}$)</td>
<td>diffusivity of the anions</td>
<td>$8 \times 10^{-10}$</td>
<td>$1.44 \times 10^{-9}$</td>
<td>$1.54 \times 10^{-9}$</td>
<td>$1.65 \times 10^{-9}$</td>
<td>[52]</td>
</tr>
</tbody>
</table>

### Table 6. Human cartilage properties after full-immobilized joint periods of four, six, eight and 11 weeks.

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>four weeks</th>
<th>six weeks</th>
<th>eight weeks</th>
<th>11 weeks</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ (MPa)</td>
<td>Young’s modulus</td>
<td>0.74</td>
<td>0.74</td>
<td>0.74</td>
<td>0.49</td>
<td>[53]</td>
</tr>
<tr>
<td>$c_F^*$ (mEq ml$^{-1}$)</td>
<td>initial FCD</td>
<td>0.185</td>
<td>0.12</td>
<td>0.119</td>
<td>0.118</td>
<td>[53]</td>
</tr>
</tbody>
</table>
(v = −0.05) much lower than that obtained experimentally (v = −0.208), whereas this difference is much less when RPG is considered (v = −0.165 in the computational simulation and v = −0.208 experimentally). It is of importance to note that, in simulation results for both models (with and without RPG effects), external bath concentrations lower than 0.15 M showed an insignificant shrinking phenomenon. Whereas in the experimental work reported by Lai et al. [10], a sharp contraction of the sample was observed. Physically, the outgoing of water occurs gradually. When small changes in bath concentration occur, only the water in the upper layer of the cartilage goes to the external solution and subsequently almost null shrinking is expected. However, when higher changes in the bath concentration, with respect to the reference state (v = 0), are imposed, significant shrinking is reached. Accordingly, this fact together with the lack of precision of the measurement techniques used in the last decades, led us to conclude that simulations showed more consistent results in the earlier stage of the shrinking phenomena.

3.2. Comparison of cartilage free swelling with and without considering proteoglycan aggregans repulsion effects

To mimic physiological cartilage free swelling, a different experimental case is considered where the external salt concentration is decreased from 0.15 to 0.125 M. Under this assumption, the model shows how the sample, with and without RPG, typically exhibit three differentiated phases: (i) a massive entrance of water to the sample during the first 200 s of simulation; (ii) saturation of the sample reaching a maximum value at 1200 s; and (iii) no entrance of water is observed and the upper surface displacement is null which indicates a new equilibrium state. Although the tendency when considering RPG phenomenon is similar to that when neglecting it, the upper surface displacement results are considerably higher when including RPG, reaching a maximum value of $u_z = 8.76 \times 10^{-9}$ m, at 1200 s. In contrast, we obtain $u_z = 5.71 \times 10^{-10}$ m, only when RPG is neglected (figure 4).

3.3. Osteoarthritis severity effects in free cartilage swelling

OARSI has established different grades for OA progression as a function of the degeneration state of the tissue and its associated dysfunction, ranging from grade 0 corresponding to healthy tissue to grade 3 severely deteriorated cartilage (table 4). To fully understand the effect of cartilage deterioration in tissue behaviour, the tissue properties measured at different phases of OA disease were introduced into the presented three-dimensional computational model (table 5). Simulation results show how swelling of the sample increases according to the progression of the OA.

3.3.1. Z-displacement

When using physiological properties of healthy knee cartilage, the model displays a maximal displacement of 1.65 $\times 10^{-7}$ m (grade 0) and 2.45 $\times 10^{-7}$ m for early OA (grade 1) within 800 s of simulated time; subsequently, these values remain constant for 3600 s. For properties corresponding to advanced OA stages (grades 2 and 3), the results present higher z-displacement ranging from 3.09 $\times 10^{-7}$ to 1.3 $\times 10^{-6}$ m after 800 s of simulated time. In all cases, after reaching its maximum z-displacement, the tissue maintains its morphology until the end of the simulation (3600 s; figure 5). It is important to remark that the results obtained are consistent with recent experimental observations where abnormal swelling was detected when the tissue presented the first symptoms of disease [20,55,56].

3.3.2. Cation fluxes

The influence of OA progression in cation fluxes and distribution in the tissue is also considered. Simulation results are shown after 200 s of swelling, corresponding the period of maximal water entrance (200 s of simulation time). Results demonstrate that, similar to swelling, important disturbances...
in this flux appear in the advanced phases of the OA. As mentioned above, the external solution is diluted to 0.125 M of NaCl which generates an imbalance between the inner and the outer medium of the sample. Consistently, cation fluxes in healthy and grade 1 OA show higher values at the upper surface, $2.10^{-4}$ and $1.89 \times 10^{-4}$ mol s$^{-1}$, respectively. When the disease progresses this pattern is altered and lower values within the whole sample, especially in the upper surface, are found, $2 \times 10^{-5}$ mol s$^{-1}$ for grade 2 (intermediate OA) and $2 \times 10^{-5}$ mol s$^{-1}$ for grade 3 (advanced OA). It is of importance to note that in the case of advanced OA, simulation results also show severe alterations in the cation flux. The middle part of the sample presents an almost null outgoing flux, whereas the bottom area exhibits values of cation flux near to $2 \times 10^{-4}$ mol s$^{-1}$ (figure 6). Biologically, when OA progresses the collagen structure suffers a progressive deterioration and a sharp PG loss. Thus, the capacity of the collagen network to retain water is reduced, and the cation flux is clearly overpassed by the intake water flux, which leads to subsequent substrate swelling [20,55]. Note that for normal human knee cartilage, cation fluxes exceed $2 \times 10^{-5}$ mol s$^{-1}$.

3.3.3. Cation distribution

Under these conditions, the cation gradient was also monitored after 200 s of free swelling (figure 7) and again compared with the results obtained for healthy cartilage. Cation fluxes directly correlate with ion distribution within the sample. The results showed a progressive decrease of cation concentration in the direction of the upper surface of the sample ($z = 0.5 \text{ mm}$) in healthy and primary stages of the disease (grades 1 and 2) ranging from 50 mol m$^{-3}$ at $z = 0 \text{ mm}$ to 230 mol m$^{-3}$ at $z = 0.5 \text{ mm}$. However, for OA

\[ \text{Figure 4. Comparison of the upper surface displacement in the free swelling test from the model with and without proteoglycan repulsion effects in cartilage behaviour. The dark area represents the saturation phase. (Online version in colour.)} \]

\[ \text{Figure 5. Surface displacement obtained with the three-dimensional computational model considering RPG for a healthy cartilage, early, intermediate and advanced OA. Healthy and osteoarthritic model parameters are included in table 5. The darker area represents the saturation phase. (Online version in colour.)} \]
grade 3, this distribution is heterogeneous, with a peak in the middle of the sample depth (z = 0.3–0.4 mm) and a minimum at the surface (figure 7).

### 3.4. Effects of immobilization time in cartilage free swelling

In this study, the model has been applied to analyse the effect of different immobilization periods in cartilage behaviour. Similar to the OA progression study, z-displacement of the upper surface, cation fluxes and cation distribution in the sample were also monitored and analysed at four, six, eight and 11 weeks of patient full joint immobilization.

#### 3.4.1. Z-displacement

Considering the properties of the cartilage measured after four, six, eight and 11 weeks of full joint immobilization (table 6), we are able to quantify the influence of degradation on tissue swelling. Results demonstrated that healthy tissue and tissue immobilized for a short period (four weeks) yielded a maximum displacement in the upper surface of the sample within the 200 s of computational simulation of 1.65 × 10−7 and 1.59 × 10−7 m, respectively. Longer joint immobilization periods (six and eight weeks) led to proportionally lower maximal displacements, 5.75 × 10−8 and 1.98 × 10−9 m, respectively. Interestingly, several researchers have reported an increment in matrix stiffness when cartilage suffers periods of flux interruption (and subsequent nutrient reduction) lower than eight weeks [53]. Surprisingly, when this flow deficiency becomes permanent (here represented as 11 weeks of immobilization), collagen fibres are degraded and PGs depletion occurs [16,53]. Therefore, as in OA, the network becomes weakened, and a massive inflow of water occurs—both phenomena were visualized by the swelling of the samples. In agreement with this observation, figure 8 shows how the model captures the visualized by the swelling of the samples. In agreement with this observation, figure 8 shows how the model captures the

#### 3.4.2. Cation flux

Cation fluxes at different periods of tissue immobilization were again simulated during 3600 s. Three-dimensional computational simulation results were divided into five stages corresponding to the duration of the immobilization period. Results showed similar values of outgoing cation fluxes for four, six and eight weeks of immobilization as well as for healthy cartilage after 200 s of computational simulation (figure 9). The typical flux pattern exhibited by the samples clearly highlights the upper zone as the one with higher outflow zone, with

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**Figure 6.** Cation fluxes obtained with the present three-dimensional computational model in a healthy and OA cartilage (early, intermediate and advanced OA) at 200 s of simulated time. Note that positive flux in each stage refers to the entrance of cations into the sample.

**Figure 7.** Cation concentration (c−) distribution in the free swelling test from the model considering RPG in healthy, early, intermediate and advanced OA samples, after 200 s of swelling. The darker area represents the healthy cartilage range c+ (Online version in colour.)
at flux. In contrast, the part located in the upper surface peaked (figure 8), indicating a middle part with minimum cation immobilization offer non-monotonic gradients of cation fluxes cement study, results of cation flux in patients with 11 weeks of healthy tissue. When the immobilization period increases periods of immobilization. Results associated with four

\[ 
\text{J}^+ = 2 \times 10^{-4} \text{ mol s}^{-1} \]

This value is reduced along the sample’s thickness reaching a minimum value (almost null), at the bottom surface. In accordance with the previous z-displacement study, results of cation flux in patients with 11 weeks of immobilization offer non-monotonic gradients of cation fluxes (figure 8), indicating a middle part with minimum cation flux. In contrast, the part located in the upper surface peaked at \( |\text{J}^+| = 2 \times 10^{-5} \text{ mol s}^{-1} \), considerably lower than for short-term immobilization periods (\( |\text{J}^+| = 2 \times 10^{-4} \)).

3.4.3. Cation distribution

Finally, the gradient of cations was also monitored after 200 s of free swelling simulation, and again compared with the results obtained for healthy cartilage. At this time point, the three-dimensional model clearly showed a progressive decrement of cation concentration towards the upper surface (\( z = 0.5 \text{ mm} \)). In the healthy tissue, it ranged from 200 mol m\(^{-3}\) at 0 mm depth (significantly low compared with the initial value of 280 mol m\(^{-3}\) imposed in the external solution at 0 s) to 98 mol m\(^{-3}\) at 0.5 mm depth. This tendency remains similar for the rest of cases corresponding to longer periods of immobilization. Results associated with four weeks of immobilization closely resembled those obtained for healthy tissue. When the immobilization period increases to six and eight weeks, a wider range of cation concentration within the sample is obtained, ranged from 270 at the bottom surface of the sample (\( z = 0 \text{ mm} \)) to 140 mol m\(^{-3}\) at the upper surface (\( z = 0.5 \text{ mm} \)). Similar to z-displacement and cation flux, the case of 11 weeks of immobilization presents values in cation distribution along thickness, far from the previous healthy patterns. Figure 10 shows how the range in this situation is reduced from 270 at \( z = 0 \text{ mm} \) to 220 mol m\(^{-3}\) at \( z = 0.5 \text{ mm} \). Furthermore, for 11 weeks of immobilization, the resulting cation concentration is clearly higher than that for lower periods of immobilization.

4. Conclusion

This study presents an extension of a previous three-dimensional mechano-electrochemical model to analyse and quantify the effects of cartilage degradation in tissue behaviour [38]. The main novelty of this model is the incorporation of the repulsion phenomenon between the negative charges bound to proteoglycan molecules [14,15]. The obtained results demonstrate that with this inclusion the model is able to capture PG depletion and abnormalities in diffusive-mediated processes that occur in cartilage degenerative diseases such as OA and in patient that suffer periods of full immobilization of their joints.

The validation of the model is performed by comparing the results obtained with those reported by Lai et al. [10] for healthy cartilage. They experimentally studied the
swelling of a healthy cartilage after changing its external solution concentration. With that improvement, our model seems to be much closer to the experimental models. In the earlier stage of the shrinking process, lower than 0.15 M of external solution concentration, computational results exhibit almost null shrinking phenomena, whereas this was not the case in the experimental results collected by Lai et al. [10]. This may be due to the lack of precision in measurement techniques used in past decades; in this sense, more accurate and reproducible experiments should be carried out.

The presented model has been applied to study two typical cartilage degenerative situations (OA and immobilized articulations). In the OA study, simulation results showed an increment of cartilage swelling for advanced grades of OA disease. This has recently been confirmed experimentally [2,56], and associated with weakening of the collagen network and subsequent reduction of PG content. The results obtained here show that changes in mechano-electrochemical properties of the matrix may serve as a biomarker for progression of cartilage degeneration, making the present model a helpful tool to predict the behaviour and therefore the degree of tissue degeneration, thus avoiding complex chemical analyses of the subsequent extraction of cartilage.

In addition, changes in outgoing fluxes and cation distributions are predicted, which might contribute to abnormal nutrient delivery and diffusion. This phenomenon may enhance the progression of the degenerative process.

In the second case studied, the model has been employed to monitor and analyse the cartilage behaviour of patients with short and long periods of full immobilization of the joint. For four weeks of immobilization, cartilage swelling is similar to that observed in healthy tissue. For longer periods of immobilization, six and eight weeks, the tissue exhibits a higher network stiffness which prevents tissue swelling. By contrast, when immobilization is longer than 11 weeks the tissue experiences higher swelling.

The present model shows how cartilage immobilized for more than 11 weeks presents a similar behaviour to that in earlier stages of OA disease, the upper surface maximum z-displacement \( u_z = 2.51 \times 10^{-7} \) m is coincident with the one obtained for early OA (grade 1 following OARSI indications). A similar situation occurs in the outgoing flux and distribution of cations which also present altered patterns as those obtained for early phases of OA. This indicates that patients that suffer long-term full immobilization of their joints may need preventive treatments to avoid early cartilage degeneration.

With all this, we consider that this model represents a valuable tool in several practical applications. For instance, as a biomarker in sports medicine to predict joints with low tissue quality where a specific support aid can be recommended. As well as in competition animals where a comparative analysis of joint cartilage between breeds and individuals within the same species could establish lower probability of injury. Moreover, this model could help in the optimization of cartilage-mimicking biomaterials. In summary, the here presented three-dimensional model is capable of capturing the different behaviours of healthy and/or degenerated cartilage, helping to establish the appropriate correlation between sample swelling, outgoing fluxes and cation distribution with the degree of cartilage deterioration, obtaining typical patterns for healthy and pathological tissue. Owing to the complexity and/or impossibility to perform experimental assays to directly measure these cartilage variables, this model is presented as a predictive tool to analyse and study different physiological and pathological processes that take place in cartilage. This, together with the capability of the model to display the results as clinically interpretable three-dimensional images, presents this model as an interesting novel tool for diagnosis, monitoring and efficacy evaluation of potential therapies.

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