Structured modeling & simulation of articular cartilage lesion formation: development & validation

Xiayi Wang
University of Iowa

Copyright 2015 Xiayi Wang

This dissertation is available at Iowa Research Online: https://ir.uiowa.edu/etd/1927

Recommended Citation
https://doi.org/10.17077/etd.w5m7jp1y

Follow this and additional works at: https://ir.uiowa.edu/etd

Part of the Applied Mathematics Commons
STRUCTURED MODELING & SIMULATION OF ARTICULAR CARTILAGE
LESION FORMATION: DEVELOPMENT & VALIDATION

by

Xiayi Wang

A thesis submitted in partial fulfillment of the
requirements for the Doctor of Philosophy
degree in Applied Mathematical and Computational Sciences
in the Graduate College of
The University of Iowa

August 2015

Thesis Supervisor: Associate Professor Bruce P. Ayati
This is to certify that the Ph.D. thesis of

Xiayi Wang

has been approved by the Examining Committee for the thesis requirement for the Doctor of Philosophy degree in Applied Mathematical and Computational Sciences at the August 2015 graduation.

Thesis committee: __________________________
Bruce Ayati, Thesis Supervisor

__________________________
Colleen Mitchell

__________________________
Victor Camillo

__________________________
Keith Stroyan

__________________________
James Martin

__________________________
Gideon Zamba
First of all, I would like to express my deepest gratitude to my advisor, Prof. Bruce Ayati, for his excellent guidance, caring, patience and support. I appreciate his vast knowledge and skills, good humor and great assistance in writing and computer programming. I would like to thank Prof. James Martin for providing me financial support and advice on the biological knowledge of articular cartilage. I also thank Prof. Gideon Zamba to explore my interest in biostatistics that has helped me extend my research. I also acknowledge the collaboration with Prem Ramakrishnan and Marc Brouillette, who have provided the biological insights and experimental data for my research. Besides my advisors and collaborators, I would like to thank the rest of my committee: Prof. Colleen Mitchell, Prof. Victor Camillo, and Prof. Keith Stroyan, for the encouragement, insightful comments and great questions.

I would also like take this opportunity to express gratitude to all of the department faculty members for their help and support. I thank my co-members in Ayati-group for the inspiring discussions in group meetings and the fun we had in the last three years. I thank all my friends for always being for me through my good times and bad.

I am grateful to my parents, parents-in-law and the rest of my family for their love, support and sacrifices they’ve made on my behalf. Finally, I give most thanks to my husband Colin for his unconditional love and support. He has been a constant source of strength and inspiration that ultimately encouraged me to finish this work.
ABSTRACT

Traumatic injuries lead to articular cartilage lesion formation and result in the development of osteoarthritis. Recent research suggests that the early stage of mechanical injuries involve cell death (apoptosis and necrosis) and inflammation. In this thesis, we focus on building mathematical models to investigate the biological mechanism involving chondrocyte death and inflammatory responses in the process of cartilage degeneration.

Chapter 1 describes the structure of articular cartilage, the process of cartilage degeneration, and reviews of existing mathematical models. Chapter 2 presents a delay-diffusion-reaction model of cartilage lesion formation under cyclic loading. Computational methods were used to simulate the impact of varying loading stresses and erythropoietin levels. The model is parameterized with experimental results, and is therefore clinically relevant. Due to numerical limitations using delay differential equations, a new model is presented using tools for population dynamics. Chapter 3 presents an age and space-structured model of articular cartilage lesion formation under a single blunt impact. Age structure is introduced to represent the time delay in cytokine synthesis and cell transition. Numerical simulations produce similar temporal and spatial patterns to our experimental data. In chapter 4, we extend our model under the cyclic loading setting. Chapter 5 builds a spatio-temporal model adapted from the former models, and investigates the distribution of model parameters using experimental data and statistical methods. Chapter 6 concludes.
Articular cartilage is a connective tissue that overlays the ends of joints. Healthy articular cartilage can provide a lifetime of support for joint function. However, many causes such as sports injuries, obesity, and aging can induce cartilage degeneration, which may eventually lead to osteoarthritis (OA). Post-traumatic osteoarthritis (PTOA) is the OA that results from traumatic injury.

A challenge for clinical research is the development of biological treatments. Most research has focused on treating osteoarthritis in the middle or late stages of disease. A better understanding of the biological response to acute injuries may provide opportunities for early intervention, which could prevent or delay the development of PTOA.

The early stages of cartilage degeneration includes chondrocytes death (apoptosis and necrosis) and inflammatory responses. In this thesis, a series of mathematical models are developed to investigate the relationship between cell death and inflammation under two scenarios: injuries resulting from a single blunt impact, and excessive loading. The mathematical models bring insight to cartilage degeneration, provide helpful information that cannot be easily obtained from biological experiments, and lead to suggestions for clinical therapies.
TABLE OF CONTENTS

LIST OF TABLES ............................................................... vii

LIST OF FIGURES ........................................................... viii

CHAPTER

1 INTRODUCTION ............................................................ 1

1.1 Articular Cartilage ..................................................... 1

1.2 Osteoarthritis ........................................................... 3

1.2.1 Cartilage lesion formation process ......................... 4

1.2.2 Pro-inflammatory & anti-inflammatory cytokines .......... 5

1.3 Mathematical models of articular cartilage ..................... 6

2 A REACTION-DIFFUSION-DELAY MODEL OF THE EFFECTS OF CYCLIC LOADING ............................................. 8

2.1 The effects of mechanical loading in articular cartilage ...... 8

2.2 One dimensional model with mechanical loading .......... 9

2.2.1 Variables and biological scheme .............................. 10

2.2.2 Mathematical equations .......................................... 15

2.2.3 Cell strain function ................................................. 19

2.2.4 Parameterization .................................................... 21

2.2.4.1 Parameters related to ECM degradation ............... 22

2.2.4.2 The release rate of chemicals by chondrocytes ....... 24

2.2.4.3 The natural decay rate of chemicals .................... 25

2.2.4.4 Other parameters ............................................... 26

2.2.5 Numerical simulations ............................................. 29

2.2.5.1 Methods and results ........................................... 29

2.2.5.2 Sensitivity analysis ............................................ 36

2.2.5.3 Numerical methodology ..................................... 37

2.3 Conclusion .................................................................. 40

3 AN AGE-STRUCTURED MODEL OF THE EFFECTS OF A SINGLE BLUNT IMPACT .................................................. 42

3.1 Introduction of the age-structured model ...................... 42

3.2 Schematic of articular cartilage lesion formation due to a single blunt impact .................................................. 44

3.3 Mathematical model and numerical simulation .............. 47
LIST OF TABLES

Table

2.1 Table of parameter values ........................................... 28

2.2 Table of variable ranges at day 10 (a) The case of low EPO (b) The case of high EPO ........................................... 31

2.3 Table of sensitivity values ......................................... 36

2.4 Table of errors (a) Errors in the $\infty$-norm with low levels of EPO (b) Errors in the 2-norm with low levels of EPO ........................................... 38

2.5 Table of errors (a) Errors in the $\infty$-norm with high levels of EPO (b) Errors in the 2-norm with high level of EPO ........................................... 39

3.1 Table of parameter values ........................................... 55

3.2 Table of relative errors in the 2-norm ........................................... 61

3.3 Sensitivity table ........................................... 62

5.1 Parameter estimations ........................................... 101

5.2 Empirical distribution of the parameters ........................................... 101
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>(a) Original model dimensions (b) Model with radial symmetry and homogenized depth</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>Cell states</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic of the articular cartilage lesion formation process under cyclic loading</td>
<td>15</td>
</tr>
<tr>
<td>2.4</td>
<td>The relationship between cell death rate and the axial tissue compressive strain by cyclic loading [17]</td>
<td>20</td>
</tr>
<tr>
<td>2.5</td>
<td>Population of healthy, catabolic and EPOR-active cells with 30% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO</td>
<td>32</td>
</tr>
<tr>
<td>2.6</td>
<td>Population of healthy, catabolic and EPOR-active cells with 40% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO</td>
<td>33</td>
</tr>
<tr>
<td>2.7</td>
<td>Population of healthy, catabolic and EPOR-active cells with 60% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO</td>
<td>34</td>
</tr>
<tr>
<td>2.8</td>
<td>Population of healthy, catabolic and EPOR-active cells with 80% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO</td>
<td>35</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic of the articular cartilage lesion process under the effect of a single blunt impact</td>
<td>46</td>
</tr>
<tr>
<td>3.2</td>
<td>Cell densities (a) The densities of healthy cells (b) The densities of healthy, catabolic, and EPOR-active cells</td>
<td>57</td>
</tr>
<tr>
<td>3.3</td>
<td>The densities of live cells</td>
<td>58</td>
</tr>
<tr>
<td>3.4</td>
<td>The density of ECM</td>
<td>58</td>
</tr>
</tbody>
</table>
3.5 The densities of IL-6 and EPO

3.6 The density of DAMPs

3.7 The density of ROS

3.8 (a) Slides of articular cartilage 1, 7, 14 days after a single blunt impact stained for EPO, the squares in each image is what is magnified in Fig. 3.8b
(b) The zoomed areas in Fig. 3.8a

3.9 Concentrations of EPO as a function of “positive” cells (a) Day 1 at the impact site (b) Day 7 at the impact site (c) Day 14 at the impact site (d) Day 1 at a distance 2 mm from the impact site (e) Day 7 at a distance 2 mm away from the impact site (f) Day 14 at a distance 2 mm away from the impact site

3.10 The marked cells in image processing to count the number of “positive cells” in the cartilage slide (a) EPO sample 14 days after impact (b) IL-6 sample 7 days after impact

3.11 (a) The concentration of EPO measured experimentally (b) The concentration of IL-6 measured experimentally (c) The concentration of EPO simulated from the model (d) The concentration of IL-6 simulated from the model. The different line types (red + solid line + square, green + solid line + circle and black + dashed line + diamond) represent the EPO/IL-6 stained 14, 7, and 1 day after impact

4.1 Schematic of articular cartilage lesion formation under cyclic loading

4.2 The evolution of cell populations under 30% strain (a) Healthy cells (b) Healthy, catabolic and EPOR-active cells

4.3 The change of chemical concentrations under 30% strain (a) TNF-α & EPO (b) ROS

4.4 The evolution of cell populations under 40% strain (a) Healthy cells (b) Healthy, catabolic and EPOR-active cells

4.5 The change of chemical concentration under 40% strain (a) TNF-α & EPO (b) ROS

4.6 The evolution of cell population under 60% strain (a) Healthy cells (b) Healthy, catabolic and EPOR-active cells
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7</td>
<td>The change of chemical concentrations under 60% strain (a) TNF-α &amp; EPO (b) ROS</td>
<td>87</td>
</tr>
<tr>
<td>4.8</td>
<td>The evolution of cell populations under 80% strain (a) Healthy cells (b) Healthy, catabolic and EPOR-active cells</td>
<td>88</td>
</tr>
<tr>
<td>4.9</td>
<td>The change of chemical concentrations under 80% strain (a) TNF-α &amp; EPO (b) ROS</td>
<td>89</td>
</tr>
<tr>
<td>5.1</td>
<td>Stained slides</td>
<td>93</td>
</tr>
<tr>
<td>5.2</td>
<td>The concentration of (a) IL6 (b) EPO</td>
<td>95</td>
</tr>
<tr>
<td>5.3</td>
<td>Simplified schematic of cartilage lesion formation due to single blunt impact</td>
<td>96</td>
</tr>
<tr>
<td>5.4</td>
<td>The test of normality assumption (a) The histograms of bootstraps (b) The qqplots of bootstraps</td>
<td>102</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

1.1 Articular Cartilage

Cartilage is a connective tissue found in many parts of the body, including the nose, ear, and joints. It is softer than bone, but stiffer than muscle. Articular cartilage is a relatively soft tissue that covers the end of long bones within the synovial environment. It is found in the knee, shoulder and hip joints, where it supports joint function and distribute physiological loads.

Even though articular cartilage is only 2 to 4 mm thick, it has a complex structure, in which more than 90% of its volume is extracellular matrix (ECM) with a sparse population of chondrocytes (specialized cells cartilage cells). The extracellular matrix is mainly composed of collagen, proteoglycan, and water. Bulk chemical analyses find proportions of more than 70% water, 15% proteoglycan, and another 15% collagen in wet mass [32]. There are four distinct zones (superficial, transitional, radial, calcified). The superficial zone consists of collagen fibers that are parallel to the cartilage surface, and takes up 5-10% of the volume inside the matrix. The collagen structure provides the superficial zone with tensile strength to resist shear stresses on the cartilage surface. The transitional zone occupies 40-45% of the matrix volume, and is composed of pseudo-random oriented collagen fibers, which gradually transiting from a horizontal to a vertical orientation. The radial/deep zone is roughly the same size as the transitional zone. The collagen fibers insert vertically
into the tidemark which distinguishes the radial zone from the calcified zone. Both the transitional and the radial zone have a high concentrations of proteoglycan, which provides resistance to compressive forces. Finally, the calcified layer is the transition zone between cartilage and bone. It anchors the collagen fibrils in the deep zone to subchondral bone [41].

Collagen is the most abundant component in ECM besides water, and it accounts for 60% of ites dry weight of the ECM. More than 90% of collagen in cartilage is Type II, with smaller portions of Type I, IV, V, VI, IX and XI. Collage fibrils are composed of aggregated molecules consisting of three polypeptide chains wound around one another to form a triple helix [31]. The triple helix structure forms the frame and provides stability to the ECM and provides tensile strength to articular cartilage [36].

Proteoglycans are another important component in ECM, consisting of 25%-35% of the dry weight [26]. Proteoglycans consist of a protein core attached by side chains of aggrecans containing large numbers of glycosaminoglycan (GAG) with sulphates bound to the central region [41]. Most aggrecans are bound to hyaluronic acid, so the molecular weights may be as high as 200 million daltons [18]. The function of proteoglycans is like the “cushion”, which maintain the integrity of ECM and protects the cartilage from the outside forces.

The cells specified in articular cartilage are known as chondrocytes, which are located inside the matrix of collagen and proteoglycans. Chondrocytes are responsible for the maintenance of the matrix, and regulate cartilage through the synthesis and
release of enzymes such as alarmins, cytokines and oxidants. Chondrocytes differ across zones. In the superficial zone, chondrocytes are small, flat, and aligned along their long side parallel to the surface. The cells in the transitional and deep zones are rounder and have larger lacunae. Unlike other cells, chondrocytes are trapped within their own matrix and do not generally migrate to adjacent areas. Chondrocytes have limited capacity of to reproduce [41], which leads to the inability of self-repair after injury.

Overall, articular cartilage is a highly heterogeneous, anisotropic and multi-phase biomaterial. It is difficult to capture all the properties in a single mathematical model. Compared to other zones, the superficial zone is specialized to resist tensile stresses and reduce surface friction. Among all the 4 layers of cartilage, superficial zone is the most important zone in cartilage degeneration. The first phase of OA starts in the superficial zone. Besides, it is the zone where most cell and chemical activities occur [21]. Rather than building a model for all zones, we focus on the properties of the superficial zone, and assume the solid cartilage matrix to be a homogeneous system.

1.2 Osteoarthritis

Osteoarthritis (OA) is a major cause of disability and chronic pain affecting approximately 250 million people worldwide (3.6% of the population), with 27 million in the United States [45]. It is estimated that 80% of the population will have radiographic evidence of OA by the age of 65, and 60% among this group show phys-
ical symptomatic [23]. Increasing average lifespans suggest that the incidence rate of osteoarthritis will increase dramatically, leading to a significant health care problems with tremendous costs. Due to the high prevalence and incidence rate, it is necessary to understand the cartilage degeneration process, and develop realistic models that can suggest long-term effective treatments.

1.2.1 Cartilage lesion formation process

The causes for OA include genetic reasons, aging and trauma. In general, cartilage degeneration may be initiated by physical injury, inflammation and metabolic perturbations. Many changes occur in the early stages of degeneration before the development of clinical OA. Those changes include the disorganization of collagen (mainly in the superficial zone), cell cloning and necrosis, followed by a dramatic loss of proteoglycan. The growth of matrix-degrading enzymes accelerates the progress of ECM degeneration. At the same time, chondrocytes may express an attempt for self-repair by synthesizing more matrix components, and increasing catabolic activities. However, chondrocyte repair response declines with age, resulting in clinically apparent OA.

OA resulting from joint trauma or excessive loading stress is called post-traumatic osteoarthritis (PTOA). It is estimated that 10-15% of OA is of post-traumatic type. PTOA occurs in patients experiencing joint fractures and dislocations, ligament and cartilage injuries in car crashes, or sports accidents. Damage is initiated by high loading rates (>20 MPa/s) and high peak stress amplitudes (>
20 MPa), and spreads across the joint surfaces. There is evidence that superficial chondrocyte death is strongly associate with the early stage of PTOA. Preventing cell death immediately after injury is the main target of most therapies for PTOA.

Chondrocyte death and survival are closely related to ECM integrity and cartilage degeneration. Cell death in cartilage can be divided into apoptosis and necrosis. Apoptosis (programmed cell death) is a process in which the dysfunctional cells are eliminated, whereas necrotic cells are caused by external injury and mechanical damage. Cell necrosis stimulates the inflammation response, and leads to the chain reactions of cartilage degradation.

1.2.2 Pro-inflammatory & anti-inflammatory cytokines

Mechanical injury is often followed by inflammation responses with varying degrees of severity. Though the role of the inflammation processes in the pathogenesis of OA remains unclear, current research indicates that the cytokine network may play an important role in the degeneration of cartilage. A better understanding of the dynamic balance among inflammatory cytokines may aid the use of antibodies and other recombinant biological factors as therapy to interrupt the degeneration and halt the disease at an early stage.

Cytokines can be divided into two broad categories: pro-inflammatory and anti-inflammatory. The function of pro-inflammatory cytokines is relatively well established in the literature, whereas the role of anti-inflammatory cytokines is still
under active investigation. Pro-inflammatory cytokines are the main reason for cell apoptosis and cause severe aggrecan depletion. They also decrease the synthesis of ECM components. In addition, pro-inflammatory cytokines may simulate the release of proteolytic enzymes, including metalloproteinases family MMPs and ADAMTS, which further decompose cartilage. Common pro-inflammatory cytokines are IL-1β, TNF-α, IL-6 and IL-8. Anti-inflammatory cytokines inhibit the synthesis of pro-inflammatory cytokines (particularly IL-1β and TNF-α), lead to increased synthesis of proteoglycan, inhibit cell apoptosis, and decrease secretion of metalloproteinase [49]. Recent research suggests that anti-inflammatory cytokines such as erythropoietin (EPO) play a role in counteracting the effect of inflammation and stop the spread of a lesion [16]. The “balancing act” between pro-inflammatory and anti-inflammatory cytokines play an essential role in the cartilage degeneration process and determines the spread of the cartilage lesion [22].

1.3 Mathematical models of articular cartilage

OA is a painful and chronic disease which affects many people. Present research on cartilage biology, biochemistry, and biomechanics provides a basis for enhanced clinical treatment. However, the exact cause of OA remains unclear, and current treatments can only temporarily relieve the symptoms [18]. Mathematical models of articular cartilage can help to build a sound understanding of the cartilage degradation process, find quantitative solutions which are difficult to be obtained in experimental studies, and bring insights and predictions.
In the past decades, Mow and his group made great contributions in the area of biomechanics [34], [33] and [28]. However, most mathematical models have focused on the mechanical properties of articular cartilage deformation using elastic, viscoelastic, and biphasic models, while ignoring cell and chemical aspects.

As we discussed in the preceding sections, chondrocyte death and inflammatory cytokines play an important role in cartilage lesion formation of PTOA, along side the biomechanics. A greater understanding of these biological processes may open the possibility of new therapy. Graham et al. developed a mathematical model to describe the biological interactions between chondrocytes and cytokines after a single blunt impact [22]. Another model was build by Wang et al. to represent the cartilage lesion process under extensive cyclic loading [46] and age structure was introduced in more recent research [47]. This body of work establishes a basis for further research on the cartilage degeneration process, with an emphasis on chondrocyte depletion and the balancing act of the inflammatory cytokines.
CHAPTER 2
A REACTION-DIFFUSION-DELAY MODEL OF THE EFFECTS OF CYCLIC LOADING

2.1 The effects of mechanical loading in articular cartilage

Articular cartilage in both the hip and the knee experiences cycles of loading and unloading every day through normal activities (walking, running and jumping). There is evidence that the physical forces in the joint influence the chondrocytes’ viability and the matrix synthesis inflammation process. However, chondrocyte deformation in response to mechanical loading is unclear. Cartilage is a hypoxic tissue, so there are very few mitochondria in cartilage. Even though the number of mitochondria is low, the development of osteoarthritis is correlated with the dysfunction of the mitochondria [19] and [15]. Mechanical loading leads to the production of reactive oxygen species (ROS) by mitochondria, and the extensive loading can lead to the production of superoxide ($O_2^-$), which is lethal to the chondrocytes. Interestingly, normal cyclic loading helps produce oxidants to maintain glycolytic ATP synthesis, but intensive loading induces superoxides and causes cell death. Experiments are needed to determine the ranges of ROS production and chondrocyte viability and thus determine if the range of strain due to cyclic loading is “safe” or “dangerous”.

The study conducted in [17] demonstrated that semi-confined static compressive stress will induce superoxide release and cell death. A relationship between static compressive strain and cell viability is determined by the experiments. When below 20%, the strain is considered to be “safe” or “healthy”. This means that the static
compressive stress is under 0.1 MPa, and the loading maintains the normal function of articular cartilage. Strains below 40% (stress below 0.25 MPa) lead to very little chondrocyte death, whereas strains above 60% cause high rates of cell death.

Although a lot of progress has been made in _in vitro_ experiments to understand the short-term responses of cartilage tissue and chondrocytes, the long-term effects involving the development of osteoarthritis are still unknown. The chronic effects of overloading can be studied in _in vivo_ experiments, but the compression stresses on the experimental animals are difficult to control. Mathematical models can help to extrapolate the short-term _in vitro_ data to OA-relevant time frames and shed light on the lesion process.

### 2.2 One dimensional model with mechanical loading

Mechanical loading plays a central role in the dynamics of articular cartilage. It stimulates biosynthetic activity and is essential for homeostasis. Although the mechanotransduction process is unclear, there is strong evidence that mechanical loading on articular cartilage can cause chondrocyte deformation and initiate special intracellular processes such as cell death, matrix degradation, and eventually inflammation. Studying the environment of chondrocytes through mathematical modeling can help explain this mechanical-biological phenomenon. A reaction-diffusion-delay model [22] was built to describe the chondrocyte dynamics after an initial severe traumatic impact with no further loading. In the model in this chapter, we assume that the articular cartilage has sustained no initial damage, but instead experiences cyclic
Figure 2.1. (a) Original model dimensions (b) Model with radial symmetry and homogenized depth

compressive loading applied to a small part of the cartilage (Fig. 2.1a). Cell apoptosis induced by cyclic loading is expressed by deformation terms in the system of partial differential equations.

2.2.1 Variables and biological scheme

We assume radial symmetry and only model the lesion formation on the surface of the cartilage cylinder. The system is reduced to a one-dimensional model with respect to space (Fig. 2.1b). The components of the system depend on radius \( r \) (\( 0 \leq r \leq 2.5 \) cm) and time \( t \) (\( 0 \leq t \leq 10 \) days). We assume the oscillating load is inside the region with radius between 0 to 0.25 cm from the origin.

Before the introduction of the mathematical equations, we first define the
variables used in the model. We have two categories of variables in our system, cells and chemicals. The cellular variables represents different cell states of chondrocytes. Different states of chondrocytes play different roles during injury response. We define three general cell types: healthy cells, sick cells and dead cells. The variables are

- $C(r, t)$: population density (cells per unit area) of healthy chondrocytes.
- $S_T(r, t)$: population density of "catabolic" chondrocytes. Catabolic chondrocytes are signaled by alarmins, also called damage associated molecular patterns (DAMPs), and start to synthesize pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Healthy cells ($C$) signaled by DAMPs or TNF-α enter into the catabolic state ($S_T$). Catabolic cells ($S_T$) start to synthesize TNF-α and reactive oxygen species (ROS).
- $S_A(r, t)$: population density of EPOR-active chondrocytes. EPOR-active chondrocytes are cells signaled by TNF-α and express a receptor EPOR for erythropoietin (EPO). There is a time delay of 8-12 hours before a cell expresses the EPO receptor after being signaled to be EPOR active [16]. EPOR-active cells may switch back to the healthy state ($C$) after being signaled by EPO.
- $D_A(r, t)$: population density of apoptotic chondrocytes. TNF-α causes irreversible cell injury; subpopulations of catabolic ($S_T$) and EPOR-active ($S_A$) cells become apoptotic. Apoptotic cells play no role in the system, but are tracked explicitly for the conservation of cell populations.
- $D_N(r, t)$: population density of necrotic chondrocytes. Necrosis occurs due to the internal mechanical loading, so the cells in the loading area become necrotic
and release alarmins (DAMPs) to trigger the cartilage injury response.

Catabolic and EPOR-active cells are classified as “sick cells”, whereas necrotic and apoptotic cells are classified as “dead cells”. The transition of cells is shown in Fig. 2.2. Healthy cells receive alarmins (DAMPs) and become sick under the effects of pro-inflammatory cytokines, such as TNF-α. Sick cells can either die due to DAMPs and TNF-α, or recover to be healthy under the effect of anti-inflammatory cytokines, such as EPO. There are two time delays ($\tau_1$, $\tau_2$). The first delay $\tau_1$ represents the gap between the time when catabolic cells become EPOR-active and the time EPOR-active cells expresses the EPO receptor. The second delay $\tau_2$ represents the gap between when healthy cells are signaled by ROS and when they produce EPO.

Besides the variables for cell states, the chemical variable for alarmins (DAMPs), pro-inflammatory cytokines, such as TNF-α, anti-inflammatory cytokines, such as
EPO, and ROS are

- $R(r, t)$: concentration of reaction oxygen species (ROS) released by catabolic cells ($S_T$). In our model we only assume that ROS affects the production of EPO by healthy cells. There is a time delay of 20-24 hours before a healthy cell signaled by ROS start to produce EPO.

- $M(r, t)$: concentration of alarmins (DAMPs) released by necrotic cells ($D_N$) and extracellular matrix ECM. DAMPs trigger the whole system by signaling healthy cells to enter the catabolic states, which will produce TNF-α and cause further lesions in the cartilage.

- $F(r, t)$: concentration of tumor necrosis factor (TNF-α) released by catabolic cells ($S_T$). The “balancing act” between TNF-α and EPO is the main driver of our system. The main function of TNF-α is:
  - causes healthy cells to become catabolic,
  - causes catabolic cells to enter the EPOR-active state $[16]$, 
  - causes apoptosis of catabolic and EPOR-active cells,
  - causes degradation of extracellular matrix (ECM), which will in turn release more DAMPs into the system,
  - limits the production of EPO by healthy cells $[16]$.

- $P(r, t)$: concentration of EPO released by healthy cells ($C$). EPO causes EPOR-active cells to switch back to the healthy state. In the model, EPO helps stop the spread of inflammation and the recovery of the system. EPO also limits
the switch from the healthy state to the catabolic state under the effect of TNF-α. However, the ability of EPO to limit the effect of TNF-α depends on the concentration of EPO \([16]\). For convenience, we assume that EPO may shut down the transition between healthy cells and catabolic cells when its concentration is higher than \(P_c\).

In addition to cellular and chemical components, we also introduce a variable to represent the density of extracellular matrix.

- \(U(r, t)\): density of ECM. In our model, we assume that ECM is degraded by TNF-α, and releases DAMPs in the degradation process.

We illustrate the inflammatory process under cyclic loading in Fig. 2.3

Mechanical loading degrades healthy cells \((C)\) in the loading area due to the activity of necrotic cells \((D_N)\). Necrotic cells release alarmins (DAMPs) and cause healthy cells \((C)\) to become sick and enter the catabolic state \((S_T)\). Catabolic cells start to release inflammatory cytokines TNF-α, which causes more healthy cell \((C)\) to become catabolic \((S_T)\). At the same time, catabolic cells \((S_T)\) signaled by TNF-α either enter the EPOR-active \((S_A)\) state after half day’s delay or become apoptotic \((D_A)\). EPOR-active cells \((S_A)\) may switch back to the healthy state \((C)\) in the presence of anti-inflammatory cytokines, such as EPO. We assume that chondrocytes are fixed in matrix, so all states have negligible motility.
2.2.2 Mathematical equations

In order to build the mathematical model, we first assume that the chemicals can diffuse, causing the spread of the lesion. However, the chondrocytes themselves are fixed inside the matrix, so there is very little cell motility. We can build the model by including diffusion terms in the chemical equations, while using ordinary differential equations for cell populations. Adapting from [22], an equations for a chemical concentration has the form
change in concentration of chemical

\[ \text{change in concentration of chemical} = \text{chemical diffusion} + \text{the production of this chemical by cells} - \text{natural decay of this chemical}. \]

An equation for a cell density population has the form

\[ \text{change in population density of the cells in this state} = \text{population density of cells in other states that switch into this state} + \text{population density of cells in this state that switch out to other states}. \]

The reaction rate of chemicals is modeled by Michealis-Menten kinetics. We define the Heaviside function,

\[ H(s) = \begin{cases} 0, & s < 0, \\ 1, & s \geq 0. \end{cases} \]

The Heaviside function is used to describe the phenomenon that the effects of DAMPs and TNF-\(\alpha\) can be shut down by EPO when its concentration exceed \(P_c\).

We assume that ROS is only produced by catabolic cells \((S_T)\), so the change of concentration in ROS equals its production by \(S_T\) plus the diffusion term and minus the natural decay,

\[ \partial_t R = \nabla \cdot (D_R \nabla R) - \delta R + \sigma_R S_T. \] (2.1)
TNF-α is solely released by catabolic cells ($S_T$) in our model,
\begin{align}
\partial_t F &= \nabla \cdot (D_F \nabla F) - \delta F + \sigma_F S_T. \tag{2.2}
\end{align}

DAMPs is either produced by necrotic cells ($D_N$) or released from the degradation process of ECM under the effect of TNF-α,
\begin{align}
\partial_t M &= \nabla \cdot (D_M \nabla M) - \delta M + \sigma_M D_N + \sigma_U U \frac{F}{\lambda_F + F}. \tag{2.3}
\end{align}

EPO is released by healthy cells signaled by ROS after a delay of $\tau_2$. The production of EPO is under the constraints of TNF-α, i.e. the higher the level TNF-α is, the less EPO can be produced,
\begin{align}
\partial_t P &= \nabla \cdot (D_P \nabla P) - \delta P + \sigma_P C \frac{R(t - \tau_2)}{\lambda_P + R(t - \tau_2) \Lambda + F}. \tag{2.4}
\end{align}

Healthy cells ($C$) will enter into catabolic state ($S_T$) under the effect of TNF-α and DAMPs. The transition can be stopped when the EPO level exceeds the threshold level $P_c$, so the Heaviside function is introduced in the equation. At the same time, healthy cells ($C$) become necrotic ($D_N$) due to the intensive cyclic loading. On the other hand, EPOR-active cells ($S_A$) may switch back to healthy ($C$) under the effect of EPO,
\begin{align}
\partial_t C &= \alpha S_A \frac{P}{\lambda_P + P} - \beta_1 C \frac{M}{\lambda_M + M} H(P_c - P) \tag{2.5} \\
&\quad - \beta_2 C \frac{F}{\lambda_F + F} H(P_c - P) - \Gamma(\epsilon, U, r) \cdot C.
\end{align}

While the healthy cells ($C$) enter into the catabolic state ($S_T$), catabolic cells ($S_T$) themselves will either enter into the EPOR-active ($S_A$) state under the effect of EPO
after $\tau_1$ delay, or degrad when exposed to TNF-\(\alpha\) and DAMPs and become apoptotic. Meanwhile, cyclic loading will also cause catabolic cells to become necrotic,

$$\partial_t S_T = \beta_1 C \frac{M}{\lambda_M + M} H(P_c - P) - \beta_2 C \frac{F}{\lambda_F + F} H(P_c - P)$$

(2.6)

$$- \gamma S_T(t - \tau_1) \left( \frac{F(t - \tau_1)}{\lambda_F + F} \right) - \nu S_T \frac{F}{\lambda_F + F} \frac{M}{M + M}$$

$$+ \Gamma(\epsilon, U, r) \cdot S_T.$$

EPOR-active cell ($S_A$) either switch back to be healthy ($C$) or become apoptotic ($D_A$) under the effects of TNF-\(\alpha\). Like other cells, $S_A$ become necrotic under loading,

$$\partial_t S_A = \gamma S_T(t - \tau_1) \frac{F(t - \tau_1)}{\lambda_F + F} - \alpha S_A \frac{P}{\lambda_P + P}$$

(2.7)

$$- \mu S_A \frac{F}{\lambda_F + F} + \Gamma(\epsilon, U, r) \cdot S_A.$$

Cyclic loading causes healthy and sick cells to become necrotic ($D_N$), and necrotic cell will decay,

$$\partial_t D_N = -\mu D_N D_N + \mu S_T S_T - \Gamma(\epsilon, U, r) \cdot (C + S_T + S_A).$$

(2.8)

The population of apoptotic cells comes from sick cells degrading under the influence of inflammatory cytokines, such as TNF-\(\alpha\),

$$\partial_t D_A = \mu S_A \frac{F}{\lambda_F + F} + \nu S_T \frac{F}{\lambda_F + F} \frac{M}{M + M}.$$  

(2.9)

ECM is degraded by TNF-\(\alpha\), and this process can be shut off if the level of EPO exceeds the threshold level $P_c$,

$$\partial_t U = -\delta U \frac{F}{\lambda_F + F} H(P_c - P).$$

(2.10)
We take our temporal and spatial domains to be $t > 0$ and $0 \leq r \leq r_m$, where $r_m = 2.5\text{cm}$ is the radius of cartilage sample. The initial conditions for chemicals when $t = 0$ are $R(r, t) = M(r, t) = F(r, t) = P(r, t) = 0 \text{ nM (nanomolars)}$.

We use homogeneous Neumann boundary conditions for the chemical concentrations:

$$\frac{\partial R}{\partial r} \bigg|_{r=0} = \frac{\partial M}{\partial r} \bigg|_{r=0} = \frac{\partial F}{\partial r} \bigg|_{r=0} = \frac{\partial P}{\partial r} \bigg|_{r=0} = 0,$$

and

$$\frac{\partial R}{\partial r} \bigg|_{r=r_m} = \frac{\partial M}{\partial r} \bigg|_{r=r_m} = \frac{\partial F}{\partial r} \bigg|_{r=r_m} = \frac{\partial P}{\partial r} \bigg|_{r=r_m} = 0.$$

The initial conditions for the cells when $t = 0$ are $S_T(r, t) = D_N(r, t) = S_A(r, t) = 0$ (cells/cm$^2$), and

$$C(r, t) = \begin{cases} 1 \times 10^5 \text{cells/cm}^2, & 0 \leq r \leq r_l \\ 0, & r > r_l. \end{cases}$$

The initial condition for ECM is $U(r, t) = 30\text{mg/cm}^2$.

### 2.2.3 Cell strain function

The cell strain function $\Gamma(\epsilon, U, r)$ is essential to our cyclic loading model. Experiments\cite{17} demonstrated the relationship between the semi-confine static compressive stress and the chondrocytes death. Cyclic loading in our case is continuous and gradually leads to a steady-state strain. When the static strain is below 40%, very few cells will become necrotic. On the contrary, strains above 60% will cause widespread chondrocyte death. When the static strain is between 40% and 60%, the cell death rate increases exponentially as the static strain increases (Fig\cite{24}).
Cyclic loading can also lead to extracellular matrix (ECM) degradation, which may lower the ability for articular cartilage to resist the damage caused by cyclic loading. The rate of cell death may increase when the mass of ECM decreases.

Our goal is to build a mathematical model to describe the effects of cyclic loading in the process of articular cartilage lesion formation. The function $\Gamma(\epsilon, U, r)$ we use is simple but appropriate for this first effort,

$$\Gamma(\epsilon, U, r) = \begin{cases} 24 \ast (1 - 0.01 \ast p_0(\exp(K_U \cdot \epsilon) - \exp(20 \cdot K_u))) \ast \frac{\lambda u}{\lambda u + \mu}, & 0 \leq r \leq r_t, \\ 0, & r > r_t \end{cases}$$

where $\Gamma$ is non-negative, and the strain $\epsilon$ ranges from 0 to 100%. When the strain $\epsilon$ is below 20%, the loading can be considered to be healthy, so there is no cell death.
The function $\Gamma(\cdot)$ is based on recent experiment results [17], and shows the cell viability rates as a function of equilibrium strain and ECM mass. The data used for modeling are based on equilibrium strain, which has some limitation. Further results are needed to build a function with respect to both strain and time. It is possible that cells may not keep dying but stay at a steady state even though the loading continues. The function is only applicable for the 1D situation and steady strain. In future study, elastic equations may be more appropriate for describing the elastic properties of articular cartilage under loading, and more suitable for 2D and 3D models.

2.2.4 Parameterization

Parameterization is needed before we can conduct numerical simulations. Some parameters can be estimated from published articles, especially those with explicit biological meanings and that can be measured in in vivo or in vitro experiments. However, some parameters have no clear biological meanings, or are difficult to determine from experiments. The values of these parameters can be approximated for modeling purposes and tested by sensitivity analyses. The main goal is to develop a minimal mechanistic mathematical model that can be used to investigate the mechanism of cyclic loading in articular cartilage. In this section, we will mainly discuss the parameters that can be estimated from references. The values of the parameters are in the Table 2.1.
2.2.4.1 Parameters related to ECM degradation

As we discussed before, the density of ECM can effect the cell death rate of cyclic loading (Eq. 2.11), so the equation for $U$ is relevant to cell death. In this model we assume TNF-α causes the degradation of ECM, which release DAMPs (Fig. 2.3). The damage to ECM can be expressed by the decrease in proteoglycan concentration in ECM, which can be measured in terms of the decrease concentration of SO$_4$. The sulfate groups decorating the aggrecan proteins are the groups that matter in ECM degradation, because the aggrecan protein is just an elaborate means to keep sulfates in the solid phase in ECM (so call “fixed charges”) [32].

The decay coefficient of ECM due to TNF-α is denoted by $\delta_U$. The decay rate of SO$_4$ under 25 ng/ml of TNF-α is about 16% per week [30]. The real decay rate under cyclic loading may be lower, so we assume that the decay rate of SO$_4$ is around 10% per week (0.1/7 % per day). The way to calculate the decay coefficient $\delta_U$ is the following. First, $\delta_U$ should satisfy

$$\frac{\partial U(r,t)}{\partial t} = -\delta_U \frac{F}{\lambda_F + F}$$

(2.12)

When $F = 25$ ng/ml, $U(r,t = 1 \text{ day}) = (1 - 0.1/7) \cdot U_0$, where $U_0$ is the initial mass of ECM (SO$_4$). The molecular weight of TNF-α is around 17kDa ($17 \times 10^3$ g/mol), so $F = 25$ ng/ml $= \frac{25 \text{ ng/ml}}{17 \times 10^3 \text{ g/mol}} = 1.4706 \text{ nM}$ (nanomolar). We choose $\lambda_F = 0.5$ nM and plug it into Eq. 2.12 to get

$$U(r,t = 1 \text{ day}) = U_0 \cdot e^{-\delta_U \frac{F}{\lambda_F + F} \cdot 1 \text{ day}} = U_0 \cdot e^{-\delta_U \frac{F}{\lambda_F + F}} = (1 - 0.1/7) \cdot U_0$$

$\Rightarrow e^{-\delta_U \frac{F}{\lambda_F + F}} = (1 - 0.1/7) \text{ day} \Rightarrow -\delta_U \frac{F}{\lambda_F + F} = \ln(1 - 0.1/7) \text{ day}$
\[
-\delta_U \frac{1.4706}{0.5 + 1.4706} = -0.0144 \text{ day}
\]

so

\[
\delta_U = 0.0193 \frac{1}{\text{day}}
\]

The coefficient for the release rate of DAMPs from ECM during the degradation is denoted as \(\sigma_U\). We assume 30 mg/cm\(^3\) of ECM (SO\(_4\)) may release 10 ng/ml of DAMPs when expose to 25 ng/ml of TNF\(_\alpha\). One of the most common species of DAMPs in articular cartilage is the chromatin-associated protein high-mobility group box 1 (HMGB1), so we use HMGB1 in our model to represent the DAMPS family. The molecular weight of HMGB1 is 29 kDA. The change of concentration in DAMPS released by ECM under the effect of TNF-\(\alpha\) can be expressed by \(\sigma_U \cdot \frac{F}{\lambda_F + F} \cdot U(r,t)\), so we can build the following relationship:

\[
\text{Change in concentration in DAMPs} = \sigma_U \cdot \frac{F}{\lambda_F + F} \cdot U(r,t).
\] (2.13)

When \(U(r,t) = 30\ \text{mg/cm}^3\), and \(F = 25\ \text{ng/ml}\),

\[
\sigma_U \cdot \frac{F}{\lambda_F + F} \cdot U(r,t) = \text{Change in concentration of DAMPs}
\]

\[
= 10\ \text{ng/ml} \times \frac{1}{\text{day}} = \frac{10 \times 10^{-9} \text{g/cm}^3 \cdot \text{day}}{29 \times 10^3 \text{g/mol}}
\]

\[
= 0.3448 \times 10^{-9} \text{mol/(L} \cdot \text{day)}.
\]

So we have

\[
\sigma_U \cdot \frac{1.4706}{0.5 + 1.4706} \times 30\ \text{mg/cm}^3 = 0.3448 \times 10^{-9} \text{mol/(L} \cdot \text{day)}
\]

\[
\Rightarrow \sigma_U = 0.3448 \times 0.7463/30 \times 10^9 \frac{\text{mol/L} \cdot \text{day}}{\text{mg/cm}^3},
\]
\[
\sigma_u = 0.0154 \text{nanomolar} \cdot \text{cm}^3 \text{mg}^{-1} \cdot \text{day}^{-1}
\]

**2.2.4.2 The release rate of chemicals by chondrocytes**

The release rate of a chemical is proportional to the production of that chemical per cell. So we can divide the change of chemical concentration by the density of cells which produce this chemical.

Let \( \sigma_R \) denote the release rate of ROS (\( R \)) by catabolic cells. We assume 1-2\% oxygen consumed in cartilage is converted to superoxides (ROS). The maximum oxygen consumption rate is around 10 nMoles per million cells per hour in normal conditions (5-21\% oxygen) \[50\],

\[
\sigma_R = \frac{0.01 \times 10 \text{nMoles}}{10^6 \text{cells} \times 1 \text{hour}} = \frac{0.1 \times 24 \times 10^3 \text{nMoles}}{10^6 \text{L}} \text{cm}^3 \text{day}^{-1} \cdot \text{cells}^{-1}
\]

\[
= 0.0024 \text{nanomolar} \cdot \text{cm}^3 \text{cells}^{-1} \cdot \text{day}^{-1}
\]

Let \( \sigma_F \) denote the release rate of TNF-\( \alpha \) (\( F \)) by catabolic cells. We assume that 100 pg/ml TNF-\( \alpha \) can be released by \( 5 \times 10^4 \text{cells/ml} \) per half day \[43\],

\[
\sigma_F = \frac{100 \times 10^{-12} \times 10^3 \text{g} \cdot \text{cm}^3/\text{L}}{5 \times 10^4 \times 0.5 \times 17 \times 10^3 \text{(g/mol)} \cdot \text{cells} \cdot \text{days}}
\]

\[
= 2.35 \times 10^{-7} \text{nanomolar} \cdot \text{cm}^3 \text{cells}^{-1} \cdot \text{day}^{-1}
\]

Let \( \sigma_M \) denote the release rate of HMGB1 (\( M \)) by necrotic cells (\( D_N \)). We
assume that 3 pg/ml HMGB1 can be released by $2 \times 10^5$ cells/ml per day [43],

$$\sigma_M = \frac{3 \times 10^{-9} \times 10^3 \text{g} \cdot \text{(cm}^3\text{/L)}}{2 \times 10^5 \times 29 \times 10^3 \text{(g/mol)} \cdot \text{cells} \cdot \text{days}}$$

$$= 5.17 \times 10^{-7} \frac{\text{nanomolar} \cdot \text{cm}^3}{\text{cells} \cdot \text{day}}$$

(2.16)

Let $\sigma_P$ denote the release rate of EPO ($P$) by healthy cells ($C$). We assume that 18 ng/ml EPO can be released by $10^5$ cells/cm$^2$ per 4 days [16]. Note that the molecular rate for EPO is 34 kDa. Then

$$\sigma_P = \frac{18 \times 10^{-9} \times 10^3 \text{g} \cdot \text{(cm}^3\text{/L)}}{(10^5)^{(3/2)} \times 4 \times 34 \times 10^3 \text{(g/mol)} \cdot \text{cells} \cdot \text{days}}$$

$$= 4.2 \times 10^{-7} \frac{\text{nanomolar} \cdot \text{cm}^3}{\text{cells} \cdot \text{day}}$$

(2.17)

We also conduct the numerical simulation for the case of a high production rate of EPO, $\sigma_P = 0.0033$, which was used in [22]. Though this value is not a “natural” production rate, it can produce enough EPO to trigger the Heaviside function $H(\cdot)$, so that the level of EPO exceeds $P_c$ and shuts off the inflammation response.

2.2.4.3 The natural decay rate of chemicals

The natural decay rate of a chemicals can be obtained by knowing the half life of that chemical. We have that

$$\text{natural decay rate} \times \text{half life} = -\ln(1/2)$$

The natural half lives are estimated from experimental results in [48] and [25] by using the N-end rule [44].

The natural half life of TNF-\(\alpha\) in articular cartilage is around 100 hours [48],
so the natural decay rate of TNF-α $\delta_F$ can be calculated as

$$\delta_F = -\frac{24 \text{ hours}}{100 \text{ hours}} \ln\left(\frac{1}{2}\right) = 0.1664 \frac{1}{\text{day}}$$ (2.18)

The natural half life of EPO in articular cartilage is around 30 hours [20], so the natural decay rate of EPO $\delta_P$ can be calculated as

$$\delta_P = -\frac{24 \text{ hours}}{30 \text{hours}} \ln\left(\frac{1}{2}\right) = 0.5545 \frac{1}{\text{day}}$$ (2.19)

The natural half life of HMGB-1 in articular cartilage is around 30 hours [25], so the natural decay rate of DAMPs $\delta_M$ can be calculated as

$$\delta_M = -\frac{24 \text{ hours}}{30 \text{hours}} \ln\left(\frac{1}{2}\right) = 0.5545 \frac{1}{\text{day}}$$ (2.20)

The natural half life of ROS is around 14 hours at 0.1 nanomolar concentration of ROS, so $\delta_R = 1.1883 \frac{1}{\text{day}}$. However, under the superoxide dismute (SOD), ROS might disappear instantaneous. However, we do not know when this reaction will happen since it depends on the SOD concentration in articular cartilage and it is hard to measure. So we assume the coefficient $\delta_R = 60 \frac{1}{\text{day}}$ in our model, which means the half life of ROS is less than 20 minutes.

### 2.2.4.4 Other parameters

The parameters $K_U = 0.0545$ and $p_0 = 1 \frac{1}{\text{day}}$ in the strain-cell function (2.11) are obtained from experiments ([17]). Diffusion coefficients $D_R$, $D_M$, $D_P$ and $D_F$ were obtained from Leddy et al. 2004 [29], and delay times $\tau_1$, $\tau_2$ are from Brines and Cerami 2008 [16]. Except for these parameters and those in the section above, the other parameters in the model were approximated so that the simulation results
give quantitatively reasonable cell population densities and chemical concentrations.

The goal is to make our model and simulation results relevant to understanding the
inflammation process. The parameters taken from experimental measurements in the
literature are

- $\alpha$: cell transition rate from EPOR-active $S_A$ to healthy $C$ under EPO,
- $\beta_1$: cell transition rate from healthy $C$ to catabolic $S_T$ under DAMPs,
- $\beta_2$: cell transition rate from healthy $C$ to catabolic $S_T$ under TNF-\(\alpha\),
- $\gamma$: cell transition rate from catabolic $S_T$ to EPOR-active $S_A$ under TNF-\(\alpha\),
- $\nu$: the rate of catabolic $S_T$ decaying to apoptotic $D_A$ under TNF-\(\alpha\) and DAMPs,
- $\mu_{SA}$: the rate of cell transition from catabolic $S_A$ to apoptotic $D_A$ under TNF-\(\alpha\),
- $\mu_{DN}$: the decay rate of necrotic cell $D_N$,
- $\lambda_{R}$: concentration of ROS at which the reaction rate is at half-maximum,
- $\lambda_{P}$: concentration of EPO at which the reaction rate is at half-maximum,
- $\lambda_{M}$: concentration of DAMPs at which the reaction rate is at half-maximum,
- $\lambda_{F}$: concentration of TNF-\(\alpha\) at which the reaction rate is at half-maximum,
- $\lambda_{U}$: the coefficient to lessen the cell death rate depending on ECM density,
- $\Lambda$: the coefficient to limit the production of EPO depending on TNF-\(\alpha\).

Even though these parameters may all have physical meanings, they cannot
be tested easily from experiments, so the way we choose them is to make sure that we
have reasonable simulation results. We need to conduct the stability of our system
by checking whether the small perturbations of parameters lead to big alterations.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_R$</td>
<td>0.1</td>
<td>cm$^2$/day</td>
<td>Determined from [29]</td>
</tr>
<tr>
<td>$D_M$</td>
<td>0.05</td>
<td>cm$^2$/day</td>
<td>Determined from [29]</td>
</tr>
<tr>
<td>$D_P$</td>
<td>0.005</td>
<td>cm$^2$/day</td>
<td>Determined from [29]</td>
</tr>
<tr>
<td>$D_F$</td>
<td>0.05</td>
<td>cm$^2$/day</td>
<td>Determined from [29]</td>
</tr>
<tr>
<td>$\delta_R$</td>
<td>60</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\delta_M$</td>
<td>0.5545</td>
<td>$1$/day</td>
<td>Approximated by [25]</td>
</tr>
<tr>
<td>$\delta_F$</td>
<td>0.1664</td>
<td>$1$/day</td>
<td>Approximated by [48]</td>
</tr>
<tr>
<td>$\delta_P$</td>
<td>0.5545</td>
<td>$1$/day</td>
<td>Approximated by [20]</td>
</tr>
<tr>
<td>$\delta_U$</td>
<td>0.0193</td>
<td>$1$/day</td>
<td>Approximated by [30]</td>
</tr>
<tr>
<td>$\sigma_R$</td>
<td>0.0024</td>
<td>nanomolar·cm$^2$/day·cells</td>
<td>Approximated by [50]</td>
</tr>
<tr>
<td>$\sigma_M$</td>
<td>$5.17\times10^{-7}$</td>
<td>nanomolar·cm$^2$/day·cells</td>
<td>Approximated by [43]</td>
</tr>
<tr>
<td>$\sigma_F$</td>
<td>$2.35\times10^{-7}$</td>
<td>nanomolar·cm$^2$/day·cells</td>
<td>Approximated by [43]</td>
</tr>
<tr>
<td>$\sigma_P$</td>
<td>$4.2\times10^{-5}/3.3\times10^3$</td>
<td>nanomolar·cm$^2$/day·cells</td>
<td>Approximated by [16]</td>
</tr>
<tr>
<td>$\sigma_U$</td>
<td>0.0154</td>
<td>nanomolar·cm$^2$/day·cells</td>
<td>Approximated by [30]</td>
</tr>
<tr>
<td>$\Lambda$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_R$</td>
<td>10</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_M$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_F$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_P$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_U$</td>
<td>1</td>
<td>mg/cm$^2$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$K_U$</td>
<td>0.0545</td>
<td>proportion</td>
<td>Taken from [17]</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>10</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>5</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\nu$</td>
<td>0.05</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$p_0$</td>
<td>1</td>
<td>$1$/day</td>
<td>Taken from [17]</td>
</tr>
<tr>
<td>$\mu_{SA}$</td>
<td>0.1</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\mu_{DN}$</td>
<td>0.05</td>
<td>$1$/day</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\tau_1$</td>
<td>0.5</td>
<td>days</td>
<td>Taken from [16]</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>1</td>
<td>days</td>
<td>Taken from [16]</td>
</tr>
</tbody>
</table>

Table 2.1. Table of parameter values
2.2.5 Numerical simulations

Using the mathematical model in Eq. 2.1 - 2.10 and the parameters in Table 2.1 we can simulate our system. We computed the results for four values of the steady-state strain, $\epsilon = 0.3, 0.4, 0.6$ and $0.8$, crossed with two values of the EPO production rates $\sigma_P$. The “low” value $\sigma_P = 4.2 \times 10^{-5}$ obtained from \[16\] represents the natural production of EPO in articular cartilage, so the EPO level is not high enough to stop the inflammation process in articular cartilage and help the system to recover. The “high” value $\sigma_P = 3.3 \times 10^{-3}$ leads to high production of EPO which can trigger the Heaviside function in our system and shut off the inflammation process. Though the high production of EPO may not be the natural process, the simulation may help us understand lesion abatement and the potential use of EPO as a therapy for cartilage injury. In order to be consistent with the experiment settings, we choose the radius range to be $0 \leq r \leq 2.5$ cm and the time range to be $0 \leq t \leq 10$ days.

2.2.5.1 Methods and results

To solve the system Eq. 2.1 - 2.10 we first obtain a semi-discrete system of these delay-differential equations by using the scheme for discretizing the symmetric diffusion operator. The semi-discretization methods is from appendix C of \[1\]. Then the semi-discrete system can be solved in MATLAB using dde23 \[38, 39, 37\]. Our simulation results are shown in Fig. 2.5a - 2.8b.

Fig. 2.5a - 2.8a show the evolution of healthy and penumbra (both catabolic and EPOR active) cell populations over 10 days at the low production rate of EPO,
while Fig. 2.5b - 2.8b show the evolution of healthy and penumbra (both catabolic and EPOR-active) cell populations over 10 days at the high production rate of EPO. The simulation results show that cells in the loading area become necrotic at day 2, and cells adjacent to the loading area start to become sick. As loading continues, the lesion starts to spread radially. The higher strain leads to more penumbra cells and a larger area of inflammation. Comparing the figures at different EPO production levels with the same strain, we see that initially cells densities are similar in this two scenarios due to the time lag for EPO production and EPOR receptor expression. In the low EPO scenario, the healthy cell populations start to be decimated and the lesion starts to spread outside the loading area around day 4. The transition from healthy to catabolic cells is limited in the high EPO scenario, and the inflammation process is stopped at around day 6. When the production rate of EPO is high, the change of strain fails to make much difference in cell populations, because the inflammation is terminated in the early stage. Necrosis happens in the loading areas in both scenarios, and the higher strain leads to more cell death.

Table 2.2a and 2.2b show the range of variables at $t = 10$ days for strains $\epsilon = 0.3, 0.4, 0.6$ and 0.8. The percentages of necrotic cells are around 10%, 30%, 60% and 85%. These percentages reflect the cell death rates caused by cyclic loading, which is consistent with experiments results [17]. The expected ECM degradation should be very mild because 10 days is a short time period for ECM to have significant degradation, consistent with simulation results. The simulations also show a preponderance of catabolic over EPOR-active cells, a useful insight which is difficult
to measure in experiments. Our model brought together the experiments results and piece-wise knowledge of articular cartilage lesion under cyclic loading and formed a more holistic understanding of the inflammatory process.

<table>
<thead>
<tr>
<th>variables range</th>
<th>strain= 0.3</th>
<th>strain= 0.4</th>
<th>strain= 0.6</th>
<th>strain= 0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$ min</td>
<td>$1.374 \times 10^4$</td>
<td>$2.58 \times 10^3$</td>
<td>71.02</td>
<td>0</td>
</tr>
<tr>
<td>$S_T$ max</td>
<td>$7.254 \times 10^4$</td>
<td>$8.627 \times 10^4$</td>
<td>$8.909 \times 10^4$</td>
<td>$9.07 \times 10^4$</td>
</tr>
<tr>
<td>$S_A$ max</td>
<td>$2.79 \times 10^5$</td>
<td>$5.524 \times 10^4$</td>
<td>$1.105 \times 10^4$</td>
<td>$1.533 \times 10^4$</td>
</tr>
<tr>
<td>$D_A$ max</td>
<td>22</td>
<td>64</td>
<td>180</td>
<td>302</td>
</tr>
<tr>
<td>$D_N$ max</td>
<td>$1.213 \times 10^4$</td>
<td>$2.888 \times 10^4$</td>
<td>$6.35 \times 10^4$</td>
<td>$8.616 \times 10^4$</td>
</tr>
<tr>
<td>$U$ min</td>
<td>29.933</td>
<td>29.894</td>
<td>29.862</td>
<td>29.824</td>
</tr>
<tr>
<td>$F$ max</td>
<td>0.0257</td>
<td>0.0332</td>
<td>0.0411</td>
<td>0.0476</td>
</tr>
<tr>
<td>$M$ max</td>
<td>0.0253</td>
<td>0.0428</td>
<td>0.0663</td>
<td>0.0744</td>
</tr>
<tr>
<td>$P$ max</td>
<td>0.3234</td>
<td>0.3159</td>
<td>0.3038</td>
<td>0.3006</td>
</tr>
<tr>
<td>$R$ max</td>
<td>2.7323</td>
<td>3.3031</td>
<td>3.479</td>
<td>3.493</td>
</tr>
</tbody>
</table>

(a)

<table>
<thead>
<tr>
<th>variables range</th>
<th>strain= 0.3</th>
<th>strain= 0.4</th>
<th>strain= 0.6</th>
<th>strain= 0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$ min</td>
<td>$8.029 \times 10^4$</td>
<td>$5.74 \times 10^4$</td>
<td>$1.01 \times 10^4$</td>
<td>0</td>
</tr>
<tr>
<td>$S_T$ max</td>
<td>$4.778 \times 10^3$</td>
<td>$7.527 \times 10^3$</td>
<td>$2.78 \times 10^4$</td>
<td>$9.022 \times 10^4$</td>
</tr>
<tr>
<td>$S_A$ max</td>
<td>21.29</td>
<td>38.28</td>
<td>118</td>
<td>355</td>
</tr>
<tr>
<td>$D_A$ max</td>
<td>0.099</td>
<td>0.39</td>
<td>1.648</td>
<td>7</td>
</tr>
<tr>
<td>$D_N$ max</td>
<td>$1.213 \times 10^4$</td>
<td>$2.887 \times 10^4$</td>
<td>$6.35 \times 10^4$</td>
<td>$8.616 \times 10^4$</td>
</tr>
<tr>
<td>$U$ min</td>
<td>29.999</td>
<td>29.9988</td>
<td>29.9987</td>
<td>29.98</td>
</tr>
<tr>
<td>$F$ max</td>
<td>0.0027</td>
<td>0.0037</td>
<td>0.005</td>
<td>0.0073</td>
</tr>
<tr>
<td>$M$ max</td>
<td>0.0082</td>
<td>0.0168</td>
<td>0.0349</td>
<td>0.0411</td>
</tr>
<tr>
<td>$P$ max</td>
<td>7.919</td>
<td>9.2917</td>
<td>13.718</td>
<td>21.28</td>
</tr>
<tr>
<td>$R$ max</td>
<td>0.189</td>
<td>0.2967</td>
<td>0.5139</td>
<td>0.699</td>
</tr>
</tbody>
</table>

(b)

Table 2.2. Table of variable ranges at day 10 (a) The case of low EPO (b) The case of high EPO
Figure 2.5. Population of healthy, catabolic and EPOR-active cells with 30% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO
Figure 2.6. Population of healthy, catabolic and EPOR-active cells with 40% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO
Figure 2.7. Population of healthy, catabolic and EPOR-active cells with 60% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO
Figure 2.8. Population of healthy, catabolic and EPOR-active cells with 80% strain (a) Cell population with low concentration of EPO (b) Cell population with high concentration of EPO
2.2.5.2 Sensitivity analysis

In this section, we study the sensitivity of those estimated parameters which were not taken from the published literature. We vary the values of $\alpha$, $\beta_1$, $\beta_2$, $\gamma$, $\nu$, $\mu_{SA}$, $\mu_{DN}$, $\lambda_F$, $\lambda_P$, $\lambda_M$, $\lambda_U$, and $\Lambda$ one at a time and observe how the perturbations of these parameters affect the simulation results. We conducted simulations with both high and low EPO production at strains of 0.3, 0.4, 0.6 and 0.8. The values we varied for each parameter are in Table 2.3.

<table>
<thead>
<tr>
<th>variables range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha \in {0.1, 0.5, 1, 1.5, 2}$</td>
</tr>
<tr>
<td>$\beta_1 \in {1, 5, 10, 15, 20}$</td>
</tr>
<tr>
<td>$\beta_2 \in {1, 2.5, 5, 7.5, 10}$</td>
</tr>
<tr>
<td>$\gamma \in {0.1, 0.5, 1, 1.5, 2}$</td>
</tr>
<tr>
<td>$\lambda_F \in {0.1, 0.25, 0.5, 0.75, 1}$</td>
</tr>
<tr>
<td>$\lambda_M \in {0.1, 0.25, 0.5, 0.75, 1}$</td>
</tr>
<tr>
<td>$\lambda_P \in {0.1, 0.25, 0.5, 0.75, 1}$</td>
</tr>
<tr>
<td>$\lambda_R \in {1, 5, 10, 15, 20}$</td>
</tr>
<tr>
<td>$\lambda_U \in {0.1, 0.5, 1, 1.5, 2}$</td>
</tr>
<tr>
<td>$\mu_{DN} \in {0.01, 0.025, 0.05, 0.075, 0.1}$</td>
</tr>
<tr>
<td>$\mu_{SA} \in {0.01, 0.05, 0.1, 0.15, 0.2}$</td>
</tr>
<tr>
<td>$\Gamma \in {0.1, 0.25, 0.5, 0.75, 1}$</td>
</tr>
</tbody>
</table>

Table 2.3. Table of sensitivity values

Most parameters are robust which means there are only quantitative, rather than qualitative, differences in the simulation results. The responses of cell populations and chemical concentrations have the same pattern for all the strain levels and both of the EPO production rates. The parameters which have the most sensitivity...
are the Michaelis-Menten coefficients $\lambda_F$, $\lambda_M$ and $\lambda_R$. In our model, the values 0.1 - 1 for these parameters are small respectively, so the Michaelis-Menten functions is close to a constant. The results change non-monotonically near the boundary of the loading area. This indicates that the Michaelis-Menten forms constitute switches. However, because the range of parameters is low, there are no qualitative differences in results.

### 2.2.5.3 Numerical methodology

Applying the semi-discretization scheme in space [1], we obtained the semi-discrete system of delay-differential equations and then solved the system in MATLAB by using dde23 [37]. Computational results are shown in Fig. 2.5a - 2.7b and the computational convergence studies for the variables (cell population and chemical concentration) are shown in Tables 2.4a - 2.5b. The relative errors are around 1-10% which are reasonable considering the experimental or real world measurement error, and the maximum errors occurs near the sharp front where the cyclic loading is applied and accumulate when time increases, so the numerical simulation results do not qualitatively differ from the true solutions.

Even though the numerical methods provide reasonable simulation results, the relative errors are somewhat to be high. The solver dde23 uses an explicit time integration method to solve the differential equations, so it has difficulty with stiff systems. Our model is a stiff system with two variables subjected to delay, so dde23 takes much smaller time steps than truncation error would dictate when the complex-
Table 2.4. Table of errors (a) Errors in the $\infty$-norm with low levels of EPO (b) Errors in the 2-norm with low levels of EPO

<table>
<thead>
<tr>
<th>variable</th>
<th>Strain=30%</th>
<th>Strain=40%</th>
<th>Strain=60%</th>
<th>Strain=80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.0242</td>
<td>0.0314</td>
<td>0.0244</td>
<td>0.0220</td>
</tr>
<tr>
<td>$S_T$</td>
<td>0.0230</td>
<td>0.0225</td>
<td>0.0219</td>
<td>0.0212</td>
</tr>
<tr>
<td>$S_A$</td>
<td>0.0383</td>
<td>0.0352</td>
<td>0.0304</td>
<td>0.0272</td>
</tr>
<tr>
<td>$D_A$</td>
<td>0.0525</td>
<td>0.0460</td>
<td>0.0371</td>
<td>0.0318</td>
</tr>
<tr>
<td>$D_N$</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>ECM</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>0.0224</td>
<td>0.0204</td>
<td>0.0204</td>
<td>0.0191</td>
</tr>
<tr>
<td>EPO</td>
<td>0.0240</td>
<td>0.0244</td>
<td>0.0319</td>
<td>0.0323</td>
</tr>
<tr>
<td>DAMPs</td>
<td>0.0219</td>
<td>0.0204</td>
<td>0.0184</td>
<td>0.0171</td>
</tr>
<tr>
<td>ROS</td>
<td>0.0230</td>
<td>0.0226</td>
<td>0.0838</td>
<td>0.1085</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>variable</th>
<th>Strain=30%</th>
<th>Strain=40%</th>
<th>Strain=60%</th>
<th>Strain=80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.0102</td>
<td>0.0132</td>
<td>0.0122</td>
<td>0.0110</td>
</tr>
<tr>
<td>$S_A$</td>
<td>0.0140</td>
<td>0.0131</td>
<td>0.01237</td>
<td>0.0118</td>
</tr>
<tr>
<td>$S_T$</td>
<td>0.0206</td>
<td>0.0186</td>
<td>0.0153</td>
<td>0.0134</td>
</tr>
<tr>
<td>$D_A$</td>
<td>0.0261</td>
<td>0.0230</td>
<td>0.0180</td>
<td>0.0151</td>
</tr>
<tr>
<td>$D_N$</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>ECM</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>0.0148</td>
<td>0.0122</td>
<td>0.0204</td>
<td>0.0114</td>
</tr>
<tr>
<td>EPO</td>
<td>0.0134</td>
<td>0.0144</td>
<td>0.0170</td>
<td>0.0175</td>
</tr>
<tr>
<td>DAMPs</td>
<td>0.0152</td>
<td>0.0137</td>
<td>0.0118</td>
<td>0.0107</td>
</tr>
<tr>
<td>ROS</td>
<td>0.0140</td>
<td>0.0133</td>
<td>0.0166</td>
<td>0.0235</td>
</tr>
</tbody>
</table>

ity of the system increases. The delay-equation solvers need to keep the past history information in memory, so the numerical method will eventually lead to “out of memory” issues. One way to solve this issue is to use a different time discretization, so we simulated our results in a “stiff” delay differential equation (DDE) solver using R [40]. We obtained similar simulation results but this solver was not faster than dde23.
<table>
<thead>
<tr>
<th>variable</th>
<th>Strain=30%</th>
<th>Strain=40%</th>
<th>Strain=60%</th>
<th>Strain=80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.0006</td>
<td>0.0013</td>
<td>0.0045</td>
<td>0.0141</td>
</tr>
<tr>
<td>$S_T$</td>
<td>0.0253</td>
<td>0.0305</td>
<td>0.0219</td>
<td>0.0420</td>
</tr>
<tr>
<td>$S_A$</td>
<td>0.0375</td>
<td>0.0362</td>
<td>0.0304</td>
<td>0.0416</td>
</tr>
<tr>
<td>$D_A$</td>
<td>0.0430</td>
<td>0.0588</td>
<td>0.0371</td>
<td>0.0559</td>
</tr>
<tr>
<td>$D_N$</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>ECM</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.0213</td>
<td>0.0204</td>
<td>0.0203</td>
<td>0.0185</td>
</tr>
<tr>
<td>EPO</td>
<td>0.0249</td>
<td>0.0246</td>
<td>0.0306</td>
<td>0.0372</td>
</tr>
<tr>
<td>DAMPs</td>
<td>0.0188</td>
<td>0.0212</td>
<td>0.0220</td>
<td>0.0192</td>
</tr>
<tr>
<td>ROS</td>
<td>0.0253</td>
<td>0.0249</td>
<td>0.0743</td>
<td>0.1144</td>
</tr>
</tbody>
</table>

(a)

<table>
<thead>
<tr>
<th>variable</th>
<th>Strain=30%</th>
<th>Strain=40%</th>
<th>Strain=60%</th>
<th>Strain=80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.0002</td>
<td>0.0003</td>
<td>0.0008</td>
<td>0.0015</td>
</tr>
<tr>
<td>$S_A$</td>
<td>0.0138</td>
<td>0.0134</td>
<td>0.0127</td>
<td>0.0116</td>
</tr>
<tr>
<td>$S_T$</td>
<td>0.0164</td>
<td>0.0158</td>
<td>0.0143</td>
<td>0.0119</td>
</tr>
<tr>
<td>$D_A$</td>
<td>0.0191</td>
<td>0.0192</td>
<td>0.0200</td>
<td>0.0167</td>
</tr>
<tr>
<td>$D_N$</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>ECM</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.0128</td>
<td>0.0115</td>
<td>0.0204</td>
<td>0.0104</td>
</tr>
<tr>
<td>EPO</td>
<td>0.0130</td>
<td>0.0126</td>
<td>0.0135</td>
<td>0.0158</td>
</tr>
<tr>
<td>DAMPs</td>
<td>0.0124</td>
<td>0.0120</td>
<td>0.0115</td>
<td>0.0105</td>
</tr>
<tr>
<td>ROS</td>
<td>0.0135</td>
<td>0.0132</td>
<td>0.0148</td>
<td>0.0247</td>
</tr>
</tbody>
</table>

(b)

Table 2.5. Table of errors (a) Errors in the $\infty$-norm with high levels of EPO (b) Errors in the 2-norm with high level of EPO

We could write tailor-made compiled code for our model, but we would still need to solve the challenge of storing all the time histories to compute the delay terms. As the complexity of the system increases, the memory issues seem unavoidable. For further work, we need a more efficient approach.
2.3 Conclusion

In this chapter, we presented a minimal mechanistic mathematical model to describe the spread of a lesion in articular cartilage under extensive cyclic loading, based on observed mechanisms for the chondrocyte populations and chemical concentrations. We created a function to represent the relationship between cell death and steady strain. In the parameterization section, we made reasonable assumptions for the release and decay rates of chemicals based on published articles, and quantitatively estimated the parameters. Our model links the mathematical equations and the existing experimental data. Future experiments are needed for deeper insight about the process of articular cartilage lesion and model validation. By solving the system numerically, our model may provide a tool for studying the degeneration of articular cartilage under cyclic loading with different levels of strains. In the long run, the model might be useful to predict the consequences on human joints of being exposed to stresses, and identify the thresholds of the stress/strain which might lead to the risk of developing OA.

The delay differential equations seem to be a reasonable approach to describe the cells and cytokine responses in articular cartilage lesion formation. Even though the delay term is easy to understand, this approach does have its limitations in computation efficiency. To solve the problem, we could convert the delay into a physiological property of individual cells: the time since the exposure to the relevant cytokine, which we call “age”. We include an additional independent variable and derivative tern for “age” structure in the cell populations which needed the delay
terms. Although an additional dimension may seem like it adds complexity, the retention of time histories in the delay differential system can quickly become larger in memory than the storage need for the additional age dimension. Age structure seems like a more complex approach, but it may be more computationally efficient. In the next chapter, we will explore this approach.
3.1 Introduction of the age-structured model

In this chapter, we use an age-structured model to describe the inflammation process in articular cartilage after a single blunt impact. Simple ordinary differential equation models for a population treat all individuals identically. ODEs cannot provide an adequate description of the prototypical dynamics of many biological populations. Age is one of the most important characteristic in population modeling. Individuals in different ages may have different behaviors, such as different reproduction capacities, death rates and infection rates. In the inflammatory process of articular cartilage, the different cell states represent the hierarchical structure of the cell population, so it is natural to introduce age into the modeling.

One way of modeling an age-structured population is to add an independent variable age $a$ in the partial differential equations, known as a McKendrick equation. The nonlinear density dependence model was built by [24]. The age-structured population can be described by a population density function $\rho(t, a) \geq 0$, where $t$ is time and $a$ is chronological age. Thus $\int_{a_1}^{a_2} \rho(t, a) da$ is the population of individuals between ages $a_1$ and $a_2$ at time $t$, and $P(t) = \int_0^{\infty} \rho(t, a) da$ is the total population at time $t$ of age $a$. Let $\mu(P, a)$ be the death modulus, which reflects the death rate individuals of age $a$ per unit time, and $\beta(P, a)$ be the birth modulus, which reflects the birth rate by individuals of age $a$ per unit time. Here both $\mu$ and $\beta$ depend on $P$, the total
population of individuals over all ages. The nonlinear Gurtin-MacCamy model is

\[ \partial_t \rho(t, a) + \partial_a \rho(t, a) = -\mu(P, a) \rho(t, a), \quad t > 0, a > 0, \]

(3.1)

\[ \rho(t, 0) = \int_\infty^0 \beta(P, a) \rho(t, a) da, \quad t > 0, \]

(3.2)

\[ \rho(0, a) = \rho^0(a), \quad a \geq 0, \]

(3.3)

where \( \rho^0(a) \) is the initial age distribution.

We can introduce a spatial variable for the radius \( r \) into the age-dependent population model, by simply defining the population density to be \( \rho(r, t, a) \). Because chondrocytes are fixed in matrix, there is no diffusion terms in their population equations.

There is a half-day delay before a cell expresses the EPO receptor after being signaled to become EPOR active, and a 1-day delay for a healthy cell to be susceptible to becoming catabolic after being signaled by ROS. We can represent these delays using age-structured equations. Catabolic cells \( (S_T) \) can be considered to be early age sick cells and EPOR-active cells \( (S_A) \) are in the late age. When age is larger than 1/2 day, catabolic cells \( S_T \) become EPOR-active cells \( S_A \), so we can simply add an additional dimension for age \( a \) into the catabolic cell population and write it as \( S_T(r, a, t) \). In order to introduce “age” in the healthy cells, we separate healthy cells into three states, healthy normal cells \( (C_U) \), healthy pre-catabolic cells \( (C_T) \) and healthy EPO producing cells \( (C_E) \) (see Fig. [3.1]). Healthy pre-catabolic cells \( (C_T) \) can be considered to be in an early age state and healthy EPO producing cells \( (C_E) \) in a late age state. When age is larger than 1 day, \( C_T \) cells become \( C_E \) cells, so we add
age \( a \) into the variable of healthy pre-catabolic cells and write it as \( C_T(r, a, t) \). The diagram of the inflammation process is in Fig. 3.1. We will discuss how we define the death and birth modulus in the following section.

In this chapter, we present the age-structured mathematical model of articular cartilage lesion formation due to a single blunt impact, solve the model equations numerically, and then discuss the computational results and the experimental validation.

3.2 Schematic of articular cartilage lesion formation due to a single blunt impact

As we discussed in previous chapters, there are various ways to cause injury to articular cartilage. All of them are related to high loading rates and high peak stress amplitudes, which initiate the damage that can cause the spread of a cartilage lesion and eventually lead to post-traumatic osteoarthritis (PTOA). The strain due to a single blunt impact can easily exceed 40\%, which is deadly to chondrocytes and ECM. We can assume that chondrocyte death inside the superficial zone is directly caused by this impact. Once the degradation of the cartilage matrix happens, it is permanent and painful. Thus the main target for most therapy for PTOA is to prevent cell death in the area adjacent to the impact site immediately after the injury. Necrosis is regularly observed at the site of impact, and apoptosis is induced by “caspase” (cysteine-aspartic proteases). Caspase inhibitors can be used \textit{in vivo} to reduce the spread rate of cell depletion and matrix degradation.
Last chapter we presented a reaction-diffusion-delay model of articular cartilage lesion formation due to cyclic loading. To mitigate some of the computational limitations from using delay terms, we present in this chapter an age-structured model of articular cartilage lesion formation due to a single blunt impact. The model in this chapter modifies the reaction-diffusion-delay model in [22], with the goal of being able to extend the model to more biological and biomechanical complexity down the road. Towards this end, we introduce age structure to replace the delay terms for the cell transitions. Numerical simulations show a successful capture of the chondrocyte behavior and chemical activities associated with the cartilage lesion after the initial injury. Our model constitutes an in silico study of the cartilage damage and aims to provide information that may not be easily obtained through in vivo or in vitro studies. Compared to the delay differential model, the age-structured model is more efficient computationally, while adding flexibility in terms of when the delays occur.

The chondrocyte depletion and ECM degradation process for the single blunt impact is illustrated in Fig. 3.1. Cartilage damage initiates the production of alarmins, such as damage associated molecular pattern molecules (DAMPs) [14], which can induce the release of pro-inflammatory cytokines such as TNF-α and IL-1α and β. TNF-α might be the early mediator, however IL-1β is the cytokine that sustains the degradation [32]. For modeling simplicity, we use only IL-6 to represent the pro-inflammatory cytokine family. Other cytokines such as IL-1β can be easily added. Pro-inflammatory cytokines are the main reason for cell apoptosis. Moreover, pro-inflammatory cytokines cause severe glycosaminoglycan (GAG) loss which is the hall-
Figure 3.1. Schematic of the articular cartilage lesion process under the effect of a single blunt impact

mark of proteoglycan depletion \[30\]. The loss of “sGAG” can be measured by the decrease in concentration of aggrecan protein. The sulfate groups (SO$_4$) decorating the aggrecan protein is essentially what is measured \[35\]. The aggrecan protein is just a elaborate means of keeping sulfates in the solid phase and in place within the matrix (so called “fixed charges”). Although limited, chondrocytes still have some self-repair ability. Anti-inflammatory cytokines such as erythropoietin (EPO) can antagonize the effect of TNF-$\alpha$, which reduces cell apoptosis and ECM degradation. The “balancing act” \[22\] between inflammatory and anti-inflammatory cytokines is
the essential mechanism of the articular cartilage lesion process.

3.3 Mathematical model and numerical simulation

In this section, we describe the age-structured model for the inflammation response after a single blunt impact. The cartilage lesion caused by a single severe traumatic event was modeled using reaction-diffusion-delay equations in [22]. For reasons stated above, we instead use age-structure instead of delays to model the lagged transitions between cell states.

3.3.1 Variables and equations

As in previous models in this dissertation, we assume radial symmetry so that the system can be reduced to a one-dimensional model with respect to space. The components of the system depend on radius \(0 \leq r \leq 2.5\) cm, time \(0 \leq t \leq 14\) days, and age \(a\). The time delays for cell transitions are \(\tau_1\) and \(\tau_2\). We also assume that the single blunt impact occurs on a small region near the origin with radius smaller than 0.25 cm.

We separate healthy cells into the following three states:

- \(C_U(r, t)\): healthy normal cell, population density (cells per unit area) at a given time and location of healthy cells not yet signaled by ROS.
- \(C_T(r, a, t)\): healthy pre-catabolic cell, population density of healthy cells signaled by ROS and set to become catabolic.
- \(C_E(r, t)\): healthy EPO producing cell, population density of healthy cells signaled by ROS and that have begun to produce EPO (20-24 hours after being
Because there is a 1-day delay for cells in the $C_T$ state to transit to the $C_E$ state, $C_T$ has the “age” dimension in $a$. We use a smooth transition kernel. When $a < \tau_2 = 1$ for cells in the $C_T$, very few of them will enter the $C_E$ state. As age $a$ approaches $\tau_2 = 1$ day, the rate of cells $C_T$ transiting to $C_E$ will increase rapidly until they reach the maximum transition rate, which they then maintain. The transition from catabolic ($S_T$) to EPOR-active ($S_A$) is modeled with a similar smooth transition, which is more biological and not possible with a delay term. Catabolic cells $S_T$ wait $1/2$ days to express the EPO-receptor, after being signaled to be EPOR-active.

Our other model components are represented similarly to those in Chapter 2. As before, we do not have diffusion or other motility terms for the chondrocytes because they are fixed in the matrix. The single blunt impact kills cells inside the impact area, in particular they become necrotic ($D_N$). The cells in the adjacent area transit from the “healthy” ($C_U, C_T, C_E$) to the “sick” ($S_T, S_A$) and then to the “dead” (in particular, apoptotic $D_A$) state. Since apoptotic cells ($D_A$) don’t play an explicit role in the system, they are not expressed explicitly in our mathematical model, but instead are represented by the sink terms in the $S_T$ and $S_A$ equations.

As before, we assume that the chemicals diffuse throughout the section of cartilage. The diffusion coefficients were estimated in [22] and [29]. Chemicals decay after a certain time, and the decay rate can be approximated by their half lives [25], [18] and [20]. However, the decay of ROS is almost instantaneous under the superoxide dismutate SOD, so its decay rate needs to be adjusted for the mathematical
model. Inflammatory cytokines, such as TNF-α, are the main cause of the spread of a cartilage lesion. EPO plays an opposing role by helping cell recovery and limiting the inflammation \[16\]. The model in this chapter captures the balance between the pro-inflammatory and anti-inflammatory cytokines.

In Fig. 3.1, the single blunt impact causes the cells in the impacted region to become necrotic. Before cells become necrotic, they release a small amount of DAMPs \((M)\) to trigger the transition \(C_U \rightarrow C_T\). The transition \(C_T \rightarrow C_E\) starts 20-24 hours after healthy cells \(C_T\) are signaled by ROS, and cells in the \(C_E\) state start to produce EPO \((P)\). DAMPs \((M)\) triggers \(C_T \rightarrow S_T\), and \(S_T\) produces TNF-α \((F)\) and ROS. TNF-α drives the \(S_T \rightarrow S_A\) transition after 8-12 hours, and causes more healthy cells \(C_T\) to become catabolic, \(S_T\). TNF-α \((F)\) will cause sick cells (both catabolic and EPOR-active) to become apoptotic \((D_A)\), will degrade ECM, and will block the production of EPO. Degraded ECM releases DAMPs, which along with TNF-α drives transition \(S_T \rightarrow D_A\). Following Fig. 3.1, we obtain the following model equations for the chemicals:

\[
\begin{align*}
\frac{\partial}{\partial t} R(r,t) &= \frac{1}{r} \frac{\partial}{\partial r} (r D_R R) - \delta_R R + \frac{\sigma_R S_T}{}, \\
\frac{\partial}{\partial t} M(r,t) &= \frac{1}{r} \frac{\partial}{\partial r} (r D_M M) - \delta_M M + \frac{\sigma_M D_N}{}, \\
\frac{\partial}{\partial t} F(r,t) &= \frac{1}{r} \frac{\partial}{\partial r} (r D_F F) - \delta_F F + \frac{\sigma_F S_T}{},
\end{align*}
\]
\[
\frac{\partial_t P(r,t)}{\text{EPO}} = \frac{1}{r} \frac{\partial_r (rD_P P_r)}{\text{diffusion}} - \frac{\delta_P P}{\text{natural decay}} + \sigma_P C_E \frac{R}{\Lambda} \frac{1}{\lambda + \rho + \Phi},
\]  

(3.7)

with initial and boundary conditions

\[
\frac{\partial_r R(0,t)}{\partial_r M(0,t)} = \partial_r F(0,t) = \partial_r P(0,t) = 0,
\]

\[
R(r,0) = M(r,0) = F(r,0) = P(r,0) = 0.
\]

The equation for the ECM is

\[
\frac{\partial_t U(r,t)}{\text{ECM}} = -\delta_U U \frac{F}{\lambda + \Phi} H(P_c - P),
\]

(3.8)

with initial condition

\[
U(r,0) = 30\text{mg}.
\]

ECM is assumed to be degraded by inflammatory cytokines such as TNF-\( \alpha \) and IL-6, and measured in terms of decreased proteoglycan concentration in matrix. When ECM is intact, the sulfate groups are kept inside ECM. The release of sulfate groups is an alarm of ECM degradation, which can be estimated by the decrease in concentration of SO\(_4\). The average concentration of SO\(_4\) in normal undamaged cartilage is 30 g/L \[35\], which is the initial weight of ECM in this model. EPO concentration can also affect ECM degradation. The function,

\[
H(P_c - P) = \begin{cases} 1 & P \leq P_c \\ 0 & P > P_c \end{cases}
\]
represents the fact that ECM degradation can be terminated when the level of $P$ exceeds $P_c$.

For the three healthy cell populations we have the equations

$$
\frac{\partial}{\partial t} C_U(r,t) = \frac{P}{\lambda_P + P} + \frac{\alpha_3 H(P - P_c) C_E}{\lambda_M + M} - \beta_{13} C_U \frac{M}{\lambda_M + M}, \quad (3.9)
$$

$$
\frac{\partial}{\partial t} C_T(r,a,t) + \frac{\partial}{\partial a} C_T(r,a,t) = -\beta_{11} \frac{M}{\lambda_M + M} H(P_c - P) C_T(r,a,t) - \beta_{12} \frac{F}{\lambda_F + F} H(P_c - P) C_T(r,a,t)
$$

$$
- \kappa_2 \gamma(a - a_2) \frac{R}{\lambda_R + R} C_T(r,a,t), \quad (3.10)
$$

$$
\frac{\partial}{\partial t} C_E(r,t) = \int_0^\infty \kappa_2 \gamma(a - a_2) \frac{R(r,t)}{\lambda_R + R(r,t)} C_T(r,a,t) \, da - \alpha_2 H(P - P_c) C_E, \quad (3.11)
$$

with initial conditions

$$
C_U(r,0) = \begin{cases} 
0, & 0 \le r \le 0.25 \text{cm} \\
100000 \text{ cells/cm}^2, & \text{otherwise}. 
\end{cases}
$$

$$
C_T(r,0,t) = \beta_{13} C_U \frac{R}{\lambda_R + R}, \quad (3e)
$$

$$
C_T(r,a,0) = C_E(r,0) = 0. \quad (3f)
$$
For the two sick cell populations, we have

\begin{equation}
\partial_t S_T(r,a,t) + \partial_a S_T(r,a,t) = \tag{3.12}
\end{equation}

\[
- \mu_{ST} \frac{F}{\lambda_F + F} \frac{M}{\lambda_M + M} S_T(r,a,t) - \eta \cdot \gamma(a - \tau_1) \frac{F}{\lambda_F + F} S_T(r,a,t),
\]

\[
S_T \xrightarrow{F, DAMPs} D_A
\]

\[
S_T \xrightarrow{F} S_A
\]

\[
\partial_t S_A(r,t) = \int_0^\infty \eta \cdot \gamma(a - \tau_1) \frac{F(r,t)}{\lambda_F + F(r,t)} S_T(r,a,t) da - \alpha_1 S_A \frac{P}{\lambda_P + P},
\]

\[
S_T \xrightarrow{F} S_A
\]

\[
S_A \xrightarrow{P} D_A
\]

\[
S_A \xrightarrow{EPO} C_U
\]

\[
S_A \xrightarrow{S} S_T
\]

\[
S_T \xrightarrow{P} S_A
\]

\[
S_T(r,a,0) = S_A(r,0) = 0.
\]

\[
(4d)
\]

For the dead cells we only track the necrotic cell population,

\[
\partial_t D_N(r,t) = - \mu_{DN} D_N, \tag{3.14}
\]

with initial condition

\[
D_N(r,0) = \begin{cases} 
100000 \text{ cells/cm}^2 & 0 \leq r \leq 0.25 \text{cm} \\
0 & \text{otherwise}
\end{cases}
\]

The main difference between our model and the reaction-diffusion-delay model in [22] is that we use age-structure to represent the time delays for cells to become...
EPO-producing and EPOR-active instead of using delay terms. In particular, we include age structure \( a \) in the equations for \( C_T \) and \( S_T \). Following [4], we use terms with the general form

\[ \gamma(a - a_{\text{max}}) = \frac{\gamma_0}{\sigma} \left( \tanh\left( \frac{a - a_{\text{max}}}{\sigma} \right) + 1 \right) \]

for the transition modulus, where \( \sigma \) is the spread parameter and \( \gamma_0 \) is the height parameter. For a fixed \( \gamma_0 \) and letting \( \sigma \to 0 \), cells in the states \( C_T \) and \( S_T \) switch their states to \( C_E \) and \( S_A \) all at the same age \( a_{\text{max}} \). When \( \sigma \) is small but not zero, only a few cells in the \( C_T \) and \( S_T \) states enter to their next state for a small. The transition rate of cells increases rapidly when age \( a \) approach \( a_{\text{max}} \), and remains high thereafter.

In particular, we choose \( a_{\text{max}} = 1 \) for \( C_T \to C_E \) and \( a_{\text{max}} = \frac{1}{2} \) for \( S_T \to S_A \).

### 3.3.2 Numerical methods and results

Most parameters are taken from [22] and [46], including the diffusion coefficients \( D_R, D_M, D_F, D_P \), the decay coefficients \( \delta_R, \delta_M, \delta_F, \delta_P \), and the release rate of chemicals \( \sigma_R, \sigma_M, \sigma_F, \sigma_P \). Other parameters are adjusted to ensure reasonable simulation results. We present our parameters in Table 3.1. The other parameters \( D_R, D_M, D_P, D_F, \delta_R, \delta_M, \delta_F, \delta_P \) and \( \delta_U \) are the same as in Table 2.1.

For the numerical simulations, we assume a two dimensional domain with radial symmetry. For the spatial variable, we discretize the domain \( r = [0, 2.5 \text{ cm}] \) by a uniform partition and compute fluxes using upwind differencing [4]. We applied the moving-grid Galerkin methods developed and analyzed in [2, 4, 8, 10] to solve the age- and space-structured differential equations. This computational methods
were proved to be effective and efficient in the modeling and simulation of biofilms [11, 12, 27, 7], avascular tumor invasion [13], and *Proteus mirabilis* swarm colony development [3, 5, 6]. By using the moving-grid Galerkin method, we discretized the age dimension with less data storage and computation that what was required by the delay terms of previous models. In particular, we used only 129 age intervals in our simulations. The time discretization is adaptive [9] and controlled by a tolerance parameters. Figures 3.2a - 3.7 show the simulation results, including cell populations and chemical concentrations.

3.3.3 Simulation results

We simulate the evolution of chondrocyte population densities per cm$^2$ for a fourteen-day period after an initial cartilage injury in the center of a disk with a radius of 2.5 cm. The radius of the impact area is 0.25 cm. We assume the initial cell density is 100000 cells/cm$^2$. When $t = 0$, a population of entirely necrotic cells occupies the center disk of radius of 0.25 cm. Fig. 3.2a-3.7 show the changes of cell populations and chemical concentrations over a period of 14 days, plotted at day 0, 1, 5, 7, 10, and 14. Fig. 3.2a shows the evolution of the three states of healthy cells. Fig. 3.2b represents the evolution of healthy, catabolic and EPOR-active cells. Fig. 3.3 the combined live cell densities (healthy and sick). Fig. 3.4 shows the degradation of ECM. Fig. 3.5, 3.6, and 3.7 show the concentrations of the chemicals.

After the initial injury, the cells adjacent to the impact area quickly sense the danger (the releasing of ROS) and start to switch states. At day 1, DAMPs
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_R$</td>
<td>0.0024</td>
<td>nanomolar·cm²/day-cells</td>
<td>Approximated by [50]</td>
</tr>
<tr>
<td>$\sigma_M$</td>
<td>$5.17 \times 10^{-7}$</td>
<td>nanomolar·cm²/day-cells</td>
<td>Approximated by [13]</td>
</tr>
<tr>
<td>$\sigma_F$</td>
<td>$2.35 \times 10^{-7}$</td>
<td>nanomolar·cm²/day-cells</td>
<td>Approximated by [13]</td>
</tr>
<tr>
<td>$\sigma_P$</td>
<td>$4.2 \times 10^{-5}$</td>
<td>nanomolar·cm²/day-cells</td>
<td>Approximated by [16]</td>
</tr>
<tr>
<td>$\sigma_U$</td>
<td>0.0154</td>
<td>nanomolar·cm²/day-cells</td>
<td>Approximated by [30]</td>
</tr>
<tr>
<td>$\Lambda$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_R$</td>
<td>10</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_M$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_F$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_P$</td>
<td>0.5</td>
<td>nanomolar</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\lambda_U$</td>
<td>1</td>
<td>mg/cm²</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>1</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>1</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\beta_{11}$</td>
<td>100</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\beta_{12}$</td>
<td>50</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\beta_{13}$</td>
<td>10</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\kappa_1$</td>
<td>10</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\kappa_2$</td>
<td>10</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$P_c$</td>
<td>1</td>
<td>nanomolar</td>
<td>Approximated by [16]</td>
</tr>
<tr>
<td>$\mu_{SA}$</td>
<td>0.1</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\mu_{DN}$</td>
<td>0.05</td>
<td>$\frac{1}{\text{day}}$</td>
<td>Approximated</td>
</tr>
<tr>
<td>$\tau_1$</td>
<td>0.5</td>
<td>days</td>
<td>Taken from [16]</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>1</td>
<td>days</td>
<td>Taken from [16]</td>
</tr>
</tbody>
</table>

Table 3.1. Table of parameter values

released by necrotic cells start to trigger cells in the $C_T$ state to become catabolic ($S_T$). Catabolic cells $S_T$ start to produce more DAMPs, TNF-α and ROS, that causes further inflammation. Because of the delay of the $C_T \rightarrow C_E$ and $S_T \rightarrow S_A$ transitions, cells can neither produce anti-inflammatory cytokines EPO nor express a receptor for EPO until day 1. In Figures 3.2a, 3.2b and 3.3 we see the population density of healthy cells $C_U$ start to decline and switch to $C_T$ and $S_T$. We can observe
a significant amount of \( C_T \), \( C_E \) and catabolic cells at day 5. Starting from day 5, there is cell death adjacent to the impact area followed by an unabated spread of the cartilage lesion. Due to the low release rate of EPO by \( C_E \) cells, \( C_E \) cells do not convert to the \( C_U \) state, nor do EPOR-active cells \( S_A \) recover to become healthy. By day 14, there remain a fair number of \( C_E \) and EPOR-active cells \( S_A \) cells waiting for more EPO to finish their conversions. Figure 3.4 shows the degradation of ECM. In the period of 14 days, we hardly notice a decrease of ECM density. This is because ECM degradation is a much slower process than the apoptosis of cells. A longer time period is needed to have a big mass loss of ECM. Significant degradation leads to changes in the mechanical properties of ECM, which can result in the dysfunction of the tissue. Fourteen days is an appropriate length of time to guarantee that the mechanic property of ECM is stable, so that the degradation due to TNF-\( \alpha \) is at a constant rate. Further study is needed to model ECM degradation in more details and over a longer time period.

Figures 3.5, 3.6 and 3.7 show the concentration of chemicals. Although the concentration of EPO increases during the fourteen-day-period, it is too low to trigger the anti-inflammatory process efficiently [16]. The concentration of IL-6, ROS and DAMPs increases in the beginning, reaches its peak around day 5, and starts decreasing at day 7. The low concentration of ROS is in accord with its biological property [17].
Figure 3.2. Cell densities (a) The densities of healthy cells (b) The densities of healthy, catabolic, and EPOR-active cells
Figure 3.3. The densities of live cells

Figure 3.4. The density of ECM
Figure 3.5. The densities of IL-6 and EPO

Figure 3.6. The density of DAMPs
3.3.3.1 Numerical methodology

Numerical efficiency is the main reason that we use an age-structured model rather than delay differential equations. The stability constraints of the semi-discretization limit the time steps chosen when using Matlab’s dde33, which in turn requires us to keep in memory much more past history information.

Because of the limitations of delay differential equation, we convert the delays into a physiological property of individual cells. Although we make an apparent sacrifice to include an additional age dimension and age derivative, the resulting system is more computationally tractable than one using delay differential equations. The relative errors are given in Table 3.2. For each dependent variable in the model, we show the relative 2-norm error over all spatial and temporal nodes. Relative errors
<table>
<thead>
<tr>
<th></th>
<th>node101</th>
<th>node201</th>
<th>node401</th>
<th>node801</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Normal ($C_U$)</td>
<td>4.11E-04</td>
<td>2.24E-05</td>
<td>2.82E-06</td>
<td>9.53E-07</td>
</tr>
<tr>
<td>Healthy Pre-catabolic ($C_T$)</td>
<td>1.03E-03</td>
<td>1.19E-04</td>
<td>8.53E-05</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>Healthy EPO-producing ($C_E$)</td>
<td>1.43E-03</td>
<td>7.76E-05</td>
<td>1.06E-05</td>
<td>3.15E-06</td>
</tr>
<tr>
<td>Catabolic ($S_T$)</td>
<td>1.70E-03</td>
<td>1.87E-04</td>
<td>4.17E-05</td>
<td>6.81E-05</td>
</tr>
<tr>
<td>EPOR Active ($S_A$)</td>
<td>1.80E-03</td>
<td>1.09E-04</td>
<td>1.28E-05</td>
<td>4.65E-06</td>
</tr>
<tr>
<td>ECM ($U$)</td>
<td>5.50E-07</td>
<td>6.95E-08</td>
<td>2.83E-08</td>
<td>1.57E-08</td>
</tr>
<tr>
<td>IL6 ($F$)</td>
<td>8.53E-04</td>
<td>7.13E-05</td>
<td>1.54E-05</td>
<td>5.97E-06</td>
</tr>
<tr>
<td>EPO ($P$)</td>
<td>1.83E-03</td>
<td>1.11E-04</td>
<td>1.92E-05</td>
<td>4.68E-06</td>
</tr>
<tr>
<td>DMAPs ($M$)</td>
<td>1.87E-03</td>
<td>8.50E-05</td>
<td>2.85E-05</td>
<td>6.35E-06</td>
</tr>
<tr>
<td>ROS ($R$)</td>
<td>2.18E-03</td>
<td>5.21E-04</td>
<td>1.21E-04</td>
<td>3.05E-05</td>
</tr>
</tbody>
</table>

Table 3.2. Table of relative errors in the 2-norm

are calculated by comparing the solution using $n$ spatial intervals with the solution using $2n$ spatial intervals. All the simulations have the relative errors under 0.3%, even for the lowest node numbers. The error goes down when the node number increases.

3.3.4 Sensitivity analysis

As we did in Chapter 2, we examine the sensitivity of the approximated parameters. The approximated parameters are $\alpha_1$, $\alpha_2$, $\beta_{11}$, $\beta_{12}$, $\beta_{13}$, $\kappa_1$, $\kappa_2$, $\Lambda$, $\lambda_R$, $\lambda_M$, $\lambda_F$, $\lambda_P$, $\mu_{S_T}$, $\mu_{S_A}$ and $\mu_{D_N}$. The value ranges for the sensitivity analysis are shown in Table 3.3. To conduct our sensitivity analysis, we hold all other parameters to their base value in Table 3.1 and perturb the parameter under scrutiny.

The parameter $\alpha_2$ doesn’t effect the system in the simulation because the level of EPO is too low to make $H(P - P_c)$ nonzero; we include it in the model for generality and completeness. The changes due to perturbations are once again quantitative
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perturbed Value</th>
<th>Robust Level</th>
<th>Suggested Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_1 )</td>
<td>{0.1, 0.5, 1, 1.5, 2}</td>
<td>robust-</td>
<td>(0.1, 2)</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>{0.1, 0.5, 1, 1.5, 2}</td>
<td>robust++</td>
<td>(0.1, 2)</td>
</tr>
<tr>
<td>( \beta_{11} )</td>
<td>{50, 75, 100, 125, 150}</td>
<td>robust-</td>
<td>(60, 70)</td>
</tr>
<tr>
<td>( \beta_{12} )</td>
<td>{40, 45, 50, 55, 60}</td>
<td>robust</td>
<td>(40, 60)</td>
</tr>
<tr>
<td>( \beta_{13} )</td>
<td>{1, 5, 10, 15, 20}</td>
<td>robust-</td>
<td>(5, 20)</td>
</tr>
<tr>
<td>( \kappa_1 )</td>
<td>{1, 5, 10, 15, 20}</td>
<td>robust-</td>
<td>(5, 20)</td>
</tr>
<tr>
<td>( \kappa_2 )</td>
<td>{1, 5, 10, 15, 20}</td>
<td>robust-</td>
<td>(5, 20)</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>{0.1, 0.3, 0.5, 0.7, 0.9}</td>
<td>robust</td>
<td>(0.1, 0.9)</td>
</tr>
<tr>
<td>( \lambda_R )</td>
<td>{1, 3, 5, 7, 9}</td>
<td>not robust</td>
<td>(3, 7)</td>
</tr>
<tr>
<td>( \lambda_M )</td>
<td>{0, 1, 0.3, 0.5, 0.7, 0.9}</td>
<td>not robust</td>
<td>(0, 0.7)</td>
</tr>
<tr>
<td>( \lambda_F )</td>
<td>{1, 3, 5, 7, 9}</td>
<td>not robust</td>
<td>(0, 0.7)</td>
</tr>
<tr>
<td>( \lambda_P )</td>
<td>{0.1, 0.3, 0.5, 0.7, 0.9}</td>
<td>robust-</td>
<td>(0.1, 0.9)</td>
</tr>
<tr>
<td>( \mu_{S_T} )</td>
<td>{0.1, 0.3, 0.5, 0.7, 0.9}</td>
<td>robust+</td>
<td>(0.1, 0.2)</td>
</tr>
<tr>
<td>( \mu_{S_A} )</td>
<td>{0.01, 0.05, 0.1, 0.15, 0.2}</td>
<td>robust+</td>
<td>(0.01, 0.2)</td>
</tr>
<tr>
<td>( \mu_{DN} )</td>
<td>{0.01, 0.03, 0.05, 0.07, 0.09}</td>
<td>robust+</td>
<td>(0.01, 0.09)</td>
</tr>
</tbody>
</table>

Table 3.3. Sensitivity table

rather than qualitative, with some exception. The most sensitive parameters are again the Michaelis-Menten coefficients \( \lambda_R \), \( \lambda_M \) and \( \lambda_F \). When these parameters become relatively small compared to their base value, solutions that were monotone become non-monotone and oscillatory. Based on these results, we provide appropriate ranges of parameter values for our model in Table 3.3. More specifically, we found that

- when \( \alpha_1 \) increases, the population of EPOR-active cell (\( S_A \)) and the density of ECM increase, but all the other chemicals and cell populations decrease,

- when \( \beta_{11} \) increases, the population of catabolic and EPOR-active cell (\( S_T, S_A \)) and the concentration of DAMPs, IL-6 and ROS increase, but the population of all healthy cell (\( C_U, C_T, C_E \)), the density of ECM, and the concentration of
EPO decrease,

- when $\beta_{12}$ increases, the population of catabolic and EPOR-active cell ($S_T, S_A$) and the concentration of DAMPs, IL-6 and ROS increase, but the population of healthy cell ($C_U, C_T, C_E$) and the concentration of EPO decrease,

- when $\beta_{13}$ increases, the population of all cells except $C_U$ cells and the concentration of all chemicals increase, however the density of ECM decreases,

- when $\kappa_1$ increases, the population of healthy cell ($C_U, C_E$) and EPOR-active cell ($S_A$) and the concentration of EPO increase, but healthy cell ($C_T$), catabolic cell ($S_T$), EPOR-active cell ($S_A$), and the concentrations of chemicals IL-6, ROS, and DAMPs decrease,

- when $\kappa_2$ increases, the population of healthy cell ($C_U, C_E$), catabolic cell ($S_T$), and the concentration of EPO increase, but the population of healthy cell ($C_T$), EPOR-active ($S_A$) cells, and the concentrations of IL-6, ROS, and DAMPs decrease,

- when $\lambda_R$ increases, all the cell populations increase except for EPOR-active cell ($S_A$) cell, and all the chemical concentrations increase except DAMPs,

- when $\lambda_M$ increases, the populations of healthy cell ($C_U, C_T, C_E$) and the concentration of EPO increase, but the populations of sick cell ($S_T, S_A$) and the concentrations of IL-6, ROS, and DAMPs decrease,

- when $\lambda_F$ increases, all the cell populations increase except for EPOR-active cell ($S_A$) cell, and all the chemical concentrations increase except DAMPs,
• when $\lambda_P$ increases, the population of healthy cell ($C_E$), EPOR-active cell1 ($S_A$), and the concentrations of EPO increase, but the population of healthy cell ($C_U$, $C_T$) cells, catabolic cell ($S_T$), and the concentrations of IL-6, EPO, DAMPs decrease,

• when $\mu_{DN}$ increases, the populations of healthy cell $C_E$ and sick cells ($S_A$, $S_T$) increase, but the populations of healthy cell ($C_U$, $C_T$), catabolic cell ($S_T$) and the concentrations of all chemical decrease.

3.4 Experimental validation

3.4.1 Experimental materials and methods

The aim of the experiments is to validate whether our predictions can qualitatively capture the biological properties. Marc Brouillette$^{12}$ and James Martin$^{12}$ designed the validation experiments. Annelises Heiner$^1$ conducted the tissue culture experiments that generated the sample sets. Marc conducted the immunohistochemical experiments on those sample sets, prepared and scanned those cartilage slide for further validation.

3.4.1.1 Generation of cartilage specimens

Osteochondral explants were surgically excised from bovine lateral tibial plateaus ($25 \text{ mm} \times 25 \text{ mm} \times 10 \text{ mm}$). All specimens were allowed to equilibrate in a low oxygen environment (5% O$_2$, 5% CO$_2$) culture media for two days. At day 0, we either

$^1$Department of Orthopedics and Rehabilitation, University of Iowa
$^2$Department of Biomedical Engineering, University of Iowa
impact the explants with a 5.5 mm rounded edge indenter from a drop tower with an imparting energy of 2.18 J/cm² or let the explants undergo a sham impact procedure. We removed the unimpacted explants at day 0, and preserve and embed them in paraffin. Replace the impacted explants in fresh culture media and remove them at 1, 7, 14 day for preservation and paraffin embedding. Culture media is changed every two days for explants cultured out to day 7 or 14.

3.4.1.2 Immunohistochemistry

Cartilage explants were cut into 5 µm thick sections on glass slides and processed for immunohistochemical detection of interleukin-6 (IL-6), erythropoietin (EPO), and erythropoietin receptor (EPOR). For each stain, all cartilage sects were processed in batch at the same time under the same conditions. All the sections were deparaffinized, quenched of peroxidase actives and under antigen retrieval using a 0.01 M citric acid solution for 4 hours at 65°C. Then block the non-specific anti-gen binding for one hour with PBS containing 10% goat serum, 1% bovine serum albumin (w/v), and 0.1% tween 20 to dilute the primary anti-bodies against IL-6 (Abcam ab193799), EPO (Abcam ab65394), and the EPOR (Abcam ab83696) at a ratio of 1:100 from their starting concentration. The slides were incubated in the solution containing the primary antibody overnight at 4°C, and then were rinsed and applied blocking solution for 30 minutes at room temperature. Incubated a biotinylated secondary antibody against the primary antibody on the slides at a 1:250 dilution (Vector Labs), and then the slides were rinsed and incubated with the VECTASTAINÆ ABC Reagent
(Vector Labs) for 30 minutes at room temperature. After another rinsing, the slides were incubated in DAB in (3,3-diaminobenzidine) HRP substrate solution (Vector Labs DAB substrate kit) for 2-10 minutes (depending on primary antibody). The slides were rinsed for the last time, counter stained with eosin, dehydrated, attached a glass coverslip and sealed with permount (Fisher).

Slides were scanned digitally on an Olympus VS110 with the software to control the slide stage in all axes and automatically focused/captured high resolution images (322 nm/pixel). The scanned images were tiled together, resulting in full explant immunohistochemically labeled images. Tiff images were exported by reducing the resolution to 20% of their original size for analysis.

Validation data was obtained by analyzing 12 cartilage slides. There are six categories for the slides: EPO at day 1, 7, 14 and IL-6 at day 1, 7, 14, with two slides per category. Each slide is approximately 1" (2.5cm) long, with a sham impact placed in the middle which formed a dent Fig 3.8a. The nucleus of the cells in those slides can be stained by the antibodies of the targeting chemical (EPO or IL-6) if the concentration of this chemical there is significant. The stained nucleus of the cells were shown in the zoomed images 3.8b. The cells whose nuclei can be stained by the antibodies are consider to be “positive” for this chemical. The number of “positive” cells per area in the cartilage slides corresponds to the relative concentration of EPO and IL-6.

The concentration of EPO as a function of “positive” cells is shown in Fig. 3.9. Each scale bar is 100 µ. The immunohistochemistry images were analyzed for “posi-
tive” cells. Those cells are above the background thresholding technique and within our cell size criteria. The concentration of EPO shows less constitutive expression at day 1, but increases at day 7 and 14.

3.4.2 Experimental validation

For image processing, each cartilage slide was separated into two half-slices, divided at the point of impact. We further subdivided each half-slide into 6 pieces, and each piece is 0.15 cm long. The pieces correspond roughly to the radius intervals $[0, 0.15]$ cm, $[0.15, 0.3]$ cm, $[0.3, 0.45]$ cm, $[0.45, 0.6]$ cm, $[0.6, 0.75]$ cm, and $[0.75, 0.9]$ cm from the impact site.

For each piece, we cropped an $800 \times 800$ pixel sample image from each piece and estimated the cell numbers in each sample. The pixel width of the images is approximately 1.610 $\mu$m giving a pixel area of 2.5921 $\mu$m$^2$. Thus $800 \times 800$ pixel sample image has an area of approximately 0.0166 cm$^2$. We have four sample images per radius interval. We performed cell segmentation using both EM-algorithm and K-mean methods for the cell segmentation. The upper threshold for the positive cell diameter is 50-150 $\mu$m$^2$ (approximately 20-60 pixels), while the lower threshold is 25-30 $\mu$m$^2$ (approximately 10-15 pixels). Our cell segmentation algorithm will identify the cell nuclei, estimate the center and radius of the nuclei, and draw a circle around the nuclei to mark the cells Fig.3.10a 3.10b, and counted the total cell counts in each image. The average cell number counts is used as an estimate of the number of “positive cells”, which gives us a qualitative estimate of chemical concentrations.
Figure 3.8. (a) Slides of articular cartilage 1, 7, 14 days after a single blunt impact stained for EPO, the squares in each image is what is magnified in Fig. 3.8b (b) The zoomed areas in Fig. 3.8a
Applying the tool of image processing, we obtained the cell number counts for each image. As we described before, there are 6 categories (EPO at day 1, 7, 14 and IL-6 at day 1, 7, 14), and each category has six radius intervals ([0, 0.15] cm, [0.15, 0.3] cm, [0.3, 0.45] cm, [0.45, 0.6] cm, [0.6, 0.75] cm, and [0.75, 0.9] cm) with four samples per interval. Averaging the cell counts for the 4 samples, we obtain an average cell counts for every interval and category. The data is plotted in Fig. 3.11a and 3.11b. From our validation results, the concentration of EPO increases late in the time interval.
Figure 3.10. The marked cells in image processing to count the number of “positive cells” in the cartilage slide (a) EPO sample 14 days after impact (b) IL-6 sample 7 days after impact.

The concentration of IL-6 at day 7 is bigger than the concentration of day 1 and 14. Compared to the experimental results, our simulated results (Fig. 3.11c and 3.11d) qualitatively match the unimodel distributions of EPO. Fig. 3.11c shows the increase
Figure 3.11. (a) The concentration of EPO measured experimentally (b) The concentration of IL-6 measured experimentally (c) The concentration of EPO simulated from the model (d) The concentration of IL-6 simulated from the model. The different line types (red + solid line + square, green + solid line + circle, and black + dashed line + diamond) represent the EPO/IL-6 stained 14, 7, and 1 day after impact.
of EPO concentration, and Fig. 3.11d shows IL-6 has the highest concentration in day 7, which agreed with the experimental data.

Our current experimental techniques cannot quantitatively measure the concentration of cytokines, and we therefore use the estimated numbers of chondrocytes expressing each cytokine at a detectable level by immunohistochemistry (“positive cells”). While there are limitations to only counting “positive cell” numbers, the immunohistochemistry analysis catches the relative rises and declines in the concentration of EPO and IL-6 present in and around the individual chondrocytes, so it is sufficient for the purpose of validating in this thesis.

3.5 Conclusion

In this chapter we presented an age-structured model to simulate the scenario of articular cartilage lesion after a single blunt impact. Our model is based on a general understanding of the biological mechanisms of articular cartilage supported by lab experiments. We simplified the model by making it radially symmetric, which reduces the spatial dimension to 1-D. Based on the current understanding, we assume that the consequences of a single initial impact in articular cartilage depends on the competition between the chondrolytic and chondroprotective responses of local chondrocytes. The path of cartilage damage has been regarded as a complex biological process. We provide a framework to model the complex biological behavior by investigating the “balancing act” between the pro- and anti-inflammatory cytokines. The accompanying immunohistology experiments suggest that the expression of IL-6
and EPO plays an important role in the system. The pro-inflammatory cytokine IL-6 plays a significant role in causing the spread of cartilage lesion, whereas the anti-inflammatory cytokine EPO play an antagonistic role to IL-6 by blocking the effect of IL-6 and limiting the spread of lesion.

Instead of using delay differential equations, we used age structure to represent the delays in the cell-state transitions. Although it is more transparent to represent delay terms using delay differential equations, the age-structured model is more computationally efficient and more flexible in representing variance in the delay times.
CHAPTER 4
AN AGE-STRUCTURED MODEL OF THE EFFECTS OF CYCLIC LOADING

4.1 Age-structured model with mechanical loading

In Chapter 2, we presented a reaction-diffusion-delay model \[46\] to investigate the cartilage lesion formation process under the effect of cyclic loading. In this chapter, we present the age- and space-structured model for the inflammation response under the effect of cyclic loading, and then present the simulation results using the same computational methods described in Chapter 3.

4.1.1 Variables and biological scheme

We assume radial symmetry to reduce the model to one dimension in space with components that depend on radius \(r\), age \(a\) and time \(t\). We assume that the loading was applied on a small region near the origin with a radius of 0.25 cm. The total radius is 2.5 cm, and total time is 14 days. We have the same components in our mathematical model as we used in Chapter 3. A schematic of the system is in Fig. 4.1.

Under cyclic loading, healthy cartilage is impacted by mechanical loading, which starts the lesion process. Loading causes necrosis inside the loading site. As opposed to the case of an initial single impact (Chapter 3), the population of necrotic cells starts at 0 and accumulates as time progresses. Healthy cells are separated into three states: healthy normal cells \(C_U\), healthy pre-catabolic cells \(C_T\) and
Figure 4.1. Schematic of articular cartilage lesion formation under cyclic loading

Healthy EPO-producing cells ($C_E$). Extensive cyclic loading causes both healthy and sick cells ($C_U, C_T, C_E, S_T, S_A$) to become necrotic ($D_N$) and produce DAMPs. Healthy cells ($C_U$) signaled by DAMPs enter into the state $C_T$. These healthy pre-catabolic cells ($C_T$) enter the catabolic state ($S_T$) under the effect of DAMPs and EPO. Catabolic cells ($S_T$) begin to synthesize TNF-$\alpha$ and ROS. Signaled by ROS, healthy pre-catabolic cells ($C_T$) become healthy EPO-producing cells ($C_E$) after a 20-24 hour delay, and $C_E$ cells begin to produce EPO. Catabolic cells ($S_T$) that are signaled by TNF-$\alpha$ will express a receptor (EPOR) for EPO and become EPOR-active
cells denoted by $S_A$. There is also a time delay of 8-12 hours before a cell expresses the EPO receptor after being signaled. Since EPOR active cells express a receptor for EPO, $S_A$ cells may switch back to the healthy normal state ($C_U$) after the reception of EPO. When EPO is abundant in the system, $C_E$ cells may stop producing EPO and become healthy normal cells ($C_U$) again. Meanwhile, the effect of inflammatory cytokines such as TNF-α may degrade the sick cells and turn them into apoptotic cells ($D_A$). We also assume that TNF-α limits production of EPO, whereas high levels of EPO may shut off the degradation process effected by TNF-α and prevent healthy cells from becoming sick. The “balancing act” between anti-inflammatory cytokines such as EPO and pro-inflammatory cytokines such as TNF-α determines the inflammatory process and the spread of an articular cartilage lesion.

The schematic in Fig. 4.1 translates to the following system of structured partial differential equations:

The equations for the chemicals are

$$\frac{\partial t}{\partial} R(r, t) = \frac{1}{r} \frac{\partial}{\partial r} (r D_R R_r) - \delta_R R + \sigma_R S_T \quad \text{(4.1)}$$

$$\frac{\partial t}{\partial} M(r, t) = \frac{1}{r} \frac{\partial}{\partial r} (r D_M M_r) - \delta_M M + \sigma_M D_N + \delta_U U \frac{F}{\lambda_F + F} \quad \text{(4.2)}$$

$$\frac{\partial t}{\partial} F(r, t) = \frac{1}{r} \frac{\partial}{\partial r} (r D_F F_r) - \delta_F F + \sigma_F S_T \quad \text{(4.3)}$$

$$\frac{\partial t}{\partial} P(r, t) = \frac{1}{r} \frac{\partial}{\partial r} (r D_P P_r) - \delta_P P + \sigma_P C_E \frac{R}{\lambda_R + R \Lambda + F} \quad \text{(4.4)}$$
with initial and boundary conditions

$$\partial_r R(0,t) = \partial_r M(0,t) = \partial_r F(0,t) = \partial_r P(0,t) = 0,$$

$$R(r,0) = M(r,0) = F(r,0) = P(r,0) = 0.$$

The equation for ECM is

$$\partial_t U(r,t)_{ECM} = -\delta U \frac{F}{\lambda_F + F} H(P_c - P),$$

with initial conditions

$$U(r,0) = 30 \text{mg}.$$

The function $H$ is as in Chapter 3 and is used to reflect that ECM degradation can be terminated when the level of $P$ exceed $P_c$.

The healthy cell equations are

$$\partial_t C_U(r,t) = \alpha_1 S_A \frac{P}{\lambda_P + P} + \alpha_2 H(P - P_c) C_E - \beta_{13} C_U \frac{M}{\lambda_M + M} - \Gamma(\epsilon, U, r) \cdot C_U,$$

$$\partial_t C_T(r,a,t) + \partial_a C_T(r,a,t) =$$

$$-\beta_{11} \frac{M}{\lambda_M + M} H(P_c - P) C_T(r,a,t) - \beta_{12} \frac{F}{\lambda_F + F} H(P_c - P) C_T(r,a,t)$$

$$- \kappa_2 \gamma(a - \tau_2) \frac{R}{\lambda_R + R} C_T(r,a,t) - \Gamma(\epsilon, U, r) \cdot C_T.$$
\[ \partial_t C_E(r, t) = \int_0^\infty \kappa_2 \gamma(a - \tau_2) \frac{R(r, t)}{\lambda_R + R(r, t)} C_T(r, a, t) da - \alpha_2 H(P - P_c) C_E \] (4.8)

\[ - \Gamma(\epsilon, U, r) \cdot C_E, \]

with initial conditions

\[ C_U(r, 0) = \begin{cases} 0, & 0 \leq r \leq 0.25 \text{cm}, \\ 100000 \text{ cells/cm}^2, & \text{otherwise}. \end{cases} \]

\[ C_T(r, 0, t) = \beta_{13} C_U \frac{R}{\lambda_R + R^2 \lambda}, \]

\[ C_T(r, a, 0) = C_E(r, 0) = 0. \]

The sick cell equations are

\[ \partial_t S_T(r, a, t) + \partial_a S_T(r, a, t) = \] (4.9)

\[ - \mu_{ST} F M S_T(r, a, t) - \eta \gamma(a - \tau_1) F M S_T(r, a, t) \]

\[ - \Gamma(\epsilon, U, r) \cdot S_T, \]

\[ \partial_t S_A(r, t) = \int_0^\infty \eta \gamma(a - \tau_1) F(r, t) \lambda_F + F(r, t) S_T(r, a, t) da - \alpha_1 S_A P \] (4.10)

\[ - \mu_{SA} F S_A - \Gamma(\epsilon, U, r) \cdot S_A, \]
with initial conditions

\[
S_T(r, 0, t) = \int_0^\infty \left( \beta_{11} \frac{M}{\lambda_M + M} H(P_c - P) + \beta_{12} \frac{F}{\lambda_F + F} H(P_c - P) \right) C_T(r, a, t) da,
\]

\[
S_T(r, a, 0) = S_A(r, 0) = 0.
\]

The necrotic cell population is

\[
\partial_t D_N(r, t) = - \mu_D N + \Gamma(\epsilon, U, r) \cdot (C_U + C_T + C_E + S_T + S_A),
\]

with initial condition

\[
D_N(r, 0) = \begin{cases} 
100000 \text{ cells/cm}^2, & 0 \leq r \leq 0.25 \text{ cm}, \\
0, & \text{otherwise}.
\end{cases}
\]

The cell-strain function \(\Gamma(\epsilon, U, r)\) is adapted from Chapter 2 and is based on the experimental results from [17],

\[
\Gamma(\epsilon, U, r) = \begin{cases} 
24 \ast (1 - 0.01 \cdot p_0(\exp(K_U \cdot \epsilon) - \exp(20 \cdot K_u))) \frac{\lambda_U}{\lambda_U + U}, & 0 \leq r \leq r_l, \\
0, & r > r_l,
\end{cases}
\]

where \(\epsilon\) represent the strain, and \(U\) is the density of ECM. The parameters are chosen to be consistent with Chapter 2, with \(r_l = 0.25\) cm, \(K_U = 0.0545\) and \(p_0 = 1\). The rate of cell death grows exponentially while the strain \(\epsilon\) increases. When strain \(\epsilon\) is below 20\%, which is considered to be “healthy loading”, there is no cell death.
4.1.2 Numerical methods and results

Equations 4.1 - 4.12 express the relationship between the static strain and the inflammation process. The spatially 1D system was solved using the same parameter sets and numerical methods presented in Chapters 2 and 3. We computed the results for four values of the steady-state strain, $\epsilon = 0.3$, 0.4, 0.6 and 0.8 (Fig. 4.2 - 4.9). Fig. 4.2 shows the evolution of cell populations and the change of chemical concentrations with a strain of 30%. When the strain is 30% and 40%, Fig. 4.2a, 4.2b, 4.4a, 4.4b each show a minor spread of the lesion. At day 14, there is less than 20% cell death under 30% strain and around 50% cell death under 40% strain. When strain is greater than 60%, there are significantly more sick cells compared to the low strain cases. At day 14, the cell death rate reaches more than 80% and the lesion spreads to the entire domain. The concentrations of EPO and TNF-α (or IL-6) increases as time processes, while the concentrations of ROS and DAMPs reach their peak between day 5 and day 7. Chemical concentrations follows the same pattern for all strain levels, except that there are higher chemical concentrations for higher strain levels. In addition, we notice more ECM degradation in the high strain case.

The figures show agreement with our current understanding of the cartilage lesion formation process under cyclic loading. When the loading strain is above 40%, chondrocyte death increases significantly and leads to widespread cartilage damage. ROS production is correlated with the cartilage deformation (strain), which is also in agreement with experimental results [17]. Further experimental investigation is needed to validate the cell viability and ROS production, which will be discussed
in the next sections. In general, the simulation results represent well the dynamics of articular cartilage under mechanical loading, giving us insight into how a lesion spreads from the loading site.

The simulation results are consistent with the results in Chapter 2 (Fig. 2.5-2.8) within the loading area. However, the population of each cell type is different. The simulation results in Chapter 2 present a much higher population of sick cells with catabolic cells dominating. However, Fig. 4.2b, 4.4b, 4.6b and 4.8b show a dominant population of EPOR-active cells. Nonetheless, we used most of the same parameters in the models in this chapter and in Chapter 3, and the two models returned qualitatively similar results, particularly when the strain is high. Comparing Fig. 3.2, 3.5 and 3.7 to Fig. 4.8 and 4.9, the effect of cyclic loading under 80% strain rate is almost the same as the effect of a single blunt impact.

4.2 Conclusion

In this chapter we presented an age-structured model of cartilage lesion formation under mechanical loading. This model extends the model in Chapter 2 by replacing delay terms with age structure. Both models show qualitatively similar predictions of load-induced responses of articular cartilage. The cell death rate of the impact area is directly proportional to strain level, which agrees with experiment results [17]. Simulation results of these two models share similar patterns of cell death, but differ in the quantitative populations of each cell type. Further experimental work is needed to investigate the distribution of chemical concentrations, as well as
Figure 4.2. The evolution of cell populations under 30% strain (a)
Healthy cells (b) Healthy, catabolic and EPOR-active cells
Figure 4.3. The change of chemical concentrations under 30% strain

(a) TNF-α & EPO  (b) ROS
Figure 4.4. The evolution of cell populations under 40% strain (a)
Healthy cells (b) Healthy, catabolic and EPOR-active cells
Figure 4.5. The change of chemical concentration under 40% strain

(a) TNF-α & EPO (b) ROS
Figure 4.6. The evolution of cell population under 60% strain (a)
Healthy cells (b) Healthy, catabolic and EPOR-active cells
Figure 4.7. The change of chemical concentrations under 60% strain

(a) TNF-α & EPO (b) ROS
Figure 4.8. The evolution of cell populations under 80% strain (a)
Healthy cells (b) Healthy, catabolic and EPOR-active cells
Figure 4.9. The change of chemical concentrations under 80% strain

(a) TNF-α & EPO (b) ROS
cell populations over the fourteen-day-period, so that the predictive model is closer to the real system.

The age-structured model is computationally relatively efficient, giving an approximate reduction of computational time by a factor of 10. The benefit of this efficiency is that ability to extend the model to higher dimensions.

The cell-strain function $\Gamma(\cdot)$ in Eq. 4.12 assumes that strain remains constant across the whole loading area, which is reasonable for a 1D model. However, articular cartilage is a binary mixture having characteristics of both a linearly elastic solid and an incompressible viscous fluid [34]. Combining our biomathematical model with a biomechanical model for the matrix requires more spatial dimensions.
CHAPTER 5
A DYNAMIC SPATIO-TEMPORAL MODEL FOR ARTICULAR CARTILAGE

5.1 Spatio-temporal data description

5.1.1 Background

In the previous three chapters, we built three mathematical models to describe the cartilage lesion formation due to different causes of injury. Those mathematical models qualitatively captured the biological processes of cartilage degeneration. Although the numerical simulations are of the first two weeks after injury, outcomes over a longer time period can be simulated, which enable us to investigate the possible long-term effects of injuries on patients. Mathematical models may thus provide useful information which may be difficult or impossible to obtain in \textit{in vivo} or \textit{in vitro}. What’s more, OA is a chronic disease, and may take many years to develop. There might be a long gap between the time of injury and the onset of the clinical symptoms. Early diagnosis may enable clinicians to prevent further injury. However, there is still no effective means of early diagnosis. Additionally, tremendous time and money is needed to conduct a clinical trial. Mathematical methods can simulate the long-term results and predict the possible outcomes for patients in various scenarios before the trials are conducted, which might help give treatment suggestions and reduce the trial budget.

Our goal is to build a “true” predictive model that is clinically relevant. As we discussed in Chapter 4, the choice of parameters may decide the simulation results.
One way to make our work closer to the “true model” is to reshape our parameters based on the existing experimental data. In this chapter, we present a spatio-temporal model based on our biomathematical models to investigate the distributions of model parameters using the data obtained from the experiments discussed in Chapter 3. Due to the limited data, we cannot conduct parameter estimations for all the parameters in our models. We therefore simplify our model and focus on the balancing act between IL-6 and TNF-α. Our statistical model predicts cytokine concentrations over time. Based on the predictions, we further discuss the possibility of using IL-6 as a potential biomarker for early diagnosis, and using EPO as a potential therapy.

5.1.2 Experimental methods & data description

Statistical analysis requires a large data set. To build our data set, we re-conduct the image processing using the same image slides from the experiments in Chapter 3. We have six categories of cartilage slices: EPO at day 1, 7, and 14, and IL-6 at day 1, 7, and 14. Each category has three slides, which are the same as the slides we described in Chapter 3 (Fig. 3.8). The sham impact was placed in the middle of each cartilage slide, so we split each slide into a half-slide by dividing it at the point of impact. We have six half-slides to analyze for each category. We further subdivided each half-slide into equal width (approximately 300 pixels) and radius regions (Fig. 5.1), and separate each image into superficial, transitional, radial, and calcified zones following the ratios 5%, 27%, 42%, 26%. We count the positive cell number for each radius region with respect to different zones, and divide the cell
counts by the area of each radius region. The average cell count among all zones is used as an estimate of the number of positive cells per region. The EM algorithm and K-mean method are applied for cell segmentation, with $50-150 \, \mu m^2$ as the upper threshold, and $25-30 \, \mu m^2$ as the lower threshold of the positive cell size.

Time (days) and space (radius region) are the two main factors of the experimental results, and we therefore build a spatio-temporal model to interpret our data. Because we are describing the reaction-diffusion characteristics of cytokines, we can derive our model from continuous differential equations. Using the image processing

![Figure 5.1. Stained slides](image)

we discussed above we obtained the data set described in the following:

- Days: day 1, day 7, day 14
- Radius region (17 in total): $(0,0.49)$, $(0.049, 0.098)$,⋯, $(0.784, 0.833)$ cm
- Group (cartilage slide replicate of experiment): 1, 2, 3, 4, 5, 6
- Slice: The name of cartilage slides in experiments
• T (cell number per $\mu m^3$) : total cell counts in each radius region

• P (cell number per $\mu m^3$) : positive cell counts for EPO in each radius region

• F (cell number per $\mu m^3$) : positive cell counts for IL-6 in each radius region

<table>
<thead>
<tr>
<th>slice</th>
<th>day</th>
<th>group</th>
<th>radius</th>
<th>T</th>
<th>P</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.0490</td>
<td>247.75</td>
<td>1.1458</td>
<td>1.3984</td>
</tr>
<tr>
<td>2 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.0980</td>
<td>206.75</td>
<td>1.3191</td>
<td>1.2808</td>
</tr>
<tr>
<td>3 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.1470</td>
<td>174.25</td>
<td>1.6553</td>
<td>1.2479</td>
</tr>
<tr>
<td>4 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.1960</td>
<td>206.75</td>
<td>1.4491</td>
<td>1.3880</td>
</tr>
<tr>
<td>5 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.2450</td>
<td>217.50</td>
<td>1.3886</td>
<td>1.3551</td>
</tr>
<tr>
<td>6 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.2940</td>
<td>255.50</td>
<td>1.8172</td>
<td>1.5166</td>
</tr>
<tr>
<td>7 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.3430</td>
<td>291.25</td>
<td>1.8598</td>
<td>1.8101</td>
</tr>
<tr>
<td>8 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.3920</td>
<td>288.50</td>
<td>1.7676</td>
<td>1.7398</td>
</tr>
<tr>
<td>9 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.4410</td>
<td>327.75</td>
<td>1.7492</td>
<td>1.8557</td>
</tr>
<tr>
<td>10 1433R.tif</td>
<td>14</td>
<td>1</td>
<td>0.4900</td>
<td>313.50</td>
<td>1.8857</td>
<td>2.2668</td>
</tr>
</tbody>
</table>

... 

5.2 Stochastic spatio-temporal model

In this section, we describe a simplified mathematical model to describe the concentration of IL-6 and EPO in articular cartilage after a single severe traumatic event.

5.2.1 Components of the system

We assume radial symmetry to reduce the model to one-dimension in space. The components of the system depend on radius $r$ ($0 \leq r \leq 2.5 cm$) and time $t$ ($0 \leq t \leq 14 days$). The schematic of the system is presented in Fig. 5.3. The variables are

• $F(r, t)$ : concentration of IL-6 (positive cell counts per area per week)

• $P(r, t)$ : concentration of EPO (positive cell counts per area per week)
Figure 5.2. The concentration of (a) IL6 (b) EPO

- \( S(r,t) \): sick cell density (sick cell counts per area per week)
- \( C(r,t) \): healthy cell density (healthy cell counts per area per week)
- \( D_A(r,t) \): apoptotic cell (dead cell)
- \( D_N(r,t) \): necrotic cell (dead cell)

The parameters are:

- \( \kappa_F(\kappa_P) \): concentration of IL6 (EPO) diffusing out of the region per week.
- \( \delta_F(\delta_P) \): reduction in concentration of IL6 (EPO) per week.
- \( \eta_F(\eta_P) \): production of IL6 (EPO) per sick (healthy) cell per week.

As shown in Fig. 5.3, the blunt impact triggers a sequence of reactions that promote the inflammatory process. Initial injury causes the chondrocytes inside the
impact area to become necrotic ($D_N$). Necrotic cells ($D_N$) release alarmins (DAMPs) to alarm the system. Cells adjacent the injury area start switching from the healthy state ($C$) to the sick state ($S$). Sick cells ($S$) begins to produce inflammatory cytokines IL-6 ($F$). IL-6 ($F$) drives more healthy cells ($C$) to sick ($S$) and causes sick cells ($S$) to become apoptotic ($D_A$). On the other hand, healthy cells ($C$) begin producing anti-inflammatory cytokines EPO ($P$), which can drive sick cells ($S$) back to the healthy state ($C$), and support the recovery of the system. However, the signaling of EPO ($P$) is partially blocked by IL-6 ($F$), which limits the self-repair of the cartilage
system. Based on Fig. 5.3, we can build our system of differential equations.

5.2.2 Model equations

The model equations corresponding to Fig. 5.3 are

\[ \partial_t F(r, t) = \kappa_F \partial_r (r F_r(r, t)) - \delta_F F(r, t) + \eta_F S(r, t) \]  
\[ \text{IL-6 diffusion natural decay production by } S \]  
\[ (5.1) \]

\[ \partial_t P(r, t) = \kappa_P \partial_r (r P_r(r, t)) - \delta_P P(r, t) + \eta_P C(r, t) \frac{\lambda_F}{\lambda_F + F(r, t)} \]  
\[ \text{EPO diffusion natural decay production by } C \text{ controlled by } F \]  
\[ (5.2) \]

\[ \partial_t C(r, t) = \alpha S(r, t) \frac{P(r, t)}{\lambda_P + P(r, t)} - \beta C(r, t) \frac{F(r, t)}{\lambda_F + F(r, t)} \]  
\[ \text{Heathy } S \rightarrow C \text{ production by } C \rightarrow S \]  
\[ (5.3) \]

\[ \partial_t S(r, t) = \beta C(r, t) \frac{F(r, t)}{\lambda_F + F(r, t)} - \alpha S(r, t) \frac{P(r, t)}{\lambda_P + P(r, t)} - \mu S(r, t) \frac{F(r, t)}{\lambda_F + F(r, t)} \]  
\[ \text{Sick } C \rightarrow S \text{ production by } S \rightarrow C \text{ and } S \rightarrow D_A \]  
\[ (5.4) \]

5.2.3 Equation discretization

In order to convert the differential equations, Eq. 5.1 and 5.2, to a linear model we discretized the PDEs both temporally and spatially using the first order difference method in time, and the radially symmetric finite difference method in space.
Eq. 5.1 can be approximated as:

\[
\begin{align*}
\frac{F(r_0,t+\Delta t) - F(r_0,t)}{\Delta t} &= \frac{2\kappa_F}{r_1^2 \delta x} F(r_0,t) - \frac{2\kappa_F}{r_1^2 \delta x} F(r_0,t) + \eta_F S(r_0,t) \\
\vdots \\
\frac{F(r_i,t+\Delta t) - F(r_i,t)}{\Delta t} &= \frac{2\kappa_F}{(r_{i+\frac{1}{2}}^2 - r_i^2 \frac{1}{2}) \delta x} F(r_i-1,t) - \frac{2\kappa_F}{(r_{i+\frac{1}{2}}^2 - r_i^2 \frac{1}{2}) \delta x} F(r_i,t) + \eta_F S(r_i,t) \\
\vdots \\
\frac{F(r_n,t+\Delta t) - F(r_n,t)}{\Delta t} &= \frac{2\kappa_F}{(r_n^2 - r_{n-1}^2 \frac{1}{2}) \delta x} F(r_{n-1},t) - \frac{2\kappa_F}{(r_n^2 - r_{n-1}^2 \frac{1}{2}) \delta x} F(r_n,t) + \eta_F S(r_n,t) 
\end{align*}
\]

(5.5)

From Eq. 5.5 we define:

\[
Y_F = [F(r_0,t) - F(r_0,t-1), F(r_1,t) - F(r_1,t-1), \ldots, F(r_n,t) - F(r_n,t-1)]'
\]

(5.6)

\[
X_{F1} = \begin{bmatrix}
\frac{2(F(r_1,t-1) - F(r_0,t-1))}{r_1^2 \delta x} \\
\vdots \\
\frac{2(F(r_{i+1},t-1) - F(r_i,t-1))}{(r_{i+\frac{1}{2}}^2 - r_i^2 \frac{1}{2}) \delta x} - \frac{2}{r_i^2 \delta x} F(r_i,t-1) \\
\vdots \\
\frac{2(F(r_{n-1},t-1) - F(r_n,t-1))}{(r_n^2 - r_{n-\frac{1}{2}}^2 \frac{1}{2}) \delta x}
\end{bmatrix}
\]

(5.7)

\[
X_{F2} = [F(r_0,t), F(r_1,t), \ldots, F(r_n,t)]'
\]

(5.8)

\[
X_{F3} = [S(r_0,t), S(r_1,t), \ldots, S(r_n,t)]'
\]

(5.9)

We obtain a linear equation for IL-6,

\[
Y_F = \kappa_F X_{F1} - \delta_F X_{F2} + \eta_F X_{F3}
\]

(5.10)
Applying the same discretization to Eq. 5.2, we obtain a linear equation for EPO,

\[ Y_P = \kappa_P \mathbf{X}_{P1} - \delta_P \mathbf{X}_{P2} + \eta_P \mathbf{X}_{P3} \quad (5.11) \]

where \( \mathbf{X}_{P1}, \mathbf{X}_{P2} \) are similar to \( \mathbf{X}_{F1}, \mathbf{X}_{F2} \), replacing \( F(r_i, t) \) with \( P(r_i, t) \). We have

\[ \mathbf{X}_{P3} = [C(r_0, t), C(r_1, t), \cdots , C(r_n, t)]' \quad (5.12) \]

### 5.2.4 Healthy cell and sick cell density

Due to the limitations of current experiments, we can only obtain total cell density \( T \) for each radius region at days 1, 7 and 14. The total cell density equals the healthy cell density plus the sick cell density. The density of \( C \) and \( S \), using Eq. 5.3 and Eq. 5.4, is given by

\[ C(r_i, t) + S(r_i, t) = T(r_i, t) \quad (5.13) \]

which is known from the experimental data for \( t = 1, 7, \) and 14 days. However, the values of parameters \( \lambda_F, \lambda_P, \alpha, \beta \) and \( \mu_s \) are taken from Chapter 3, and may not exactly match the experimental data.

### 5.2.5 Linear model and the results

Because of the discretization and measure error, we add a mean-zero, random error component \( \mathbf{\epsilon} = [\epsilon_1, \cdots , \epsilon_{n+1}]' \) to the right-hand side of Eq. 5.10 and Eq. 5.11. We obtain two linear equations,

\[ Y_F = \kappa_F \mathbf{X}_{F1} - \delta_F \mathbf{X}_{F2} + \eta_F \mathbf{X}_{F3} + \mathbf{\epsilon}_F \quad (5.13) \]

\[ Y_P = \kappa_P \mathbf{X}_{P1} - \delta_P \mathbf{X}_{P2} + \eta_P \mathbf{X}_{P3} + \mathbf{\epsilon}_P \quad (5.14) \]

where \( \{\epsilon_i\}, i = 1, 2, \cdots , n + 1 \) are iid.
Using OLS we construct estimates of parameters, which can be found in Table 5.1. The estimates show that all of the parameters differ significantly from zero. In order to check the normality assumption, we conduct cross-validation using Monte Carlo bootstrapping. We randomly select 10 observations from the original data set (constructed by sample group 1-5), and replace these 10 observations by the observations from a validation set (the 6th sample group), the we repeat the process of parameter estimation 1000 times. The bootstrap results are shown in Fig. 5.4a and 5.4b. The results shows that the normality assumption of the random error is reasonable. We present the empirical distribution of the parameters in Table 5.2. From the table, the mean of diffusion rate for IL-6 is around 4.2e-04 per region per week, which is bigger than the diffusion rate of EPO, which is about 3.2e-04 per region per week. The mean of delay rate for IL-6 is around 0.38 per region per week, which is smaller than the delay rate of EPO, which is about 0.56 per region per week. The mean of production rate of IL-6 is around 0.0032 per region per week, which is smaller than the production rate of EPO which is about 0.27 per region per week. The standard deviations of all parameters are small compared to the means, so we have relatively narrow 95% confidence intervals.

5.3 Conclusion

We present a statistical model to predict the formation of an articular cartilage lesion after a single blunt impact. We use a simplified model that is radially symmetric to obtain the empirical distribution of our model parameters. The distribution of
Table 5.1. Parameter estimations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\hat{\kappa}$</th>
<th>$\hat{\delta}$</th>
<th>$\hat{\eta}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 $F$</td>
<td>0.0003100</td>
<td>0.5146000</td>
<td>0.0041190</td>
</tr>
<tr>
<td>EPO $P$</td>
<td>0.0002883</td>
<td>0.5850904</td>
<td>0.0280677</td>
</tr>
</tbody>
</table>

Table 5.2. Empirical distribution of the parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mean</th>
<th>sd</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\hat{\kappa}_F$</td>
<td>4.2260e-04</td>
<td>5.4195e-05</td>
<td>(0.0003164, 0.0005288)</td>
</tr>
<tr>
<td>IL-6 $\hat{\delta}_F$</td>
<td>3.8264e-01</td>
<td>4.4585e-02</td>
<td>(0.2953000, 0.4700300)</td>
</tr>
<tr>
<td>$\hat{\eta}_F$</td>
<td>3.1830e-03</td>
<td>3.0000e-04</td>
<td>(0.0025950, 0.0037710)</td>
</tr>
<tr>
<td>$\hat{\kappa}_P$</td>
<td>3.2200e-04</td>
<td>2.9909e-05</td>
<td>(0.0002634, 0.0003810)</td>
</tr>
<tr>
<td>EPO $\hat{\delta}_P$</td>
<td>5.6017e-01</td>
<td>1.4935e-02</td>
<td>(0.5309000, 0.5894400)</td>
</tr>
<tr>
<td>$\hat{\eta}_P$</td>
<td>2.7091e-01</td>
<td>5.4200e-04</td>
<td>(0.0260290, 0.0281530)</td>
</tr>
</tbody>
</table>

These parameters help us investigate the diffusion, decay and production rate of IL-6 and EPO after the mechanical trauma, and provides insight into the distribution of cytokine concentrations. Although our results are based on two weeks of data, the model opens up the possibility of predicting cytokine concentrations over longer time.
Figure 5.4. The test of normality assumption (a) The histograms of bootstraps (b) The qqplots of bootstraps
periods, which may not be easily obtained through *in vivo* or *in vitro* studies.

Due to the ethics of conducting experiments on humans, experimental cartilage research is mostly based on the use of animal experimentation. In this paper we base our model on bovine cartilage because human and bovine cartilage share common structural and compositional features [42]. Further study is needed to establish the relationship between human and bovine IL-6 and EPO levels following mechanical injuries.

Since the measurements in the experiments are taken at only three time points (1, 7, and 14 days), we are restricted in our choice of parameter estimation technique. If we are able to obtain more time points in the future, we can build a spatio-temporal model by converting the partial differential equation system into a VAR(1) model. More advanced methods such as EHM (empirical hierarchical modeling) or BHM (Bayesian hierarchical modeling) can be applied for parametrization.

Current experiments only measure the total cell density, and are unable to distinguish different cells in different states within our cartilage samples. More advanced experiments are needed to directly measure the healthy and sick cell densities. Due to the cost of experiments, the concentration level of IL-6 and EPO are measured by counting the positive cell numbers, which can only qualitatively represent chemical concentrations. More accurate measurements would lead to better results.
In this thesis we have built mathematical representations of articular cartilage degeneration using reaction-diffusion-delay equations, age- and space-structured equations, and spatio-temporal equations. We qualitatively captured the features of chondrocyte behavior and the balancing act between pro- and anti-inflammatory cytokines following traumatic injury. This work consisted of minimal mathematical models that provide the best means of capturing the biological response to mechanical loads, and introduces population dynamics to cartilage modeling. Experimental and numerical results shed new light on the pathogenesis of osteoarthritis.

We extended the delay differential equation model to a scenario of cartilage lesion formation under excessive cyclic loading. Our new model relates the bio-mechanical properties of cartilage deformation under mechanical compression to cellular and chemical reactions, and proves to be useful for predicting the long-term consequences of mechanical loading under various stress levels. From a clinical point of view, a beneficial feature of our work is that our choices of parameter values are based, in most cases, on prior experimental results that can be found in the literature.

Due to the computational challenges of delay differential equation models, we also used age- and space-structured models to simulate cartilage lesion formation under the effect of the single blunt impact as well as cyclic loading. We adapted existing computational methods for age- and space-structured differential equations to develop a solver for our model that is numerically efficient. Our simulation results
confirm our assumption that the behavior of cartilage degeneration is influenced by pro- and anti-inflammatory activities of cytokines. The expression of IL-6 and EPO in immunohistochemical experiments suggests that targeted cytokines may play a significant role in the system.

Based on our mathematical equations and experimental data we also presented a stochastic spatio-temporal model. Our model further validates the significant correlation between cartilage degeneration and cytokine activity. We are able to construct empirical distributions for clinically relevant model parameters, which in principle could lead to quantitative model predictions, although we do not undertake that task here.

In summary, in this thesis we present a mathematical approach to understanding the inflammation response relevant to cartilage lesion formation. Further research is needed to capture the spatial heterogeneity of cartilage structure. In addition, the elastic properties of cartilage suggest that finite element models may be required to fully capture the essential properties of cartilage deformation under stress. Detailed experimental work is also needed to improve parameterization and reconcile the disparity between our predictive model and biological reality.
REFERENCES


