Biochemical and Biomechanic Integrated Modeling of Bone

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Dedicated to my Family.
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Resumo

O tecido ósseo está constantemente em processo de renovação. Este processo engloba a reabsorção, por parte dos osteoclastos, seguida de formação de novo osso pelos osteoblastos. O desenvolvimento de modelos matemáticos e computacionais, capazes de replicar os processos bioquímicos e biomecânicos envolvidos na remodelação óssea são extremamente úteis para comparar os comportamentos do osso em estados saudáveis e patológicos, servindo como sistemas de suporte para a tomada de decisões clínicas no que respeita à implementação de terapias e avaliações prognósticas. Modelos ósseos desenvolvidos anteriormente consistem num sistema de equações diferenciais ordinárias que relacionam as interações entre os osteoclastos e os osteoblastos, permitindo determinar não só a dinâmica da população de células, mas também as alterações na massa óssea em locais específicos de remodelação óssea afetados pela doença do mieloma. Este trabalho integra esses modelos com um modelo de remodelação biomecânico, de forma a poder avaliar a resposta do osso a estímulos mecânicos. Assim, um código Matlab foi implementado com o objetivo de reproduzir os processos bioquímicos envolvidos na remodelação óssea, bem como a correspondente resposta biomecânica do osso, sendo o estímulo caracterizado pela densidade de energia de deformação obtida através do código de elementos finitos ABAQUS. Os resultados para um modelo bidimensional simplificado do fémur mostraram a influência da carga mecânica na concentração de osteoclastos e osteoblastos e, consequentemente, na distribuição de densidades. O modelo provou ser uma boa representação do ambiente ósseo, uma vez que os resultados se encontram em concordância com a situação biológica. A expansão do modelo para incluir os efeitos do mieloma também levou à obtenção de resultados que estão de acordo com o esperado, com um aumento do número de osteoclastos, acompanhado por uma acentuada diminuição no número de osteoblastos e uma consequente diminuição da massa óssea, característica desta patologia.

Palavras-chave: Remodelação Óssea, Mecânica Computacional, Biomecânica, Mieloma
Abstract

Bone tissue is in a constant state of turnover. The remodeling process encompasses resorption, by osteoclasts, followed by formation of new bone by osteoblasts. Mathematical and computational models that replicate the biomechanical and biochemical processes involved in bone remodeling are very useful to compare the behavior of healthy and pathological states, serving as clinical decision support systems for the implementation of therapeutic regimes and prognostic assessments. Previous bone models consist on a system of ordinary differential equations relating the interactions between osteoclasts and osteoblasts, which allow the calculation of the dynamics of cell population, as well as changes in bone mass at discrete sites of bone remodeling in myeloma bone disease. This work integrates these models with a biomechanical remodeling model in order to evaluate the bone’s response to mechanical stimuli. A Matlab code was implemented, reproducing the biochemical processes involved in bone remodeling, as well as the corresponding bone mechanical environment, which is characterized by the strain energy density that was obtained using the finite element code ABAQUS. The results for a simplified two-dimensional femur bone model showed the influence of the load in the concentration of osteoclasts and osteoblasts and, consequently, in density distribution. The model proved to be a good representation of the bone environment, as the results were in agreement with the biological situation. The expansion of the model to include the effects of myeloma bone disease also lead to results according to expected, with an increase in the number of osteoclasts, followed by a decrease in the number of osteoblasts and a consequent decrease of bone mass, characteristic of this disease.

Keywords: Bone Remodeling, Computational Mechanics, Biomechanics, Myeloma Bone Disease
Contents

Acknowledgments ................................................. v
Resumo .............................................................. vii
Abstract ........................................................... ix
List of Tables ...................................................... xiii
List of Figures ..................................................... xvii
Nomenclature ...................................................... xx

1 Introduction .................................................... 1
   1.1 Motivation .................................................. 1
   1.2 Thesis outline .............................................. 2

2 Biomedical Concepts ........................................ 3
   2.1 Anatomical concepts ....................................... 3
      2.1.1 Structure of long bones .............................. 4
      2.1.2 Histology of bone ................................... 5
   2.2 Bone remodeling process ................................. 5
   2.3 Mechanical Stimulus ...................................... 8
   2.4 Myeloma bone disease ................................... 10

3 State of the art ............................................... 13

4 Model Development ........................................... 19
   4.1 Mathematical Model ....................................... 19
      4.1.1 Biochemical Model .................................... 19
      4.1.2 Biomechanochemical Model ......................... 21
      4.1.3 Cancer Cells ......................................... 22
   4.2 Computational Model ..................................... 23

5 Results ......................................................... 25
   5.1 Biochemical Model ........................................ 25
   5.2 Biomechanochemical Model ............................... 28
   5.3 Cancer Cells ............................................... 39
List of Tables

5.1 Initial values and parameters of the model. .................................................. 26
A.1 Tests for $k_C$ and $k_C$ parameters. .......................................................... 57
A.2 Initial values and parameters of the biomechanichemical model. .................. 58
A.3 Initial values and parameters of the model with myeloma bone disease. ....... 58
List of Figures

2.1 Structure of a long bone. ................................................. 4
2.2 Histology of a long bone. .............................................. 6
2.3 Schematic representation of osteoclast and osteoblast autocrine and paracrine interactions. 8
2.4 Schematic representation of the influence of the mechanical stimulus on the process of bone remodeling ................................................................. 9
2.5 Schematic representation of the effects of myeloma on the bone remodeling process. ... 11
4.1 Two-dimensional finite element femur model ......................... 23
4.2 Two-dimensional femur model and side plate. ......................... 24
5.1 Comparison between the bone mass evolution obtained with Komarova’s bone mass equation and the equation proposed for this model. ......................... 26
5.2 Results obtained with the original model proposed by Komarova et al. [1]. ............ 27
5.3 Results obtained with the new equation of bone mass 4.5. .............. 27
5.4 Bone mass distribution in a biological bone. .......................... 29
5.5 Tests of parameters for equation 4.7: $\xi_{BC} = -0.1, \xi_{BB} = 0$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 30
5.6 Tests of parameters for equation 4.7: $\xi_{BC} = -0.2, \xi_{BB} = 0$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 30
5.7 Tests of parameters for equation 4.7: $\xi_{BC} = 0, \xi_{BB} = 0.1$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 31
5.8 Tests of parameters for equation 4.7: $\xi_{BC} = 0, \xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 31
5.9 Tests of parameters for equation 4.7: $\xi_{BC} = 0, \xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 33
5.10 Tests of parameters for equation 4.7: $\xi_{BC} = -0.1, \xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 33
5.11 Tests of parameters for equation 4.7: $\xi_{BC} = -0.1, \xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 34
5.12 Tests of parameters for equation 4.8: $O_{C} = -0.01, O_{B} = 0$. Each line corresponds to one of the 1292 nodes of the finite element model. ................................................. 34
5.13 Tests of parameters for equation 4.8: \(OC = 0, OB = 0.1\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 36
5.14 Tests of parameters for equation 4.8: \(OC = 0, OB = 1\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 36
5.15 Tests of parameters for equation 4.8: \(OC = 0, OB = 2\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 37
5.16 Tests of parameters for equation 4.8: \(OC = 0, OB = 5\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 37
5.17 Tests of parameters for equation 4.8: \(OC = -0.01, OB = 1\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 38
5.18 Tests of parameters for equation 4.8: \(OC = -0.01, OB = 2\). Each line corresponds to one of the 1292 nodes of the finite element model. .................................................. 38
5.19 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 2, \xi_{BC} = -0.1, \xi_{BB} = 0.3\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days. 40
5.20 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 2, \xi_{BC} = -0.1, \xi_{BB} = 0.2\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days. 40
5.21 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 1, \xi_{BC} = -0.1, \xi_{BB} = 0.3\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days. 41
5.22 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 2, \xi_{BC} = -0.05, \xi_{BB} = 0.3\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days. .................................................. 41
5.23 Location of the two tumor cases on the femur model. .................................................. 42
5.24 Evolution of the tumor. .................................................. 42
5.25 Results for equation 4.11, without the mechanical stimulus: \(OC = 0, OB = 0, \xi_{BC} = 0, \xi_{BB} = 0, r_{CC} = 0.005\). .................................................. 44
5.26 Reference results for tumor case 1. .................................................. 44
5.27 Reference results for tumor case 2. .................................................. 45
5.28 Reference results for tumor case 1 and 2. .................................................. 45
5.29 Results for equations 4.11 with tumor case 1 and \(r_{CC} = 0.005\). .................................................. 46
5.30 Results for equations 4.11 with tumor case 2 and \(r_{CC} = 0.005\). .................................................. 46
5.31 Results for equations 4.11 with tumor case 1 and 2 and \(r_{CC} = 0.005\). .................................................. 47
5.32 Results for equations 4.11 with tumor case 1 and \(r_{CC} = 0.2\). .................................................. 47
5.33 Results for equations 4.11 with tumor case 2 and \(r_{CC} = 0.2\). .................................................. 49
5.34 Results for equations 4.11 with tumor case 1 and 2 and \(r_{CC} = 0.2\). .................................................. 49

B.1 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 2, \xi_{BC} = -0.1, \xi_{BB} = 0.3\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days. 59
B.2 Tests of parameters for equation 4.9: \(OC = -0.01, OB = 2, \xi_{BC} = -0.1, \xi_{BB} = 0.2\). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days. 60
B.3 Tests of parameters for equation 4.9: \( O_C = -0.01, O_B = 1, \xi_{BC} = -0.1, \xi_{BB} = 0.3 \). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.

B.4 Tests of parameters for equation 4.9: \( O_C = -0.01, O_B = 2, \xi_{BC} = -0.05, \xi_{BB} = 0.3 \).
Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.

B.5 Results for equations 4.11 with tumor case 1 and \( r_{CC} = 0.005 \). Results for 350 days.

B.6 Results for equations 4.11 with tumor case 1 and \( r_{CC} = 0.02 \).

B.7 Results for equations 4.11 with tumor case 2 and \( r_{CC} = 0.02 \).

B.8 Results for equations 4.11 with tumor case 1 and 2 and \( r_{CC} = 0.02 \).

B.9 Results for equations 4.11 with tumor case 1 and \( r_{CC} = 0 \).

B.10 Results for equations 4.11 with tumor case 2 and \( r_{CC} = 0 \).

B.11 Results for equations 4.11 with tumor case 1 and 2 and \( r_{CC} = 0 \).
Nomenclature

Greek symbols

$\alpha_B$ Activity of osteoblast production.
$\alpha_C$ Activity of osteoclast production.
$\beta_B$ Activity of osteoblast removal.
$\beta_C$ Activity of osteoclast removal.
$\gamma_T$ Tumor growth constant.
$\Psi$ Threshold for the strain energy density.
$\Psi$ Strain energy density.
$\xi_{BB}$ Weight of the mechanical stimulus to the $g_B B$ parameter.
$\xi_{BC}$ Weight of the mechanical stimulus to the $g_B C$ parameter.
$\xi_{CB}$ Weight of the mechanical stimulus to the $g_C B$ parameter.
$\xi_{CC}$ Weight of the mechanical stimulus to the $g_C C$ parameter.

Roman symbols

$\overline{B}$ Number of osteoblasts at steady-state.
$\overline{C}$ Number of osteoclasts at steady-state.
$B$ Number of osteoblasts.
$C$ Number of osteoclasts.
$L_T$ Maximum tumor size.
$O_B$ Influence of the osteocytes response to mechanical stimulus on osteoblasts.
$O_C$ Influence of the osteocytes response to mechanical stimulus on osteoclasts.
$T$ Density of tumor cells.
$g_{BB}$ Osteoblast-derived autocrine factor.
\( g_{BC} \) Osteoblast-derived paracrine factor.

\( g_{CB} \) Osteoclast-derived paracrine factor.

\( g_{CC} \) Osteoclast-derived autocrine factor.

\( k_B \) Normalized activity of bone formation.

\( k_C \) Normalized activity of bone resorption.

\( t \) Time instant.

\( z \) Total bone mass.
Chapter 1

Introduction

1.1 Motivation

Bone remodeling is a tightly regulated process, in which bone resorption is always followed by bone formation in a site-specific manner, and plays an important role in maintaining the integrity of the skeleton and repairing micro-fractures. Several diseases, such as osteoporosis, Paget's disease and myeloma, influence the remodeling cycle, disturbing its equilibrium. The development of models that integrate the knowledge from bone physiology and that can replicate the biomechanical and biochemical processes involved in bone remodeling is of extreme importance, since these allow the comparison between healthy and pathological states, serving as clinical decision support systems for the implementation of therapeutic regimes and prognostic assessments.

There are several studies of bone remodeling models that replicate the biochemical interactions between bone cells, and several factors, for instance, the parathyroid hormone, were considered as triggers [1, 2, 3]. Some of these models were adapted to include the influence of bone metastases or myeloma bone disease in the remodeling cycle [4, 5]. However, they do not include the influence of the mechanical stimulus in the process. On the other hand, biomechanochemical models that consider the mechanical stimulus as a trigger of the process of bone remodeling have the disadvantage of considering a large number of variables, making it difficult to separately test the influence of the parameters [6]. Furthermore, these models seldom include the influence of diseases that affect bone remodeling.

The objective of this work is to develop a model with a simpler description of the biochemical interactions between bone cells in bone remodeling, in which the process is triggered by an external mechanical stimulus. This model is further extended to include the myeloma bone disease. One major advantage of this new model is that it has fewer state variables than the current models that consider the influence of the mechanical stimulus. This new approach makes it easier to test the influence of each variable, isolated, which is important for the development of more efficient treatments.
1.2 Thesis outline


Chapter 2 includes a description of all the phenomenons and concepts that are important for understanding the work. Firstly, the anatomical concepts of the skeletal system are given, and the macro and micro structure of long bones is explained. After, the bone remodeling process is explained, with a special insight on the biochemical factors that are responsible for triggering and controlling the process. The influence of the appliance of an external mechanical stimulus on the process of bone remodeling and on the effectiveness of the biochemical factors is also explained here. Finally, the myeloma bone disease is described and its influence in bone remodeling is explained.

Chapter 3 is the state of the art, in which several groups that developed mathematical and/or computational models of bone and bone remodeling are mentioned, and their work shortly explained.

Chapter 4 has the description of the model presented for this work. It starts with a detailed description and explanation of the biochemical model used as a starting point for this work. Few alterations to this model were made, which are also presented here. Then, it is explained how the influence of the mechanical stimulus was introduced in the previous equations and which assumptions had to be made. To finish, the equations were expanded to include the presence and influence of the myeloma bone disease.

Chapter 5 follows the same organization of chapter 4. The results for the biochemical model are presented, followed the results obtained after few alterations were made. After, the results for the biomechananochemical model are showed, with several parameters tested, and the influence of the mechanical stimulus, as well as the influence of the parameters is discussed. Finally, the results for the myeloma bone disease are presented, with and without the presence of the mechanical stimulus, in order to evaluate the influence of this factor on the cancer cells.

Chapter 6 has the conclusions drawn from this work, and says how the objectives for this work were fulfilled. In addition, it has some comments and ideas of future work that can be made, in order to improve the current model, as well as to expand it to include other bones, disease and treatments.
Chapter 2

Biomedical Concepts

2.1 Anatomical concepts

The skeletal system is the framework of the body and it is comprised by bones and their associated connective tissue, including cartilage, tendons and ligaments. The major functions of the skeletal system include:

1. **Support.** The rigid, strong bones play an important role in maintaining the shape of the body and supporting its weight. Their cartilaginous ends form joints, which are fixed by ligaments that serve as lever arms around joints holding the bones together. Cartilage also provides a flexible, yet firm, support to certain structures as the nose, external ear, thoracic cage and trachea.

2. **Protection.** Bones protect several vital organs, for example: the skull encloses and protects the brain, the vertebrae surround the spinal cord and the rib cage protects the heart, lungs and other organs of the thorax.

3. **Movement.** Tendons are strong bands of connective tissue that attach the skeletal muscles to bones. The jointed bones are moved and at the same time stabilized by the contraction of skeletal muscles via the tendons. The smooth cartilage that covers the ends of bones, within some joints, allows the bones to move freely with minimum friction. The ligaments are important to prevent excessive movement.

4. **Storage.** Bones are important mineral reserves in the calcium and phosphorous metabolisms of the organism. If the blood levels of these minerals decrease, they are released from the bone into the blood.

5. **Blood cell production.** Certain bones are a significant part of the hemopoetic system, as they contain cavities filled with red bone marrow, which is responsible for the production of blood cells and platelets.

In summary, bones play protective, mechanical and metabolic roles in the body [7, 8]. According to their function, bones present different shapes, that can be grouped in four categories: long bones, which
are most of the bones of the upper and lower limbs (for example, the femur bone); short bones, like the bones from the wrist and ankle; flat bones, like the ribs and certain skull bones and irregular bones, like the vertebrae and facial bones, which have shapes that do not fit in the other categories [7]. For the purpose of this work, only the structure and histology of long bones will be detailed.

2.1.1 Structure of long bones

Long bones consist of two ends, each called epiphysis, separated by a central shaft called diaphysis (Figure 2.1). When the long bone is still growing, it contains a structure between the diaphysis and each epiphysis called epiphyseal plate (or growth plate), which is composed of cartilage, allowing the bone to grow in length. When the bone stops growing, the cartilage is replaced by bone and becomes an epiphyseal line. At the end of the epiphysis, where the bone articulates with other bones, there is a thin layer of articular cartilage, which reduces the movement friction and plays an important role in absorbing the energy released in the joint movements.

Within the diaphysis, bone contains a large cavity, called medullary cavity, filled with a soft tissue called bone marrow (Figure 2.1). There are essentially two types of marrow: yellow marrow, which consists mostly of adipose tissue, and red marrow, which consists of blood-forming cells. As the person ages, the amount of red marrow in bones decreases, being replaced by yellow marrow. In fact, in adults, red marrow is confined to the bones in the central axis of the body and in the most proximal epiphysis of the limbs, and is the only site of blood formation. Endosteum, a thin connective tissue membrane lines the surface of the medullary cavity. The outer surface of the bone is covered by the periosteum: a dense connective tissue that contains blood vessels and nerves (Figure 2.1) [7].

![Figure 2.1: Structure of a long bone.](image-url)
2.1.2 Histology of bone

Osteoblasts are bone forming cells, present in both the periosteum and the endosteum, which are important for the processes of bone formation, repair and remodeling. When osteoblasts become surrounded by matrix, they are referred to as osteocytes. Bone consists of thin sheets of extracellular matrix called lamellae, between which there are spaces, called lacunae, that contain osteocytes (Figure 2.2). Cell processes, started in osteocytes, extend across the extracellular matrix of the lamellae, through small channels called canaliculi.

Based on their histological structure, there are essentially two types of bone: compact bone, which consists mostly of solid matrix and cells, and spongy bone (or cancellous bone), which is a porous network of bone with many small marrow-filled spaces.

Most of the diaphysis of long bones are formed by compact bone (Figure 2.1). The lamellae of compact bone are organized forming concentric rings, which surround a central canal called haversian canal that contain the blood vessels that run parallel to the long axis of the bone. The haversian canal, together with the lamellae and osteocytes surrounding it, forms the haversian system, also called osteon. The blood vessels in the haversian canal are connected to blood vessels in the periosteum and endosteum. The nutrients that leave the blood vessels of the haversian canal diffuse to the osteocytes through the canaliculi, while waste products diffuse in the opposite direction (Figure 2.2).

The epiphyses, on the other side, consist mostly of spongy (or trabecular) bone (Figure 2.1). Trabecular bone is formed by interconnecting plates or trusses of bone called trabeculae, which add strength to the bone without adding weight (which would happen if it was a solid mineralized matrix). Each trabecula consists of several lamellae with osteocytes between them, and the spaces between trabeculae are filled with marrow. As there are no blood vessels penetrating the trabeculae, the nutrients exit the vessels in the bone marrow and pass by diffusion through canaliculi to the osteocytes of the trabeculae [7].

2.2 Bone remodeling process

As a dynamic tissue, bone undergoes continual adaptation throughout life, changing its internal structure by removing the old bone and replacing it with newly formed bone in a process called remodeling, which occurs in spatially and temporal discrete sites [9, 2, 3]. While bone modeling is a process related to bone growth, in which bone is selectively added or removed from the surfaces altering the size and shape of bones, bone remodeling plays an important role in maintaining the integrity of the skeleton, repairing micro-fractures that may lead to macroscale fatigue fractures under repeated cyclic loading [10, 11]. It also influences the mineral homeostasis, by providing access to stores of calcium and phosphate [10]. The major difference between bone modeling and bone remodeling is that while the first involves bone resorption (by the activation of osteoclasts) or bone formation (by the activation of osteoblasts), but not both at the same location, the remodeling is a tightly regulated process in which bone resorption is always followed by bone formation in a site-specific manner [11, 12].

The process of bone remodeling is usually triggered by an external stimulus, which can take several
forms, for example, direct mechanical strain that results in structural damage, or hormone action on bone cells in response to systemic changes in homeostasis. Osteoblasts and osteocytes sense these signals and recruit osteoclasts that start removing trenches of bone from the surface of trabecular bone, or forming tunnels into cortical bone. The trenches and tunnels are then filled by osteoblasts, which lay down successive layers of new (unmineralized) bone matrix, called osteoid, which becomes mineralized by the influence of vitamin D and other factors. These clusters of bone-resorbing osteoclasts and bone-forming osteoblasts are arranged within temporary anatomical structures known as basic multicellular units (BMUs). The BMU can be seen as a mediator mechanism, as it bridges individual cellular activity to whole bone morphology [9, 11, 12].

The remodeling is then achieved by the balanced activities of bone’s constituent cell types: bone-forming osteoblasts, which produce the organic matrix and aids its mineralisation, also playing a role in sensing the stimulus that trigger the remodeling; bone-degrading osteoclasts, which dissolve bone mineral and degrades extracellular matrix proteins; osteocytes, known for being the major mechanosensors and bone lining cells, which play a role in coupling bone resorption to bone formation [13]. The activities of bone cells, more specifically the activities of bone resorption and formation are said to be coupled to one another, ensuring that where bone is removed, new bone will be restored. This relationship is maintained by receptor-ligand interactions between osteoclasts and osteoblasts that activate bidirectional signaling, which means that the interactions between the ligand and the receptor induce signaling in both the receptor-expressing and the ligand-expressing cells. The switch from bone resorption to bone
formation is controlled by the result of this bidirectional signaling [2, 11, 13].

The coupling between bone resorption and bone formation involves the regulation of a number of soluble factors produced by both osteoclasts and osteoblasts that can be either paracrine (produced by one cell type to influence the other cell type) or autocrine (produced by one cell type to influence its own cell type), as illustrated in Figure 2.3 [13, 1]. The number of factors involved in this process is enormous and so, for the purpose of this work, only the most relevant ones will be mentioned and considered.

**Osteoblast paracrine factors (ObPF)**

Osteoblasts paracrine factors are factors produced by osteoblasts that influence osteoclasts, and include factors like the receptor activator of nuclear factor \(\kappa\)B ligand (RANKL) and osteoprotegerin (OPG), which bind to the receptor activator of nuclear factor \(\kappa\)B (RANK), present in the surface of osteoclast precursor cells [1]. When RANKL binds to RANK it stimulates the differentiation and activation of osteoclasts. OPG, on the other side, is a soluble decoy receptor that binds to RANKL preventing its binding with RANK, leading to a negative regulation of RANKL activity. The RANKL/OPG ratio determines the degree of osteoclast differentiation and function [9, 2, 14].

**Osteoblast autocrine factors (ObAF)**

Examples of factors produced by osteoblasts that have an influence in osteoblasts are the insulin-like growth factor (IGF), responsible for recruiting osteoblasts to sites of bone resorption and \(W_{nt}\), which is indispensable for osteoblast differentiation and activation. \(W_{nt}\) co-receptors activate \(\beta\)-catenin, leading to an upregulation of transcription factors that are crucial for osteoblast differentiation. In differentiated osteoblasts, \(W_{nt}\)-signaling also plays an important role in stimulating OPG and inhibiting RANKL, thereby negatively regulating osteoclast formation [9, 13, 1, 15].

**Osteoclast paracrine factors (OcPF) and osteoclast autocrine factors (OcAF)**

The transforming growth factor (TGF)-\(\beta\) is the predominant factor produced by osteoclasts and is an example of both osteoclast paracrine and autocrine regulation. Bone matrix is the largest source of TGF-\(\beta\) in the body. It is released by osteoclasts during the process of bone resorption, and is capable of stimulating the recruitment of osteoblasts and the migration and proliferation of osteoblast precursors. At the same time, TGF-\(\beta\) induces osteoclast apoptosis [2, 1, 16].

Several diseases, like osteoporosis, Paget's disease and some cancer-related bone diseases affect the rate of bone remodeling and the number of remodeling sites in the skeleton, and can be responsible for a disruption of a biochemical or cellular link of this finely organized network [2]. The net amount of old bone removed and new bone formed in one remodeling cycle is a quantity called bone balance. While coupling rarely is affected, bone balance can vary quite widely in many disease states [11]. An accurate and detailed understanding of the process of bone remodeling is of extreme importance not only to identify and understand pathological states, but also for treatment assessment.
2.3 Mechanical Stimulus

Bone tissue is able to adapt to changes in its mechanical environment: normal and excessive physical activities generate mechanical forces through muscles, which influence bone remodeling and bone architecture in general, optimizing bone's morphology to withstand its mechanical demand. Bone is able to respond not only to an overuse, stimulating bone remodeling with bone formation dominating bone resorption in order to increase bone mass (as seen, for example, in the serving arms of professional tennis players) but also to a disuse or lack of loading, which causes an acceleration of bone turnover, with bone resorption dominating bone formation, leading to loss of bone mass (as observed in astronauts experiencing zero gravity and in immobilized patients) [2, 11, 12, 13]. Robling et al. [11] states that the effects of loading in bone follow a U-shaped curve since both disuse (insufficient loading) and overuse (overloading) stimulate bone remodeling, but there is a range of loading, called physiological range, within which bone remodeling is minimized. All the cells responsible for bone remodeling (osteoclasts, osteoblasts, osteocytes and bone lining cells) have the potential for sensing mechanical strains and translate this forces into biochemical signals, although their sensitiveness and the effects of their response have distinct intensities.

Bone is fundamentally a hydrated tissue and, when a mechanical force is applied to its surface, extracellular fluid flows are induced, being the primary loading-induced fluid motion through the lacunar-canalicular network, where osteocytes inhabit [11, 17, 18]. For this reason, and due to their three-dimensional distribution throughout both trabecular and cortical bone and extensive interconnectivity, osteocytes are considered to be the major cell type responsible for sensing mechanical strain and respond with signals of resorption and formation. In addition, osteocytes are able to relate the intensity of the strain signals, as well as its distribution throughout the bone, into signals to regulate the process of remodeling [19, 20]. When subjected to fluid shear stress, osteocytes expression of OPG increases, decreasing the RANKL/OPG ratio and, consequently, down-regulating osteoclastogenesis. Furthermore,
mechanically challenged osteocytes release nitric oxide (NO), which is a strong inhibitor of bone resorption that suppresses the expression of RANKL and increases the expression of OPG, and TGF-β, also contributing to the decrease in osteoclastogenesis. Osteocytes also play a role in stimulating osteoblasts formation and activation by inducing β-catenin signaling, which leads to the expression of Wnt targets [11, 13, 17, 18, 19, 20]. However, osteocytes also respond to unloading: osteocytes that are not exposed to the appropriate physical signals shift the balance of secreted factors to favor resorption [18].

Osteoblasts are the second major type of cell to sense and respond to mechanical stimulus, though its response is very similar to the one from osteocytes. When exposed to strain, osteoblasts reduce the expression of RANKL, decreasing osteoclast number. Like osteocytes, osteoblasts also release NO, responsible for decreasing the RANKL/OPG ratio, downregulating osteoclast formation and activation. In addition, osteoblasts subjected to mechanical strains increase the production of Wnt, upregulating its own differentiation and activation [11, 17, 18, 20, 21].

Summarizing, when mechanically stimulated, osteocytes and osteoblasts respond sending messengers that downregulate osteoclasts action (bone resorption) and upregulate osteoblasts action (bone formation). Mechanical stimulus also affects osteoclasts but, although these effects are not yet well understood, they seem to be indirect, adding another layer of control by which mechanical force might limit bone resorption [11, 20]. Also, mechanical loading reduces the rates of programmed cell death (apoptosis) in osteocytes and active osteoblasts [11].

Figure 2.4: Schematic representation of the influence of mechanical stimulus on the process of bone remodeling. The blue arrows represent effect of the mechanical stimulus: the activity of osteoclasts is downregulated, while the activity of osteoblasts is upregulated.
Multiple myeloma (MM) is a hematological malignancy, associated with clonal expansion of malignant plasma cells within the bone marrow, being the most frequent cancer to involve bone [12, 4, 22]. Bone marrow infiltration by plasma cells induces osteolytic lesions (or osteoclastic lesions) in 80-90% of patients, which appear in x-rays like holes in the bone, and lead to severe and debilitating bone pain, pathological fractures, hypercalcemia (high levels of calcium in blood) and spinal cord compression, being the major source of morbidity and mortality in patients with MM [12, 4, 22, 23, 24]. Further, bone lesions in patients with myeloma rarely heal, even when the patients are in prolonged complete remission [12].

The major difference between myeloma bone disease and bone diseases caused by other types of tumors is that, while both myeloma and other osteolytic metastases induce increased osteoclastic bone resorption, osteoblast activity in myeloma is severely decreased or absent, uncoupling the process of bone remodeling in areas adjacent to myeloma cells: there are large numbers of osteoclasts with no reactive new bone formation and no osteoblastic response (Figure 2.5) [12, 4, 23, 24, 25, 26]. In addition, interactions between myeloma cells and cells of the bone marrow microenvironment induce myeloma growth and survival (Figure 2.5), and stimulate the development of osteolytic lesions [4, 27]. Despite any bone can be affected by MM, it is most common in sites of red marrow, such as vertebral bodies and ribs [12].

In MM, osteoclast activity is increased due to the release of osteoclastogenic factors, produced by MM cells, in the bone marrow microenvironment, which act decreasing the production of OPG and stimulating the production of osteoclastogenic cytokines, such as RANKL. This phenomena lead to an increase in the RANKL/OPG ratio, which was discribed above (section 2.2) as being the major factor to control osteoclast differentiation and activation [12, 25, 27, 28, 29, 30]. Besides RANKL, which may further contribute to bone destructive process, MM cells are responsible for promoting two other factors that seem to have a very important role in promoting osteoclastogenesis: macrophage inflammatory protein-1α (MIP-1α), which is a chemokine produced by MM cell to stimulates the production of RANKL [12, 30] and IL-3, which enhances the effects of RANKL and MIP-1α [12, 31]. As the increase of osteoclast activity is verified only in a region of bone adjacent to MM cells, the bone destruction in MM is considered to be a local event [12, 24]. In addition, IL-3 and other growth factors [30] released during the bone destructive process are able to stimulate MM cell growth, creating what Abe et al. [32] describes as a "vicious cycle", in which the growth factor released during the bone resorptive process increase MM tumor burden, which in turn increases bone destruction [12, 28, 32].

Despite there is significantly less information related to the influence of MM cells on osteoblasts, it is known that myeloma cells decrease the activity of Wnt by producing a Wnt antagonist, which downregulates osteoblast differentiation and activation [33]. Also, as Wnt signaling has the potential to upregulate OPG expression and downregulate RANKL expression, it indirectly increases osteoclastogenesis [12]. Other studies [12, 30] also suggest that the production of TGF-β, an inhibitor of osteoblast differentiation, is increased in MM patients. IL-3 might also play an important role in inhibiting osteoblast
differentiation [31]. It is interesting to notice that the influence of the myeloma cells on the interactions between bone cells is opposite to the one triggered by the mechanical stimulus.

Treatments for MM require not only management of the underlying malignancy, but also of the suppressed osteoblast activity and bone formation and of the increased bone resorption [12]. The majority of current treatments include chemotherapy, which reduces the tumor mass in bone marrow, but does not heal bone lesions [34]. The control of bone lesions is acquired with a combination of biphosphanate therapy (to inhibit osteoclast activity), localized radiation (for control of bone pain and treatment of fractures) and/or surgery. Some therapies also include the administration of drugs that inhibit osteoclast activity by blocking RANKL which, at the same time plays a role in inhibiting MM cell growth and survival [28, 27]. Examples of these drugs are the Denosumab drug, which is a monoclonal antibody that bonds to RANKL with high affinity and specificity, reproducing the effects of OPG, and Bortezomib, which is a proteasome antagonist that induces MM cell apoptosis and decreases RANKL activities by altering osteoblast and osteoclast activities [12]. The administration of drugs that downregulate TGF-β would also be important, since it would inhibit MM cell growth and stimulate osteoblast activity [30].
Chapter 3

State of the art

The development of models that integrate the knowledge from bone physiology and can replicate the biomechanical and biochemical processes involved in bone remodelling is of extreme importance, since they allow the comparison between healthy and pathological states, serving as clinical decision support systems for the implementation of therapeutic regimes and prognostic assessments.

In 2003, Komarova et al. [1] created a model that describes the population dynamics of bone cells. This model arrives from the proposition that cells can interact with each other through effectors that are released or activated by bone cells, and can act in a paracrine (affecting the other cell type) or autocrine (affecting the cell type of origin) manner. The paracrine and autocrine factors can only regulate the rates of production of osteoclasts and osteoblasts, being the rates of their removal proportional to the current number of corresponding cells. A power law approximation was used to summarize the effect of local factors on the rates of cell population, due to the high non-linearity of the system, according to the model proposed by Savageau [35]. Bone degradation, caused by osteoclasts, and posterior bone formation, by osteoblasts, results in the temporal evolution of bone mass. By varying the initial conditions, this model allows not only the representation of a single remodeling cycle, but also the representation of a periodic behavior, with specific amplitude and frequency.

Lemaire et al., in 2004 [2], proposed a mathematical model to explain the interactions between osteoblasts and osteoclasts, based on the idea that the relative proportions of immature and mature osteoblasts control the degree of osteoclastic activity and that osteoclastic control of osteoblasts is dependent on osteoclast level of differentiation. One major advantage of this model is that it is the first to include the RANK-RANKL-OPG pathway, considered to be the primary mechanism for regulation of osteoclast formation. In addition, the obtained results were to be used to explain experimental observations in bone biology and to explore failures of the biochemical control network that may lead to bone disease, such as osteoporosis. Parathyroid hormone (PTH) was considered as the trigger to the process of bone remodeling, since it is (in concert with vitamin D) the most important hormone regulating calcium homeostasis and bone remodeling, being involved in numerous clinical trials as an anabolic agent for the treatment of low bone mass in osteoporosis. Another advantage of this model is that it can easily be adapt to simulate skeletal disease, by inserting dysfunctional connections in the coupling network.
to explore different disease hypotheses. The results obtained with this model were in agreement with experimental observation, corroborating all behaviors of bone remodeling system, including the coupling between osteoclasts and osteoblasts, the effects of PTH administration and the actions of RANKL and OPG. The model also presented good results regarding the simulation of metabolic bone diseases, such as vitamin D deficiency. Potential routes for therapeutic interventions were also determined.

In 2005, Komarova [3] extended her previous model [1] to include the effects of PTH at a single site of bone remodeling. PTH is secreted when the calcium levels in the plasma drop. PTH primarily affects osteoblasts, which have PTH receptors, and upregulates RANKL expression while downregulating OPG, leading to an increase in the RANKL/OPG ratio, which increases osteoclastogenesis and bone resorption. As a result, calcium levels in plasma are increased. As PTH affects osteoclasts indirectly, by altering RANKL production by osteoblasts, it was considered that PTH only affects osteoblasts paracrine factor, which is no longer constant but dependent on the level of PTH. The results of this study showed that a continuous PTH stimulation leads to bone loss because bone resorption by osteoclasts always precedes bone formation. However, with a withdrawal in PTH, osteoclasts are removed and osteoblasts rebuild bone back to normal levels. On the other hand, on certain levels of PTH activation, osteoblasts overcompensate, leading to net gain of bone mass. The major disadvantage of this model is that, as it only represents a single site of bone remodeling, the results obtained for some levels of PTH administration are not coherent to what is observed in vivo.

Later, in 2009, Ryser et al. [36], developed a mathematical model describing the spatio-temporal evolution of a BMU. The objective of this model is to describe the experimental observed dynamics of the BMU and to assess how taking into account different temporal and spatial dynamics of RANKL and OPG affects the progression of the BMU. Starting with the equations described by Komarova et al. [1], the group extended the model to include a two dimensional spatial component, resulting in a novel nonlinear model, comprising a system of differential equations which include five state variables: densities of osteoclasts and osteoblasts, concentrations of OPG and RANKL, and the local bone mass. It was assumed that (1) osteocytes surrounding a microfracture produce RANKL, attracting osteoclasts, (2) OPG and RANKL are produced by osteoblasts and diffused through bone, (3) RANKL is eliminated by binding to OPG and RANK, (4) osteoblasts are coupled to osteoclasts through paracrine factors. The results obtained show that the RANKL field was higher at the microfracture in front of the BMU, while the peak of OPG was verified at the back of the BMU, resulting in the formation of a RANKL/OPG gradient, which strongly affects the rate of BMU progression and its size. A parameter estimation and sensitivity analysis of this model is presented in [37].

Ayati et al., in 2010 [4], extended Komarova’s equations of bone remodeling, proposed in 2003 [1] to include the influence of myeloma bone disease. The group adapted the previous model [1], in order to describe the influence of tumor growth on bone remodeling and, in particular, how the tumor influences osteoblast and osteoblast autocrine and paracrine signaling. At first, the model consists of a dynamical system with zero explicit space dimensions and with a dependent variable that represents the evolution of bone mass in time. For the case of myeloma bone disease, an independent variable that represents the evolution of tumor in time was added. The model was also adapted to include some therapeutic
approaches (proteasome therapy) for the treatment of myeloma, which affect both tumor cells (myeloma cells) and cells of the bone marrow microenvironment (osteoclasts and osteoblasts). The group expanded the model to simulate a one-dimensional spatial distribution, by developing a diffusion model in a second spatial domain ($\Omega$). It was assumed that both osteoclasts and osteoblasts diffuse in $\Omega$. The results showed that, in the case of normal bone, the process of bone remodeling is represented as stable regular oscillations, while in the case of myeloma the regular cycles are destabilized with an increase in osteoclasts typical of this disease, and with corresponding destruction of bone mass and progressive tumor growth. The treatment with proteasome was found to significantly reduce tumor burden and prevent myeloma bone disease.

In 2012, Ryser et al. [38] extended his previous model [36] to include the effect of bone metastases in bone remodeling and to study the ambiguous role of OPG in the system. It was assumed that OPG produced by cancer cells causes a local reduction in RANKL levels, including an accentuated RANKL gradient away from the tumor and towards the bone tissue, leading to faster resorption and tumor expansion. In this model, the tumor size is included as a variable, along with the PTH related protein (PTHrP), which binds to osteoblasts increasing RANKL production. It was also assumed that the tumor growth is dependent on the activity of osteoclasts, in a way that it fills the cavity resulting from bone resorption. The results demonstrated that, at lower expression rates, tumor-derived OPG enhances RANKL gradient and osteolysis, but at higher expression rates OPG broadly inhibits RANKL and decreases osteolysis and tumor burden.

More recently, in 2013, Scheiner et al. [6] developed a mathematical model describing the process of bone remodeling at the macroscopic level of cortical bone by combining, for the first time, bone cell population kinetics with multiscale bone mechanics. By using the output of previously proposed bone cell population models as an input for bone micromechanics formulations and extending the state-of-the-art to micromechanically quantified strain stimulus, the group aimed to understand if bone remodeling, which is often associated with some “mechanostat paradigm”, can be explained solely by combined effects of multiscale mechanics and bone cell population kinetics, which are exclusively based on physical properties such as chemical concentrations, volume fractions, geometrical shapes and mechanical properties. The key variables of the model are the concentrations of bone cells (osteoclasts, osteoblasts and their progenitors), biochemical factors (RANK, RANKL, OPG, PTH and TGF-$\beta$) and mechanical strains, both at the level of cortical bone (macroscale) and at the level of the extravascular bone matrix (microscale). It was assumed that the relationship between the macroscopic strains resulting from the loads and the microscopic strains, which modulate the expression or proliferation behavior of the extravascular bone matrix cells is delivered by multiscale bone mechanics. The model provides reasonable estimates of the stiffness changes of cortical bone and its load-carrying capacity, driven by biochemically and/or mechanically regulated bone cell activities. In addition, it is able to explain the experimentally observed evolution of bone mass in postmenopausal osteoporosis and under microgravity conditions, and to reproduce physically observed key features such as rapid bone loss due to unloading and slower bone gain after re-establishment of the normal mechanical loading. The major disadvantage of this model is its extensive number of variables and equations.
Bone cells can only remove and replace bone at the bone surface, whose microscopic availability depends on the everchanging bone microstructure. Focusing on the potential regulatory mechanism of bone cells that involve the morphology of the microstructure of bone, Pivonka et al. [39], in 2013, developed a mathematical model of bone cell interactions that takes into account biochemical, biomechanical and geometrical regulations. The objectives of this model are to investigate at which stage of the bone remodeling sequence geometrical feedback has the strongest effect, study the interactions between geometrical and mechanical feedback and the impacts, in terms of bone porosity and bone stiffness, of geometrical feedback in osteoporosis. The biochemical regulatory factors considered were the RANK-RANKL-OPG pathways, the action of TGF-β, the macrophage colony-stimulating factor (MCSF) and PTH. On the other hand, biomechanical regulation of bone formation and bone resorption is mediated by the microscopic strain energy density (SED) of the bone matrix. A phenomenological relationship between the specific surface and the vascular porosity obtained from various types of bones is used to elucidate the geometrical feedback due to microscopic bone surface availability. The results obtained suggest that geometrical regulation of the activation of new remodeling events is significant for bone porosity and stiffness in osteoporosis. In addition, it was possible to conclude that the development of osteoporosis is accelerated by the geometrical regulation in cortical bone, but slowed down in trabecular bone.

Later in the same year, Idhammad et al. [40] performed numerical simulations on a proximal femur using an elastic-damage theory for small displacements and including the presence of mechanical stimulus. The mathematical model was based on a system of nonlinear ordinary differential equations, and it was implemented into two-dimensional femur model, obtained using a finite element method, in order to investigate the effect of the damage. The effects of both strain and damage in bone structure were studied and the results showed that bone stiffness drops in damage bone structure under mechanical loading.

Coelho et al., in 2016 [5], extended the model proposed by Komarova et al. [1] to include the influence of PTH, assumed as capable of triggering and regulating the process of bone remodeling. The model also considers the secretion of PTH-related protein (PTHrP) by cancer cells, which upregulates RANKL production by osteoblasts, leading to an activation of osteoclasts and a consequent increase in bone resorption. The increase in bone resorption releases growth factors entrapped in the bone matrix, which induce tumor growth, giving rise to a self-perpetuating cycle known as the vicious cycle of bone metastases. In addition, the model describes how the presence of metastases contributes to the decoupling between bone resorption and bone formation. The model was also extended to include the effects of anti-cancer and anti-resorptive treatments, through chemotherapy and bisphosphonates or denosumab, along with their pharmacokinetics and pharmacodynamics. The results showed that the model is able to describe bone remodeling cycles, the growth of bone metastases and the efficiency of treatment in reducing the tumor burden on bone, while preventing the loss of bone strength.

The work presented here consists on a mathematical and computational model of bone remodeling, in which the process of bone remodeling is triggered by an external mechanical stimulus. The interactions between bone cells are described by a set of ordinary differential equation (ODEs) and a
finite element model (FEM) of a proximal femur was created to obtain the mechanical stimulus. The biochemical interactions between bone cells during the process of bone remodeling were described by the equations proposed by Komarova et al. [1]. The biomechanical processes were introduced in those equations according to the processes described by Rubin et al [20], which assumes that an increase in the mechanical stimulus is responsible for an increase in osteoblasts autocrine factors and a decrease in osteoblasts paracrine factors. Also, being osteocytes the cells primarily responsible for sensing and responding to the mechanical stimulus, their influence was included in the equations in a way that the number of osteoclasts is decreased while the number of osteoblasts is increased. The model was then expanded to include the influence of myeloma bone disease on the previously studied bone remodeling cycles. This influence was introduced in the equations according to the formulation described by Ayati et al. [4].
Chapter 4

Model Development

To replicate the biochemical processes involved in bone remodeling, as well as the corresponding bone mechanical environment, a set of differential equations were defined, and solved using a numerical integration algorithm in Matlab. A finite element model was then created using ABAQUS in order to include the mechanical stimulus and observe the density distribution in the bone.

4.1 Mathematical Model

4.1.1 Biochemical Model

According to the model described by Komarova et al. [1], and later reviewed by Ayati et al. [4], the dynamics of cell populations at the bone remodeling site is described by the following system of differential equations

\[
\frac{dC(t)}{dt} = \alpha_C C(t) g_{CC} B(t) g_{BC} - \beta_C C(t) \tag{4.1a}
\]

\[
\frac{dB(t)}{dt} = \alpha_B C(t) g_{CB} B(t) g_{BB} - \beta_B B(t) \tag{4.1b}
\]

where \( t \) is the instant of time, \( C(t) \) and \( B(t) \) are, respectively, the number of osteoclasts and osteoblasts; \( \alpha_i \) is the activity of cell production (\( \alpha_C \) - production of osteoclasts, \( \alpha_B \) - production of osteoblasts) and \( \beta_i \) is the activity of cell removal (\( \beta_C \) - removal of osteoclasts, \( \beta_B \) - removal of osteoblasts). The parameters \( g_{ij} \) represent the net effectiveness of osteoclast- or osteoblast-derived autocrine or paracrine factors (\( g_{CC} \) - osteoclast-derived autocrine factor, \( g_{CB} \) - osteoclast-derived paracrine factor, \( g_{BB} \) - osteoblast-derived autocrine factor, \( g_{BC} \) - osteoblast-derived paracrine factor), which reflect the amount of effector produced per donor cell as well as the responsiveness of the target cell. It was considered in this model that both osteoclast-derived autocrine and paracrine factors are positive (\( g_{CC} > 0 \) and \( g_{CB} > 0 \)), which means the factors produced by osteoclasts lead to an increase in both osteoclasts and osteoblasts. On the contrary, osteoblast-derived paracrine factors are negative (\( g_{BC} < 0 \)), leading to a decrease in the number of osteoclasts. Osteoblast-derived autocrine factors are considered to be zero (\( g_{BB} = 0 \)) [1].
The model includes a third equation describing the changes in bone mass. To build this equation it was assumed that the populations of osteoclasts and osteoblasts under steady-state conditions consist of differentiated cells that are able to participate in autocrine and paracrine signaling, but unable to resorb or build bone. Proliferation and differentiation of precursors into mature cells, which are able to remove or build bone, was considered to lead to an increase in cell numbers above steady-state levels. It was also assumed that there is a proportionality between the rates of bone resorption and formation and the number of osteoclasts and osteoblasts, respectively, exceeding steady-state levels [1]. Therefore, the third equation is written as

$$\frac{dz(t)}{dt} = -k^0_C \max[0, C(t) - C] + k^0_B \max[0, B(t) - B]$$

(4.2)

where $z(t)$ is the total bone mass, $k_i$ is the normalized activity of bone ($k^0_C$ - bone resorption, $k^0_B$ - bone formation), the max conditions represent the number of cells actively resorbing or forming bone, and $i$ are the number of cells at steady-state, given by

$$C = \left( \frac{\beta_C}{\alpha_C} \right)^{1-g_{BB}} \left( \frac{\beta_B}{\alpha_B} \right)^{g_{BC}}$$

(4.3a)

$$B = \left( \frac{\beta_C}{\alpha_C} \right)^{g_{CB}} \left( \frac{\beta_B}{\alpha_B} \right)^{1-g_{CC}}$$

(4.3b)

where

$$\gamma = g_{CB}g_{BC} - (1-g_{CC})(1-g_{BB})$$

(4.4)

Analyzing equation 4.2, it is possible to see that, if both the number of osteoclasts and osteoblasts decrease to values below the steady-state, there will be no alteration in the value of bone mass, which may not correspond to what happens in biological environment. To overcome this limitation, equation 4.2 was replaced by the following equation

$$\frac{dz(t)}{dt} = (-k_CC(t) + k_BB(t))S(z(t))$$

(4.5)

where

$$S(z(t)) = z(t)(1.73 - z(t))$$

(4.6)

In this equation, the number of active cells is no longer given by the maximum between zero and the difference between the actual number of cells and the number of cells at steady-state. Instead, the number of active cells is considered to be a percentage of the total number of cells and, this way, the bone mass is affected by both increases and decreases in the number of activated cells. The parameters $k_C$ and $k_B$ had to be readjusted in order to recover the original behavior of the system. In addition, the equation was multiplied by a surface function, $S(z(t))$, because cellular actions begin at the surface of the bone, so its potential is directly dependent on the surface [41]. This function equals zero when the
bone density is zero or when it assumes its maximum value \((S(0) = 0\) and \(S(1.73) = 0\)), and reaches a maximum value when the density equals half its maximum.

### 4.1.2 Biomechanochemical Model

As discussed above (section 2), the mechanical loading (in this case considered as strain energy [42]) influences the net effectiveness parameters \((g_{ij}\) in equation 4.1) and, for this reason, a term dependent on the strain energy density (SED) was added to these parameters as follows

\[
\frac{dC(t)}{dt} = \alpha C(t)^{g_{cc} + \xi_{cc}} B(t)^{g_{bc} + \xi_{bc}} \left( \frac{\Psi(t)}{\Psi} - 1 \right) - \beta C(t) \quad (4.7a)
\]

\[
\frac{dB(t)}{dt} = \alpha B(t)^{g_{cc} + \xi_{cc}} B(t)^{g_{bc} + \xi_{bc}} \left( \frac{\Psi(t)}{\Psi} - 1 \right) - \beta B(t) \quad (4.7b)
\]

where \(\xi_{ij}\) are constants that represent the weight of the mechanical term to the respective \(g_{ij}\) parameter, \(\Psi\) is the SED at time \(t\) and \(\Psi\) is a threshold value. It is easy to see that, when \(\Psi(t)\) equals \(\Psi\), \(\frac{\Psi(t)}{\Psi} = 1\), and the mechanical components become zero, having no influence in the system. The mechanical stimulus is known to increase the osteoblast autocrine factors (leading to an increase in the number of osteoblasts) and, for this reason, \(\xi_{BB}\) assumes a positive value \((\xi_{BB} > 0)\). Osteoblast paracrine factors, on the contrary, are decreased when the mechanical stimulus increases, and so \(\xi_{BC}\) has a negative value \((\xi_{BC} < 0)\). The mechanical stimulus has a little influence in osteoclasts, reason why the parameters \(\xi_{CC}\) and \(\xi_{CB}\) are very small and, for the purpose of this work, they are assumed to be zero \((\xi_{CC} = 0\) and \(\xi_{CB} = 0)\).

In addition, it was also discussed in section 2 that osteocytes are the cells primarily responsible for sensing the mechanical stimulus, producing a response that can affect both osteoclasts and osteoblasts. Given this, a term related to the influence of the osteocytes response to the mechanical stimulus was added to equations 4.1 as follows

\[
\frac{dC(t)}{dt} = \alpha C(t)^{g_{cc} + \xi_{cc}} B(t)^{g_{bc} + \xi_{bc}} \left( \frac{\Psi(t)}{\Psi} - 1 \right) + O_C \left( \frac{\Psi(t)}{\Psi} - 1 \right) \quad (4.8a)
\]

\[
\frac{dB(t)}{dt} = \alpha B(t)^{g_{cc} + \xi_{cc}} B(t)^{g_{bc} + \xi_{bc}} \left( \frac{\Psi(t)}{\Psi} - 1 \right) + O_B \left( \frac{\Psi(t)}{\Psi} - 1 \right) \quad (4.8b)
\]

where \(O_i\) are constants that represent the influence of the osteocytes response. As osteocytes are responsible for upregulating osteoblasts differentiation and inhibiting osteoclasts, \(O_B\) was assumed to be positive \((O_B > 0)\) and \(O_C\) negative \((O_C < 0)\). The final biomechanochemical model of bone remodeling is then given by

\[
\frac{dC(t)}{dt} = \alpha C(t)^{g_{cc} + \xi_{cc}} B(t)^{g_{bc} + \xi_{bc}} \left( \frac{\Psi(t)}{\Psi} - 1 \right) - \beta C(t) + O_C \left( \frac{\Psi(t)}{\Psi} - 1 \right) \quad (4.9a)
\]
\[
\frac{dB(t)}{dt} = \alpha_B C(t)^{g_{CN} + \xi_{CN} (\Psi(t) - 1)} B(t)^{g_{BN} + \xi_{BN} (\Psi(t) - 1)} - \beta_B B(t) + O_B \left( \frac{\Psi(t)}{\Psi} - 1 \right)
\] (4.9b)

\[
\frac{dz(t)}{dt} = (-k_C C(t) + k_B B(t)) S(z)
\] (4.9c)

4.1.3 Cancer Cells

Equations 4.9 are the final biomechanical model of bone remodeling for normal cells. However, as was discussed in section 2.4, cancer cells, affected by myeloma, behave differently and cannot be described by the same model. Ayati et al.[4] modeled the influences of tumor growth on bone remodeling, with a special insight on how the tumor influences autocrine and paracrine signaling in the osteoclast and osteoblast cell populations. The following equations were purposed

\[
\frac{dC(t)}{dt} = \alpha_C C(t)^{g_{CC} + g_{C}r_{CC} \frac{T(t)}{T_T}} B(t)^{g_{BC} + g_{BC}r_{BC} \frac{T(t)}{T_T}} - \beta_C C(t)
\] (4.10a)

\[
\frac{dB(t)}{dt} = \alpha_B C(t)^{g_{CN} + \left[ \frac{g_{BN}}{1 + r_{BN} \frac{T(t)}{T_T}} - g_{CN} \right]} B(t)^{g_{BN} - r_{BB} \frac{T(t)}{T_T}} - \beta_B B(t)
\] (4.10b)

\[
\frac{dT(t)}{dt} = \gamma_T T(t) \log \left( \frac{L_T}{T(t)} \right)
\] (4.10c)

where \(C(t)\) and \(B(t)\) are, respectively, the number of osteoclasts and osteoblasts, as before, and \(T(t)\) is the density of tumor cells at time \(t\). The tumor equation (equation 4.10c) is of Gompertz form \([43]\) with growth constant \(\gamma_T\) (positive and independent of bone loss) and maximum tumor size \(L_T\). In equations 4.10a and 4.10b it is possible to see that the presence of the tumor leads to an increase in autocrine promotion of osteoclasts \((g_{CC} + g_{C}r_{CC} \frac{T(t)}{T_T}) > g_{CC}\), since \(g_{CC} > 0\)), and a reduction in paracrine inhibition of osteoclasts \((g_{BC} + g_{BC}r_{BC} \frac{T(t)}{T_T}) < g_{BC}\), since \(g_{BC} < 0\)), in the paracrine promotion of osteoblasts \(\frac{g_{CN}}{1 + r_{CN} \frac{T(t)}{T_T}} < g_{CB}\), since \(g_{CB} > 0\)) and in the autocrine promotion of osteoblasts \((g_{BB} - r_{BB} \frac{T(t)}{T_T} < g_{BB})\). The bone mass variation is describe by equation 4.9c as before.

The final equations for cancer cells, with the influence of the mechanical stimulus, are

\[
\frac{dC(t)}{dt} = \alpha_C C(t)^{g_{CC} + g_{C}r_{CC} \frac{T(t)}{T_T} + \xi_{CC} (\Psi(t) - 1)} B(t)^{g_{BC} + g_{BC}r_{BC} \frac{T(t)}{T_T} + \xi_{BC} (\Psi(t) - 1)} - \beta_C C(t) + O_C \left( \frac{\Psi(t)}{\Psi} - 1 \right)
\] (4.11a)

\[
\frac{dB(t)}{dt} = \alpha_B C(t)^{g_{CN} + \left[ \frac{g_{BN}}{1 + r_{BN} \frac{T(t)}{T_T}} - g_{CN} \right] + \xi_{BN} (\Psi(t) - 1)} B(t)^{g_{BN} - r_{BB} \frac{T(t)}{T_T} + \xi_{BB} (\Psi(t) - 1)} - \beta_B B(t) + O_B \left( \frac{\Psi(t)}{\Psi} - 1 \right)
\] (4.11b)
\[
\frac{dT(t)}{dt} = \gamma T(t) \log \left( \frac{L_T}{T(t)} \right) \quad (4.11c)
\]

\[
\frac{dz(t)}{dt} = (-k_C C(t) + k_B B(t)) S(z) \quad (4.11d)
\]

### 4.2 Computational Model

The dynamic behavior of the system of equations 4.9 and 4.11 was analyzed using numerical integration by a fourth-order Runge-Kutta algorithm in Matlab, which is an iterative method used to obtain the approximate solutions of ODEs. A finite element model (FEM) of a 2D femur bone (Figure 4.1), with 2403 linear triangular elements (1292 nodes), was created using ABAQUS in order to obtain the mechanical stimulus and strain energy value in each node. The forces \( F_1 \) and \( F_2 \) \((F_{1x} = -224N, F_{1y} = -2246N, F_{2x} = 768N, F_{2y} = 1210N \) [44]) were applied according to Figure 4.1, with an uniform distribution, while the bottom of the model was fixed. A side plate (Figure 4.2) was built to create an interaction between the diaphysis wall, that would be connected in a 3D situation.

The routine implemented in Matlab computes the number of bone cells (osteoclasts and osteoblasts), as well as the bone mass for each node of the model, meaning that the number of equations to be solved is determined by the number of elements of the model. In this mathematical model, bone is considered an isotropic material, with two material properties: Poisson's coefficient \( (\nu = 0.3) \) and Young's modulus, determined according to the following power law.
Figure 4.2: Model of the side plate.

\[ E = 3790z^3 \]  \hspace{1cm} (4.12)

where \( E [MPa] \) is the Young’s Modulus and the density, \( z \), varies between 0 and 1.73gcm\(^{-3}\).
Chapter 5

Results

The results obtained with the equations described in Chapter 4 are presented here, as well as some tests to better understand the influence of the parameters of the equations. Firstly, the results obtained with the original equations of Komarova et al. [1] (equations 4.1 and 4.2) are presented, followed by tests to determine the new $k_C$ and $K_B$ parameters for equation 4.5. Afterwards, the mechanical stimulus is introduced in the equations (equations 4.9) and tests to parameters $\xi_{BC}$, $\xi_{BB}$, $O_C$ and $O_B$ are performed. Finally, the influence of the myeloma bone disease is introduced (equations 4.11) and results for different values of the parameter $r_{CC}$ are presented.

5.1 Biochemical Model

The results for the original model proposed by Komarova et al [1] (equations 4.1 and 4.2) are presented in Figure 5.2 being, respectively, Figure 5.2(a) the number of osteoclasts, Figure 5.2(b) the number of osteoblasts and Figure 5.2(c) the bone mass in each node of the femur model presented in Figure 4.1. Figure 5.2(d) shows the bone mass distribution in the same model. The initial values and parameters for these equations are presented in table 5.1, and are according to [1].

In this case, the bone remodeling process was triggered by an initial increase in the number of osteoclasts, with respect to the steady-state condition. It is possible to see that both the number of cells and the bone mass are the same for each node of the model, as Figures 5.2(a), 5.2(b) and 5.2(c) present only one line, which is, in fact, an overlap of the 1292 lines (one for each node of the model). In addition, Figure 5.2(d) shows that the bone mass distribution is homogeneous along the femur model. It is also possible to see that, after the initial perturbation in the number of osteoclasts, the solution converges for the steady-state conditions after 150 days. These results are in agreement with the ones obtained by Komarova et al. [1].

As described in Section 4.1.1, equation 4.2 was replaced by equation 4.5, and the parameters $k_C$ and $k_B$ had to be determined. $k_C$ and $k_B$ had to obey the following relation:

$$-k_C C + k_B B = 0$$  \hspace{1cm} (5.1)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{initial}$</td>
<td>0 days</td>
</tr>
<tr>
<td>$t_{final}$</td>
<td>150 days</td>
</tr>
<tr>
<td>$C(0)$</td>
<td>11.06 cells</td>
</tr>
<tr>
<td>$B(0)$</td>
<td>212.13 cells</td>
</tr>
<tr>
<td>$z(0)$</td>
<td>0.865 g cm$^{-3}$</td>
</tr>
<tr>
<td>$\alpha_C$</td>
<td>3 cells day$^{-1}$</td>
</tr>
<tr>
<td>$\alpha_B$</td>
<td>4 cells day$^{-1}$</td>
</tr>
<tr>
<td>$\beta_C$</td>
<td>0.2 day$^{-1}$</td>
</tr>
<tr>
<td>$\beta_B$</td>
<td>0.02 day$^{-1}$</td>
</tr>
<tr>
<td>$g_{CC}$</td>
<td>0.5</td>
</tr>
<tr>
<td>$g_{CB}$</td>
<td>1.0</td>
</tr>
<tr>
<td>$g_{BC}$</td>
<td>-0.5</td>
</tr>
<tr>
<td>$g_{BB}$</td>
<td>0.0</td>
</tr>
<tr>
<td>$k_0^C$</td>
<td>0.004152 g cm$^{-3}$ cell$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$k_0^B$</td>
<td>0.00002941 g cm$^{-3}$ cell$^{-1}$ day$^{-1}$</td>
</tr>
</tbody>
</table>

Table 5.1: Initial values and parameters of the model.

Being $C$ and $B$ the steady-state concentration of osteoclasts and osteoblasts, respectively. This relation results from the need to impose a steady-state situation identical to the one obtained with equation 4.2. The values tested for these parameters are presented in table A.1. Figure 5.1 represents the differences between the evolution of bone mass obtained with Komarova’s equation (equation 4.2), with the original values for $k_0^C$ and $k_0^B$, and the new bone mass equation (equation 4.5) for different values of $k_C$ and $k_B$.

$k_C$ was assumed to be 0.0037 ($k_C = 0.0037, k_B = 0.0000185$), as it was the value that presented the smaller variation. Figure 5.3 shows the results obtained with the new equation of bone mass (equation 4.5) and the values of $k_C$ and $k_B$. For all the other parameters and conditions, the values are as presented in table 5.1. The results are very similar to the ones presented in Figure 5.2, with a slightly decrease in bone mass. The results show that the initial increase in the number of osteoclasts leads to a decrease in the bone mass, to which the system responds increasing the number of osteoblasts to
Figure 5.2: Results obtained with the original equations of Komarova et al. [1] (equations 4.1 and 4.2).

Figure 5.3: Results obtained with the original bone cell equations described by Komarova et al. [1] (equations 4.1) and the new bone mass equation (equation 4.5), with $k_C = 0.0037$ and $k_B = 0.0000185$. 
form new bone and reestablish the equilibrium.

5.2 Biomechanochemical Model

In this section, the results after introducing the mechanical stimulus in the biochemical model are presented, and a comparative analysis of parameters is performed. The section starts with an analysis of the influence of the mechanical stimulus on the net effectiveness parameters, according to equations 4.7, testing different for the parameters $\xi_{BC}$ and $\xi_{BB}$. After, the influence of the response of osteocytes to the mechanical environment is studied, according to equations 4.8, comparing results for different values of parameters $O_C$ and $O_B$. The range of values admitted for the parameters in test take into account two main aspects: (1) the order of magnitude of the other terms of the equations, as the $g_{ij}$ parameters; (2) its influence on the convergence and stability of the solution. Lastly, the influence of the mechanical stimulus on both the net effectiveness parameters and the osteocytes is tested, according to equations 4.9.

**Net effectiveness parameters**

Figures 5.5 to 5.11 represent the results obtained with the system of equation 4.7 and 4.5, with different values for parameters $\xi_{BC}$ and $\xi_{BB}$. As stated before (section 2.3) $\xi_{CC}$ and $\xi_{CB}$ were assumed to be zero (Table A.2). The values of $k_C$ and $k_B$ are as determined above: $k_C = 0.0037$ and $k_B = 0.0000185$, and $C(0)$ equals its steady state value ($C(0)=1.06$ cells). The value $\bar{\Psi}$ was assumed to be an average value of the SED of each node, obtained with Komarova’s model (equations 4.1 and 4.2). The remaining parameters of the model assume the values presented on table 5.1.

The first conclusion to withdraw is that the introduction of the mechanical stimulus leads to a solution that is no longer homogeneous, in which each node has a different concentration of osteoclasts and osteoblasts and the bone mass is distributed according to the demands of the mechanical environment. Several parameters were tested and the goal is to obtain a distribution of bone mass comparable to the biological situation (Figure 5.4), in which the diaphysis walls correspond to regions with higher bone mass, between which there is the medullary channel with a bone density close to zero. Also, it should be possible to identify the Ward’s triangle near the femur’s head.

The values tested for parameters $\xi_{BC}$ and $\xi_{BB}$ were beneath the range of values admitted by Komarova et al., in order to assure the stability of the equation. Figures 5.5 are the results obtained with $\xi_{BC} = -0.1$ and $\xi_{BB} = 0$. The evolution of the number of cells and bone mass showed to be smooth, reaching a steady-state at around 100 days. The initial variation in the number of osteoclasts seems to be more pronounced, which may be due to the fact that the parameter in test, $\xi_{BC}$, has a direct effect on osteoclasts. When comparing these results with the ones from Figure 5.6, in which $\xi_{BC} = -0.2$ and $\xi_{BB} = 0$, it can be seen that increasing, in modulus, the value of $\xi_{BC}$ leads to an increase in the number of osteoclasts. Consequently, as the number of osteoblasts is dependent on the number of osteoclasts, the number of osteoblasts also increases, though this increase is less pronounced. There were also some changes in the values of bone mass after 150 days: the higher values are slightly increased, due
to the increase of osteoblasts, and there is a notable decrease of the lower values, due to the increase of osteoclasts. Despite the small range of values of bone mass observed in Figures 5.5(d) and 5.6(d), it is possible to see that the distribution of bone mass is according to what was expected: regions of higher bone mass are forming in the diaphysis walls, with a region of lower bone mass between them.

In Figure 5.7, $\xi_{BC}$ was considered to be zero and $\xi_{BB}$ was assumed as 0.1 ($\xi_{BC} = 0$, $\xi_{BB} = 0.1$). In this case, the variation of osteoclasts is smoother as the parameter in test does not have a direct influence on these cells. However, when comparing Figure 5.7(b) with Figures 5.5(b) and 5.6(b), it is possible to see that there was a decrease in the number of osteoblasts, with an increase in the number of nodes that have a concentration of these cells below the initial value. Figure 5.7(d) shows that the range of values for bone mass is larger in this case when compared with Figures 5.5(d) and 5.6(d), but Figure 5.7(c) shows that 150 days were not enough for bone mass to reach a steady-state.

Increasing the number of $\xi_{BB}$ (Figure 5.8, with $\xi_{BC} = 0$ and $\xi_{BB} = 0.2$) leads to a not so regular evolution of the concentration of osteoblasts in some nodes. In addition, the maximum values of the concentrations of both osteoclasts and osteoblasts are higher, while the minimum values are lower, when compared to the previous analysis (Figure 5.7). The bone mass is still not converging after 150 days, but Figure 5.8(d) shows that the maximum value after 150 days is higher when compared to Figure 5.7(d), and the minimum value is lower.

In Figure 5.9, with $\xi_{BC} = 0$ and $\xi_{BB} = 0.3$, the conclusions to withdraw are the same as for the previous analysis (Figure 5.8): in some nodes the concentration of cells is not stabilized after 150 days, and the bone mass is also not converging to a steady-state after 150 days. There is also an increase in the maximum values and a decrease in the minimum values of both the concentration of cells and the bone mass.

Introducing the influence of mechanical stimulus in both osteoblast paracrine and autocrine factors, with $\xi_{BC} = -0.1$ and $\xi_{BB} = 0.2$ (Figure 5.10), showed that the concentrations of bone cells seem to reach a steady-state after 150 days, but the bone mass is not yet stabilized. Figure 5.10(b) shows that, while in some nodes the concentration of osteoblasts is increased above the initial value in the
Figure 5.5: Tests of parameters for equation 4.7: $\xi_{BC} = -0.1$, $\xi_{BB} = 0$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.6: Tests of parameters for equation 4.7: $\xi_{BC} = -0.2$, $\xi_{BB} = 0$. Each line corresponds to one of the 1292 nodes of the finite element model.
Figure 5.7: Tests of parameters for equation 4.7: $\xi_{BC} = 0$, $\xi_{BB} = 0.1$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.8: Tests of parameters for equation 4.7: $\xi_{BC} = 0$, $\xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model.
beginning of the bone remodeling cycle, evolving to values close or even below the initial value after a few days, there are other nodes that behave in the opposite way: the concentration of osteoblasts starts by decreasing below the initial value and evolves to values close or even higher than the initial value. Despite the evolution of osteoclasts, in Figure 5.10(a), also shows some nodes with a similar behavior to the one described for osteoblasts, this phenomenon seems to be much less pronounced. Figure 5.10(d) shows that the solution is getting similar to the biological situation (Figure 5.4), as the walls of the diaphysis correspond to the regions with higher bone mass, reaching values of $1.161 \text{gcm}^{-3}$, with a medullary channel forming between them, corresponding to the lower values of bone mass ($z = 0.231 \text{gcm}^{-3}$) and a Ward’s triangle forming in femur’s neck.

When the value of $\xi_{BB}$ is increased (Figure 5.11, with $\xi_{BC} = -0.1$ and $\xi_{BB} = 0.3$), the initial increase in the number of osteoblasts seems to be more smooth in Figure 5.11(b) when compared to Figure 5.10(b), but the variations seem to be amplified for the case of osteoclasts, when comparing Figure 5.11(a) with Figure 5.10(a). In addition, the maximum value of osteoblasts concentration increases with the increase of $\xi_{BB}$, while the minimum value decreases. In the case of osteoclasts concentration, there is an increase in the maximum value, but the minimum value seems not to suffer significant changes. Although the bone mass is still not stabilizing after 150 days, there was also an increase in its maximum value, and a decrease in the minimum.

**Osteocytes**

Figures 5.12 to 5.18 represent the results obtained with the system of equations 4.8 and 4.5, for different sets of parameter $O_C$ and $O_B$. Figure 5.12 shows the results for the test of the influence of parameter $O_C$. In this case, $O_C$ was assumed to be -0.01 and $O_B$ was considered to be zero ($O_C = -0.01$ and $O_B = 0$). This value of $O_C$ was chosen in order to allow the model to converge, as with all the other values tested for this parameter the model did not seem to converge. The results show that both the concentration of bone cells and the bone mass have a very regular evolution along the 150 days, converging to a steady-state after few days. Figure 5.12(a) shows that there is an initial perturbation in osteoclasts concentration, which was expected since the parameter in test has a direct influence on osteoclasts. It is also possible to see that, while both the number of osteoclasts and osteoblasts have a higher number of nodes with values above the initial value, there is a higher number of nodes with bone mass below the initial value. In addition, the small range of variation in both bone cells and bone mass, mainly visible in Figure 5.12(d), suggests that $O_C$ has a small influence on the system.

In Figure 5.13, the parameter $O_C$ is set to zero, and the influence of osteocytes on osteoblasts is tested, assuming $O_B = 0.1$. Though $O_B$ was set one order of magnitude higher than $O_C$ in Figure 5.12, the results show that the range of variation of bone cells is even smaller than in the previous case, suggesting that the influence of $O_B$ in the number of bone cells is lower than the influence of $O_C$. However, contrary to what is verified in Figure 5.12(b), in Figure 5.13(b) there is a higher number of nodes with a concentration of osteoblasts below the initial value. In addition, Figure 5.13(c) shows that the values of bone mass did not stabilize after 150 days, contrary to what happened in Figure 5.12(c).

Increasing the value of $O_B$ by one order of magnitude (Figure 5.14, with $O_C = 0$ and $O_B = 1$) increased the range of values between which the bone mass varies, though it still does not reach a
Figure 5.9: Tests of parameters for equation 4.7: $\xi_{BC} = 0$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.10: Tests of parameters for equation 4.7: $\xi_{BC} = -0.1$, $\xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model.
Figure 5.11: Tests of parameters for equation 4.7: $\xi_{BC} = -0.1$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.12: Tests of parameters for equation 4.8: $O_C = -0.01$, $O_B = 0$. Each line corresponds to one of the 1292 nodes of the finite element model.
steady-state after 150 days. Comparing also with Figure 5.15, in which $O_C = 0$ and $O_B = 2$, it is notable that the range of values between which the number of cells vary is increasing, and the same is verified for the bone mass. Figure 5.15(d) shows that a medullary channel is forming, however, there is yet no formation of a Ward’s triangle.

In Figure 5.16 $O_B$ was set to 5 ($O_C = 0, O_B = 5$). Figures 5.16(a) and 5.16(b) show that the evolution of the number of cells is not so regular for some nodes as it was in the previous analysis, suggesting that the value of $O_B$ is probably too high and is starting to destabilize the system. It is also possible to see that the range of variation of bone cells and bone mass is much wider when compared to Figures 5.14 and 5.15. This suggests that the increase in the range of variation is not linear, since increasing $O_B$ by one order of magnitude (from Figure 5.13 to Figure 5.14) lead to much smaller changes than an increase by some units (from Figure 5.14, to Figure 5.15 and then Figure 5.16). Figure 5.16(c) shows that an increase in $O_B$ does not have any significant effect in the convergence of bone mass after 150 days. One conclusion that is interesting to withdraw from the previous analysis is that when the influence of the mechanical stimulus is introduced only in the parameters that directly affect osteoblasts ($\xi_{BB}$ and $O_B$), the evolution of bone mass seems to be slowed down, and the results do not converge after 150 days, fact that is not verified when the influence of the mechanical stimulus is introduced solely on the parameters that have a direct influence on osteoclasts ($\xi_{BC}$ and $O_C$).

Testing the influence of the mechanical stimulus in both $O_C$ and $O_B$ (Figure 5.17, with $O_C = -0.01$ and $O_B = 1$) leads to a very smooth evolution in the number of cells (Figures 5.17(a) and 5.17(b)) and in the bone mass (Figure 5.17(c)), though the last one is still varying after 150 days. The range of values between which the bone cells and bone mass vary is smaller when compared to the tests in which the mechanical stimulus is only influencing $O_B$. However, the distribution of the number of cells follows a pattern similar to the analysis of $O_B$: there are more node with a number of osteoclasts above the initial value and a number of osteoblasts below the initial value, which consequently leads to more nodes with bone mass below the initial value. Though the range of variation of bone mass is very small, in Figure 5.17(d) it is visible that the bone mass is being addressed to the expected places.

Figure 5.18, in which $O_C = -0.01$ and $O_B = 2$ does not present significant differences when compared to Figure 5.17, beyond the wider range of variation of bone cells and bone mass. However, this set of parameters was considered to be the most acceptable, moreover, the number of osteoclasts is about 200 times higher than the number of osteoblasts, which is the relative value between the parameters $O_B$ and $O_C$.

**Net effectiveness parameters and osteocytes**

Figures 5.19 to 5.22 are the results obtained with the system of equations 4.9, for different sets of parameters $O_C$, $O_B$, $\xi_{BC}$ and $\xi_{BB}$. Figure 5.19 are the results obtained with $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$ and $\xi_{BB} = 0.3$. Figure 5.19(a) shows that the range of variation in the number of osteoclasts lays between a minimum value that is close to 0 and a maximum value close to 5. It is visible that, initially, there is a higher number of nodes for which the bone mass starts increasing. However, after a few days, a group of these nodes show a decrease in the number of osteoclasts to values close of even below the initial value. On the other hand, some of the nodes that showed an initial decrease
Figure 5.13: Tests of parameters for equation 4.8: \( O_C = 0, O_B = 0.1 \). Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.14: Tests of parameters for equation 4.8: \( O_C = 0, O_B = 1 \). Each line corresponds to one of the 1292 nodes of the finite element model.
Figure 5.15: Tests of parameters for equation 4.8: $O_C = 0$, $O_B = 2$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.16: Tests of parameters for equation 4.8: $O_C = 0$, $O_B = 5$. Each line corresponds to one of the 1292 nodes of the finite element model.
Figure 5.17: Tests of parameters for equation 4.8: $\theta_C = -0.01$, $\theta_B = 1$. Each line corresponds to one of the 1292 nodes of the finite element model.

Figure 5.18: Tests of parameters for equation 4.8: $\theta_C = -0.01$, $\theta_B = 2$. Each line corresponds to one of the 1292 nodes of the finite element model.
in the number of osteoclasts present an increase, a few days later, to values close or above the initial value. The number of osteoblasts, in Figure 5.19(b), presents a behavior similar to the one described for osteoclasts: some nodes have an increase in the number of osteoblasts right after the beginning of the bone remodeling cycle, reaching values close to 350 cells, and then decreasing to values close or below the initial value, while other nodes show the opposite behavior. It is important to notice that, while in the case of osteoclasts the majority of the nodes show an initial increase of the number of cells, followed by a decrease after a few days, in the case of osteoblasts the majority of the nodes show an initial decrease in the number of cells. In the case of bone mass (Figure 5.19(d)) it is possible to see that the range of values of bone mass variation is closer to what would be expected, with the minimum value very close to zero and a maximum value of $1.332gcm^{-3}$ (which is closer to the expected value of $1.73gcm^{-3}$). In addition, the results show formation of a medullary channel, with higher values of bone mass in the diaphysis walls and values close to zero between them, and the formation of the Ward’s triangle. Figure 5.19(c) shows that the bone mass is not stabilized after 150 days.

Decreasing the value of $\xi_{BB}$ to 0.2 (Figure 5.20, with $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$ and $\xi_{BB} = 0.2$) decreases both the range of variation of bone cells and bone mass. In addition, their evolution seems to be slower, when compared to Figure 5.19, since the phenomena described for the variation of osteoclasts and osteoblasts seem to be less accentuated, and the variation of bone mass seems to be smoother.

In Figure 5.21, the $O_B$ parameter was decreased with respect to Figure 5.19. For this case, the parameter were set as: $O_C = -0.01$, $O_B = 1$, $\xi_{BC} = -0.1$ and $\xi_{BB} = 0.3$. As happened for Figure 5.20, the range of variation of both bone cells and bone mass is smaller when compared to Figure 5.19, however it is not as small as in Figure 5.20, which suggests that $\xi_{BB}$ has a stronger influence on the system than $O_B$. It is important to notice that some parameters have a cross-influence, being able to counteract the tendency verified when the tests were performed individually in each parameter.

In Figure 5.22 the parameter $\xi_{BC}$ was decreased, in modulus, to 0.05 ($O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.05$ and $\xi_{BB} = 0.3$). The results show that the range of variation of osteoclasts decreases in Figure 5.22(a) when compared to Figure 5.19(a), but the range of variation of osteoblast, in Figure 5.22(b), is wider when compared to Figure 5.19(b). In addition, the initial increasing bump on osteoblasts number for some nodes present in the previous analysis is not present in Figure 5.22(b). In the case of the bone mass, Figure 5.22(c) seems to show a smoother variation when compared with Figure 5.19(c), and Figure 5.22(d) shows an increase in the maximum value, when compared to Figure 5.19(d), but also an increase in the minimum value. Figures B.1, B.2, B.3 and B.4 correspond to the analysis presented, respectively, in Figures 5.19, 5.20, 5.21 and 5.22, for 250 days. The results show that after 250 days the bone mass still did not reach a steady-state.

5.3 Cancer Cells

To test the influence of the myeloma bone disease, together with the influence of the mechanical stimulus, two cases of tumor were considered (Figure 5.23), separately and combined. The system of
Figure 5.19: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days.

Figure 5.20: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$, $\xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days.
Figure 5.21: Tests of parameters for equation 4.9: \( O_C = -0.01 \), \( O_B = 1 \), \( \xi_{BC} = -0.1 \), \( \xi_{BB} = 0.3 \). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days.

Figure 5.22: Tests of parameters for equation 4.9: \( O_C = -0.01 \), \( O_B = 2 \), \( \xi_{BC} = -0.05 \), \( \xi_{BB} = 0.3 \). Each line corresponds to one of the 1292 nodes of the finite element model. Results for 150 days.
equations 4.11 describes the interactions between bone cells, with the influence of myeloma cells and the mechanical stimulus. The values assumed for the parameters of these equations are the same as before (Table A.2), plus: \( O_C = -0.01, O_B = 2, \xi_{BC} = 0.1, \xi_{BB} = 0.2 \). The cancer parameters are \( \gamma_T = 0.05, L_T = 100, r_{BC} = 0, r_{CB} = 0, r_{BB} = 0.2 \) and \( r_{CC} = 0.005 \) (Table A.3), which were set according to what is described by Ayati et al. [4], and the analyses were performed for 250 days, as the group considers that, under the influence of myeloma bone disease, a bone remodeling cycle takes about 200 days. Figure 5.24 shows the evolution of the tumor cells after 250 days, and it is possible to see that it reaches 100% of its size a few days after the remodeling cycle begins.

![Figure 5.23: Location of the two tumor cases on the femur model. Figure (a) corresponds to tumor 1 and Figure (b) to tumor 2.](image)

Figure 5.25 shows the solution of equations 4.11, without the influence of the mechanical stimulus \( (O_C = 0, O_B = 0, \xi_{BC} = 0 \) and \( \xi_{BB} = 0) \). As happened in Figures 5.2 and 5.3, the number of cells and the bone mass have the same value for each node. However, in this case, two situations are distinguishable: the cells that are not affected by myeloma, for which the evolution of bone cells and bone mass are constant and equal to the initial value through all the process, and the myeloma cells, for which it is verified an increase in the number of osteoclasts and a decrease in the number of osteoblasts, as was expected, and a consequent decrease in the bone mass.

![Figure 5.24: Evolution of the tumor.](image)
Figures 5.26, 5.27, and 5.28 are presented as a reference for each tumor case (Figure 5.26 for tumor case 1, Figure 5.27 for tumor case 2, and Figure 5.28 for both tumor cases). These analyses correspond to the same analysis presented in Figure 5.20, but in this cases the cells that will suffer the influence of the myeloma are highlighted in order to facilitated a posterior comparison of their behavior with, and without the influence of myeloma, and withdraw some conclusions about the effects of the disease.

Figures 5.29, 5.30, and 5.31 are the results for the system of equations 4.11, with the initial parameters presented in Table A.3 and $r_{CC} = 0.005$, which was one of the values proposed by Aytaì et al. [4] for this parameter. Comparing Figure 5.29, which has the results for the first tumor case, with Figure 5.26, it is possible to see that the number of osteoclasts in the nodes affected by the tumor (Figure 5.29(a)) increased, and the number of osteoblasts suffered a significant decrease (Figure 5.29(b)). Consequently, the values of bone mass for these nodes are lower in Figure 5.29(c) when compared to Figure 5.26(c). These results are according to what was expected, since the myeloma bone disease stimulates the formation and activation of osteoclasts, while inhibiting osteoblast action. Figure 5.29(d) shows a region of low density (close to zero) in the place where the tumor was set. This low density region corresponds to what was described in section 2.4 as the osteolytic bone disease. As in Figure 5.29(a), the number of osteoclasts in some nodes is still increasing after 250 days, analysis were preformed for 350 days (Figure B.5) and the results show that the number of osteoclasts starts stabilizing a few days later.

The results in Figure 5.30 correspond to the second tumor case. Comparing these results with the ones from Figure 5.27, the conclusions to withdraw are the same as the previous analysis: there is an increase in the number of osteoclasts in the nodes affected by the tumor, a decrease in the number of osteoblasts and a consequent decrease in the bone mass for the same nodes. However, in this case, there are more nodes reaching a higher number of osteoclasts (Figure 5.30(a)), than in the previous analysis (Figure 5.29(a)). In addition, the decrease in bone mass seems to be more pronounced. This suggests that the location of the second tumor leads to more devastating consequences than the location of the first tumor.

Figure 5.31 is a combination of both Figure 5.29 and 5.30. Comparing Figure 5.31(d) with Figure 5.28(d), it is notable the presence of two osteolytic lesions. Aytaì et al. [4] also preformed tests with $r_{CC} = 0.02$. The tests performed with this value (Figures B.6, B.7 and B.8 in Appendix) did not show significant differences when compared to the tests preformed with $r_{CC} = 0.005$, suggesting that the influence of this parameter in the system is weak. To support this hypothesis, tests were preformed with $r_{CC} = 0$ (Figures B.9, B.10 and B.11 in Appendix) and, once again, no significant alterations were noticed when compared to the results with $r_{CC} = 0.005$. However, increasing $r_{CC}$ to 0.2 (Figures 5.32, 5.33 and 5.34, with $r_{CC} = 0.2$) lead to some alterations in the results.

In the case of the first tumor, hen comparing Figure 5.32(a) with Figure 5.29(a), it is possible to see that the number of osteoclasts of the nodes affected by the tumor reaches higher values. On the other hand, the decrease in osteoblasts (Figure 5.32(b)) seems to be less accentuated than in the previous case (Figure 5.29(b)). Nevertheless, there seems not to be significant changes regarding the bone mass.

Comparing Figures 5.33, corresponding to the second tumor case, and 5.34, which is the combina-
Figure 5.25: Results for equation 4.11, without the mechanical stimulus: $O_C = 0$, $O_B = 0$, $\xi_{BC} = 0$, $\xi_{BB} = 0$, $r_{CC} = 0.005$.

Figure 5.26: Reference results for tumor case 1.
Figure 5.27: Reference results for tumor case 2.

Figure 5.28: Reference results for tumor case 1 and 2.
Figure 5.29: Results for equations 4.11 with tumor case 1 and $r_{CC} = 0.005$.

Figure 5.30: Results for equations 4.11 with tumor case 2 and $r_{CC} = 0.005$. 

(a) Osteoclasts number 
(b) Osteoblasts number 
(c) Bone mass 
(d) Bone mass distribution
Figure 5.31: Results for equations 4.11 with tumor case 1 and 2 and $r_{CC} = 0.005$.

Figure 5.32: Results for equations 4.11 with tumor case 1 and $r_{CC} = 0.2$. 

(a) Osteoclasts number
(b) Osteoblasts number
(c) Bone mass
(d) Bone mass distribution

(a) Osteoclasts number
(b) Osteoblasts number
(c) Bone mass
(d) Bone mass distribution
tion of both tumor cases, with Figures 5.30 and 5.31, respectively, it is possible to notice that the increase in osteoclasts is higher when $r_{CC} = 0.2$ (Figures 5.33(a) and 5.34(a)), and the decrease in osteoblasts is less accentuated (Figures 5.33(b) and 5.34(b)), similarly to what happened in Figure 5.32. However, in Figures 5.33(d) and 5.34(d), it is noticeable that the minimum value of the bone mass is lower when compared to Figures 5.30(d) and 5.31(d), which supports the conclusion that the location of the second tumor leads to stronger perturbations in the system than the location of the first tumor.
Figure 5.33: Results for equations 4.11 with tumor case 2 and $r_{CC} = 0.2$.

Figure 5.34: Results for equations 4.11 with tumor case 1 and 2 and $r_{CC} = 0.2$. 
Chapter 6

Conclusions

The development of models that reproduce the biochemical and biomechanical interactions between bone cells during the process of bone remodeling is important to better understand the behavior of bone under different circumstances, for instance, abnormal load carrying situations or diseases. The model proposed by Komarova et al. [1] proved to be a good representation of the biochemical environment of bone and of the activity of bone cells and their interactions. However, since this model does not include the influence of the mechanical stimulus, it fails in addressing the bone mass to places of higher demand, leading to a homogeneous distribution of bone density that does not happen in the biological environment.

The introduction of the mechanical stimulus on Komarova’s equations lead to a solution that was no longer homogeneous, with bone mass being addressed according to the mechanical demand. In these results it was possible to observe that bone was forming a medullary channel, with higher values of bone density in regions correspondent to the diaphysis walls, and values close to zero between them, which is in agreement with the biological situation. In addition, in most of these results, it was possible to observe the formation of Ward’s triangle on the femur’s neck. The tests performed adding the influence of the mechanical stimulus on the net effectiveness parameters and adding the influence of the osteocytes response to mechanical stimulus showed that, for some sets of parameters, the number of osteoclasts and/or osteoblasts follow a not so regular evolution and that, when this influence is added in parameters that directly affect osteoblasts, the evolution of bone mass is slowed down and does not seem to converge. More tests and biological studies would be important to understand these phenomena, and to understand how the concentration of bone cells evolve, throughout the whole process of bone remodeling, in different sites of the mechanically stimulated bone.

For the cases of tumors tested, the myeloma bone disease showed to have the expected influence on the affected nodes: there was an increase in the number of osteoclasts, followed by a substantial decrease in the number of osteoblasts, which consequently lead to a decrease in the bone mass. The analyses preformed suggested that the influence of the osteoclast autocrine cancer parameter in the system is weak, however, as mentioned for the mechanical stimulus, it would also be important to perform tests to the osteoclasts and osteoblasts autocrine and paracrine cancer parameters in order to
understand their isolated influence on the system. Additionally, the theory states that not only the cells of myeloma affect both osteoclasts and osteoblasts, but also osteoclasts have an important influence in the activation and proliferation of myeloma cells and, for this reason, the addition of a term dependent on the number of osteoclasts to the tumor equation should be considered. Moreover, as the myeloma cells seem to have a direct influence on both osteoclasts and osteoblasts, the effects of the addition of a term dependent on the myeloma cells, in the equations of bone cells, should be tested. The model should also be expanded to include the influence of the treatments for myeloma bone disease.

One limitation of this model is that it does not consider the possible phenomena of the migration of bone cells to different sites of bone. As future work, it would also be interesting to adapt the model to include the diffusion of both bone cells and tumor cells, similarly to what was done by Ayati et al. [4]. Moreover, it would be interesting to test the equations in different types of bones as, for example, the vertebral bodies or the ribs, as they are sites of red marrow and are the bones most commonly affected by multiple myeloma. Lastly, it would be important to test the influence of multiple loads on the bone.
Bibliography


Appendix A

Model Parameters

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Table A.1: Tests for $k_C$ and $k_C$ parameters.
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Table A.2: Initial values and parameters of the biomechanical chemical model.

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Table A.3: Initial values and parameters of the model with myeloma bone disease.
Appendix B

Additional Results

Figure B.1: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.
Figure B.2: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.1$, $\xi_{BB} = 0.2$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.

Figure B.3: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 1$, $\xi_{BC} = -0.1$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.
Figure B.4: Tests of parameters for equation 4.9: $O_C = -0.01$, $O_B = 2$, $\xi_{BC} = -0.05$, $\xi_{BB} = 0.3$. Each line corresponds to one of the 1292 nodes of the finite element model. Results for 250 days.

Figure B.5: Results for equations 4.11 with tumor case 1 and $r_{CC} = 0.005$. Results for 350 days.
Figure B.6: Results for equations 4.11 with tumor case 1 and $r_{CC} = 0.02$. 

Figure B.7: Results for equations 4.11 with tumor case 2 and $r_{CC} = 0.02$. 

(a) Osteoclasts number  
(b) Osteoblasts number  
(c) Bone mass  
(d) Bone mass distribution
Figure B.8: Results for equations 4.11 with tumor case 1 and 2 and $r_{CC} = 0.02$.

Figure B.9: Results for equations 4.11 with tumor case 1 and $r_{CC} = 0$. 

(a) Osteoclasts number
(b) Osteoblasts number
(c) Bone mass
(d) Bone mass distribution

(a) Osteoclasts number
(b) Osteoblasts number
(c) Bone mass
(d) Bone mass distribution
Figure B.10: Results for equations 4.11 with tumor case 2 and $r_{CC} = 0$.

Figure B.11: Results for equations 4.11 with tumor case 1 and 2 and $r_{CC} = 0$. 