PREDICTIVE MODELING FOR STUDYING THE DISEASE PROCESS OF OSTEOPOROSIS

by

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Background:

Osteoporosis exerts a burden on the national health services. Hip fractures cause significant pain, functional disability, and lengthy inpatient treatment. Therefore, prevention is of utmost importance for patients and physicians, as well as insurance payers and insurance providers [1].

Significance:

There are gaps in knowledge about the complex interactions among the multiple factors which control the disease process. Computational modeling can aid in understanding the intermingled relations between the various factors at different levels and provide further insight into the disease development mechanism.

Methodology:

In the first aim, we built a computational model using Agent-Based Modeling (ABM) to investigate osteoporosis disease's progression by simulating the interactions among the cellular and biochemical factors within the BMU and external factors such as weight, and physical activity. In the second aim, we added a therapeutic agent to predict the changes in patients who are receiving that treatment. In the third aim tested the model's ability to estimate the bone

density without the use of an initial DXA Scan reading. In the three aims, we validated the model by performing statistical tests to compare the model's predictions and the DXA scan readings from the patients.

Results:

The Paired Sample T-test results was statistically not significant $t(16) = -1.6$, $p = 0.12$ in first aim and $t(42) = 8.1$, $p = 0.28$ and in the second aim. The sensitivity was between 85.7% and 100%, and the specificity was 90% to 100%. In the third aim, the model successfully predicted the bone density in the first group (40-50 years age group) of patients Wilcoxon-sign (13), $p=0.196$. The model was not able to estimate the bone density for the other age groups.

Conclusion:

We successfully built an ABM that can predict the bone density changes in osteoporosis patients and patients who are receiving alendronate drug treatment. The model, however, requires further improvement and testing to be able to estimate the bone density as a diagnostic tool. We conclude our ABM model can be used in research for studying the process of osteoporosis and has the potential to be developed into a clinical diagnostic tool.

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1.0 INTRODUCTION

Osteoporosis and related fractures are a significant and growing cause of morbidity in the United States population and have been a significant health concern worldwide for a long time [1]. According to National Health and Nutrition Examination Survey (NHANES), adults of age 50 years and over suffer from osteoporosis of Femur neck & Lumbar spine in 5% and 6% respectively [2]. Osteoporosis exerts quite a burden on the national health services. Hip fractures, for example, causes significant pain, functional disability, and eventually extended inpatient treatment. Therefore, prevention is of utmost importance for patients and physicians as well as insurance payers and insurance providers [1].

The annual cost of osteoporosis-related fractures in the US elderly was estimated using a nationally based study to be $16 billion, using the same methodology to calculate the projected cost; the cost was expected to reach $22 billion in 2008 [3]. Predictive modeling also called "Computational modeling" is a collection of mathematical techniques performed for building a mathematical relationship between two or more factors [4]. Alternatively, predictive agent-based techniques use information extracted from basic scientific methods to create mechanistic relations among agents that ultimately simulate the mechanism and outcomes within that particular system [5]. Predictive modeling has been utilized for commercial purposes, for example: as analytical tools to analyze big data, companies can predict customers' preferences or

improve their profitability. Also, Analytical tools are now routinely employed in sales, marketing, and supply chain optimization [6].

In healthcare predictive modeling has been used to predict the incidence, mechanism, and progression of genetic and infectious diseases [7-11]. Also, predictive modeling has also been utilized for diagnosis, prognosis, and treatment of various diseases and health-related conditions [7, 10-16].

Dual-energy x-ray absorptiometry (DXA) is considered to be the “Gold standard” for diagnosing osteoporosis [17, 18].By building an agent-based model and using the DXA scan for validating the models performance, this model can aid healthcare professionals in predicting the risk of developing osteoporosis and changes in bone density. This model can serve as a tool to study the internal mechanisms of osteoporosis. It also can perform various virtual experiments to investigate the effects of therapeutic interventions on the internal factors of bone matrix or bone density. After validating the model, it can be introduced as a clinical tool to predict changes in bone density.

1.1 RESEARCH AIMS

This dissertation has three aims:

- **Aim 1:**

  In the first stage, we collect sufficient information about the disease mechanism and the factors involved in the disease process from the literature. Then we build a model using an agent-based modeling technique to simulate the disease progression. After designing a functioning model, we test the model's validity by comparing the results of the model's output with actual individual patients DXA scan bone reports. Also, during this stage, we will test the model's accuracy, sensitivity, specificity, and reliability.

- **Aim 2:**

  In the second stage, we aim to introduce a therapeutic agent into the model. Then, we will validate the model and test its ability to predict the effect of that therapeutic intervention on bone density changes. We will perform accuracy, sensitivity, specificity, and reliability tests to ensure that the model is stable and reliable after modification.

- **Aim 3:**

  In the last stage, we will test the model's ability to predict the first reading, in other words, to test the model's ability to predict the expected bone density based on the inputs that we feed it. There are no valid computational models' -as far as we know- that provide an estimation of the bone density and that can be used an alternative in the absence of DXA-scan machines. We will test validation of the model by statistically comparing its outputs to those obtained from DXA-scan reports from real cases. If the

model proves to be valid, we will perform other tests to measure accuracy, sensitivity, specificity, and reliability of the model.

1.2 RESEARCH SIGNIFICANCE

While the disease mechanism has been studied extensively through *in vivo* and *in-vitro* experiments, there are gaps of knowledge about the complex interactions among the multiple factors which control the disease process. Primary osteoporosis is a disease that is related to aging mainly, but other factors influence the disease progression in different patients. While some researchers concentrate on cellular and biochemical agents in the BMU as the main factors that control the bone density, other factors such as Gender, nutrition, weight, and physical activity can significantly affect the course of the disease [19-29].

Dual-energy x-ray absorptiometry (DXA) is considered to be the “Gold standard” for diagnosing osteoporosis and is recommended by the WHO for screening and diagnosing osteoporosis [30-32]. However, DXA scan only provides a snapshot of the bone health at the time of the exam and does not predict the progression of the disease. Also, the DXA scan or alternatives such as QCT or MRI machines are not available to all clinicians everywhere. Wet-labs experiments require a lot of funding, resources, and time. Also, unapproved medications cannot be tested on human subjects especially if the benefit is questionable with the risk present. There is a need for an alternative; a tool that can be used for testing the effects of experimental interventions and exploring the changes that occur in the bone while avoiding any harm to human subjects.

A computational model can be used to study and test theories about the interactions and roles of different factors in the BMU. The use of a computational model does not waste resources, and the experiment can be performed and completed within minutes. The model can perform any experiment using different interventions without the risk of harming animals or human subjects and with minimal financial cost. With the aid of computational modeling, the disease macro and micro processes can be analyzed virtually. While computational simulation is not a replacement for wet lab experiments, it indeed can provide valid results in much shorter time and less cost.

Computational modeling can also aid in understanding the intermingled relations between the various factors at different levels and provide further insight into the disease development mechanism [5, 33, 34].

This model after being validated clinically can also be used as a clinical tool that aids clinicians to predict the disease status and progression in specific patients. Such tool can be easy to use, inexpensive and be installed easily in most clinical settings. There were many efforts to create computational models to simulate the bone remodeling process, the bone healing process, and disease progression in specific conditions applying different technics: mathematical, statistical and agent-based modeling [35-42].

This model is unique because results of the simulations and cell numbers can direct researchers to target a specific mediator -such as MCSF or OPG -or proper time to start preventive measures or maximize the effects of therapeutic interventions’.

Osteoporosis as a disease depends on the outcome of the reaction between the cells within the BMU. Although most researchers agree about the importance of the roles of the BMU's different cell types in influencing the bone health, there are different opinions about the inflammatory mediators that control the cellular actions. Cytokines such as IL-1, IL-6, IL-17, and TNF-α were included in the literature each with supporting evidence that suggests a critical role for that cytokine in the disease process [43-47].

Our model, when validated, can be modified to test the theories behind each of those cytokines separately or in combination to see which one is the most important in the disease development. The model also can be used -when the proper values are available- to provide a threshold or a serum level in which that cytokine becomes actively involved in the bone remodeling process.

2.0 LITERATURE REVIEW

2.1 LITERATURE SEARCH PROCESS

The literature review done in this dissertation was divided in two stages. The following subsections explain how each stage was completed.

2.1.1 General Information About Osteoporosis Epidemiology & Histopathology

In this stage, our focus was to locate, and review information related to osteoporosis; to understand the disease mechanism and behavior. Since we aim to create a model to study the behavior of osteoporosis, it is mandatory for us to review detailed medical and scientific reviews that describe the disease epidemiology and explain the histopathology of the disease mechanism. We conducted a literature search using Google Scholar, and PubMed from 1990 to 2015, to identify studies examining bone histology, bone physiology, epidemiology, and histopathology of primary osteoporosis.

In our literature search, we used the terms “Osteoporosis pathogenesis,” and, “bone histology,” “bone physiology,” “Osteoporosis,” “Osteoporosis Epidemiology,” and “osteoporotic fractures.” We restricted the search to articles in English language only, to avoid confusion that

may happen after translations. Also, we used cross-referencing, by going through articles from relevant original research or review articles.

We also searched the mentioned search engines using the previous terms separately and using conjunction operators (OR & AND). Primary sources for those reviews and reports were scientific journals and specialized professional associations' including but not limited to WHO, NIH, CMS, and the CDC.

2.1.2 Information About the Use of Modeling for Medical Purposes in General And Osteoporosis Specifically

We searched google, google scholar, and PubMed using the search terms: "modeling in healthcare," "computerized modeling in healthcare," "agent-based modeling & healthcare," "Osteoporosis in-Silico" and, "agent-based modeling for osteoporosis".

We also examined the above search engines using the previous terms separately and using the conjunction operators (OR & AND). Some essential references were located and chosen through cross-referencing from relevant papers or reports that were selected from search results.

Selection criteria: The following table includes the selection criteria that we applied for choosing references that were used in the literature review:

Table 1: Reference selection criteria for step 2.

Criteria	Purpose
1. Article or Report is published in a scientific journal or a well-known professional source. (Mandatory)	To ensure credibility and authenticity of any information used in the project.
2. Selection of the most recent publications (past five years). (Mandatory)	Including up to date information.
3. Cross-referencing (Preferred)	To choose papers that are highly cited which provide rich and valuable information.

2.2 BONE HISTOLOGY AND PHYSIOLOGY

In this section, we will review basic bone histology and physiology information which is essential to better understanding the mechanism of the natural bone tissue development in the human body.

2.2.1 General Information About the Bone

The human skeletal system is formed mainly by the bone that provides the framework and serves for supporting the weight of the body as well as protecting the vital internal organs. The bone houses the red marrow that produces all kind of blood cells through hematopoiesis [48].

There are four general categories of bones: long bones such as, femur and tibia; Short bones include the carpal and tarsal bones. While Flat bones examples include the skull, and mandible; and Irregular bones like vertebrae, sacrum, and coccyx [49]. The bone weight approximately constitutes 15% of total body weight for males and 12% of females [50].

The bony skeleton is vital for many functions including:

1. Physical supporting function for the whole body frame.
2. Works in tandem with the muscular system to allow for precise and smooth body movements.
3. Shields the internal vital and sensitive organs.
4. It also works as storage for minerals, growth factors, and inflammatory mediators.
5. Protects the main tissue that performs hematopoiesis that is the red marrow [49].

2.2.2 Bone Histology

The bone at a histological level is built into two structural organizations: cortical bone, and trabecular bone. Bones in the body are formed from both types of bones in varying ratios.

The "osteon" which is the primary functional and building unit of the bone constitutes both the cortical and trabecular bone. Cortical bone and trabecular bone are naturally developed in a lamellar formation, where collagen fibrils are arranged in alternating alignments. Perhaps the alternating alignments of the collagen contents are the reason behind the significant sturdiness of the bone [49].

2.1.1.1 Bone various histological structures: Cortical bone forms 80% of the total bone structure. Dense and stable in composition, the cortical bone basic building unit – or osteon – is called the Haversian system. Haversian systems are cylindrically shaped structures that form a branching framework within the cortical bone [49]. The Haversian systems wall is

designed of lamellae aligned and encircling the Haversian canal. It also serves as a tunnel that protects nerve fibers and the accompanying blood vessels to the interior of the bone's cavity [49, 51]. Amplified cortical remodeling results in porosity and reduction in cortical bone mass, naturally occurring in aging healthy individuals' where the bone cortex suffers thinning accompanied by increased porosity [49].

Trabecular bone composes 20% of the total bony skeleton. Trabecular bone has greater elasticity and turnover, but less density than cortical bone. The trabecular bone osteons also called (packets) are built in a semilunar organization, with multiple layers of concentric lamellae [49, 52].

2.1.1.2 The Bone Matrix: Type I collagen is the core organic component of the bone matrix by constituting 90% of the organic composition. Collagen fibers are organized either in parallel fashion in trabecular lamellae or concentric manner encircling the Haversian canal as a part of the Haversian system [52]. The bone matrix also contains a multitude of none-collagenous proteins that were found recently to have significant roles –mechanism still under research - in bone mineralization [53]. Calcium **hydroxyapatite** crystals are deposited between the collagen fibers to strengthen and stabilize the bone. About 99% of the calcium in the body is stored in the bone [22, 25, 51, 52, 54-56].

2.1.1.3 Basic Multicellular Unit BMU: BMU is the structural and functional unit in the bone in charge of bone formation and resorption. It is comprised of osteocytes, osteoblasts,

and osteoclasts [22, 28, 44, 45, 51, 54, 57, 58]. It was estimated that normally there are about 1 million active BMUs in an adult human skeleton [59].

2.1.1.4 Osteocytes: They are basically osteoblasts that are entrapped within the osteoid. Osteocytes compose 90% of the cellular matrix but have a limited role in matrix production. Osteocytes have an essential role in bone homeostasis, through adaptation of bone to mechanical stress and hormone-mediated mineralization [52, 54, 60-63].

2.1.1.5 Osteoblasts: Osteoblasts are the primary building units of the bone, producing most of the bone matrix components. Osteoblasts are organized in clusters along the lining of the layer of bone matrix which they create [52, 54]. Osteoblasts secrete essential growth factors including insulin-like growth factors (IGF), transforming growth factor beta (TGF-β), and bone morphogenetic proteins (BMP). Those growth factors regulate the activity of the osteoblasts. Osteoblasts surface receptors include receptors for hormones such as the Parathyroid, Thyroid, and Progesterone hormones. Significant nuclear steroid receptors are also located on the osteoblasts that have a major role in the bone health such as vitamin D3 and estrogen and androgen surface receptors [52].

Bone formation is an ongoing process that runs through three main phases: production, matrix maturation, and matrix mineralization. In the normal physiological state, those three phases run at a consistent rate. Osteoblasts build the osteoid by rapid secretion and deposition of collagen. Immediately after that, the mineralization starts within the collagen fibers. In the

maturation phase, collagen fibers synthesis comes to a halt while the mineralization continues until the bone tissue is properly mineralized [52, 54].

2.1.1.6 Osteoclasts: Osteoclasts are multinucleated giant cells; their diameter can reach up to 100 mm. Osteoclasts are the main cells responsible for bone resorption [52, 54]. Osteoclasts attach to the bone surface by small active binding structures called (Podosomes) through the actions of integrin [64, 65]. After binding of Osteoclasts to the bone matrix, they produce lysosomal enzymes such as acid phosphatase and Cathepsin-K, which marks the initiation of bone resorption by breaking down the hydroxyapatite crystals which are covering the collagen fibrils [52, 54, 64]. Next, collagen fibrils are digested by Cathepsin-K and activated collagenases [52, 54]. Osteoclasts activity and bone resorption are regulated locally by cytokines and systemically by hormones [52, 54].

2.2.3 Bone Physiology

The bone reacts to physiological or mechanical stress by changing its shape through the modeling process. Bones are modified in width and become sturdier due to the activities of osteoblasts' or osteoclasts to tolerate the stress of biomechanical forces [49, 52].
The active periosteal surface is vital for the process of bone growth and repair. Bone growth typically exceeds bone resorption; consequently, with aging bones naturally increase in thickness. The endosteum, however, has greater remodeling activity than the periosteum; which is probably due to the greater exposure to cytokines produced in the neighboring Morrow, as

well as the mechanical stress. In contrast to the periosteum, the endosteum Bone resorption rate exceeds the bone formation causing marrow space to expand with aging [49, 52].

2.1.1.7 Bone remodeling: Bone remodeling is an ongoing physiological process. Damaged or old bone is continuously replaced by a newly built healthy bone [51, 52]. Between 5 and 10% of the human skeleton is replaced per year [66].

Remodeling is a cyclical process that runs through three stages: resorption followed by a reversal and finally bone formation. Osteoblasts and osteoclasts work in balance with the aid of cytokines and hormones to continue running the remodeling cycle. Undifferentiated Pre-osteoclasts migrate to the area targeted for remodeling where they join forming giant multinucleated osteoclasts that start resorption of the bone, a stage that may extend up to two weeks. After the resorption process is complete, the reversal stage starts by pre-osteoblasts migration into the targeted area and differentiation into active osteoblasts; this stage typically lasts from four to five weeks. Osteoblasts start building new bone tissue (formation stage) until they replaced all of the resorbed bone; bone formation is the longest stage as it extends up to four months after which the process is complete, and then bone enters a resting stage that continues until the next remodeling cycle [52, 54].

2.3 OSTEOPOROSIS

2.3.1 Information About The Disease

Osteoporosis is a systemic condition that affects the whole bone skeleton weakening the bone mass and microarchitecture and resulting in bone fragility and many cases, fractures [19, 23, 57, 67]. Osteoporosis can either be primary osteoporosis that is due to aging-related hormone sex hormonal deficiency, or secondary; which occurs secondary to other systemic inflammatory diseases such as rheumatoid arthritis or inflammatory bowel disease [19, 45, 55, 68].
It is estimated that more than 200 million osteoporosis case among women worldwide [58]. In the United States and Europe, approximately 40% of Caucasian postmenopausal females suffer from osteoporosis, with the life-long elevated risk of spine fractures up to 50% [23, 69]. Males aged 50, and above have an increased risk of 20% for suffering an osteoporosis-induced fracture. Osteoporosis prevalence in the state Pennsylvania has reached -according to the C.M.S- 7.5% of the total state population in 2012 [70].

2.3.2 Osteoporosis Disease Mechanism

Bone formation and resorption is a process that runs continuously within the Basic multicellular unit (BMU). In the ordinary setting, bone formation and resorption are operating in balance, which results in removing the old bone and replacing it with a new healthy bone. When that balance is disturbed in osteoporosis, the resorption overwhelms the formation, because of that; the bone becomes fragile and brittle or Osteoporotic. Several factors trigger and stimulate

osteoporosis by disturbing the mentioned balance in the absence of secondary systemic conditions, including the acute phase of estrogen deficiency during early stages of menopause, medications that suppress sex hormones in both genders, and smoking [19, 23, 28, 57, 67, 71]. Activation of Osteoclasts is usually accomplished through the interactions of hematopoietic precursors with osteoblasts or by inflammatory cells, such as leukocytes or T-lymphocytes. Macrophage colony-stimulating factor or (M-CSF) which is either membrane-bound or secreted by inflammatory cells, stimulates the differentiation of osteoclasts progenitor-monocytes in the bone marrow into pre-osteoclasts. Pre-osteoclasts express the Receptor Activator of Nuclear Factor κ B known as (RANK). Next, the RANK receptor that can couple with a cytokine produced by osteoblasts and inflammatory cells, known as RANK Ligand or RANKL [25, 45, 52, 54, 56].

Coupling of RANK with RANKL activates osteoclasts that merge into multinucleated giant cells that produce Acid phosphatase and Cathepsin-K that break down the bone's hydroxyapatite crystals and collagen fibrils. The hormonal imbalance that stimulates the maturation of osteoclasts also reduces the rate of osteoclasts apoptosis and reduces activation of osteoblasts, in other words, extended bone resorption and limited or no bone formation[19, 22, 23, 44, 45, 52, 55-58, 68, 69, 71]

2.4 FACTORS INVOLVED IN OSTEOPOROSIS DEVELOPMENT

2.4.1 Hormones

Reduction of estrogen in females in menopause and testosterone in males after the age of 50 triggers the stimulation of bone resorption. Estradiol (a form of estrogen) has a protective effect on the bone in both genders, as it preserves the bone matrix in a healthy state [19, 44, 72-74]. In females, the risk of fracture increases with time, as the risk of fracture increases for Caucasian females from 15% at the age of 65 to 35% at the age of 85 [19, 21, 67, 75].

Estrogen also inhibits the maturation of osteoclasts and promotes osteoclasts apoptosis, on the other hand, it preserves osteoblasts by inhibiting RANK and stimulating bone formation [6, 19, 21, 45, 76, 77]. Estrogen suppresses RANKL production by osteoblasts and T helper cells; it also stimulates the production of OPG [19, 22, 54, 67].

The Parathyroid hormone (PTH) can cause severe bone fragility in hyperparathyroidism. However, when used in therapy, it was found to preserve osteoblasts and stimulate their bone formation activity [21, 54, 67, 78].

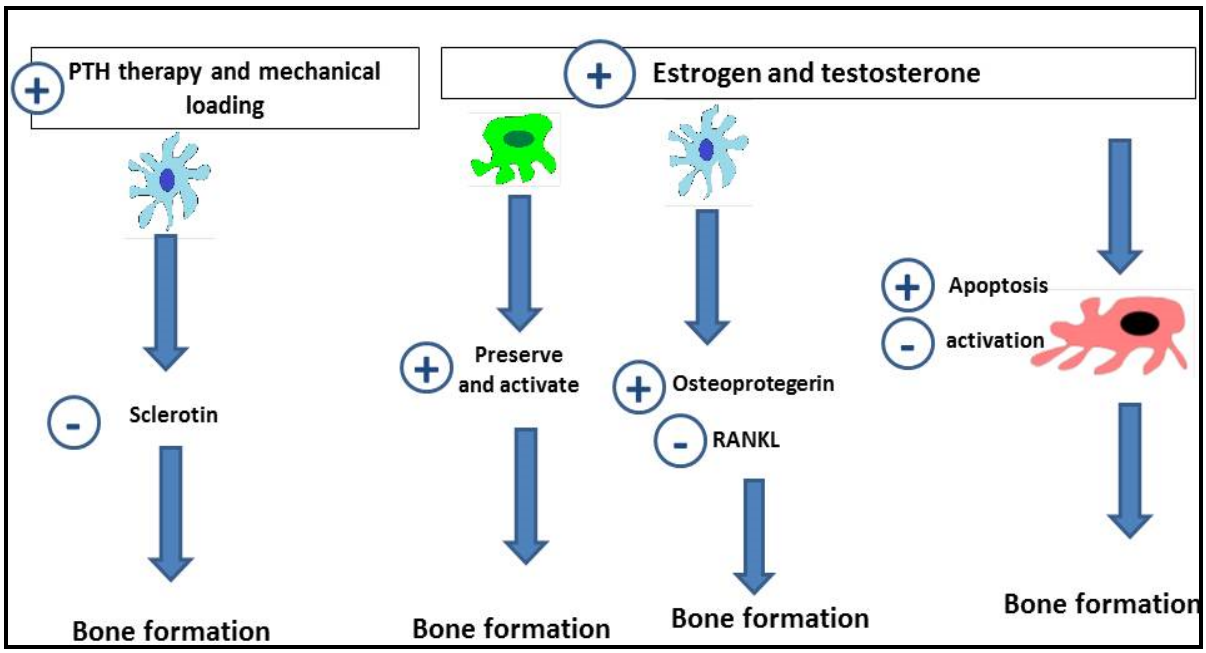

Figure 1: Mechanisms that promote bone formation: Blue cells represent osteocytes, green are osteoblasts, and pink cells are osteoclasts.

2.4.2 Chemical Agents Within The BMU

2.4.2.1 RANK, RANK-L, and OPG: Receptor activator of nuclear factor-κ B ligand (RANK-L) is a cytokine produced by the osteoblasts. RANK-L binds to its receptor activator of nuclear factor-kB (RANK) present on the surface of osteoclasts causing osteoclasts activation and ultimately bone resorption [21, 25, 44, 45, 52, 72, 77-79]. Osteoprotegerin or (OPG) is another cytokine produced by osteocytes, OPG binds with the RANK competing with RANK-L, which results in inhibitor of osteoclastogenesis [21, 25, 28, 44, 45, 52, 56, 63, 72, 77, 79]. OPG also binds to RANKL reducing its half-life and promoting RANKL shedding [80].

2.4.2.2 M-CSF : Macrophage colony-stimulating factor or (M-CSF) which is produced by Osteoblasts binds to a surface receptor present on the osteoclasts precursors promoting the maturation of osteoclasts and increasing their numbers [19, 21, 44, 52, 54, 72, 79, 81]. Serum

M-CSF level increases with age slowly as it is measured in individuals in their twenties to be about 12 ng/mL up to 20 ng/mL in the eighties of age. The age-related elevation of serum M-CSF was closely related to age-induced bone resorption rate that reached up to four folds the resorption rate of young individuals [82].

2.4.2.3 Acid phosphatase and Cathepsin-K: Activated osteoclasts release Acid phosphatase and Cathepsin-K breaking down the bone's hydroxyapatite crystals and collagen fibrils and resulting in bone resorption [23, 25, 26, 28, 44, 52, 56, 57, 68, 72, 77, 79].

2.4.3 Cellular Factors of the BMU

2.4.3.1 Osteocytes: Osteocytes are critical in regulating the process of bone resorption and formation by producing mediators that control the activity of osteoclasts and osteoblasts [21, 25, 54-56]. Osteocytes were found to produce interferon-β that inhibits osteoclasts differentiation [83]. Osteocytes produce Osteoprotegerin (OPG), an inhibitor of osteoclastogenesis [21, 54, 84-86]. Osteocytes also produce Sclerostin, which inhibits osteoblasts bone formation activity [21, 54]. Mechanical loading and PTH effect osteocytes which causes inhibition of Sclerostin secretion by osteocytes which ultimately will preserve the bone matrix [21, 54, 78, 87].

2.4.3.2 Osteoblasts: Osteoblasts are the primary cells responsible for bone formation as they build the bone matrix by producing collagen, the main protein that composes most of the organic matrix. Reduction of osteoblasts activity is directly linked to bone fragility in primary and

secondary osteoporosis [19, 21, 25, 28, 52, 55, 67]. Osteoblasts activity can be stimulated by some hormonal factors such as estrogen and parathyroid hormone (PTH) [23, 78]. Osteoblasts can be inhibited by hormones such as high levels of PTH in hyperparathyroidism, or chronic glucocorticoids treatment [23, 25, 30, 57, 67, 69].

2.4.3.3 Osteoclasts : Bone resorption is carried out by osteoclasts, in osteoporosis, the number of osteoclasts is increased and are found to be more active [19, 21, 23, 51, 55, 69].Osteoclasts secrete H+ ions that acidify the resorption compartment causing the mineral component (hydroxyapatite) of the bone matrix to dissolve. They also secrete the cathepsin K enzyme which digests the organic matrix, which is mainly built from type I collagen [25, 49, 52].Osteoclasts are negatively influenced by estrogen, as it induces apoptosis and reduces their activity [19, 22, 57, 72, 88-90].

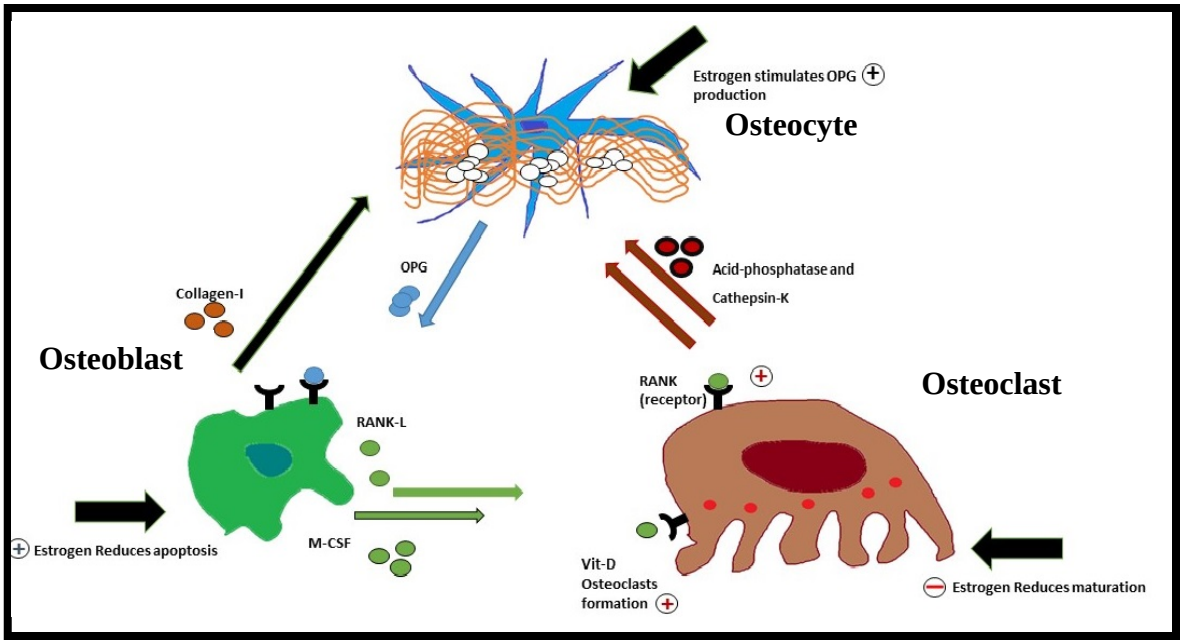

Figure 2: interaction between the cellular and chemical factors within the BMU. The Osteocyte (blue cell) surrounded by collagen fibers and calcium form the healthy bone matrix. Osteocytes controls the activity of the other two cells by reacting to damage or stress and releases factors that may increase or reduce the bone density. The active osteoblast (green cell) lay down collagen type one to build the bone matrix, but also produces factors (RANK-L,M-CSF) that stimulate the maturation and activity of osteoclasts. Osteoclasts produce enzymes that break down the bone matrix causing bone resroption.

2.4.4 Age and Gender

Bone mass density (BMD) decreases with age, which consequently causes an increased risk of osteoporotic fractures. There was an approximately a 50% difference in BMD of females who are aged between 20 and 90 years old [19, 30, 67]. The lifetime risk of sustaining an osteoporotic fracture in males is estimated to be between 13-20% while in females can reach up to 50 % [30, 67]. BMD decreased among patients aged 20 to 90 was greater in females than in males, as it was measured to be 39–55% for females and 34–46% for males [91].

2.4.5 Vitamin D

Vitamin D is essential for intestinal absorption of calcium that is used for bone mineralization and strengthening the bone density [20, 21, 25, 69, 78]. Reduction of 25-hydroxyvitamin D serum level below 30 ng/ml indicates a Vitamin D deficiency, which leads to increased PTH in secondary hyperparathyroidism, and ultimately osteoporosis [20, 29].

2.4.6 Body Mass Index

Studies found that Body mass index (BMI) is inversely related to osteoporosis and bone fracture risk [92-96]. High BMI (over 30 Kg/M^2) in patients over 70 years of age provided them with 33% reduction of femoral neck osteoporosis in comparison to their peers who have ideal or low BMI [94].

2.4.7 Serum Biomarkers

Biomarkers which are products of bone formation/resorption are measured in plasma, or urine can be used to monitor or estimate bone formation/resorption rate in patients with normal renal function [97-100]. N-telopeptide of bone type I collagen or (NTX) is raised in association with bone resorption and increased osteoclasts activity [97, 98]. Bone formation and osteoblasts activity cause elevation of bone-specific alkaline phosphatase in serum [97, 98].

Osteocalcin elevation in the serum may indicate bone formation or resorption [97, 98]. Procollagen type I amino-terminal propeptide (s-PINP) rises in serum as a reaction to bone formation [24, 99, 101-103]. Serum carboxy-terminal cross-linking telopeptide of type I collagen

(s-bCTX) is increased in relation to bone resorption [24, 99, 101, 102]. s-PINP and s-bCTX are recommended for screening by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [99, 102]. There is no biomarker that can be used as a gold standard to diagnose osteoporosis. However, the biomarkers recommended by IOF and IFCC can be used in detecting and monitoring the response of osteoporosis to treatment [99, 102-104].

2.5 DIAGNOSING OSTEOPOROSIS

2.5.1 Clinical Assessment

A clinician can identify the patient as a high risk for the disease by obtaining a detailed medical history from the patient and asking about the following risk factors: menopausal status for females or age over 50 years' old, and over 60 years old of age for males. The age when osteoporosis is diagnosed can also be related to factors such as smoking, vitamin D deficiency, poor physical activity, and other factors [27, 30, 71, 105]. The physician may ask questions about causes of secondary osteoporosis such as Hyperparathyroidism, or intake of glucocorticoid drugs [20, 106, 107].

2.5.2 Laboratory Tests

Blood tests may show low serum Vitamin-D, low serum calcium, or bone turnover markers that result from the bone remodeling process. Such tests can be used to give an idea about the bone's general health condition but cannot be used to diagnose osteoporosis or guide the treatment plan of the disease [99, 101, 102, 104, 108, 109].

2.5.3 Bone Density Tests

Radiological examinations remain the most reliable none-invasive method to determine the bone density and estimate the risk of bone fracture. Bone mass density tests that are commonly used in clinical practice include dual-energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT) [19, 23, 31, 32, 45, 57, 68, 110]. The DXA bone scan, however, is the test recommended by the world health organization and the national osteoporosis foundation because of the acceptable accuracy and reliability of the DXA test [31, 110, 111].

2.5.4 The DXA Scan

The DXA scan operates by sending radiation using a radiation source towards a detector positioned directly behind to the anatomical site to be assessed. The patient lies on a table that is placed between the radiation source and the detector. The radiation beam then passes through the targeted area and into the detector. The reduction of the radiation

beam which is due to resistance and uptake by the tissues is measured and then used for calculating the bone mineral density. The DXA scan machine produces two X-ray beams with different energy levels which are directed at the soft tissues and bone within the site for the test. Digital subtraction of the soft tissue absorption is performed; after which the BMD can be determined from the amount of radiation absorbed by the bone [110, 112].

The DXA scan report includes Bone mineral density measured by g/cm^2, the T-score, and the Z-score. The T-score compares the patient's BMD to the BMD mean of healthy 30-year-old individuals. The Z-score compares the patient's BMD to the mean BMD of peers of the same age. WHO recommended uses the T-scores as the standard for comparison in the osteoporosis classification. The T-score is the number of standard deviations closer or further from the reference value, T-score of Zero means that the subjects BMD is Ideal and equals the reference value. The T-score is calculated using the following formula: (patient's BMD – mean BMD of 30 y/o healthy subject) ÷ SD [30, 32, 110, 113, 114].

Table 2: DXA scan results and its clinical interpretation as in the WHO osteoporosis guidelines [66-68]:

DXA scan's T-score	Interpretation.
+1.0 to -1.0	Normal bone mass density
-1.0 to -1.5	Slightly lower BMD than normal
-1.5 to -2	Moderate reduction of BMD
-2 to -2.5	High reduction of BMD with Moderate risk for fracture
-2.5 and below	Severe loss of BMD with High risk for fracture

2.5.5 Online and Computer-Based Tools

Some researchers adopted the use of osteoporosis risk factors such as (age, gender, race) for creating different clinical tools that calculate the probability of bone fracture [115]. Among those tools is FRAX, which was developed by the University of Sheffield as a screening tool to estimate the risk of bone fracture in particular areas of the bony skeleton. FRAX was based on population cohorts' studies from different populations worldwide to calculate the bone fracture risk by processing several risk factors. Much simpler -still quite useful - tools were developed by researchers for screening and self-assessment purposes' that use age only or age and BMI for estimating the risk for osteoporosis [115-117].

2.6 OSTEOPOROSIS TREATMENT

2.6.1 Treatment Options

There are many treatment options available that include lifestyle adjustments, oral supplements, oral or intravenous medications. Each of the treatment options has its advantages and disadvantages. In the following subsections, we will explore the treatment options available thus far for managing osteoporosis.

2.6.2 Lifestyle Adjustment and Supplements

As previously mentioned in our literature review, smoking has adverse effects on bone health as it was found in studies to reduce the bone density. However, the damaging effect of Cigarette smoking on bone health is highly variable [27, 105, 118].

Maintaining a healthy body weight and performing weight-bearing exercise help reduce the bone resorption process. Underweight and bed-ridden or inactive individuals are more likely to have a weaker bone matrix and suffer pathological fractures [21, 28, 57, 119]. National Osteoporosis Foundation (NOF) and IOM advise the intake of 1000 mg/ day of calcium for men over 50 years old, and 1200 mg/day for women over 51 years and men over 70 years of age. An intake of Calcium over the recommended amount is not associated with documented improvements but may cause side effects such as kidney stones [17, 120]. Vitamin D is unquestionably essential for preserving the bones health and the body in general [20, 29, 57, 121-123].

As a preventive measure, a Vitamin D is an intake of 800 IU to 1000 IU per day after the age of 50 is suggested by the NOF for maintaining bones healthy [17, 120]. Vitamin D deficiency must be treated as soon as discovered to prevent further deterioration in the bones density. Vitamin D deficiency is treated according to the severity of the condition; the patient may be treated with Vitamin D injections and oral supplements in order to reach a blood level of 30 ng/ml [17, 120, 124]. The efficacy of Vitamin D as a treatment for osteoporosis is highly variable among different populations. Patients' who suffer from osteoporosis and have vitamin D- deficiency are advised to do weight-bearing physical exercise in addition to vitamin D intake to have a better outcome [29, 57, 120, 121, 123]. Vitamin D alone may

have no effect on improving the bones health in some individuals, but when Vitamin D is combined with physical exercise was found to improve the bone health between 1-2% per year [20, 29, 67, 122-125].

2.6.3 Current Medications Used

One of the most frequently used drugs groups for treating osteoporosis are Bisphosphonates. Bisphosphonates drug group operate by inhibiting the recruitment of osteoclasts and inducing osteoclasts apoptosis. This process ultimately reduces the bone matrix resorption and preserves the bone density [126]. Of the Bisphosphonates, Alendronate is the most clinically used and studied in the literature. Alendronate was proven to reduce the bone loss in post-menopausal women and to improve their bone density. However, Alendronate's efficacy for treating osteoporosis depends on the dose, duration of treatment, and the individual's response to treatment. Alendronate when used as a treatment for osteoporosis for a duration of ten years, resulted in bone density improvement by 2-3.9% [127-130].

In another study alendronate was used in different doses: 5 mg, 10mg, and 20mg daily for three years had variable improvements levels of bone density in various parts of the bony skeleton that ranged from 2.2 to 8.8 % [128]. For patients with osteoporosis, Alendronate reduced the risk of hip fracture when used for three years by 50%, by 36% in the femoral neck, and reduced the risk of vertebral fractures by 49% [120, 131, 132]. Other examples of Bisphosphonates available in the market and used for treating osteoporosis are Ibandronate, Risedronate, and zoledronate [127, 128, 133, 134].

Estrogen hormonal therapy is an effective but also a risky alternative for treating osteoporosis and preventing pathological fractures. Although Estrogen therapy was proven to reduce the risk of bone fractures by 30-50%, it is also associated with increased risk for severe medical conditions such as Myocardial infarction, pulmonary embolism, and breast cancer [120, 135, 136].

Parathyroid hormone is used for treating male and female osteoporosis patients with high risk for pathological fractures [19, 78, 133, 137]. Recombinant human parathyroid hormone (1–34) or Teriparatide (Drug name) is effective in reducing the risk of osteoporotic fractures by 53 to 65% [120, 137]. It functions by reducing the osteoblasts apoptosis, which results in higher rate of bone formation [38, 78, 133, 137, 138].

Abaloparatide which is a synthetic peptide analog of human parathyroid hormone-related protein or (hPTHrP), is used in an injectable form for treatment of severe osteoporosis with a high risk of osteoporotic fractures [139, 140].

An estrogen-like agent (Tibolone) is tissue-specific, is used for reduction and prevention of postmenopausal bone loss. Tibolone currently is not approved for use in the United States [120, 141, 142].

Another medication used for treating osteoporosis is Denosumab, which is a RANKL/RANKL inhibitor. Denosumab is a recombinant human Immunoglobulin-G2 antibody with an affinity and specificity for binding to RANK-L. As it prevents the osteoblasts RANKL/RANK interaction, it reduces the activation, activity, and lifespan of osteoclasts [52, 79,

80, 133, 143-145]. Treatment with Denosumab for three years reduces the risk of osteoporotic fractures in different areas of the skeleton by 20% to 68%[120, 133].

2.6.4 New Treatments Under Evaluation

Romosozumab is a humanized monoclonal antibody that blocks the action of Sclerostin, which will result in increased bone mass. Romosozumab still in phase 3 of the drug evaluation [87, 146-148].

Genistein which is extracted from soybeans is an isoflavone which is a class of phytoestrogens. Genistein improves the bone mass by stimulating osteoblasts activity and inhibiting osteoclasts resorptive activity [120, 149, 150].

2.7 COMPUTATIONAL MODELING APPLICATIONS IN BONE HEALTH RESEARCH

2.7.1 Predictive Computational Modeling In Different Professions

Computational modeling has been used in different professional fields for making predictions and serving many different purposes for over fifty years. Computational modeling has been in use for predicting the weather forecast since the 1950s'. The experts who were using historical information collected over decades created equations and fed the data to the computational units and computers to predict the expected changes in the weather over time. Later, computational models became more advanced, and with their rules defined and better refined, they provided

predictions with accuracy that reaches over 80% for 36 hours predictions [151]. For marketing and other commercial uses, predictive modeling has been integrated into analytical tools to analyze big data. Companies can predict customers' preferences based on their choices and create offers or send ads to users. Healthcare insurers and providers aim to maximize control of healthcare costs, so they use computational models to predict the most effective and efficient intervention based on the recorded insurance costs [152-154]. Currently, Analytical tools are now routinely employed in sales, marketing, and supply chain optimization [6].

In healthcare, predictive modeling has been used to predict the incidence, mechanism, and progression of genetic and infectious diseases [7-11]. Also, predictive modeling has also been utilized for diagnosis, prognosis, and treatment of various diseases and health-related conditions [7, 10-16]. Those models can aid practitioners and policymakers in their daily work, especially when faced with limited data, having a model to help analyze the limited data is better than having minimum raw data [153, 155, 156].

2.7.2 Computational Modeling for Studying Bone Health

There have been many research efforts in implementing computational modeling for the study of cellular bone regeneration, tissue healing, bone-specific diseases, and the mechanism of bone fracture in particular areas of the bony skeleton [35, 36, 39, 41]. Many of the research projects were directed towards understanding the effect of stress exerted on the BMU and how it orchestrates the activity of the cellular and chemical components to regenerate or enforce the bone matrix [35-37, 39, 41, 42].

Different techniques were used to build computational simulation models for studying bone health and disease processes. Mathematical, statistical, and agent-based models were applied for

predicting and studying the bone regeneration and resorption processes [37-39, 157]. There are many models created using the three approaches. We will include examples of each approach to demonstrate the uses of each approach.

2.7.3 Mathematical Models

Researchers apply the use of mathematical equations to create computational models to simulate changes in the bone matrix and the forces or chemical factors that cause those changes. Researchers created mathematical models to investigate many topics such as simulating the changes in the vertebral bone during health and disease conditions, bone adaptation to physical loading at a macroscopic level, and the effect of disruption of the horizontal trabeculae of the bone matrix on the bone strength [37, 41, 158].

2.7.4 Examples of Mathematical Models

Different hypotheses were tested, one of which was that the strength of the bone structure deteriorates due to disruption of the horizontal trabeculae of the bone matrix which results from strain-adaptive resorption. To test this hypothesis three simulations were analyzed, in the first set of simulations was involved strain-adaptive resorption of the bone, in the second set micro-damage resorption was added in the simulation, and in the third set, the damage threshold was adjusted to increase as the damage to bone matrix progresses. After running the simulations and analyzing the outcomes, Mc.Donnel and his group concluded that strain induced adaptive resorption causes the horizontal trabeculae of the bone to break down leading to perforations in

the bone matrix; these perforations cause the breakdown of the vertical trabeculae and further deterioration of the bone matrix [158].

Another group of researchers tested three different methods for studying bone remodeling: Ordinary differential equation (ODE), stochastic model of the ODE, and Piecewise Multi-affine (PMA) modification of ODE. Creating a computational model based on differential equation modeling, Bartocci and his colleagues managed to simulate the process of bone remodeling while including estimated and calculated values of the cellular and chemical factors in the BMU. They concluded that computational equation modeling could be used to simulate the bone remodeling process and when improved by adding patient-specific values, such models may be clinically useful [41].

Concentrating on mechanical strains as a central cause for osteoporotic fracture, López et. Al. Developed a computational model using partial differential equations. The model created simulated the BMD changes in subjects who are not under any therapeutic interventions and in those taking different drugs for osteoporosis. The research was focused on the neck of the femur bone. Although the model was not validated, the authors claimed that this model could be used after clinical validation and modifications for predicting bone fracture risk in specific patients or for predicting the outcome of specific therapies [159].

To evaluate the physical resilience of vertebral bone and predict the outcome of vertebral cement augmentation, a group of researchers created a computational model that analyzes the vertebral bone geometrical properties and isotropic permeability before and after the augmentation process. That model served as a guiding tool to predict the outcome of vertebroplasty where the augmented material is injected into the damaged or weakened vertebral bone to strengthen it and reduce the pain. Widmer and his colleagues successfully created a valid

three-dimensional model that simulates the changes in vertebral bone microstructure. They concluded that their model could be used to predict the long-term stabilization of the vertebra post-cement augmentation treatment and aid in choosing the best site for the therapeutic injection [160].

Based on a previous algorithm to simulate the process and outcome mechanical loading on the bone cells, Christen and colleagues improved their algorithm to test the effect of hypoparathyroidism on bone remodeling. They hypothesized that Hypoparathyroidism increases osteocytes mechanosensitivity and reduces the bone turnover. After developing the algorithm, it was validated by comparing the results with bone biopsy results collected from the iliac bone of human cadavers and participating patients who were diagnosed with hypoparathyroidism. The results confirmed their hypothesis and were consistent with clinical data. They concluded that hypoparathyroidism could increase the cellular mechanosensitivity up to 40% and that hypoparathyroidism, when accompanied by mechanical loading, explains the increased bone mass and preservation of bone tissue [161].

2.7.5 Statistical Models

Statistical techniques used in modeling are Statistical shape models (SSM), and statistical appearance models (SAM). SSM simulates the bone shape while SAM is used to simulate the density. SSM and SAM which is also called statistical density modeling can be used in combinations and be known as Statistical shape and density modeling or SSDM [162, 163]. Both SSM and SAM, depend on training using patterns of data extracted from the mapping of anatomical landmarks on a specific bone surface such as the proximal femur. Radiological images

such as X-ray or DXA, are used for training the 2-D models while CT and MRI images are used to build 3-D models. Active Shape Models (ASM) and Active Appearance Models (AAMs) are Algorithms that are developed for fitting the statistical model's shape and appearance to the shape and appearance of the images [162-165].

2.7.5.1 Statistical models' examples: group of researchers created a model that compares and fits the bone surface landmarks information from the patterns of data to QCT images obtained for the subject. The model then generates a 3-D picture of that area. The model compares the shape and density of the head of the proximal femur of individuals that suffered a fracture in that area and others that did not have a fracture; then it provides a prediction of fracture risk according to the differences in surface shape and density distribution [165].

Whitemarsh et al. created a model that simulates the shape of the bone to create a 3D image of the proximal femur by training the model to recognize the bone shape landmarks from X-ray images. This model 3D image provides a better visual presentation of the bone shape and structure to allow for better prediction of hip fracture risk [166]. Waarsing and colleagues built a model to estimate the relation between clinical osteoarthritis and the density or shape of the proximal femur bone surface. Using the DXA-scan images for the patients, they trained the model to recognize the differences in bone shape and density between individuals who have symptoms of osteoarthritis and individuals who are asymptomatic but have radiological signs of the condition [167].

2.7.6 Agent-Based Modeling

Agent-based modeling or ABM can simulate behavioral mechanisms of biochemical and cellular interactions as observed in natural biological processes. As agents interact with each other in the simulation, each group of agents interacts with the other factors or groups based on sets of rules programmed into the model. The rules used in the model are supported by values and interactions mechanisms that are directly extracted or inferred from scientific literature [5, 34, 168].

The purpose of ABM simulation is to build of groups of agents and mimic their interactions in a hypothetical (virtual) environment, in order to perform an *in-silico* experiment. ABMs do not depend on patterns of data like most the mathematical or computational biomedical models. ABMs do not apply the inductive logic of scientific inquiry because it starts with the rules for interactions and attempts to recreate the recorded or scientifically observed outcomes by computing the interaction between the agents according to the behavioral rules [33, 34, 169].

ABM employs the concept of "*parallelism*"; a concept that is built on the logic that each agent's class manifests several behavioral routes that connect to other agents' operating within the same virtual environment. Each agent within the agent class expresses a distinct sort of behavior and operates concurrently with the other agents' -in parallel routes- which will affect the result of the cumulated interactions between the agents in that trajectory. The parallelism concept is expressed by the different cells in the BMU. An example would be the osteoblasts: each cell manifests variable activity levels based on its response to estrogen among other factors.

The end result will be the amount of bone formed based on the aggregated outcomes of all the osteoblasts operating in that area [5, 19, 21, 22, 168].

ABM simulation which is an *in-silico* experimental process has an advantage over wet-lab approaches because of the ABMs' ability to create complex and large datasets in the low-cost virtual environment. A model can be designed to perform an in silico-experiment in a few minutes that simulates a similar in-vitro/in-vivo experiment that requires days or months and a much higher cost to complete [5, 33, 34, 169].

2.7.7 ABM For Bone Research

ABM was recently introduced in bone research when some researchers used ABM for creating models to study the bone fracture and healing processes. Schutte created an ABM simulation of Cellular signaling and coupling of the BMU which was used to analyze the bone regeneration processes. Two ABM models were designed and built to study osteocytes signal for controlling bone resorption, and osteoblasts coupling to osteoblasts. The models were validated by comparing Z-scores produced by the models to Z-scores obtained from cases in the literature [157].

Bayrak and colleagues focused on Mesenchymal stem cells (MSC) as a promising solution for tissue engineering applications. They applied ABM to examine the joint impacts of growth factors and biomaterials on MSC differentiation and cell survival as well as their contribution to bone density. Studying the survival of stem cells implants within the bone tissue in-vivo is challenging mainly because of the variable rate and outcomes of which the cellular

differentiation of implanted cells and vascularization of the bone tissues scaffolds. Vascular endothelial growth factor (VEGF) and bone morphogenic protein-2 (BMP-2) were selected after searching the literature as the most important factors that were included in the simulation. Building on a previous model designed by the same group to simulate endothelial cells behavior during angiogenesis, the new model included the movement of MSCs' within the polymeric scaffold and their differentiation into osteoblasts. The authors managed to create a 3D model that simulated the MSCs' behavior during the osteogenesis process, and the results were that VEGF loading improved the vascularization of the structural scaffold and contributed to stem cells survival.

They concluded that the simulations results were consistent with the information available in the literature and that ABM can be used to effectively to examine the joint stimuli of growth factors and biochemical factors on MSCs' development and survival. [170].
Other researchers used ABM to analyze hematopoietic stem cells (HSCs) behavior in order to understand bone marrow homeostasis better. HSCs' differentiate into all kinds of cells that constitute the blood and populate the bone marrow. The mechanism of cellular preservation and behavior of HSCs' is still unclear and determining such behavior can be very helpful in tissue repair research. Kurhekar and Deshpande utilized ABM to build a model to simulate the behavior of HSCs' and how it differentiates into transitive cells and how it proceeds to apoptosis. Their model was an improvement to a previous mathematical model created by Agur et al. [171].The result was a 2D model that simulated the HSC division and mobility as it populates the bone marrow. Although their model was not validated, it was an interesting step to model HSCs' which provided further insight into the HSC conversion into other blood cells and the process of HSC's apoptosis [172].

2.8 GAPS IN KNOWLEDGE

The general concept of Osteoporosis as far as the diseases mechanism, diagnosis, or prognosis is well described in the literature. However, there are many pieces of information missing that need to be uncovered for a better understanding of the disease mechanism at micro-processes levels. Cellular and chemical factors that interact within the BMU are explained by different theories and medical opinions that sometimes contradict each other. The values of each of the chemical mediators within the BMU or the bloodstream are mostly unavailable. Cellular Receptor affinities and critical levels which may cause the disease the progress or regress are unknown.

At a clinical and public health level: FRAX can provide an estimation of fracture risk for patients based on information gathered from public health studies, and there are self-assessment tools that provide patients with simplified estimation of their bone health using a small number of factors in their calculation. However, there is a need for a tool that can simulate the complex bone tissue environment and disease progression over time while bringing together the scientific theories that explain the disease process. Such tool can be used for researching new drugs, testing theories about bone remodeling factors and micro-processes. Also, after clinically validated this tool can provide an assessment or a prediction in a simplified report that can be easily understood by clinicians' and their patients'.

3.0 AIM ONE BUILDING AN AGENT-BASED MODEL TO SIMULATE OSTEOPOROSIS

3.1 INTRODUCTION

In this section, we will describe the implementation of the first aim of this dissertation, that is to create an agent-based model to simulate the osteoporosis disease process and predict the changes in the disease condition as the time passes. This section includes the model's design and justification of the design's method, agents and their behavioral rules, parameters values and validation of the model.

3.2 THE MODEL'S DESIGN

In order to create a model that simulates the progression of osteoporosis, we need to choose the proper method of modeling that can process the different variables involved in the disease process and capture the possible outcomes of interactions between those variables.

In comparison to tools that rely on patterns of data to predict the risk for bone fracture or osteoporosis in a particular area of the body based on patterns or mathematical equations, our

model should simulate the bone health using the cellular and biochemical interactions in the BMU's environment. Our model should also include the relations of those interactions to external factors that exert an indirect effect on them such as the patient's weight, physical activity in addition to others [21, 37, 61, 93]. Our model will include complex interactions between the cells and mediators in the BMU and the external factors which ultimately control the bone remodeling process and the progression or regression of bone health.

Osteoporosis is a dynamic and highly complicated process; the simulation will include several interactions within the BMU function through multiple layers of receptors activation that affect cellular activity levels. Within each type of cells, there are different possibilities of activity levels, as well as different receptor affinities and variable lifespans. The process of the disease is described explicitly in the literature. The literature, however, does not provide precise numbers/levels of the mediators within the BMU or the extent by which each mediator affects the cells involved. Therefore, we need to design our model to accommodate the wide variety of factors with different behavioral patterns. Such factors are running in a continuous manner and at different levels while applying randomness within the interacting factors and the outcomes. We chose agent-based modeling design to build our model because it is the best method that can reasonably simulate the disease process which is as complex and dynamic as osteoporosis.

3.3 DESIGN JUSTIFICATION

Agent-based modeling has been used by the researchers to simulate disease conditions and biological processes in the past decade with great success [5, 170, 172, 173]. The reason behind

that is that ABM applies many concepts that are valuable to simulate dynamic biological processes like wound healing, bone remodeling, and acute or chronic inflammations. Among those principles that provide ABM with advantages over other modeling methods are spatial incorporation, parallelism, stochasticity, and others which we will discuss in this section [5].

ABM being originally grid-based, can express simulations in a two-dimensional grid or easily create the simulation environment within three or six dimensions according to the requirements' and purpose of the model built. Because of such flexibility, clinicians who understand the process of the condition to be simulated can build models that have complicated logical relations without difficulties. ABM allows for to more instinctive knowledge translation into models or clinical tools that can be deployed, attuned to data, and then validated and used for patient care [5, 170, 172].

A significant advantage that ABM brings to our model is "parallelism" which will serve greatly in adequately simulating the changes in the bone matrix. ABM regards each agent class to have several stages as parts working together forming a whole collection of agents that interact inside a virtual, parallel processing environment. Similar to cells in the BMU, changes in factors interacting with each agent, exert direct control over the behavioral routes of involved individual agents. Also, comparable to the diverse behavior of individual cells in the BMU, agents within the Agent-based model move in various behavioral patterns which are expressed by multiple levels of agents. Agent-based modeling has the power to provide the results of the aggregated system dynamics into a meaningful visual output [5, 157, 168, 170, 172].

Stochasticity or random behavior and unpredictability is another concept that agent-based modeling is capable of simulating. Biological systems or conditions such as wound healing or bone resorption may appear to the observant eye as unpredictable, but there are deterministic rules that control that seemingly random behavior. Moreover, although such dynamic systems have rules that -if known- can be used to calculate the outcome in a mathematical equation, it is almost impossible to determine such rules from observation alone especially that the initial parameter values can be highly diverse which will also diversify the process routes and the outcomes.

Agent-based modeling solves this problem by creating populations of the different agents within the model. The probability of a specific behavior is determined for the population as a whole, which will determine the probability function for the behavior of a single member (agent) of that population, and next identify the behavioral rules for that agent. As the simulation progresses, each agent follows a unique trajectory of behavior while the behavioral rules' probability is adjusted for the whole population. This technique allows for the creating a broad range of behavioral outputs for every agent-based model, ultimately building a virtual system where agents are acting similar to their counterparts in the biological system under observation [5, 157, 170, 172, 173].

A distinct property of agent-based models is that they generate new behavioral trends through the interaction between the agents within the simulation, those innovative patterns cannot be extrapolated from inspection of the rules of the natural process. This unique property of agent-based modeling is called "emergent behavior," it can be helpful in the model's design, and validation process, but it can also break some of the processes leading the model to crash.

An example from our topic is the affinity of cells (Osteoblasts) towards a mediator (OPG), as the affinity to bind with OPG changes with the simulation due to the changes in the virtual environment and the functionality of the other agents interacting with the osteoblasts creates an unexpected behavior which results in higher osteoblasts surface receptor affinity causing the depletion of the OPG within the virtual space and resulting in the simulation to malfunction and stop [5, 157, 170, 172, 173].

An agent-based model can be designed and function properly even in with some missing information of the process under simulation. This property is highly valuable when constructing models for biological processes because there is -in many cases- a lack of complete knowledge about the details of those processes. This useful property allows the designers to simplify the rules which will make it easier later to verify the model. While mathematical modeling methods require details and exact numbers in order to create a sound simulation of a particular process, Agent-based modeling can overlook some details or exact numbers by performing qualitative verification of the possible outcomes and comparing it to the real outcome in the actual scenario.

The stochasticity and emergent behavior properties in combination with mapping the model to known details of the biological process allow for fairly simulating the biological process outcomes and the unpredictability or randomness that occurs within the processes. This property is very valuable in our topic, because there are a lot of missing details about the numbers of surface receptors, quantities of mediators, rate and range of activities for the cells within the BMU, and many other details that cannot be possibly obtained from the literature [5, 21, 34, 108, 157, 169, 170, 172, 173].

An agent-based model operates under control by rules of behavior of the agents within it; it has a “Modular design.” Having such design allows for adding a new class of agents or removing a group of established agents by modifying the rules that pertain to the group involved without going through the process of rebuilding the whole model. Such advantage is quite useful in our research because that makes our model flexible and beneficial for testing the effects of new treatments under experimentations or emerging biochemical factors that may change the scientific perception of the disease process [5, 157, 170, 172].

Biomedical researchers’ and healthcare professionals can translate their knowledge into agent-based models with minimal difficulty, even without programming skills or complex mathematical equations because agent-based modeling is object-oriented [5, 157, 173]. As mentioned previously, osteoporosis is a complex and highly dynamic biological process. Such process although well described in the literature, still, there are gaps in information about exact numbers of cells within each part of the process, the amount of chemical mediators and their cell surface receptors for each mediator, and other numbers that are age or gender specific. In addition to that, osteoporosis involves -to the observer- a high level of stochasticity, and various behavioral routes for all agents within the natural process [21, 25, 55, 71, 72, 113, 174, 175].

Based on the above attributes and advantages of ABM, we carefully chose ABM as the most suitable method for designing a computational model to simulate osteoporosis. Our agent-based model that has a modular topology will be used by researchers’ for testing therapeutic interventions in-silico, it can be easily modified later to include new interventions as (agents) or

removing involved cells or mediators according to the latest advances in disease research or changes in clinical knowledge.

3.4 MODEL'S DESIGN

In following subsections, we will discuss the software used to build the model, the agents, parameters and their values, rules of behavior for each agent, and the components in the user's interface.

3.4.1 Software

We used NetLogo version 5.2.1 which is an open-sourced freeware, developed and continuously improved by professor Uri Wilensky at the Center for Connected Learning and Computer-Based Modeling, Northwestern University [176].

NetLogo runs on Java virtual machine and is compatible with most major operating systems (Mac, Windows, Linux, and others). It provides a flexible virtual environment for modeling complex systems and simulating their progress over time. It is capable of processing simulations that involve thousands of agents, running their courses and allow the user to observe the behavior of each agent at any specific instance of the simulation. This ability allows it to express the emergent behavior which is a unique property of agent-based modeling [176, 177].

NetLogo has been used to create agent-based models to simulate biological and disease processes successfully [5, 177, 178].

3.4.2 Basic Concepts of The Model's Design

Our model simulates the progress of Osteoporosis process in distinct steps or (Ticks), each tick executed represents a simulation of the disease process in one-day duration. The simulation outcome is a result of the behavioral interaction between the biochemical factors that are (enzymes, inflammatory mediators, or hormones) and cellular components that form the BMU of the bone. Parameter values and interaction rules that we used in creating our model were based on rules and values that were extrapolated from previous osteoporosis models or calculated from values provided in the literature [25, 35, 37, 38, 54, 56, 97, 99, 170, 179].

The model simulates the behavior of a BMU in the bone matrix as it progresses with time, BMU is constituted from Osteoblasts that are the bone "constructors," their increase in number and or activity reflects means more bone density. Osteoclasts are bone "breakers," their increase in number and or activity reflects a reduction of bone mass, and Osteocytes which are the primary cells present in the bone matrix, more osteocytes mean denser matrix. Osteocytes form 90% of the cellular matrix leaving about 10% for osteoblasts and osteoclasts. It is estimated that for every osteoblast there are ten osteocytes. In the natural condition, the bone formation is in equal balance with bone resorption. Which brings the BMU ratio to 400 (osteocytes) :40 (osteoblasts) :4 (osteoclasts) [23, 25, 37, 49, 51, 54, 56].

We designed the model to simulate primary osteoporosis development in individuals who have a healthy immune system and are free of genetic or systemic conditions that affect the bone matrix or bone growth such as osteopetrosis or renal osteodystrophy [180, 181]. Each “Tick” within the model represents an advance in time of one day, with changes in agents’ values and bone health calculated and provided for the user to view at the end of each tick. In our model we assume that the BMU in the simulation represents the activity of the BMUs in the body, in other words, we assume that all the BMUs’ are acting in the same way.

Like In-Vitro methods of studying the progress of the disease, our model simulates the development of the disease within a closed environment. Our model, however, may provide a better understanding of the disease process. While In-Vitro experiments cannot explain many reactions that occur within the living organism because many cellular and biochemical factors lose their viability when removed from the natural environment. Our model simulates the disease body.

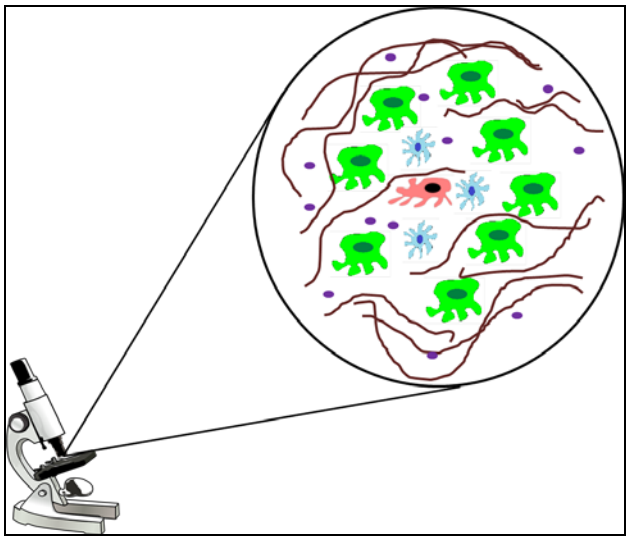

Figure 3 concept of the model: the bone multicellular unit within the bone matrix surrounded by collagen fibers and the mineral component (hydroxyapatite).

3.4.3 Agents Involved in The Simulation

We have selected a group of agents to include in the simulation; the selection was based on information from the literature about the most important factors involved in the disease process. As the mentioned in the literature, there are many mediators that are involved in the bone formation and resorption. We avoided the factors that have questionable importance or unproven relation to the disease process. The following table includes the agents involved in the simulation, their roles, and their rules of interaction with other agents in the model.

Table 3: A list of the agents included in the model.

A simplified list of the interacting agents, and external factors	
Agent	Manner of interaction
Osteocytes	• Osteocytes regulate the process of bone formation and resorption. • Osteocytes produce Sclerostin, which inhibits osteoblasts bone formation activity. • Under the effect of estrogen, Osteocytes produce OPG that inhibits bone resorption.
Osteoblasts:	• Osteoblasts are the main cells responsible for bone formation. • Activated osteoblasts produce collagen type 1 fibers. • Collagen fibers are used to build the healthy bone matrix. • Osteoblasts are buried under the newly formed matrix and convert to osteocytes. • Osteoblasts produce RANKL that promotes Osteoclastogenesis.
Osteoclasts:	• Bone resorption is carried out by osteoclasts. • Osteoclasts secrete acid phosphatase and cathepsin K enzyme which digest the organic bone matrix. • Estrogen negatively influences osteoclasts.
RANKL	• Receptor activator of nuclear factor-kB ligand (RANK-L) is a cytokine produced by the osteoblasts. • It binds to the receptor activator of nuclear factor-kB (RANK) present on the surface of osteoclasts increasing their activity. • Estrogen inhibits RANKL release.

Table 3 continued	
OPG	• Osteoprotegerin or (OPG) is a cytokine produced by osteocytes. • binds with the RANK competing with RANK-L, which results in inhibition of osteoclastogenesis. • Estrogen stimulates OPG release from osteocytes. • Binds to RANKL promoting the breakdown of RANKL and inducing shedding or inactivation of RANKL.
Sclerostin	• Osteocytes produce Sclerostin, which inhibits osteoblasts bone formation activity. • Sclerostin increases steadily with age. • In females over 60-year-old, Sclerostin increases up to 2.4 times the original level measured in the twenties of age. • In males over 60-year-old, Sclerostin increases up to 4.6 times the original level measured in the twenties of age. • The standard Sclerostin level is higher in males than females.
M-CSF	• Macrophage colony-stimulating factor or (M-CSF) which is produced by Osteoblasts • binds to a surface receptor on present on the osteoclasts precursors promoting the maturation of osteoclasts and increasing their numbers. • Estrogen reduces the M-CSF level.
Collagen-I	• Activated osteoblasts produce collagen • A key component in the organic matrix.
Acid phosphatase and Cathepsin-K	• Activated osteoclasts release Acid phosphatase and Cathepsin-K. • Those enzymes break down the bone's hydroxyapatite crystals and collagen fibrils resulting in bone resorption.
Factors	Effect on the simulation
Sex hormones: Estrogen & testosterone.	• As individuals grow in age, their sex hormones (testosterone and estrogen) drop gradually. • Sex hormones stimulate bone formation and inhibit bone resorption by increasing the activity and lifespan of osteoblasts • Reduces the activity of osteoclasts and lifespan.
BMI	Body mass index is positively correlated with bone density; higher BMI means denser bones.
Smoking	• Smoking reduces the active estrogen level in both males and females. • Smoking negates the therapeutic effect of Hormonal replacement therapy. • The effect of smoking on osteoblasts and hormonal activity is dose-dependent. • It reduces the collagen production from the osteoblasts
Physical activity	▪ Weight-bearing exercise Improve the bone density and stimulate the bone formation.

3.4.4 Parameters Values and Agents' Rules of Interaction.

In the following subsections, we will discuss details about parameters, agents and their behavioral rules for each agent within the model.

3.4.5 The Bone Mineral Density (BMD)

BMD is demonstrated in the model by a percent value as compared to that of the BMD of a healthy individual that is 100%.

The DXA-scan provides two types of measurements to demonstrate the bone mass density:

1. T-score: which compares the patient's bone density reading with that of a healthy young adult.
2. Z-scores: compares the patient's bone density reading with other people that match the patient in any of the following characteristics: age, gender, and race [111].

We used the T-scores obtained from DXA scan as the reference for validating the model's output value of BMD. Our model can be modified to simulate the changes in different areas in the bony skeleton. The version that we are working on in this dissertation simulates the changes of bone density in the proximal femur which includes the neck, Trochanteric, and Inter-trochanteric areas is also called Total-Hip [18, 182-184]. We have selected the proximal femur because 50% of it is formed of cortical bone which is typically have a lower turnover than that of the trabecular bone. Also, this area of the body is clinically important because fractures -Hip fractures- in that area causes higher morbidity and mortality [49, 185-189].

Creating a model to predict the changes in the vertebral bone is in our future plan because the vertebral bone is formed mostly from trabecular bone which makes the turnover much higher than that of the head of the femur. The high turnover of the vertebral bone, as well as the variation of response to treatment between different patients, poses a challenge for building the models rules. Such model will require sufficient data from different samples with the proper sample size.

In Our model, the agents interact with each other and with the bone mass density causing reduction or increase in the BMD. The output in our model is called “bone health” because the model simulates the progression of osteoporosis with age and provide and diagnosis or (health status) proper to that level of bone mass density. Any change in T-score by 0.1 corresponds to change by 1% in the model. The following table shows the BMD values of T-scores in the DXA-scan report and how those values are reflected in the model, and clinical condition that corresponds to that level of bone health level.

Table 4: Conversion between the T-scores used in the DXA scan report and the bone health that appears in the model's output [190].

DXA scan T-score	**+1.0 to 0**	**-0.5**	**-1.0**	**-1.5**	**-2.0**	**-2.5 or less**	**Less than -2.5+history of osteoporotic fracture**
Model interpretation	**100%**	**95%**	**90%**	**85%**	**80%**	**75%**	**Less than 75% + history of fracture**
Bone condition	**Normal from (100% to 86%)**			**Osteopenia from 85% to 76%)**		**Osteoporosis from (75% to 70%)**	**Severe Osteoporosis less than 70%.**

3.4.6 Agents in The Model

3.4.6.1 Osteoblasts: Osteoblasts in the model are in the active mature form. A fixed number of osteoblasts is set at the beginning of the simulation, however; the number of osteoblasts may increase or decrease according to the outcome of the reaction between the other factors. Considering the broad range estimation of Osteoblasts age in the literature 2-100 days, we will assume that the osteoblasts lifespan in our model is 50 days [46, 62]. One in ten osteoblasts may transform to an osteocyte per day within 20 days or continue functioning until it expires [54, 62]. Each osteoblast increases bone density by 0.25% per day [37]. It is estimated in the literature that there are ten osteocytes for each osteoblast in the bone in normal condition [62].We will assume that there are 40 active osteoblasts in each BMU.

3.4.6.2 Osteoclasts: Osteoclasts average lifespan is 14 days after which it expires [46, 190]. Osteoclasts in the model are in the mature active form. Each osteoclast reduces bone density by 2.5% per day [37]. A fixed number of osteoclasts is set at the beginning of the simulation, however; the number of osteoclasts may increase or decrease according to the outcome of the reaction between the other factors. Because of the bone production and bone breakdown are in balance, we will consider the number of osteoclasts in our model to be four cells for each BMU.

3.4.6.3 Osteocytes : Osteocytes lifespan reaches 25 years [63]. The number of osteocytes in a BMU is 369.9 ±101.0; therefore, we will consider each BMU to have 400 osteocytes [191]. More Osteocytes in our model do not signify stronger bone; it is, however, an essential part of

the BMU because they influence both osteocytes and osteoclasts. A fixed number of osteocytes is set at the beginning of the simulation. The number of osteocytes may increase or decrease according to the outcome of the reaction between the other factors.

3.4.6.4 Sclerostin : Osteocytes produce Sclerostin, which inhibits osteoblasts bone formation activity [21, 54]. The baseline value for Sclerostin serum level that we use in our study is 30 pmol/L [192]. Because Sclerostin serum level increases with age, in our model it is increased by one pmol/L every year [147, 192]. An increase of Sclerostin serum level above average by 30% is associated with increased risk for pathological fractures by 45% [193]. Sclerostin serum level more than 75 pmol/L is associated with increased risk of fracture, and the risk of fracture may increase up to 15 times compared to patients with average normal Sclerostin serum level [194].

3.4.6.5 Estrogen Premenopausal serum level 30 to 400 pg/mL [195]. Postmenopausal women with serum level under 5 pg/mL have twice the fracture risk of cases with serum level of 5-25 pg/mL [196]. Although the age at which menopause occurs is highly variable and depends on different factors, we will consider that the average age of menopause is 51 [197]. Postmenopausal normal serum level is 0 to 30 pg/mL [195]. Estrogen replacement therapy range is between 40 – 60 pg/mL [196].

3.4.6.6 Smoking: Smoking reduces the level of estrogen in the blood and diminishes the protective effect that estrogen provides for the bone. Smoking also nullifies the therapeutic effects of hormonal replacement therapy [27, 105]. Non-smokers, when compared to smokers who smoked ten cigarettes or more, had higher estrogen levels by 25-35% [105].

3.4.6.7 Age and gender : Bone mass density decreased among patients aged 20 to 90 was greater in females than in males, as it was measured to be 39–55% for females and 34–46% for males [91]. In our model, we assume that the bone density decreases by 0.7% per year for females and 0.5% for males. Bone density starts declining in the forties of age [190]. Bone mass density is reduced by 1-2% annually in postmenopausal women. This reduction is noted in women who were postmenopausal for five years [92].

3.4.6.8 Physical activity: Mechanical loading and physical activity have various positive effects on bone health, depending on the type of activity, gender, and age [54, 72, 119, 198].

3.4.6.9 Body mass index: Studies showed that underweight individuals have a greater risk of lower BMD and osteoporosis [96, 199, 200]. Each increase in BMI by one unit reduced the chance of BMD loss by 12% [92]. In Men, a BMI of > 25 is associated with more than four times lower risk of developing osteoporosis [201]. Although there is a high positive correlation between BMI and BMD, the exact mechanism by which a higher BMI contributes to higher BMI is unclear [96, 199, 200].

3.4.7 Table of parameters of agents and external factors

The following table contains detailed explanations about each agent or external factor, the parameter values, and the source references used to select the values for the model.

Table 5: Parameters used in designing the model, their range values and their sources from the literature. The rules and values in this table are the ones that we used in the latest working version of the model.

Parameter values for agents in the model and external factors that affect the simulation			
Agent description	Parameter	Reference value(s)	Source
Osteocytes	Number, lifespan	400 cells/BMU. Lifespan = 25 years. Reduction of bone health by 1% is associated with a reduction of osteocytes by the same percentage.	[46, 191]
Osteoblasts	Number, lifespan, conversion rate	40 cells/BMU. Lifespan = 50 days. Every osteoblast has a chance to convert to osteocyte by 10% for the first 20 days only. Every 50 days 40 new cells enter the BMU.	[46, 62]
Osteoclasts	Number, lifespan. RANK receptors.	Four cells/BMU, lifespan is 14 days. Each osteoclast has 2326 receptors that may bind to RANKL or OPG units. Every 14 days four new cells enter the BMU.	[38, 46, 60, 62, 65]
RANKL	Serum level per Osteoblast / Serum level BMU/ RANKL units per cell/RANKL receptors per Osteoclast.	• Average level Per BMU: 0.00006 ng/dl per BMU • Consumption per day = 0.00003900702 ng/dl per BMU. If receptors do not consume this amount, it will join the amount accumulated in the BMU. • At the start of the simulation, An amount of the 0.6*(starting level) ng/ dl enters the BMU level enters the BMU daily. The amount enters the BMU reduces with age by 1% per year. • High resorption level per BMU: (0.000064 - 0.00008 ng/dl per day). • Estrogen level is negatively correlated with RANKL: Level changes with by -/+ 1% in correspondence to every +/- 1% change of estrogen level. • Number of RANKL/OPG receptors per osteoclast = 2326. • Each RANK receptor has 70% chance to bind to either OPG or RANKL. • Each Osteoclast RANK receptor has a 65% chance of binding to a RANKL particle. • RANKL units (free or attached to receptors) have a half-life of 300 days, every 300 days 50% of the RANKL produced expires. • Every change of RANKL by ± 1% for 0.000064 ng/dl for each BMU results ± 1% receptor affinity by 1%. •	[38, 59, 80, 89, 143, 145]

Table 5 continued			
		• Every 50 RANKL surface receptors activated will reduce the affinity by 1%. • Each RANK surface receptor requires an amount of RANKL of 0.0000000064 ng/dl to be activated. • Each Osteoclast surface receptor occupied increases the production of Acid phosphatase and Cathepsin-K by 0.004% for the cell activated. • Every surface receptors of an osteoblast occupied by estrogen results in a reduction of RANKL production by that cell by 0.001%.	
OPG	Serum level per osteocyte/ Serum level BMU	• Production rate per BMU is (0.0000000765 - 0.000000191 ng/dl), High resorption rate range is (0.00000004675 - 0.00000011875 ng/dl). • Normal level Per BMU: 0.0000764 ng/dl. • Each osteocyte produces an amount of 0.0000002 ng/dl per day. • High resorption rate range per BMU is (0.0000187 - 0.0000475 ng/dl) • An amount of the 0.5*(starting level) enters the BMU daily. The amount enters the BMU reduced with age by 1% per year. • Estrogen level is positively correlated with OPG: Level changes with by +/- 1% in correspondence to every +/- 1% change of estrogen level. • Every 1% reduction in the RANKL receptor for RANK affinity results in an increase of 1% affinity towards the OPG particles. • Each Osteoclast RANK receptor has a 35% chance of binding to an OPG particle. • OPG units (free or attached to receptors) have a half-life of one week, every seven days 50% of the OPG units produced expire. • Consumption per day = 0.00002670248 ng/dl per BMU. If this amount is not consumed by receptors, it will join the amount accumulated in the BMU. • Each RANK surface receptor requires an amount of OPG of 0.0000000082 ng/dl to be activated. • Each OPG unit binds to an osteoclast RANK receptor reduces the production of Acid phosphatase by 0.008%. • Every surface receptors of an osteocyte occupied by estrogen results in an increase of OPG production by that cell by 0.01%.	[38, 59, 80, 89, 143, 145]

Table 5 continued

M-CSF	Serum level per BMU	• Normal level range is (69.44 - 90.84 ng/dl), high resorption level range is (173.1 - 303.3 ng/dl). • High resorption level per BMU is (0.0001731- 0.0003033 ng/dl). • Level increases with age by 1% every year in correspondence to estrogen reduction. As M-CSF increases so the number of active osteoclasts. • The number of M-CSF receptors per osteoclasts is estimated to be 1000 receptor. • Normal MCSF level per BMU is 0.00009084 ng/dl. • Production rate per osteoblast daily is 0.00001736 -0.000002271 ng/dl. • Each surface receptor requires an amount of 0.0000000758 ng/dl to be activated. The surface receptor required amount be dependent on the affinity. • Each receptor has a 70% chance to bind to an M-CSF particle each day. • Every increase of MCSF by 1% over 0.00001736 for each BMU results in an increase of receptor affinity by 1%. The reduction of MCSF by 1% over 0.00001736 for each BMU results in a reduction of receptor affinity by 1%. Each receptor activated will increase the osteoclast lifespan by 0.001%. M-CSF particles lifespan is one day. When bound to a receptor, that receptor remains active for three days (including the MCSF particle attached).	[82, 89, 144, 202-206]
Sclerostin	Serum level per BMU	• Normal range in males: per BMU (0.000037 - 0.000071 ng/dl), high resorption level per BMU ranges from (0.000043 - 0.000089 ng/dl). • In females, it increases with age by 5.4% per year. • Normal range per BMU in females: 0.0000018 to 0.0000019 ng/dl. • In males, it increases with age by 5.7% per year. • An amount of the 0.5* (starting level) enters the BMU daily. The amount enters the BMU reduced with age by 1% per year. • Every day 0.00005 ng/dl is introduced into each BMU. The Same amount is eliminated daily. • There are 1000 sclerostin surface receptors on each osteoblast.	[46, 59, 207, 208]

Table 5 continued			
		• Each surface receptor requires an amount of 0.0000000000048 ng/dl to be activated. • Receptor affinity is related to the level of sclerostin per BMU, every 1% increase in the level results in increased receptor affinity by 1% and every 1% of sclerostin reduction results in a reduction of affinity by 1%. • The simulation starts with a 70% affinity. • The Sclerostin particle attached to the receptor will be active for three days, after which it will expire, and the receptor will be free to bind to another particle. • Each receptor activated will reduce the osteoblast activity (collagen production) by 0.001%. • The percentage of Sclerostin that increase with age effects that amount introduced only.	
Acid phosphatase	Percentage reduction, amount produced per osteoclast.	• Bone resorption rate per osteoclast = 0.00025%/day/cell. Normal production per osteoclast per day is (Bone breakdown per osteoclast) = 78.125 ng/dl per day.	[37, 59, 89, 143]
Sex hormones: Estrogen & testosterone.	Normal serum level and age-related reduction	Females, Estrogen: • Before 35 years of age serum level: 3 to 40 ng/dL. • Before menopause, estrogen level reduces slowly with age. From the age of 35 until menopause or 51 years of age: estrogen is reduced by 1% per year. • Postmenopausal after 51 y/o age women normal serum level is 0 to 30 ng/dL. • Level Per BMU Before 35 years of age: 0.00004 + a random number from 0 to 0.000008 ng/dL • An amount of the 0.44* (starting level) enters the BMU daily. The amount enters the BMU reduced with age by 1% per year. • The half-life of estrogen is one day; when bound to a receptor that receptor remains active for three days (including the estrogen particle attached). • Osteoblasts and osteoclasts have 500 receptors per cell. Osteocytes have 1000 surface receptors. Every receptor requires an amount of 0.0000000015 ng/dl to be activated. • Every 1% number of receptors activated results in an increase of activity of the corresponding enzyme for that cell type by the same percentage.	[73, 74, 88, 105, 118, 195, 209-213]

Table 5 continued

		• For osteoblasts and osteoclasts: Receptor affinity is related to the level of estrogen per BMU, every 1% increase in the level results in increased receptor affinity by 1% and every 1% reduction in estrogen results in a reduction of affinity by 1%. • The simulation starts with a 70% affinity. • For osteocytes: • Receptor affinity is related to the level of estrogen per BMU, every 1% increase in the level results in increased receptor affinity by 1% and every 1% reduction in estrogen results in a reduction of affinity by 1%. The simulation starts with a 70% affinity. Males, Estradiol, Testosterone: • Average Testosterone level before 30 years of age is 270-1,070 ng/dl. • After the age of 30 years of age: testosterone & estrogen sustain 1% reduction per year. • The average level of estradiol in males is 1 - 4 ng/dl. • Estrogen in males has the same protective effect on the bone as in females. • Both hormones Testosterone and estradiol are important to keep the bones healthy in males. • In our model well consider the estradiol to represent the hormonal protective effect of both (Testosterone and estradiol) on bones for males. • Before the age of 30: Average level of estradiol per BMU in males is from (0.00067 to 0.00008 ng/dl). • After the age of 30 years of age: estradiol sustains a 1% reduction per year. • Osteoblasts and osteoclasts have 500 receptors per cell. Osteocytes have 1000 surface receptors. Every receptor requires an amount of 0.000000000135 ng/dl to be activated. • An amount of the 0.44* (starting level) enters the BMU daily. The amount enters the BMU reduced with age by 1% per year. • Testosterone is converted to estrogen through the aromatization process. Reduction of Testosterone in males causes the reduction of estrogen. Smoking reduces the estradiol level by 25% in moderate smokers (less than ten cigarettes per day) and 35% in heavy smokers >10 cigarettes per day)	

Table 5 continued			
Collagen-I (bone density)	Percent of BMD, amount produced per osteoblast.	• Bone formation rate per osteoblast 0.000025 %/ cell/day. Normal production of Collagen-I osteoblast (bone mass added per day) = 7.8125 ng/dl per day. • Each estrogen receptor activated will increase the osteoblast activity (collagen production) by 0.001%.	[37, 59, 89, 143]
Factors	Unit	Effect on the simulation	References
BMI	Percentage increase	• each increase of BMI by one unit over the ideal BMI for the patient will increase the bone density by 0.06% per year. In the model: • Each increase of BMI by one unit over the ideal BMI for the patient will increase the bone density by 0.06% (one time) at startup. Each decrease of BMI by one unit under the ideal BMI for the patient will reduce the bone density by 0.06% (one time) at startup.	[92]
Smoking status	Smoking Effect on estrogen (percentage)	• Smoking affects negatively the estrogen availability and action in the BMUs' as well as the proliferation rate of osteoblasts in a dose-dependent manner. • Nonsmoker (zero to less than five cigarettes a day) = no effect. • Smoking 5-10 cigarettes cause estrogens action reduction by 15 to 25% and reduces the collagen production by 5%. • Smoking 10 to 20 cigarettes per day results in estrogens action reduction by 25 to 35 % and reduces the collagen production by 10%. • Smoking one pack of cigarettes per day results diminishes the estrogen action by 45 to 55 %, and, reduces the collagen production by 15%. • Smoking two packs of cigarettes per day result in estrogens action reduction by 55 to 65 %. Moreover, reduces the collagen production by 20%.	[27, 105, 118, 214-217]

Table 5 continued			
Physical activity	Direct effect on bone health	▪ No weight bearing exercise = no effect on bone health. ▪ Weight-bearing exercise effect on bone health: ❖ over 30 years old for males' increases the bone health by 1% ❖ 30 to 50 years of age for females' increases the bone health by 0.75% • Over 50 years of age, increases the bone health by 1%	[54, 119]
Bone health	Percentage	Bone health represents the bone's general condition of the organic matrix: 90% of the bone health is composed of collagen, and 10% is composed by osteocytes. <u>Startup value:</u> If bone health is provided from a previous bone scan examination, it will be used as the startup point. If there was no previous reading the following rules would apply: • Male patients: After 30 years it will be 100 - 0.5% for every year of age. ❖ Female patients: bone health will drop by %0.25 per year from age 30 to 50 years old. After 50 years of age, it will drop by 0.75% every year if the patient is over 50 years of age.	[25, 30, 45, 51, 52, 54, 62, 91, 218]

3.4.8 Models Calibration and Values Adjustment

After selecting the parameters' values from the literature, we adjusted those values during the calibration stage to ensure the stability of the model's operation and that the model simulates the disease progression according to the expected rate and outcome documented in the literature.

We had to adjust the values numerous times during hundreds of simulations to reach this final version. During the calibration process, the model crashed because of reasons such total consumption of a mediator, or hyperactivity of agents resulting in unnatural process progression.

3.4.9 The User's Interface

We used the design functions in NetLogo to create the user's screen, which includes sliders, drop down selections, a line graph, graphic representation of the simulation, and other contents.

We edited the sliders and the drop-down contents to properly reflect the inputs required for the simulation. We Programmed the code to show the values of the internal factors such as the MCSF and the osteocytes number.

We also had to program the code to show the provisional diagnosis and the bone health percentage. The following subsection shows the detailed explanation of the user's screen sections.

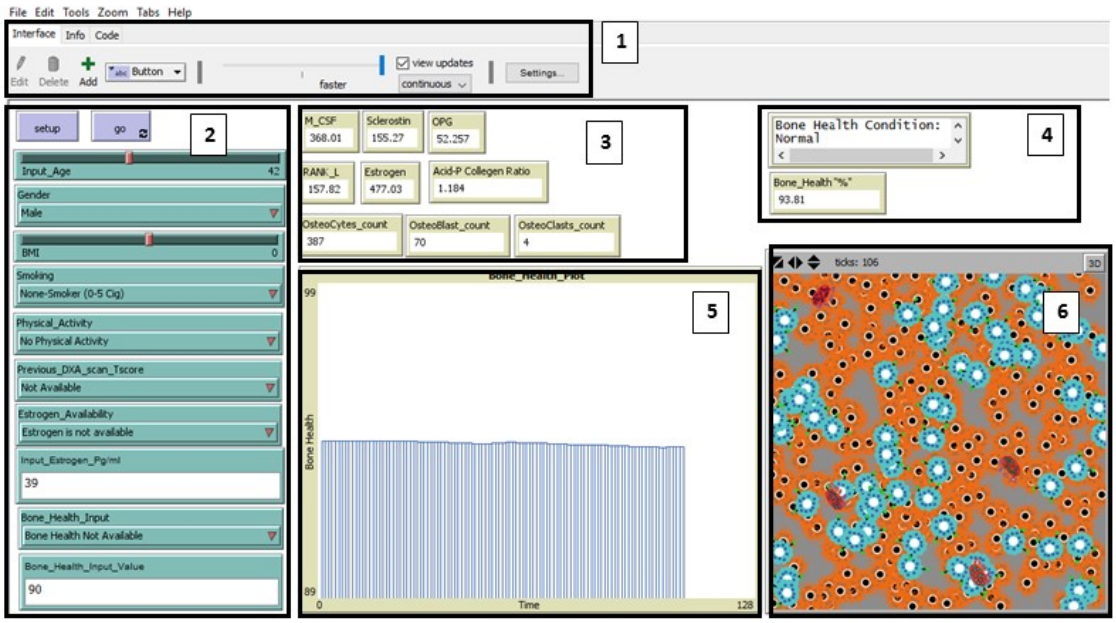

Figure 4 : A screenshot of the model's user screen

3.4.9.1 The User's screen Figure 4 above shows a screenshot of the model as it appears in windows-10. Section one includes a slider that controls the speed of the simulation process. Section two shows the sliders and drop down selection counters where the user can set startup age, gender, body mass index or BMI (where zero means the BMI is ideal), smoking status, physical activity (weight bearing is yes while no or regular activity is no physical activity). Previous DXA scan readings (T-score) if available, Estrogen level is entered manually if available by Pg/ml unit, and bone health input. The bone health input is an option where the user can specify the startup bone health percentage and run the simulation to see how the bone health changes with time.

The third section of the screen includes counters for M-CSF, Sclerostin, RANKL, and Estrogen. While those counters show numbers of the related mediators as they change with time, those numbers actually reflect the numbers of those factors as they change within the model and

not the levels within the subject's real body. Acid-P to Collagen ratio counter shows the balance between Acid-P which represents the chemical factors that break down the bone matrix and Collagen which is the main component that is used in building the new bone. A ratio of 1 indicates the balance of bone resorption and bone formation; this ratio continues to change in favor of bone resorption or bone formation according to the interaction that occurs between the various factors involved in the simulation.

Section four includes two counters: bone health condition which indicates diagnosis according to the current bone health level, and a counter that shows the bone health in percentage where 100% is perfectly healthy bone.

Section five includes a line plot that shows the progression or regression of bone health through time with the Y-axis as the bone health and X-axis as the time in days. Section six is a graphic presentation of how different cells within the bone matrix interact with each other.

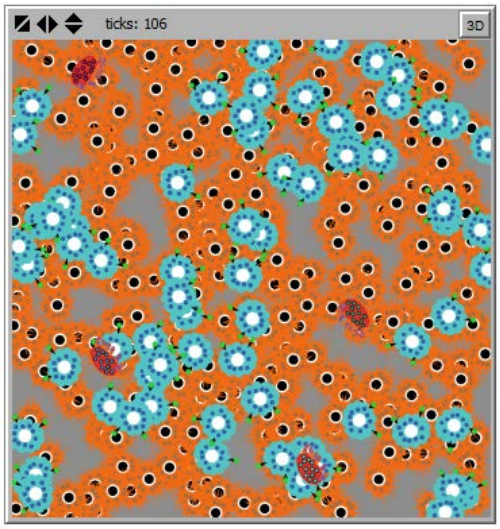

Figure 5 Graphic presentation of the simulation

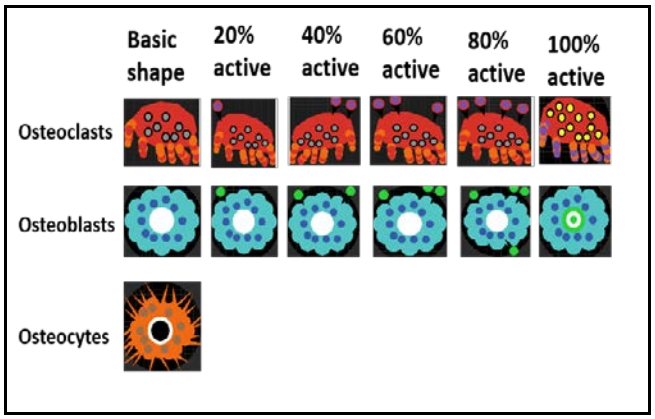

Figure 6 Different phases of each cell type.

Figure 6 demonstrates the alteration of the shape of the cells within the simulation according to the number of activated receptors. The reason there are no changes in the osteocytes -though they have active surface receptors- is that their number is much greater than both osteocytes and osteoclasts together which -with individual changes of each cell- will require greater processing power and time for processing each step. The software will have to sort through each osteocyte to measure the cell's activity and assign a shape to that cell while processing all the other calculations running in the simulation. The ability to provide a graphic presentation of the simulation is an advantage among many advantages of ABM; the graphic presentation can be quite useful as a cheaper and simpler substitute to cell cultures and individual cell's tagging in the laboratories [5, 219, 220].

The graphic presentation in our model can aid researchers that focus on the behavior of individual bone cell as these cells are exposed to certain conditions such as changes in PH because shifts in the extracellular environment may induce apoptosis [221]. Some research efforts concentrate on creating conditions that induce osteoblasts proliferation or increase their activity such bone-graft or bioactive materials transplant that operate as a scaffold for osteoblasts and supports the growth of new bone tissue [221-223]. Other current research efforts study the effect of cytokines such as IL-1β or TNF-α on an osteoblasts cell's activity, proliferation or lifespan [224].

Our model allows the user to follow any individual cell and view its activity level and surface receptors saturation until the cell expires. Instead of going through the process of extracting the cells, preparing cultures and marking individual cells, and following up the through the culture period which will cost a lot of time and funding, our model allows the user to follow the cells to follow an individual cell and view the effect of the intervention within minutes

and at a negligible cost. Our model can be modified by adding the proper rules and the agents - chemical or cellular- that are the focus of a research experiment and run the simulation to view the results the simulation in no time. Although this is not within the scope of this dissertation, those changes could be introduced in the future versions of the model.

3.5 MODEL'S VALIDATION PROCESS

In this section, we will explain the process for validating the model using data from the literature. This step includes the data selection, data description, the statistical test used for comparing the data collected from the literature with the outcomes gathered from the model, and the results of the statistical test.

3.5.1 Similar Models And Their Validation Methods.

To validate our model, we reviewed the previous models created by other researchers for modeling bone-related conditions. In the following table, we can see the different models, their designs, and their validation method.

Table 6 Different models that simulate bone related processes and their validation methods.

Number	Models purpose	BMD measurement	Validation method	Reference
1.	Simulate the bone remodeling process	None	Not validated using human data.	[41]
2.	Simulate the interactions between osteoblasts and osteoclasts	None	Not validated using human data.	[38]
3.	Graphical simulation of the bone remodeling process.	None	Not validated using human data.	[42]

Table 6 Continued				
4.	Simulate the osteocytes role in the bone formation process	None	Not validated using human data.	[60]
5.	Simulate the cellular signaling during the bone remodeling process	None	Validated using values from the literature. No case-to-case specific comparison performed.	[225]
6.	simulate the bone structure and density in the neck of the femoral bone	None	They used DXA scan images from two patients to compare the results the visual output provided by the mathematical model.	[226]
7.	Simulation of MSCs' cellular differentiation.	None	The model was validated using values from a single paper reporting ranged values obtained from mice.	[170]
8.	Simulation of bone remodeling in hypoparathyroidism	None	20 cases samples from human cadavers.	[161]
9.	Simulate the bone refilling in the BMU of the cortical bone	None	from a single article that provided animal data.	[61]
10.	Simulate the changes in vertebrae bone density to predict fracture risk.	g/cm^3	Not validated using human data.	[36]
11.	Predict the fracture risk in the proximal femur.	g/cm^2	Not validated using clinical data.	[159]
12.	Compare bone changes between osteomyelitis and osteoporosis.	Percentage	Bone density measurement was not validated	[227]
13.	Simulation of osteoporosis	Thickness in mm/ Percentage	Validated using ranged values from an article, no actual case-t-simulation specific comparison performed.	[40]
14.	Estimate the bone density in the head of the tibia bone	g/cm^3	Used different sites from a single case	[228]
15.	Estimate the bone density in the iliac bone	Percentage based on WHO classification	Used a single subject's 3D model of the pelvis bone.	[229]
16.	Simulate the head of the femurs bone density and shape in osteoarthritis and normal cases.	g/cm^3	220 cases DXA scan images for developing creating the model. No validation process for clinical use was performed.	[167]
17.	Predict the fracture risk in the proximal femur.	g/cm^3	45 (males) cases were used for validating the model. Not validated for clinical use.	[165]
18.	predict the risk of fracture in the proximal femur based using data from DXA scan	g/cm^3	7 cases of cadaveric femoral bones were used for validation. Does not provide BMD measurements that can be used in clinical setting.	[230]

We researched the methods that other researchers used for validating different mathematical, statistical and agent-based models. We can see that some models used different sample size for the validation process. In our case, the model is an agent-based model and does not require cases for training like mathematical or statistical models. Although we prefer as many cases as possible to perform the validation process, the difficulty of obtaining the data and the multiple simulations that we will perform for each case makes having a sample size of 50 cases is quite reasonable. Also, in the validation process, we are searching for a difference or an effect size, we are just comparing the readings between the model and the DXA-scan measurements to ensure that the model is simulating the changes in bone health correctly and providing a reasonably accurate prediction.

It is essential to understand that predictive modeling does not produce -nor expected to- a 100% accurate predictions and that the longer the duration of simulation the more the prediction is expected to be less accurate. Predicting the weather changes is limited to few days and mostly two weeks, even with the relatively stable pattern of weather changes over decades and the use of the most sophisticated equipment that provide real-time measurements of the weather parameters.

Predicting the changes in biological process in the human body and in our case -bone density- is a challenging task. That is why our purpose at this stage is to validate the model for use in the research setting. Indeed, the model can be modified to simulate the risk of fracture and the bone density changes in clinical setting. However, that is on of our future goals, and we expect that this model we require many modifications, calibrations, and clinical testing of thousands of cases over many years before it can be a robust, clinically trusted tool for bone density prediction and measurement.

3.6 DATA ACQUISITION AND ANALYSIS

In the following sub-sections, we will describe the data acquisition process, the sample description, and data handling.

3.6.1 Data Acquisition Process

We have obtained an IRB from the University of Pittsburgh. We have also obtained an IRB from the King Fahd Medical City (KFMC) Research Center which is located in Riyadh, Kingdom of Saudi Arabia. The data was collected from DXA scan reports and medication charts via the electronic medical record system. The data collection was performed in summer 2017.

3.6.2 Data Description

We obtained a file that includes all the patients who are registered in the osteoporosis clinic in the KFMC. The total number of patients who are enrolled in the clinic is 416 patients. We filtered the cases according to the following inclusion and exclusion criteria:

Table 7: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
1. Age ≥40. 2. DXA scan results available (two or more consecutive readings preferred).	1. Genetic bone tissue diseases. 2. Conditions that may induce secondary osteoporosis such as hyperparathyroidism or chronic intake of glucocorticoids'. 3. Patients who have received bone health treatment over the years.

The total number of cases that fulfilled the criteria is 17 cases. The reason for this small number of cases is because the patients who follow up in the clinic mostly are under treatment for osteoporosis, or were screened and did not receive treatment, so they ignored the follow up because they considered it unnecessary. For this step, we need cases that are not taking treatment to test the model's validity before inserting the therapeutic agent which will happen in the next step. Also, we need at least two consecutive readings to check the model's ability to predict the change in the bone health over time. All the cases were females, with ages between 44 and 70 years. We could not find any male cases that fulfilled the criteria; the male patients had multiple conditions that caused secondary osteoporosis.

The follow-up period between the readings ranged from six months to four years, which is useful for testing the model's ability in predicting the changes over a range of different time periods. All the cases were de-identified, and each case was assigned a serial number to be used during the data analysis process.

3.7 DATA ANALYSIS AND RESULTS

We coded the data in the SPSS as follows: Age (continuous variable), BMI (ordinal), and DXA scan's second reading and the Model's simulation for the second reading both as continuous variables. We did not code the other variables because all the cases were nonsmokers, had no record of weight-bearing physical activity, and did not have their serum estrogen level measured at the time when the DXA scan was performed.

A Kolmogorov-Smirnov test was used to for the DXA scan second readings data set to test for normality. The result was statistically not significant P = 0.2, which indicates that the data is normally distributed.

We used the Paired Sample T-test to compare the DXA-scans second reading and the Models simulations of the second readings. The test result was statistically not significant t (16) = -1.6, p = 0.12. based on the result we conclude that there was no significant difference between the DXA-scan readings and the readings obtained through the model's simulations.

3.8 DISCUSSION

The purpose of the patients' data collection and the above statistical analysis is to validate the model's ability to predict the change in bone density over time. The results indicated that model's simulations were statistically not different from the actual DXA scan readings obtained from patients during their clinic visits. Such outcome enables us to declare that the model is valid for predicting the bone density changes in human subjects. However, the sample contained only female cases, who were non-smokers. Which means that there is a need in the future to collect another sample that contains male cases, smoking individuals, and serum estrogen readings to validate the other functions in the model. At this point, this is enough proof to demonstrate model's concept and ability to encourage further research and later, the use in clinical practice.

Computerized tools predict bone fracture using simple parameters such as age, weight, history of fracture, and previous DXA scan reading. Our model also uses simple and easy to obtain information, but the model's internal mechanisms are complex and are not like using historical statistical data or fixed mathematical calculations. ABM allows the computational

model to simulate as closely as possible the biological conditions. In our model, all the factors have independent behavior and random trajectories while interacting with each other within the virtual space to provide the end outcome of that chain reaction; which is the bone density percentage.

3.8 TESTING THE MODEL'S ROBUSTNESS AND STABILITY

Now that the model has been validated, we shall proceed to test the reliability, sensitivity, specificity, and accuracy of the model's outputs.

3.8.1 Reliability Tests

We performed a reliability test to examine the model's reliability as a tool. In following subsections, we will describe the test, how data was collected, and how we performed the data analysis.

3.8.1.1 Test-Retest reliability test : In this step, we had the model run simulations for ten virtual subjects. For each subject, we had the model run twice (Test/Retest) for a total of 20 simulations each for the duration of one month (30 ticks). After organizing the data, we compared the values from the first ten test simulations to the retest simulations regarding changes observed in the agents and the outcomes to see if there were remarkable differences or minimal (acceptable) differences that fall within the normally expected limits.

Table 8: Test runs for testing the reliability of the model, including different values of the agents. for each parameter, two readings are recorded. The shaded columns represent the Re-test values. The diagnosis is either Normal (N), Osteopenia (O.P), Osteoporosis (O), and Severe Osteoporosis (S.O).

Subject	Age	Gender	Osteocytes count		Osteoblasts count	Osteoclasts count			Bone health %		Diagnoses	
1.	31	F	399	400	39	38	8	8	99.41	99.40	N	N
2.	45	M	372	371	38	39	8	8	92.4	92.41	N	N
3.	49	F	367	364	35	38	8	8	90.39	90.41	N	N
4.	54	F	352	354	36	34	8	8	86.87	86.85	N	N
5.	55	F	352	348	33	37	8	8	86.09	86.13	N	N
6.	59	F	335	339	38	34	8	8	83.14	83.1	O. P	O. P
7.	63	M	338	337	36	37	8	8	83.38	83.4	O. P	O. P
8.	68	F	309	313	37	33	8	8	76.38	76.36	O. P	O. P
9.	74	F	291	289	37	39	8	8	71.89	71.88	O	O
10.	80	F	274	271	36	39	8	8	67.38	67.4	S. O	S. O

We coded the data in the SPSS as follows: Age (continuous variable), Gender (nominal), Osteocytes Count 1st and 2nd run (continuous variable), Osteoblasts count 1st and 2nd run (continuous variable), Osteoclasts 1st and 2nd run (continuous variable), Bone health Percentage 1st and 2nd run (continuous variable), and Diagnosis for 1st and 2nd run (Ordinal variable).

For testing the test-retest reliability of the model, we used Inter-class coefficient or ICC reliability test because of it a proper approach and the commonly used method for analyzing the reliability of medical instruments measuring continuous variables [231-233]. We chose Cronbach's alpha test for testing the model's reliability through the Test-Retest approach. We performed the test for each variable separately, comparing the first run with the second run for the ten subjects.

Table 9: Cronbach's alpha values between the first run and the second run.

Variable	First run	Osteocytes Count	Osteoblasts Count	Osteoclasts Count	Bone health percentage	Diagnosis
Second run						
Osteocytes Count		**0.99**				
Osteoblasts Count			**0.8**			
Osteoclasts Count				**NA**		
Bone health percentage					**1**	
Diagnosis						**1**

The results as shown in Table 9: the interclass coefficient between osteocytes count, bone health, and diagnosis is over 0.9 which means excellent consistency. For osteoblasts, the ICC is 0.8 which is considered as a good consistency. The test cannot be performed for osteoclasts the reason for that is that there is no difference between the readings, osteoclasts numbers are limited, and they change every two weeks (the lifespan of the cells). Therefore, osteoclasts cell number is constant as estimated by the model according to the simulation rules.

3.8.2 Sensitivity, Specificity, And Accuracy Testing

We defined the sensitivity of the model to be the percentage that the model will diagnose the subject to have less than normal bone density (osteopenia or worse) when the subject, in fact, had a bone density below normal level. We defined the Specificity of the model to be the percentage that the model diagnosed the subject to have normal bone density while the subject has healthy and normal bone density. We coded the cases into the SPSS and performed the analysis. The sensitivity or true positive rate was 85.7%, and the specificity or true negative rate was calculated to be 100%.

To test the accuracy of the model we used the SPSS to plot the Receiver Operating Characteristic (ROC) Curve. In the SPSS, we assigned the cases that had normal bone density 0 indicating the absence of the condition and assigned 1 for any subjects that had lower than normal bone density diagnosis for the two datasets. We performed the ROC test, and the results are in the following table:

Table 10: Results of ROC analysis

Area Under the Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Significance	Lower Bound	Upper Bound
.929	.081	**.003**	.77	1

The area under the curve is 0.929 which indicates excellent accuracy. The asymptotic Significance is 0.00 which is less than the P value of 0.05. Therefore, we say that the results obtained from the model are more accurate than readings obtained by chance.

3.8.3 Discussion of The Results

The Test-retest reliability performed, included ten randomly selected (Virtual) subjects. The ICC values ranged from 0.8 to 1 indicating a good to very high consistency between the datasets from the test and the retest. In comparison to our model, the DXA scan test-retest reliability which ranged from 0.98 to 0.99 in the lower limb bone density measurements and scored between 0.89 to 0.97 for measurement of soft tissues in the lumbar region [234-236]. We compared our model reliability to that of the DXA scan because it is considered to gold-standard for measuring the

bone density, and because we used it as the standard for validating our model. By comparing the reliability of our model to that of the DXA scan, we can say that the model is reliable.
We calculated the model's sensitivity (true positive rate) to be 85.7%, and the specificity (true negative rate) was calculated to be 100%. The model's sensitivity and specificity were calculated by comparing the model's readings to those obtained from the golden standard tool (DXA scan).

The DXA scan, its self, has a sensitivity of 88.2 to 94.1 % and specificity of 25 to 62.5% [183]. It is quite possible to have different -and more representative- values of the model's sensitivity and specificity if a bigger sample size was used. Also, the fact that a small difference in the measurement can move the case from negative to positive which can be as little as 1% may have a significant impact on the sensitivity/specificity analysis, in particular with the small sample size. In the ROC test, the area under the curve was **0.929** which indicates an excellent level of accuracy of the model's measurement. Based on the small sample size that we used for the ROC analysis, it is fairly possible that testing the model's accuracy using data from different, or bigger -more diverse- populations may provide different levels of the Model's accuracy.

3.9 HYPOTHESIS TESTED IN THE FIRST STEP

Osteoblasts in their active form produce collagen to build up the bone density, while active osteoclasts -conversely- break down the bone matrix releasing acid-phosphatase and Cathepsin-K, and both processes are a part of the bone remodeling process [19, 25, 28, 44, 52, 56, 57, 68, 133]. In theory, more active osteoblasts and less active osteoclasts mean more bone density, but to measure that in a real situation; bone samples must be obtained from live individuals who

have variable bone density levels. Such procedure is quite difficult and may not be possible, most of the studies are done on mice and samples obtained from cadavers [43, 76, 83, 164, 230]. We will use the model to perform the simulations and explore the possible relation between the Acid phosphatase to collagen ratio and bone density in the virtual environment. The virtual space of the model is unique because it keeps the cells in their active form, unlike what happens when they are separated from their natural environment during the sampling and preservation process. Having the model validated and tested for reliability, we can perform simulations to test the hypothesis as we test our hypothesis in an In-silico experiment.

3.9.1 Simulations and Virtual Subjects

The sample consists of 30 virtual cases, all females. Age of the subjects ranged between 26 and 87 years of age with a mean of 51.4 ± 16 years. The bone density of the subjects was between 99% to 62%, which corresponds to Hip-T-scores between 0 to -3.8. We chose to use default values for physical activity and estrogen serum levels for all the subjects to avoid the confounding effect of those variables on the outcome of simulations. For each case, the simulation was performed for 60 days, during which, we record the highest value of the Acid Phosphatase to collagen. We chose to use this period -sixty days- because during hundreds of simulations we noticed that the Acid phosphatase to collagen ratio reaches a peak between 30 to 50 days and then starts dropping.

3.9.2 Data Analysis

We coded the data in the SPSS as follows: Age (continuous variable), Bone density percentage as (continuous variable), and Acid phosphatase to collagen ratio as (continuous variable). Pearsons' correlation test was used to test the correlation between the two factors: Bone density percentage and Acid phosphatase/collagen ratio.

3.9.3 Results

Pearson's correlation coefficient value was (-0.878) which is considered as a very strong negative relation. The P value was (0.000), indicating that the relation between the two factors is highly significant.

Table 11: Pearson's correlation test as it appears in SPSS.

Pearson's Correlation	**Bone density**	**AcidP/Collagen Ratio**
Bone density	**-**	**- 0.878**
AcidP/Collagen Ratio	**- 0.878**	**-**
Significant. (2-tailed test)	**0.000**	

3.9.4 Discussion

Using the model's simulations to answer a theoretical question is one of the benefits of using computational models. In our model, we chose to run simulations to test the hypothesis that there

is a relation between the AcidP/Collagen Ratio and the bone density. We opted to test this hypothesis because evidence in the literature supports that as individuals age, Osteoblasts activity is reduced and - as a consequence - the collagen production is decreased [19, 20, 22, 23, 44, 45, 52, 54-56, 62, 72, 76, 237].

The model can simulate changes that happen within the BMU in the virtual space. Even though the model needs further validation in the future, using the model to perform virtual experiments for testing the hypothesis proves the concept that this model can be employed to scientifically test hypotheses or answer possible questions that relate to changes in bone density. Especially when experimenting in a lab using live human cases is very difficult if possible at all. Our statistical analysis of the simulations revealed a highly significant correlation between the AcidP/Collagen Ratio and the bone density with a P value of (**0.000).** The correlation analysis results of our simulations showed that there is a strong negative relationship between the AcidP/Collagen Ratio and the bone density with Spearman's correlation coefficient **(-0.878).** Based on the results and the analysis, we chose to conclude there is a strong negative relation between the AcidP/Collagen Ratio within the bone matrix and the bone density.

3.9.5 Testing the Model's Usability

The usability and the user acceptance are essential factors for any tool -computational or otherwise- to achieve field success and widespread user adoption [238-240]. Our model is no exception to that rule. Therefore, we decided to perform a cross-sectional study in the form of user's survey to evaluate the user's acceptance of the model.

3.9.5.1 The Survey Design and Distribution : We used a survey which is called system usability scale or SUS [241]. We used this survey because it is a validated tool and does not involve many questions which makes it easier for the participants to complete the survey in a short time. Also, this survey is suitable for evaluating the model at its current stage. We built the questionnaire on the website surveymonkey.com and prepared it for electronic distribution.

We also prepared a ten minutes video presentation to distribute it with the questionnaire, so that the participants watch the video demonstration before answering the questionnaire. Then, we contacted the research center in the KFMC and asked permission to distribute the E-mail containing the survey and accompanying files to the clinicians who may be interested in participating in the usability study. The target population is clinicians who are expected to deal with primary osteoporosis more often than others, such as endocrinologists, geriatrics, and orthopedics. We then contacted other clinicians who work in Saudi Arabia but in other hospitals or clinics. Table 13 shows the questions used in the survey as they appear in the online survey, and the expected responses.

Table 12: the survey questions and expected responses

Question number	Question	Expected response
1.	I think that I would like to use this model frequently	Five points Likert scale from strongly disagree as one point to five points as strongly agree.
2.	I found the mode unnecessarily complex	
3.	I thought the model was easy to use	
4.	I think that I would need the support of a technical person to be able to use this model.	
5.	I found that the various functions of this model were well integrated.	
6.	I thought there was too much inconsistency in this model	
7.	I would imagine that most people would learn to use this model very quickly	
8.	I found the model very cumbersome to use	
9.	I felt very confident using the model	
10.	I needed to learn a lot of things before I could get going with this model	

Table 12 continued		
11.	Gender	Male or Female
12.	Years of working experience	Number of years.

We recorded a ten minutes video presentation to demonstrate the different functions of the model. Then we sent an email that included a link to the video and another link to the survey. In the E-mail, we explained the purpose of the survey and how to answer the survey. The System software survey has a unique grading method, and each survey must have the score calculated manually.

3.9.5.2 Statistical Analysis and Results: During the 30 days that the survey was available online, and after contacting the clinicians several times through email, the number of respondents reached eight. We consider this number sufficient because the goal of this survey was to evaluate this version of the model and give an idea about the user's acceptance of the model. Also, this survey may be repeated in the future to evaluate future versions of the model and with a bigger sample of users. The sample contained two males and six female participants. With years of working experience ranged from one to 20 years. The scores for the survey are available in the table below.

Table 13: calculated scores for the participants.

Participant	Experience (years)	Scores
1.	20	45
2.	10	55
3.	3	47
4.	5	70
5.	10	82.5
6.	1	50
7.	12	45
8.	5	50
Mean		55.5

The average of the SUS scores among the sample was 55.5 which considered an average number, meaning the model has an average usability score [242, 243]. A Kolmogorov-Smirnov test was used to for the DXA scan second readings data set to test for normality. The result was statistically significant P = 0.024, which indicates that the data is not normally distributed.

We used the Spearman rank correlation to test the relation between the experience years and the survey scores. Spearman rank coefficient value was (-0.28) which is considered as a very weak negative relation. For the relation between the age and the survey scores The P value was (0.49), indicating that the relation between the two factors is not significant.

3.9.5.3 Discussion: The results of the survey scores demonstrate average-level usability of the model among the participants of this sample. Also, the correlation test shows that there is no relationship between the participant's experience and between the usability of the model. There are many factors to consider while commenting on this result; including the small sample size, the method of the demonstration, and surveys design.

Although a sample size of 5 users is sufficient theoretically for a usability study, this sample size is not favorable for our study because the small sample size may not reflect the majority of the target population [244-246]. It is better to have a bigger sample to have a better picture of the user's opinions about the model. The method that we chose (video demonstration), was not the most ideal for seeking the users' opinions about a tool's usability; It was better to have a hands-on, one-to-one demonstration of the computational model, then ask the user to fill the survey. Unfortunately, that was not possible because our offer to provide the demonstration in a live session was ignored or declined by the clinicians that we contacted through the research center.

The survey's design its self is unique because the questions format would allow for a reasonably accurate assessment of any system's usability if and only if the participants read the questions carefully before answering the questions. If the users were planning to answer the questions with a preconception of accepting the model and plan to answer strongly agree with all the answers, the results would be average. Because the odd number questions format is different from the even numbered questions.

Therefore, we can say that at this stage, we consider the model to have an average usability level. However, this study must be repeated in the future with a larger sample and must have a live demonstration which will include a small Q and A session to explain some minor details that may be of concern to the users.

3.9.6 Conclusion Of The First Aim.

Our aim in this step was to design, implement, and validate an agent-based computational model to simulate the changes in bone density. We searched the literature and selected the most commonly accepted theories that explain the disease process. After that, we found the parameters values for the most influential factors that control the disease process and formulated their rules of interaction within the model based on their documented behavior in the literature.
Next, we calibrated the model and tested the validity of the model's ability to simulate the disease's progression over different periods of time in a group of patients' while comparing the Model's output to the documented DXA scan readings for the patients'.

We also conducted sensitivity, specificity, and reliability tests to explore the stability and reliability of the model's predictions. Although we used a small sample for performing the tests, we believe it is sufficient for us to conclude that the model at the current point of our research is valid, reliable, and with acceptable sensitivity and specificity. Therefore, we can proceed to the second aim of this dissertation.

4.0 AIM TWO INTRODUCING THERAPEUTIC AGENT INTO THE MODEL

4.1 INTRODUCTION

In the second step of this dissertation, we introduce a therapeutic agent into the model and test the model's ability to predict the changes in bone density in individuals receiving treatment. We performed a literature review to choose a drug that is clinically used for treating osteoporosis. After that, we will build the rules to integrate this agent in the model's design. Then, we perform sensitivity, specificity, and accuracy tests to measure the precision and reliability of the model's predictions. If the model proves valid and reasonably accurate, the model can be used in research to predict therapeutic interventions effects on the changes of bone density in In-Silico experiments.

4.2 SELECTING THE THERAPEUTIC AGENT

Before choosing a drug to use as the therapeutic agent in the model, we need to be confident that it is a commonly used drug for treating osteoporosis patients. The literature must have enough information about the long-term effects of the drug on bone density. Also, the drug that we use should have a clearly explained mechanism of action for changing the bone density. The sample

that we were going to collect is from KFMC, as we previously mentioned in aim one. Therefore, the drug must be a commonly used drug to treat the patients in the KFMC clinics; because we needed data from patients under treatment by the same drug for validating the model's output. We found that the most suitable drug that fulfills the previous conditions is Alendronate also known as (Fosamax) because after collecting the sample from KFMC, we noticed that Alendronate is the most commonly used drug for treatment of osteoporosis cases. Also, the literature is rich with research about alendronate's mechanism of action, clinical results, and changes in bone density over time [127-130, 247].

4.3 METHODOLOGY

In following sub-sections, we will explore the following steps: models modifications, data acquisition, data description, statistical tests used for comparing the patients' data with the outcomes collected from the model.

4.3.1 Alendronate's Mechanism of Action and The Model's Modifications

Alendronate is a drug that belongs to the Bisphosphonates group. Alendronate -like other Bisphosphonates- target's areas in the bone of high turnover and active resorption. Alendronate attaches to the Calcium hydroxyapatite crystals that are exposed on the bone surface of the bone resorption areas. Alendronate then is taken into the bone tissue where it enters the osteoclasts and initiate intercellular biochemical changes leading to a reduction of osteoclasts activity and

increased apoptosis [132, 248, 249]. Alendronate is a well-known drug that has been commonly used for treating osteoporosis for over 20 years [127, 128, 132, 248, 250, 251].

Based on alendronates effect on the osteoclasts activity and lifespan, we created the rules for alendronate as an agent and introduced it into the model. Like the all the other rules that we built before, the values that we used were inferred from values and information from research papers about Alendronate. The rules in table 15 include finalized values after the model's calibration.

Table 14: rules and values that are used for alendronate in the model.

Agent	Mechanism	Input modes and units	Rules of interaction and	references
Alendronate	Reduces osteoclasts activity (Anti-resorptive action).	5 mg, 10 mg, 20 mg/ Duration in months.	Reduction of osteoclasts activity: 5 mg per day → 0.15% to 0.4% 10 mg per day → 0.3% - 0.8% 20 mg per day → 0.45% - 1%	[127, 128, 132, 247, 250]

4.3.2 Data Acquisition Process & Data Description

We selected the patients who are registered in the osteoporosis clinic in the KFMC who fulfill the inclusion/exclusion criteria. The total number of patients who are enrolled in the clinic is 416 patients. We filtered the cases according to the following inclusion and exclusion criteria in table 16:

Table 15: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
1. Age ≥40. 2. DXA scan results available (2 or more consecutive readings preferred). 3. The patient must be receiving alendronate for the treatment of the condition at the time the DXA scan readings were performed	1. Genetic bone tissue diseases. 2. Conditions that may induce secondary osteoporosis such as hyperparathyroidism 3. Patients taking medications or substances that affect the bone density such as chronic intake of glucocorticoids'.

The total number of cases that fulfilled the criteria is 43 cases. We consider this number to be reasonable considering that many patients have multiple health conditions in addition to osteoporosis such as hypothyroidism, hyperthyroidism, or rheumatoid arthritis. There are many patients how are receiving hormonal therapy post-breast surgery as a part of breast cancer treatment. Also, we need clear documentation about the duration of treatment and that the patient was compliant with the drug treatment.

All the cases were females, with ages between 42 and 77 years. The subjects received Alendronate (Fosamax) as a unified dose which is 70 mg once per week orally. However, we could not find any male cases that fulfilled the criteria; the male patients had multiple conditions that caused secondary osteoporosis. The follow-up period between the readings ranged from six months to four years, which is useful for testing the model's ability in predicting the changes over a range of different time periods. Of the 43 cases, 29 cases subjects had two documented readings (initial and one follow up), and 14 cases had three documented readings (initial and two follow up visits). Table 17 below provides the descriptive statistics for the sample.

Table 16: Descriptive statistics of the sample

Parameter	Minimum	Maximum	Mean	SD
Age	42	77	58.72	7.6 ±
Difference from ideal BMI	-2	14	4.19	3.6 ±
Bone Density %	70	100	85.14	7.3 ±

All the cases were de-identified, and each case was assigned a serial number that we used during the data analysis process. After organizing the data, and running the simulations, we will proceed to statistical analysis.

4.3.3 Statistical Analysis and Results

We coded the data in the SPSS as follows: Age (continuous variable), BMI (ordinal), and DXA scan and the Model's simulation readings both as continuous variables. We did not code the other variables because all the cases were nonsmokers, had no record of weight-bearing physical activity, and did not have their serum estrogen level measured at the time when the DXA scan was performed.

A Kolmogorov-Smirnov test was used to for the DXA scan second readings data set to test for normality. The result was statistically not significant $P = 0.2$, which indicates that the

data is normally distributed. Based on that result, we can use a parametric test to analyze the data.

We used the Paired Sample T-test to compare the DXA-scans second reading and the Models simulations of the second readings. The test result was statistically not significant $t(42) = 8.1$, $p = 0.28$. based on the result we conclude that there was no significant difference between the DXA-scan readings and the readings obtained through the model's simulations.

We checked the normality of the DXA scan third readings obtained from the sample. We performed the Kolmogorov-Smirnov test to test for normality. The result was statistically not significant $P = 0.2$, which indicated that the data is normally distributed. Next, we performed the Paired Sample T-test to compare the DXA-scans third readings and the Models simulations of the third readings. The test result was statistically not significant $t(14) = 1.413$, $p = 0.181$. based on the result we conclude that there was no significant difference between the DXA-scan readings and the readings obtained through the model's simulations.

4.3.4 Results Discussion

The purpose of the patients' data collection and the above statistical analysis is to validate the model's ability to predict the change in bone density over time in patients who are under treatment by Alendronate. The results indicated that model's simulations were statistically not different from the actual DXA scan readings obtained from patients during their clinic visits. The results allow us to claim that the model is valid for predicting the bone density changes in patients receiving alendronate for the treatment of osteoporosis. The model managed to simulate the changes in subjects receiving the intervention over different time periods that ranged from six

months to four years. As with the sample used in the first aim, this sample used here contains only female cases, who were non-smokers. Which means that there is a need in the future to collect another sample that contains male cases, smoking individuals, and serum estrogen readings to validate the other functions in the model. At this point, this is enough proof to demonstrate model's concept and ability to encourage further research and later, the use in clinical practice.

Currently, there are no computational tools that predict the changes in bone density in patients who are receiving alendronate. This model introduced a new method to study effects of different medications on bone density. Instead of the traditional research methods that consume time and resources, this model can be modified to test experimental interventions or scientific theories via In-silico experiments. This model needs further validation using many different samples from various populations before it can be used in clinical practice.

4.4 SENSITIVITY, SPECIFICITY, AND ACCURACY TESTING

In the previous step, we tested the model's reliability. Our model is built using ABM, and one of the advantages of ABM is having a modular design. In this step, we merely added alendronate as an agent with its pertaining rules without performing any changes to the other agents in the model, which keeps the model's performance and internal mechanics unchanged and therefore do not require repeating the reliability tests. However, in this step, we have validated the model's simulations of the second and third readings obtained from the DXA scan. Therefore, we need to test the sensitivity, specificity, and accuracy for simulations of the second and third readings.

4.4.1 Sensitivity, Specificity Tests and Results

We defined the sensitivity of the model to be the percentage that the model diagnosed the subject to have less than normal bone density (osteopenia or worse) when the subject, in fact, had a bone density below the normal level. We defined the Specificity of the model to be the percentage that the model diagnosed the subject to have normal bone density while the subject has healthy and normal bone density. We coded the cases into the SPSS and performed the analysis. For simulating DXA Scans second readings, the sensitivity or true positive rate was 90%, and the specificity or true negative rate was calculated to be 91.3%. For simulating DXA Scans third readings, the sensitivity or true positive rate was 100%, and the specificity or true negative rate was calculated to be 100%.

4.4.2 Accuracy Tests and Results

To test the accuracy of the model we used the SPSS to plot the Receiver Operating Characteristic (ROC) Curve. In the SPSS, we assigned the cases that had normal bone density 0 indicating the absence of the condition and assigned 1 for any subjects that had lower than normal bone density diagnosis for the two datasets.

We performed the ROC test for measuring the accuracy of simulating DXA Scan's second readings, and the results are in the following table:

Table 17: Results of ROC analysis for the second readings simulations.

Area Under the Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Significance	Lower Bound	Upper Bound
.907	.052	**.000005**	.804	1

The area under the curve is 0.907 which indicates excellent accuracy. The asymptotic Significance is 0.000005 which is less than the P value of 0.05. Therefore, we say that the results obtained from the model are more accurate than readings obtained by chance.

For measuring the accuracy of simulating DXA Scan's third readings, and the results are in the following table:

Table 18: Results of ROC analysis for the third readings simulations.

Area Under the Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Significance	Lower Bound	Upper Bound
1	0	**.01**	1	1

The area under the curve is which indicates perfect accuracy. The asymptotic Significance is 0.01 which is less than the P value of 0.05. Therefore, we say that the results obtained from the model are more accurate than readings obtained by chance.

4.4.3 Discussion Of Results

In the second aim, we performed the sensitivity/specificity, and accuracy analysis for model's simulations of DXA Scans second and third readings of the patients. The model's sensitivity (true positive rate) ranged from 90% to 100%, and the sensitivity ranged from 91.3% to 100%. To be 85.7%, and the specificity (true negative rate) was calculated to be 100%. The area under the curve value for the model ranged between 0.9 to 1. The DXA scan, its self, has a sensitivity of 88.2 to 94.1 % and specificity of 25 to 62.5% [183]. The area under the curve ranged for the DXA scan ranges from 0.82 to 0.91 [252].

The results seem impressive (100% sensitivity and specificity) and unusually high for a computational predictive tool, but that is because of the small sample used in the analysis. Still, the results are very appealing and encourage us to use other samples to see how the model will fair when different sample sizes obtained from different populations are used for validation and accuracy testing. In the ROC test, the model's area under the receiver operating curve (AUROC) was 0.9 to 1 which is a higher score than that of the DXA scan. Again, the small sample size here is the reason for this high AUROC score, because the ROC plot is the relation between the sensitivity and 1- specificity.

4.5 CONCLUSION

In the second aim, we tested the model's ability to predict changes in bone density in patients who are under treatment by a therapeutic agent for osteoporosis. We introduced Alendronate as

the therapeutic agent into the model and performed the simulations to by using data from patients from the KFMC sample which we used in the first aim. The statistical analysis that we used to compare the model's output to the DXA Scan readings demonstrated that the model is valid, and with high specificity, and sensitivity in predicting bone density changes over time in patients who are receiving treatment for osteoporosis.

The sample size was small, which have affected the results. The results of the analysis, however, are very inspiring, which motivate us to perform further analysis of the model using larger samples obtained from different populations.

5.0 AIM THREE: TESTING THE MODEL'S ABILITY TO PREDICT THE BONE DENSITY AS A DIAGNOSTIC TOOL

5.1 INTRODUCTION

In the third aim, we will test the model's ability to estimate the bone density as a first reading. This aim introduces a new approach to diagnose osteoporosis using an agent-based computational model. We will select the cases from the KFMC sample, perform the statistical comparison, and explore the possible uses of the model as a clinical diagnostic tool for osteoporosis.

5.2 METHODOLOGY

In following sub-sections, we will explore the following steps: case selection, data description, statistical tests used for comparing the patients' data with the outcomes collected from the model, and the results of the statistical test.

5.2.1 Selection of Cases

We selected the patients who are registered in the osteoporosis clinic in the KFMC and fulfilled the inclusion/exclusion criteria. We filtered the cases according to the following inclusion and exclusion criteria:

Table 19: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
1. Age ≥40. 2. DXA scan of the total Hip available.	1. Genetic bone tissue diseases. 2. Conditions that may induce secondary osteoporosis such as hyperparathyroidism 3. Patients taking medications or substances that affect the bone density such as chronic intake of glucocorticoids'.

5.2.2 Sample Description

The total number of cases that fulfilled the criteria is 55 cases. All the cases were female cases with their ages ranged from 44 to 90 years old. All the subjects are nonsmokers, and there are no serum estrogen readings that were measured at the time of the DXA scan examination. No male cases were among the subjects selected because all the male cases had medical conditions that affected the bone density and thus were excluded.

Table 20: Descriptive statistics of the sample.

Age group	Weight (Kg)	Height (Cm)	BMI	BMI difference from normal	DXA Scan
40-50	83.41 ± 10.6	146 ± 5.6	34.5 ± 4.26	8.18 ± 4.7	0.2 ± 1.7
51-60	70 ± 13.3	154.3 ± 6.47	29.4 ± 5.27	5.3 ± 4.7	0.8 ± 0.98
61-70	68.7 ± 12.2	153.46 ± 7.2	29.5 ± 5.2	6.2 ± 6.5	-1.79 ± 0.74
71 and over	69.9 ± 17	151 ± 8.85	31 ± 6.46	6.7 ± 6.3	-2.3 ± 0.66

5.2.3 Simulations and Data Analysis

We performed the simulations using the model for each subject by entering specific parameters values that pertain to that subject such as age, BMI, or smoking status. We entered inputs to the model and run the model to see how the model's outputs compare to the initial DXA Scan readings of the cases. Also, for each case, we ran the simulation for six months to allow for proper interaction between the agents. After collection the results of the simulations, we performed the statistical tests for the sample as a whole and then for each age group separately.

5.2.4 Statistical Analysis and Results

We performed the Kolmgrove-Smirnov test to check the normality for each group and then for the whole sample. According to the results of the tests, we selected the Paired sample T-test for the groups that show normal distribution, and the Wilcoxon-sign test for the groups that are not normally distributed. The table below shows the types of tests used for each group and the test results.

Table 21: Statistical tests used the results for each group.

Group	Age group	N	Test	2-tailed P value	P value
1	40-50	13	Wilcoxon-sign test	0.196	$P > 0.05$
2	51-60	13	Wilcoxon-sign test	0.02	$P < 0.05$
3	61-70	15	Paired T-test	0.039	$P < 0.05$
4	71 and over	14	Paired T-test	0.005	$P < 0.05$
All	Total sample	55	Wilcoxon-sign test	0.000	$P < 0.05$

5.2.5 Discussion of The Results

In the third aim, we tested the limits of the model's capability by testing the model's ability to predict the proper bone density level of the subjects by feeding the model limited inputs. To our knowledge, there are no similar models that predict the bone density of an individual using such inputs.

The test for the first group did not show a significant difference between the patients DXA scan readings and the model's simulation results. However, for all the other groups the test results demonstrated a significant difference between the actual readings of bone density and model's predictions. We noticed that difference significance is increased between the groups, the older the age group, the higher the difference in the test results, however, this may be a random occurrence. We believe that a possible reason for the results is sample size.

We believe using larger samples and obtaining samples from different populations may provide very different and exciting results. Another possible reason is that the older groups generally have many other factors that may affect the bone density but is not accounted for, such malnutrition, undiagnosed conditions that may cause secondary osteoporosis or genetic factors

that may allow the patient to have higher -or lower – than average bone density levels. All the previous factors increase the level of uncertainty and unpredictability which makes it difficult for a computational model to simulate the conditions and estimate the proper bone density level of the individual.

5.3 CONCLUSION OF THE THIRD AIM

In the third step of this dissertation, we tested the model's ability to estimate the bone density of the individuals without an initial DXA scan value. The model successfully estimated the subjects bone density under fifty years of age. It was not able to predict correctly the bone density of older patients. That is possible because the factors that are not present in the simulation or not accounted for such as genetic or coexisting medical conditions during the sample collection. In future versions, We hope that we can obtain larger samples with complete details about the subjects involved in the study, and that we will be able to add other factors to the model so that the model develops into a useful tool that can be used not only to estimate the progression of the bone density but as a screening tool for osteoporosis.

5.4 POSSIBLE APPLICATIONS FOR PUBLIC HEALTH

Different computational models are currently in use for public health studies or public health projects. One notable project is MIDAS which is funded by the NIH. MIDAS – as defined by

the project developers- is a collective group of researchers and scientists who build and use computational, statistical and mathematical models to study infectious disease dynamics that aid the authorities to act proactively in detecting, eradicating and preventing various infectious disease threats [253, 254]. The models are developed to study the disease spread rate and possible communication routes and are designed to predict the possible outbreaks of communicable diseases such as measles and influenza.

Many innovative and valuable models were developed and supported by MIDAS such as Framework for Reconstructing Epidemiological Dynamics or **FRED**, Legal network analyzer **LENA,** and project **Tycho [253].** Our computational model is designed to study the disease process in individual cases, but it can be used to study the possibility of osteoporosis development in individuals from samples collected from specific age groups and use the results to predict the prevalence of osteoporosis in that specific group.

5.5 IMPACT ON THE E.H.R AND CLINICAL DOCUMENTATION

Our model in the current state operates separately from any other software. However, it can be connected to E.M.R to allow for the transfer of information directly to the patient's electronic record. It can also be improved to run the predictions periodically while being updated by data collected from the patient most visits and DXA scan reports. The nature of data that is fed to the model and the collected output data is in the form of numbers (structured data) which does not require high-speed connections. Not only our model does not require high-speed connections, but it can also operate on any computer because the NetLogo requires small processing power and

memory space; it can work on Windows XP with a 64 MB RAM. This will also make it easier for mobile devices and smartphones to run this model in the future when we develop a version for mobile devices.

5.6 RESEARCH LIMITATIONS

In the literature review step, we used scientific articles and research papers that were in the English language. This can be a limitation because there are hundreds of papers that are available in different languages that may contain useful information for our research project.

During the design phase, there were many missing values for the agents that we use in the model those values are not available in the literature. To solve this problem, we used parameter values that were calculated by other researchers for use in their models. We consider this a limitation; however, it is an acceptable limitation in modeling and specifically agent-based modeling.

The small sample size that we used in validating the model is another limitation. It was very difficult to acquire the data for validation because it was obtained from a clinical source, but we believe this small sample is sufficient at this step to validate the model at this preliminary phase and to prove the concept of the model.

In the third step of this dissertation, we tested the model's ability to predict the bone density without a starting value obtained from the DXA scan. There many factors that limit the

ability of the model to perform such prediction. Many factors that may affect the bone density cannot be accounted for in the simulation. Factors such as genetic, environmental, coexisting medical conditions, drugs or herbal medications can affect the bone health, but it is very difficult to obtain all the details from the clinical notes because of the poor handwriting, missing and unorganized data of the scanned charts.

5.7 FUTURE DIRECTIONS

Our model, Similar to FRAX®, can be more accurate in simulating the disease process for specific populations if the rules were modified to simulate disease behavior in those particular populations. FRAX® which was endorsed by the WHO and NOF as the best tool to predict the ten years' risk of osteoporotic fracture has 63 models that are used to predict the fracture risk for populations in 58 countries around the world [255].

The reason for that is that every population have different factors that may contribute or delay the progression of Osteoporosis and osteoporotic fractures; Such factors can be genetic, environmental, nutritional habits or others [21-23, 25, 30, 52, 55, 68, 69, 73, 120, 121, 256-258]. The model can be improved to predict the fracture risk by integrating the relevant agents into the model. The model can then predict the fracture risk at specific parts of the bony skeleton such as the wrist or the femoral neck bones.

Other future improvements to the model include adding different therapeutic agents including other medications in use for treatment of osteoporosis including various types of bisphosphonates, and Parathyroid hormone (teriparatide) [19, 69, 78, 120].

The model may have different versions to study conditions that cause secondary osteoporosis such as chronic intake of glucocorticoids, hyperparathyroidism, or Renal osteodystrophy [21, 30, 59, 96, 120, 133]. To create the previously mentioned changes, sufficient information about the internal mechanisms of the new agents and all the relevant parameters values must be available to develop the interactions rules and integrate the new agents into the model. Also, each version must be validated and tested for sensitivity and specificity. New medications that are under investigations can be added as agents to the model where virtual experiments can be performed numerous times to test the efficacy of the new drug and its effects on the bone cells functions and longevity.

This model will need more testing against data from different populations in clinical settings before considered as a valid diagnostic clinical tool. It will also undergo many changes and updates according to the changes in the research field regarding the disease process. New biochemical factors or inflammatory mediators may emerge as important players that can change the disease's course. Older and well-known factors may prove insignificant or of low importance in the affecting the disease mechanism. The model will be changed to accommodate the expected changes.

Osteoporosis and the complications that result from it exert a great financial burden on the healthcare providers and is a major concern of policymakers and top healthcare leaders. Medical costs in the united states in 2015 of a patient's admission who suffered from an osteoporosis fracture or related injury was estimated to be $30,550. Also, Osteoporosis fall injuries including fatal and non-fatal were calculated to be around $32 billion [259].

Such great costs could be reduced by anticipating and preventing the complications with early detection and treatment of osteoporosis. Our model could be modified and improved to perform simulations of a population then these simulations -using analytical software- can provide benchmarks for expected incidence and costs of complications. It may also perform simulations for the patients, and these simulations can be transformed to informative charts using analytical software that provide a better understanding for decision-makers to make the best decision according to the updated disease status in the served population.

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