A COMPUTATIONAL MODEL OF APOPTOTIC OSTEOCYTE CORRELATION TO
CORTICAL BONE REMODELING PARAMETERS

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Engineering

With a Specialization in Biomedical Engineering

by

Michael Stephen Alexander

December 2009

© 2009

Michael Stephen Alexander

ALL RIGHTS RESERVED

COMMITTEE MEMBERSHIP

TITLE: A Computational Model of Apoptotic Osteocyte
Correlation to Cortical Bone Remodeling Parameters

AUTHOR: Michael Stephen Alexander

DATA SUBMITTED: December 2009

COMMITTEE CHAIR: Scott Hazelwood, PhD, Professor

COMMITTEE MEMBER: Daniel Walsh, PhD, Senior Associate Dean

COMMITTEE MEMBER: Lanny Griffin, PhD, Department Chair

ABSTRACT

A Computational Model of Apoptotic Osteocyte Correlation to Cortical Bone Remodeling Parameters

Michael Stephen Alexander

The onset of osteoporosis caused by aging, disease states, and post-menopausal conditions significantly impacts patient quality of life, required healthcare funding, personal autonomy losses from increased fracture risk and the subsequent corrective surgery. Research has indicated that osteocyte apoptosis may be a key parameter in bone remodeling, raising the possibility of remodeling rate modulation for the mitigation of bone mass resorption. By developing therapies that target osteocyte apoptosis, it may be possible to prevent undesired bone remodeling activity while maintaining a healthy balance between damage formation in the form of microcracks induced by the strain environment and the removal of damaged bone facilitated by resorbing BMUs, resulting in the preservation of bone mass. This preservation in turn results in a decreased risk of bone fracture.

The purpose of the current study was to develop a computational model to predict bone density changes from empirical osteocyte apoptosis data. This was achieved by developing a simulation that correlates osteocyte apoptosis with activation frequency and mechanical stimulus, two key bone remodeling parameters. The results of the simulation show that it is possible to use osteocyte apoptosis to predict remodeling and the resulting bone porosity changes. Instances of increased osteocyte apoptosis occurred simultaneously with increases in porosity, and under-loaded force conditions were more deleterious to bone porosity than overload associated with heavy exercise, which agrees with previous models.

ACKNOWLEDGEMENTS

I would like to thank all of those who have assisted and advised me through the various phases of this thesis. I owe a great deal of gratitude to my advisor, Dr. Scott Hazelwood, who used his previous research and understanding of the field to define the project, for his expertise and ever-patient tutelage. I would particularly like to thank my parents, who have encouraged my interests and supported me from those very first clumsy steps to the (slightly better balanced) steps I will make today.

TABLE OF CONTENTS

LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER 1: INTRODUCTION.....	1
1.1 OSTEOPOROSIS: A LEADING HEALTH THREAT	1
1.2 INTRODUCTION TO BONE: PURPOSE AND ORGANIZATION	4
1.3 BONE REMODELING	6
1.4 STIMULATION OF BONE REMODELING.....	7
1.5 STRUCTURAL PROPERTIES OF BONE.....	9
1.6 FRACTURE REPAIR	11
1.7 ON VARYING INDIVIDUAL REMODELING TRIGGERS.....	12
1.8 APOPTOSIS OVERVIEW	13
1.9 INTRODUCTION TO OSTEOCYTE ANATOMY AND PURPOSE	15
1.10 OSTEOCYTE APOPTOSIS TRIGGERS.....	17
1.11 CURRENT HYPOTHESES REGARDING OSTEOCYTE FUNCTION AND APOPTOSIS.....	20
1.12 PREVIOUS WORK.....	24
1.13 STUDY GOALS.....	28
CHAPTER 2: METHODS.....	30
2.1 OVERVIEW: CODE SECTIONS	30
2.2 PRIOR CONDITIONS	31
2.3 SOURCES OF DATA	31
2.4 SIMULATION.....	32
2.5 MICRODAMAGE CREATION AND REMOVAL.....	36
2.6 SIMULATION OF APOPTOSIS - PREFACE	38
2.7 SIMULATION OF APOPTOSIS – DAMAGE CONDITIONS.....	40
2.8 SIMULATION OF APOPTOSIS – DISUSE CONDITIONS	41
2.9 BMU ACTIVATION	46
2.10 CHANGE IN BONE POROSITY	47
2.11 VALIDATION.....	49
CHAPTER 3: RESULTS	50
3.1 RESULTS INTRODUCTION: THREE- AND TWO-DIMENSIONAL OUTPUT PLOTS.....	50
3.2 OUTPUT BATTERY AT 0N	51
3.3 OUTPUT BATTERY AT 445.8N	55
3.4 OUTPUT BATTERY AT 891.6N	63
3.5 OUTPUT BATTERY AT 1600N	67
CHAPTER 4: DISCUSSION.....	73
4.1 RESEARCH SUMMARY	73
4.2 ON THE NOMENCLATURE OF DISUSE AND DAMAGE:.....	73
4.3 ON THE CONVERSION FROM PERCENT APOPTOSIS TO THE NUMBER OF APOPTOTIC OSTEOCYTES.....	74
4.4 APOPTOSIS ISSUE AT THE INTERFACE BETWEEN DISUSE AND THE EQUILIBRIUM CONDITIONS	75
4.5 THE SIMULATION OF POROSITY.....	76
4.6 ON THE APPARENT IMBALANCE BETWEEN REFILLING AND RESORBING BMUS.	77
4.7 ON THE CREATION OF DAMAGE WITH REGARD TO EXERCISE.	77

4.8 REOCCURRING PATTERNS AND LIMITATIONS THEREIN	78
4.9 ADDITIONAL LIMITATIONS	79
4.10 COMPARISONS WITH PREVIOUS WORK.	82
4.11 FUTURE WORK.....	90
CHAPTER 5: SUMMARY OF CONCLUSIONS.	91
CHAPTER 6: REFERENCES.....	92
APPENDIX A: 3D OUTPUT OF PARAMETERS VS. FORCE AND TIME.....	102
APPENDIX B: MATLAB CODE.....	117
B.1: REMODELING PARAMETER ASSIGNMENTS	117
B.2: PRIOR CONDITIONS.....	118
B.3: SIMULATION: MECHANICAL CONDITIONS	119
B.4: SIMULATION: DISUSE PORTION	119
B.5: SIMULATION: DAMAGE PORTION	120
B.6: SIMULATION: TRANSLATION FROM % TO # OF APOPTOTIC OSTEOCYTES	121
B.7: SIMULATION: BONE MASS CALCULATION.....	121
B.8: OUTPUT: 3D SURFACE PLOT GENERATOR.....	122
B.9: OUTPUT: GENERAL PLOTS.....	122
B.10: UTILITY CODE: CURVE-FITTING.....	124

LIST OF TABLES

TABLE 2.1 REMODELING PARAMETERS USED IN THE PRIOR CONDITIONS AND SIMULATION.....	31

LIST OF FIGURES

FIGURE 1.1 OSTEOPOROTIC EFFECTS ON BONE MASS. (A) HEALTHY TRABECULAR REGION. (B) OSTEOPOROTIC REGION. [8].....	2
FIGURE 1.2 OSTEOPOROSIS RISK BETWEEN GENDERS. [45]	3
FIGURE 1.3 CORTICAL AND TRABECULAR (CANCELLOUS) REGIONS. [66]	5
FIGURE 1.4 BASIC MULTICELLULAR UNIT. [25]	6
FIGURE 1.5 PHASES OF BONE REMODELING. [40].....	7
FIGURE 1.6 STRAIN VS. CYCLES PER DAY - MINIMUM REQUIREMENTS FOR BONE EQUILIBRIUM. [57]	8
FIGURE 1.7 OSTEOCYTES WITHIN OSTEOONS. [23].....	10
FIGURE 1.8 FRACTURE REPAIR PHASES. [55]	11
FIGURE 1.9 APOPTOSIS PATHWAY, WITH "BLEBBING" AND DNA DAMAGE. [56]	14
FIGURE 1.10 OSTEOCYTE WITH PROCESSES. [53].....	16
FIGURE 1.11 ITERATIVE COMPUTATIONAL TRABECULAR ALIGNMENT. [47].....	24
FIGURE 1.12 ITERATIVE APPARENT DENSITY PREDICTIONS FROM A COMPUTATIONAL MODEL OF THE FEMUR. [12].....	24
FIGURE 1.13 REPRESENTATIVE VOLUME OF BONE UTILIZED FOR THE HAZELWOOD ET AL. (2001) SIMULATION. [28] ..	27
FIGURE 1.14 BONE REMODELING CODE FLOWCHART. [28]	28
FIGURE 2.1 FLOWCHART OF CODE MECHANISMS.	34
FIGURE 2.2 BLUE CIRCLES REPRESENT DATA POINTS OF APOPTOTIC OSTEOCYTES PLOTTED AGAINST DAMAGE- CONTRIBUTING ACTIVATION FREQUENCY. RED ASTERISKS REPRESENT DATA POINTS OF CURVE-FIT EQUATION. .	41
FIGURE 2.3 DISUSE APOPTOSIS VS. Φ	42
FIGURE 2.4 CURVE FIT OF DAMAGE AND DISUSE CONDITIONS FOR $Ac.F$ CONTRIBUTION VS. PERCENT APOPTOSIS. RED POINTS INDICATE THE CURVE FIT EQUATION; BLUE POINTS REPRESENT DATA POINTS.	46
FIGURE 3.1 POROSITY OUTPUT AT FORCE = 0N.	52
FIGURE 3.2 TOTAL OSTEOCYTE APOPTOSIS AT FORCE = 0N.	53
FIGURE 3.3 TOTAL DAMAGE AT FORCE = 0N.....	53
FIGURE 3.4 ACTIVATION FREQUENCY AT FORCE = 0N.	54
FIGURE 3.5 MECHANICAL STIMULUS (Φ) AT FORCE = 0N.	54
FIGURE 3.6 POROSITY AT FORCE = 445.8N.	59
FIGURE 3.7 TOTAL OSTEOCYTE APOPTOSIS AT FORCE = 445.8N.	59
FIGURE 3.8 MAGNIFICATION OF FIGURE 3.7.	60
FIGURE 3.9 TOTAL DAMAGE AT FORCE = 445.8N.....	60
FIGURE 3.10 ACTIVATION FREQUENCY AT FORCE = 445.8N.	61
FIGURE 3.11 MAGNIFICATION OF FIGURE 3.10.	61
FIGURE 3.12 MECHANICAL STIMULUS (Φ) AT FORCE = 445.8N.	62
FIGURE 3.13 MAGNIFICATION OF FIGURE 3.12.	62
FIGURE 3.14 POROSITY AT FORCE = 891.6N.	64
FIGURE 3.15 TOTAL OSTEOCYTE APOPTOSIS AT FORCE = 891.6N.	65
FIGURE 3.16 DAMAGE AT FORCE = 891.6N.....	65
FIGURE 3.17 ACTIVATION FREQUENCY AT FORCE = 891.6N.	66
FIGURE 3.18 MAGNIFICATION OF FIGURE 3.17.	66
FIGURE 3.19 MECHANICAL STIMULUS (Φ) AT FORCE = 891.6N.	67
FIGURE 3.20 POROSITY AT FORCE = 1600N.	69
FIGURE 3.21 TOTAL OSTEOCYTE APOPTOSIS AT FORCE = 1600N.	69
FIGURE 3.22 DAMAGE AT FORCE = 1600N.....	70
FIGURE 3.23 ACTIVATION FREQUENCY AT FORCE = 1600N.	70
FIGURE 3.24 MAGNIFICATION OF FIGURE 3.23.	71
FIGURE 3.25 MAGNIFICATION OF FIGURE 3.23.	71

FIGURE 3.26 MECHANICAL STIMULUS (Φ) AT FORCE = 1600N.	72
FIGURE 4.1 POROSITY RESPONSES OVER TIME FOR HAZELWOOD ET AL. (2001) IN DISUSE SITUATIONS. [28].....	83
FIGURE 4.2 PREDICTED POROSITIES UNDER DISUSE CONDITIONS.	84
FIGURE 4.3 ACTIVATION FREQUENCY RESPONSES OVER TIME FROM HAZELWOOD ET AL. (2001) IN DISUSE SITUATIONS. [28]	86
FIGURE 4.4 ACTIVATION FREQUENCY RESPONSES OVER TIME FOR THE MODEL IN DISUSE SITUATIONS, CURRENT RESULTS.	86
FIGURE 4.5 MAGNIFICATION OF OSCILLATIONS AT 445.8N IN FIGURE 4.4.	87
FIGURE 4.6 PREDICTED AND EXPERIMENTAL POROSITY COMPARISON. ERROR BARS INDICATE ONE STANDARD DEVIATION.	88
FIGURE 4.7 PREDICTED AND EXPERIMENTAL APOPTOSIS COMPARISON. ERROR BARS INDICATE ONE STANDARD DEVIATION.	89
FIGURE 4.8 PREDICTED AND EXPERIMENTAL AC.F COMPARISON. ERROR BARS INDICATE ONE STANDARD DEVIATION.	89
FIGURE A.1 POROSITY SURFACE PLOT.	102
FIGURE A.2 DAILY BONE FORMATION SURFACE PLOT.	103
FIGURE A.3 DAILY BONE RESORPTION SURFACE PLOT.....	104
FIGURE A.4 NET DAILY CHANGE IN BONE SURFACE PLOT.....	105
FIGURE A.5 MODULUS SURFACE PLOT.	106
FIGURE A.6 STRAIN SURFACE PLOT.	107
FIGURE A.7 DAMAGE CONTRIBUTION TO ACTIVATION FREQUENCY SURFACE PLOT.	108
FIGURE A.8 DISUSE CONTRIBUTION TO ACTIVATION FREQUENCY SURFACE PLOT.	109
FIGURE A.9 TOTAL ACTIVATION FREQUENCY SURFACE PLOT.....	110
FIGURE A.10 DAMAGE FORMATION RATE SURFACE PLOT.....	111
FIGURE A.11 DAMAGE REMOVAL RATE SURFACE PLOT.....	112
FIGURE A.12 TOTAL DAMAGE SURFACE PLOT.	113
FIGURE A.13 NUMBER OF REFILLING BMUS SURFACE PLOT.	114
FIGURE A.14 NUMBER OF RESORBING BMUS SURFACE PLOT.	115
FIGURE A.15 NUMBER OF DISUSE-INDUCED APOPTOTIC OSTEOCYTES SURFACE PLOT.	116

CHAPTER 1: INTRODUCTION

In this introduction, I will explain how osteoporosis is the driving factor behind my thesis, and how this research may assist in paving the way for additional therapies. I will continue with an overview into the basic bone anatomy and functionality before detailing the importance of the osteocyte and osteocyte apoptosis, concluding with relevant information about the evolution of our knowledge of the osteocyte.

1.1 Osteoporosis: A Leading Health Threat

Osteoporosis has leapt to the stage of health concerns confronting aging Americans. A 2004 US Department of Health and Human Services report stated that 44 million Americans over the age of 50 either have osteoporosis or are at risk for developing it, and the disease results in a \$12 to \$18 billion yearly impact to the health system (USDHHS, 2004). The very nature of osteoporosis defines the severity of the issue; erosion-induced reduction in bone mass appears under the histological microscope (Figure 1.1) almost as a termite's nest appears to a carpenter – spindly, crooked arrangements of precariously thin bone act as mere caricatures of previously durable bone, and in severe cases of osteoporosis, forces as slight as those caused by a simple sneeze can induce fractures.

This chronically weak bone state results in approximately 1.5 million bone fractures a year, and cumulative circumstantial complications from an osteoporosis-induced hip fracture result in a 20% mortality rate within 1 year of the event (USDHHS, 2004) and permanent debilitation (Hannan et al., 2001). The three most common sites of osteoporotic fracture are vertebral, hip,

and wrist fractures (Johnell and Kanis, 2006) – all of which are more susceptible because they have less reinforcement from a strong cortical shell than other bones (Raisz, 2005).

Figure 1.1 Osteoporotic effects on bone mass. (A) Healthy trabecular region. (B) Osteoporotic region. [8]

Although osteoporosis is generally associated with postmenopausal women because estrogen deficiency is linked to unbalanced remodeling that decreases bone mineral density, BMD, (Goldstein et al., 1999; Demir et al., 2008), there are also many other causes of osteoporosis, including comorbidity and lifestyle choices such as exercise and diet habits (Navarro et al., 2009; Israeli et al., 2008; Borer, 2005)). As such, both men and menopausal women (Figure 1.2) are susceptible to osteoporosis (Wimalawansa, 2000) through decreased testosterone levels in men (Seeman, 1995; Wu et al., 2000), low estrogen levels in menopausal

women (though therapeutically blocking estrogen receptors via Tamoxifen for the treatment of breast cancer (Kudachadkar and O'Regan, 2005) actually results in a reduction in osteoporosis (Nakamura et al., 2007; Krum et al., 2008)), heritability (Karasik and Ferrari, 2008; Ng et al., 2006) - possibly as high as 80%, with over 30 genes currently linked to increased risk of osteoporosis (Raisz, 2005) - ancestry (Araujo et al., 2007; Tracy et al., 2005), and other inducers.

Figure 1.2 Osteoporosis risk between genders. [45]

Although there are many causes of osteoporosis, there are only a few processes directly responsible for it. The most direct cause of osteoporosis is unbalanced bone remodeling (Raisz, 2005), which is the constantly occurring process that removes damaged bone and replaces it with new bone (Martin et al., 1998). Normally, the removal of bone is orchestrated proportionally to the amount of bone added, but during imbalances caused from the aforementioned osteoporotic influences, there can be differing amounts of bone added and removed so that too much bone is removed and not enough added, or the quality of the added bone is insufficient to replace the older bone (Raisz, 2005). Since the myriad of osteoporotic macro-causes result in very few

actual micro-mechanisms, elucidating these immediate mechanisms of bone remodeling may unveil new therapies to treat osteoporosis prior to, during, or in response to it. Already, an exciting array of medications has become available as a direct result of a clearer understanding of bone remodeling. Some examples are Teriparatide (which mimics parathyroid hormone to encourage bone forming cells to deposit higher quantities of new bone to rebalance removed/added bone), and forms of strontium, which engages a higher number of bone forming cells, while discouraging correlating higher numbers of bone removing cells (Atkins et al. 2008; Lam et al. 2008). With the possible advent of even more effective treatments on the near horizon – with millions of fractures in aging baby-boomers at risk- it is imperative to tease out the secrets and intricacies of bone remodeling, and there is currently a single bone cell that may hold the key: the humble but ubiquitous osteocyte.

1.2 Introduction to Bone: Purpose and Organization

The skeleton holds much biological utility, in that it provides mechanical structure to the otherwise-gelatinous flesh, motile leverage utilized by muscles, protection from blunt trauma (in the cranial, ocular and thoracic regions), generation of formed blood elements, protective buffering to mitigate blood pH oscillations through alkaline salt modulation, and finally mineral (e.g. calcium, phosphorus) metabolism (Martin et al. 1998).

Figure 1.3 Cortical and trabecular (cancellous) regions. [66]

Bone anatomy, from a top-to-bottom perspective, begins with the differentiation between two types of osseous tissue: cortical (i.e. compact) bone, and trabecular (i.e. porous) bone (Figure 1.3). Cortical and trabecular bone are fundamentally different in both anatomy and functionality, because cortical bone is configured at a microscopic scale to act as a load-bearing lever, while trabecular bone is meant as a structurally-efficient, strut-reinforced volume that holds marrow, which is a soft tissue supplied with nutrients from a dense vascular network that fabricates formed blood elements (Martin et al. 1998). Cortical bone is comprised of long, cylindrical elements called osteons. Both the struts of trabeculae and the cylindrical osteons are oriented axially along the lines of principle strain (Hert, 1994; Petrtyl et al., 1996; Martin et al., 1998). The cortical and trabecular regions of bone are further distinguished from each other by

variations in porosity; cortical bone is usually between 5 and 10 percent porous, and trabecular bone is usually between 75 and 95 percent porous. However, both regions tend toward the extremes of the scale, and rarely does bone contain porosities in the midrange between 10 and 75 percent (Martin et al. 1998); midrange porosities are generally associated with disease states.

Figure 1.4 Basic multicellular unit. [25]

1.3 Bone Remodeling

One purpose of bone remodeling is to remove damaged bone. Cortical and trabecular regions collect damage that would become cumulative if not for a constant turnover of damaged osteons and trabeculae by teams of cells called basic multicellular units (BMUs) (Figure 1.4). BMUs are comprised of osteoclasts and osteoblasts. Osteoclasts are amorphous, multinuclear cells responsible for removing bone through a process called bone resorption, where osteoclasts demineralize the bone by absorbing ions (e.g. calcium and phosphate) into the cells' numerous vesicles. Osteoblasts are bone cells with a single nucleus that acts to create new bone material after osteoclasts have cleared old bone from an area. Osteoblasts function by secreting a substance called *osteoid*, which is composed of type 1 collagen. Minerals are then deposited onto the osteoid through a process called *mineralization*, where ions are attached to the type 1

collagen matrix. Prior to mineralization, the collagen fibers formed from the osteoid have water occupying the spaces between fibers. Approximately ten days after the osteoblasts lay down the osteoid seam, primary mineralization occurs, where minerals crystallize between the gaps in collagen fibers. The mineralization gains mass and eventually drives the water out of the collagen structures. The result is a matrix of collagen, with minerals filled in between the fibers. In the average adult human, remodeling (Figure 1.5) takes between 4 and 6 months (Martin et al. 1998).

Figure 1.5 Phases of bone remodeling. [40]

1.4 Stimulation of Bone Remodeling

Bone remodeling occurs as a response to two environmental circumstances: the presence of microcracks in the bone matrix caused by daily strain exposure, and disuse caused by the lack or decrease in strain seen by the bone. Microcrack damage occurs from normal movement, but also occurs in events that cause abnormally high strains, such as during the enrollment of a new exercise program. Disuse occurs in specific instances such as reduced-gravity environments aboard the space station, or when an animal is injured and cannot move as frequently, or any

myriad of circumstances that prevent a nominal amount of bone loading. Since disuse remodeling is a primary contributor to osteoporosis in postmenopausal women, much effort is focused on controlling the amount of disuse remodeling that bones perform. Qin et al. (1998) found a relationship (Figure 1.6) between strain magnitude and frequency that allows disuse to be predicted and suitably prevented by, at one end of the scale, exposing bone to very high strains a few times daily, and at the other end of the scale, exposing bone to lower strains many times daily.

Figure 1.6 Strain vs. Cycles Per Day – Minimum requirements for bone equilibrium. [57]

Later research by Xie et al., (2006) demonstrated that vibrations communicating small strains – below 10 microstrain, with accompanying very high frequencies between 20 and 100Hz – are able to increase bone mineral density by remodeling to add bone, while preventing bone from going into disuse remodeling, which would otherwise remove unloaded bone and escalate the

risk of fracture. This knowledge may contribute to future devices that exact strain upon bone either through exterior vibrations, or direct stimulation of adjacent nerves.

1.5 Structural Properties of Bone

The combination of the flexible, regularly structured osteoid matrix and the mineral addition results in a viscoelastic biomaterial that is both strong and flexible. The mineralization lends bone its stability, and the osteoid matrix increases the yield strength by offering enough flexibility to minimize brittle fracture. These aspects combine to provide bone with its 3-to-4 fold factors of safety, which means that many bones can withstand 3-4 times their peak physiological forces (Skerry, 2008); in the case of the human skull, factors of safety as high as 40 have been observed (Hillam et al., 1995).

As osteoblasts create osteoid material, they become buried within the matrix and cease creating bone. The buried osteoblasts are referred to as *osteocytes*, and are the most numerous bone cells. Osteocytes reside in falciform compartments called *lacunae* (Figure 1.7). The lacunae are connected to each other via cytoplasmic dendritic processes called *canaliculi* (Figure 1.7), which are hypothesized to act as a communication network between osteocytes.

Figure 1.7 Osteocytes within osteons. [23]

The skeleton must sustain daily stresses, modulate its mass to maintain a high performance-to-weight ratio, and recover from excessive forces resulting from macro fracture. As a result, it has developed the ability to create original bone, remove damaged bone, quickly repair life-threatening fractures, slowly and methodically engineer improvements to reinforce habitually stressed bone regions, and change its strength/mass relationship by adding or removing bone material. This mass modulation results in the familiar curved bone morphology seen most readily in the long bones of the appendages. Curvature is hypothesized to be beneficial because a curved structure has a predictable location of breakage due to the increased stress concentrations that the bone experiences at the apex of the curve. This may allow the bone to target that predictable apex for reinforcement, instead of having to reinforce scattered areas of bone structure (Bertram and Biewener, 1988). Consequently, as the bone sees varying force vectors during daily movement, the curved bone's predictability and ease of reinforcement is suggested to be superior to the force-minimizing, completely straight bone morphology because the straight bone could break at any point in the midshaft region, resulting in a greater area of

bone needing to be reinforced in possible anticipation of mitigating future fractures (Skerry, 2008).

1.6 Fracture Repair

When significant damage occurs to the bone, a swift physiological response (Figure 1.8) ensures that the bone is repaired as quickly as possible by creating *woven bone*, a structure that forfeits ideal parameters in favor of a “quick fix” that is found in both damaged and very young skeletons where bone growth must be expedited.

Figure 1.8 Fracture repair phases. [55]

Woven bone does not obey a specific orientation or structure, which reflects the lack of osteoblast cooperation that occurs in the slower, more organized bone remodeling. Additionally, since more osteoblasts are utilized simultaneously in woven bone creation than in normal remodeling, a higher concentration of osteocytes exists in woven bone than in normal, remodeled bone. A significant structural shift occurs after the woven bone has been created and the wound

filled or the skeleton built. Since woven bone is an intrinsically unstructured design, the bone will begin the slower process of bone remodeling to replace this quickly formed woven bone with highly structured bone called *lamellar bone*. During the transition from woven to lamellar bone, the concentration of osteocytes in the bone decreases. Lamellar bone is stronger than woven bone because it comprises concentric lamellae, where the bone structure orientation of each discrete lamella is arranged in a direction offset from the orientation of adjacent lamellar structure. The combination of neighboring lamella and their differing structural orientation results in a foundational composition that transfers force between lamellae while allowing for minute flex between lamellar sheets. The cement lines, which separate osteons from each other and from interstitial bone matrix, are concentrically weak lines in the bone matrix. As a result, microcracks that intersect a cement line will often follow it in the direction of least resistance, which is often in a lateral plane parallel to the osteon. This prevents many microcracks from going through osteons, which further protects bone from transverse breaks.

1.7 On Varying Individual Remodeling Triggers

It should be mentioned here that the state of addition or removal of bone is not triggered by the same magnitudes of damage or disuse for each individual. The relativity of bone damage and disuse magnitudes can be demonstrated by exhibiting the bones of a professional weightlifter, who loads his or her bones to greater magnitudes and frequencies than the average person. In the case of the weightlifter, reducing the daily workout will result in bones being placed in “disuse” remodeling, even though the reduced daily workout may still expose the weightlifter to far greater forces than the lower-activity person. Likewise, the average person’s bones will launch into damage-induced remodeling if the person were to suddenly attempt the weightlifter’s reduced workout. Interestingly, Skerry (2008) has hypothesized that there may be a

minimum bone density, or minimum disuse remodeling equilibrium that is genetically coded into different areas of different bones in the skeleton, based upon the historical loads that an individual's genetic predecessors had experienced. This could possibly explain why the human skull maintains its porosity, without undergoing constant disuse remodeling, despite only rarely being loaded, and with small forces. A study sought to confirm the existence of a "genetic minimum" by growing fetal bones in vitro and in vivo for time periods that allow the bone to create shapes vaguely akin to adult bones while maintaining that the fetal bones that were grown do not match the adult bones in terms of porosity, function or accurate form (Garcia-Cabezas et al., 2004; Chen et al., 1995; Miller, 2003).

1.8 Apoptosis Overview

The body has constant use for selective cell self-termination; removing defective or dangerous cells, maintaining homeostasis, and removing cellular excess and scaffolds during development.

When toxins, radiation, DNA damage and nutrient restriction occur, they frequently result in a defective cell, or a cell that has the potential to harm the body. As a result, intrinsic or extrinsic factors cause the cell to destroy itself by apoptosis, which provides an efficient and clean route to safely remove the cell. The perpetual process of replacing cells requires that the number of cells added matches the number of cells that they are replacing in order to avoid a build-up or shortage of cells (Thompson, 1995). If cells fail to apoptose at the correct time, or apoptose too frequently, tumors (Liang et al., 2001) or tissue erosion can occur, respectively. When a body component develops, more cell structure is created than the end product requires. For example, in human fetuses, distal appendage regions initialize as clubs that erode via apoptosis to sculpt the club into the final form of fingers or toes.

Figure 1.9 Apoptosis pathway, with "blebbing" and DNA damage. [56]

Although apoptosis and necrosis both result in cell death, apoptosis maintains a highly regulated, complex process (Figure 1.9) that assures that the cell is efficiently disassembled in packets ideal for removal by immune cells while avoiding both inflammation and material leakage into adjacent regions (Luthi and Martin, 2007). Additionally, while necrosis may occur through a myriad of processes, apoptosis only occurs from a single primary mechanism.

A cell conducts apoptosis in response to either extracellular or intrinsic signals.

Extracellular signals must either penetrate the cell's membrane or transduce a signal through the

membrane via a surface receptor (Ashkenazi and Dixit, 1998). The candidate cell releases intrinsic signals. Regardless of where the signal originates, there are only two known methods of activation for apoptosis: by transforming zymogens into enzymes, or by ceasing the inhibition of enzymes. In both cases, these enzymes orderly dismantle the cell's organelles and the dying cell packages the broken-down components of the organelles into packets removed by phagocytotic immune cells (Luthi and Martin, 2007).

1.9 Introduction to Osteocyte Anatomy and Purpose

Osteocytes are the most numerous of all bone cells, yet their function and processes are only vaguely understood. They have been suggested as controllers, activators or otherwise associates of remodeling, and as their importance is becoming better understood, increased research reveals more and more about their function. Osteocytes modify their behavior and longevity in direct correlation to hormone types and concentrations (Bringham, 2002; Divieti et al., 2001; Tomkinson et al., 1997) as well as to cytokines (Ahuja et al., 2003), which are a large range of proteins and glycoproteins integral to cellular signaling. Additionally, osteocytes have demonstrated a particular affinity for the influence of human parathyroid hormone (PTH), which corresponds with increased viable osteocyte densities (Divieti, 2005). There are explicit membrane signal receptors for PTH, estrogen, and others that reside on the outer surface of osteocytes. One of the current areas of interest is the correlation between a decrease in estrogen in postmenopausal women and the increase in osteocyte apoptosis (Bringham, 2002). The new focus of research on the osteocyte roles and functions have resulted in a growing body of evidence that supports the possibility of osteocytes (Figure 1.10) existing in part as mechanosensors that detect strains that occur in the bone due to loading stresses, and conduct the detection of the strains to surrounding cells that comprise the bone. In addition to sensing the

strain magnitudes, osteocytes may also respond to the strains (Rubin et al., 2006) by inducing apoptosis, which is a controlled self-digestion that removes the cell.

Figure 1.10 Osteocyte with processes. [53]

The role of osteocytes in osteoporosis has been suggested to correlate with decreased efficiency of bone remodeling induced as a response to the age-related decrease of osteocytes (Dunstan et al., 1993; Frost, 1960) from the matrix of the bone architecture, resulting in a crippled ability to remove areas of bone that contain damage in the form of microcracks. This decrease may be caused by the decrease in estrogen as aforementioned, or may be a combination of both estrogen decreases and natural age-related decreases in viable osteocytes. Additionally, new findings are shining light on additional molecular osteocyte controllers, such as the CD40 ligand, which research has indicated may protect osteocytes from apoptosing (Bonewald, 2004). Current evidence that osteocytes hold a substantial influence over the frequency of bone remodeling shows regions of high densities of apoptotic osteocytes that coincide with regions

that experience an elevated occurrence of bone resorption (Gu et al., 2005; Noble et al., 2003; Verborgt et al., 2000). Additional research raised the suggestion that the osteocyte apoptosis activity is induced in order to somehow signal osteoclasts or pre-osteoclast progenitor cells in such a way that conveys the location and magnitude of microdamage within the bone matrix. As a result, osteoclasts may use this stimulus to fixate on a target of microdamage in order to remove it (Noble et al., 2003).

1.10 Osteocyte Apoptosis Triggers

Fatigue induced bone matrix damage in the form of microcracks is one factor responsible for the occurrence of osteocyte apoptosis in the affected regions. Suitable empirical data exists to support this assertion.

Verborgt et al. (2000) arrived at three important conclusions by inducing matrix damage in adult rat ulnae. First, fatigue damage was found to result in osteocyte apoptosis. Second, spatial distributions of osteocyte apoptosis were found to occur in conjunction with regions occupied by microcracks. Third, osteoclastic resorption coincided with the presence of both osteocyte apoptosis and microcracks caused by fatigue. A 400% increase in TUNEL-detected osteocyte apoptosis was observed in bone adjacent to microcracks; in comparison, regions in the bone lacking microcracks had similar osteocyte apoptosis rates as unloaded control ulnae, reinforcing the hypothesis that osteocyte apoptosis is caused by the presence of microcracks induced by fatigue. Related work from the same laboratory found diminished osteocyte integrity in proximity to cortical resorption spaces appearing after bone fatigue (Bentolila et al., 1998), and the presence of increased osteocyte apoptosis resulting from targeted bone damage in vivo (Qiu et al., 1997). Additionally, a separate team (Noble et al., 1998) discovered an increase in

osteocyte apoptosis in a murine model resulting from the plastic deformation induced by the cyclical application of supraphysiological strains.

Tami et al. (2002) developed a computational model to predict the impact on the canicular system from three varieties of microcracks: microcracks that act to block the convection of interstitial fluid, microcracks that act to divert the convection of interstitial fluid, and microcracks that act as a reservoir to collect and slow the convection of interstitial fluid. The model predicted that microcracks blocking interstitial flow cut osteocytes off from the blood supply, resulting in an inadequate nutrient exchange rate. Microcracks that divert the convection of interstitial fluid away from the osteocytes demonstrated results similar to microcracks that act as a block in that the osteocyte is prevented from adequate new trade exchange. Microcracks that act as a reservoir to collect and slow the rate of interstitial flow through the canicular network compromised the nutrient exchange rate experienced by the osteocytes. In all three circumstances, fatigue induced microcracks were directly responsible for decreased nutrient exchange within the canicular network, resulting in differing degrees of metabolic starvation experienced by the osteocytes.

A research group (Noble et al., 2003) studying the effects of small intervals of mechanical loading with regard to osteocyte apoptosis rates in rat ulnae found that osteocyte strain survival rates followed a U-shaped response curve. At very low strains, osteocyte apoptosis occurred to a significant degree; the application of moderate strains resulted in an increased rate of osteocyte viability. At very high strains, osteocyte apoptosis also occurred significantly. The U-shaped morphology and the osteocyte apoptosis response curve indicate that osteocyte apoptosis occurs under both strain extremes: disuse and damage.

The occurrence of osteocyte apoptosis appears to be induced not only by the stimulus of strain induced by stress loads on the bone matrix, but additionally by the *lack* of strain stimulus, which is referred to as bone *disuse*. When a bone is deprived of loading stress to the point at which the lack of stress is less than a critical loading magnitude, disuse occurs. When exposed to low strains, osteocytes apoptose in a manner identical to apoptosis in response to high strains (Aguirre et al., 2006). In response to disuse-induced osteocyte apoptosis, bone remodeling units tunnel through the bone (Martin et al., 1998). However, since there is no damage to the bone placed in disuse, the remodeling units remove structurally sound bone and insert a new osteon in the volume where the disused bone was. The addition of a Haversian canal where bone used to be results in an increase in porosity in cases of long-term disuse, which in turn results in a weaker bone section. Thus, while the presence of increased strain results in the repair of damage and a corresponding increase in bone strength, the lack of strain results in decreased bone strength. Despite the reduction in bone strength, it is hypothesized that the removal of bone from unused regions serves to streamline the mass/strength relationship; by removing unnecessary bone, less resources will be required to maintain it, the materials can be used elsewhere, and less extra weight will result in a higher motile efficiency (Martin et al., 1998).

Since disuse can lead to – or further the progression of – osteoporosis, if the functionality and mechanisms behind the osteocyte-remodeling interface are elucidated, then perhaps therapies may be developed to prevent, stop, or reverse osteoporosis. Since many elderly people are not able to move as often as they should to preserve bone mass, the control of the osteocytes' apoptotic signaling could add or remove signals that would prevent “streamlining” remodeling from occurring, thus compensating for the lack of strain placed on the bones, instead of using strain application, which could cause injury in the elderly, and may not be feasible. In addition,

some of the strain-inducing therapies are difficult to target towards some of the more delicate bones that are prone to osteoporotic breakage, such as ribs and fingers. Additionally, a more thorough understanding of the osteocyte function could play a key part in developing therapies for astronauts, who are exposed to extreme disuse through occupation in zero gravity environments. If the therapies could reduce or eliminate the need to provide equilibrium levels of bone strain through time-consuming loading exercises, then the astronauts could perform with higher efficiency.

1.11 Current Hypotheses Regarding Osteocyte Function and Apoptosis

As researchers attempt to peel back the blanket of uncertainty that shrouds the functions of osteocytes, several questions arise. The first is perhaps the most important: “What role do osteocytes have in general?” From this initial question, many branching points of mystery arise, like heads on a hydra: “What role do osteocytes play in the targeting of remodeling?”, “Can osteocytes steer basic multicellular units?”, “Are osteocytes the primary points of control for bone remodeling?”, “Can osteocytes control other parts of bone functionality?”, “What does a bone without functioning osteocytes look like – what damage does osteocyte mis-coordination or malfunction exact on its native bone?”, and ultimately, “Can we –and how could we – harness the common osteocyte to do our bidding?”. As these questions breed into more discrete and esoteric bloodlines, further hypotheses seeking to elucidate increasingly convergent goals come to life. The following is a review of several current hypotheses seeking to understand the biological laws that govern osteocyte functionality with regards to disuse.

Just before the turn of the last century, Dodd et al. (1999) suggested that osteocytes transduce disuse into apoptosis by being starved for air, a condition referred to as *hypoxia*. The researchers thought that during normal loading, the bone endured subtle flexing of its internal

structure. This flexing served to displace fluid as canaliculi channels opened and closed, resulting in pressure gradients that drove nutrients to the osteocytes from the Haversian canals, and removed osteocyte waste products. The use of flexure and canaliculi to draw fluid from the Haversian canal was suggested because otherwise, the osteocytes spanning the lamellae – especially distal from the Haversian canal – are possibly too far to benefit from the passive diffusion of nutrients from the canal to the osteocyte. Dodd et al. (1999) hypothesized that under disuse conditions, this flexing did not occur, so no pressure gradients were generated, resulting in an insufficient fluid flow to supply the osteocytes with nutrients, the most immediately vital nutrient being oxygen. Using pimonidazole, a hypoxia marker, and a monoclonal antibody follow-up, the team was able to determine which osteocytes were hypoxic. Applying this approach to an avian ulna model revealed that 24 hours of disuse was a long enough interval for osteocytes to demonstrate an increased expression of hypoxia marker. The reader should note that the presence of hypoxia marker expression is not equivalent to apoptosis occurrence; instead, the marker may be the fountainhead of downstream mechanisms that result in apoptosis. A follow-up study (Gross et al., 2001) demonstrated that after the initial 24-hour increase in hypoxia marker concentration, further marker concentration increase does not occur. The team further hypothesized that since the strain densities in the bones of the skeleton are differently loaded, then what would be considered low strain for one bone would be a high relative strain in another bone at a different part of the skeleton. Considering that osteocytes reside in all bones, and complying with the theory that the osteocytes are the main regulatory elements, the team suggested that osteocytes upregulate hypoxia signals in response to *relative* changes from differing area-specific baselines. For example, a small magnitude of strain applied to a skull and not resulting in disuse remodeling could result in disuse remodeling in the femur.

A separate research team (Turner et al., 2002) hypothesized that osteocytes function similarly to a neural network because the osteocytes that the team examined appeared to become less sensitive to a particular strain level after prolonged exposure. The team argues that it is the *departure* from normal strain environments that is the catalyst for bone remodeling. They found that osteocytes “habituate” – become accustomed to – repeating mechanical loads after being exposed to 40 cycles of loading. This was suggested to explain the observed difference between baseline strain magnitudes and corresponding remodeling activity triggers in different bones of the skeleton.

Also in the same time frame as the previous studies, another team (Smit et al., 2002) similarly explored the implications of fluid flow caused by strain in the bone matrix with regard to remodeling. Their research focused on the mechanism by which a BMU may be steered by osteocyte vitality as a result of fluid flow morphology induced by correlated strain environments at different points surrounding the structure of the BMU as it tunneled through cortical bone. Using a FEM approach, they showed that the cutting-cone tip of their modeled BMU experienced an area of consistent fluid stasis resulting from the geometry of the cone and the surrounding bone matrix. As a result, the team further suggested that osteocytes located directly adjacent to this cutting-cone tip apoptosed as a result of hypoxia induced by fluid stasis in this region. This, the team stated, may act as a steering mechanism for the BMU if apoptotic osteocytes sent or inhibited signals that resulted in the BMU being attracted to the site of apoptosis. However, a separate histological BMU analysis (Hadi, 2007) revealed little correlation between BMU geometry, steering direction and osteocyte apoptosis density.

An examination by Stevens et al. (2006) found that hypoxic osteocyte concentrations did not significantly differ between loaded and unloaded bones in a murine hind limb extension

model. The team used the same hypoxia marker to assess oxygen deprivation that Dodd et al. (1999) utilized. However, the team's findings supported the hypothesis that disuse leads to lowered levels of pressure and thus lowered magnitudes of fluid flow. Known differences between murine and avian bone remodeling may be responsible for the discrepancies between the two studies, because the adult turkeys used in Dodd et al. (1999) were able to remodel, while the mice used in the Stevens et al. study were not able to remodel to any significant level. Additional differences in methodology stemming from dissimilar approaches to simulating disuse may also be responsible for the different experimental results.

Malone et al. (2008) found that osteocytes are covered with primary cilia on their exterior that may interface with the surrounding environment by transducing fluid flow into osteocyte apoptosis by translating exterior cilia movement to the internal release of pro-apoptosis signals. If true, this could replace the hypothesis that hypoxia and further nutrient restriction is the cause of osteocyte apoptosis in response to disuse conditions.

Future research of osteocyte function may produce accelerated returns, as more gene knockout research protocols are utilized to target and remove specific gene expressions, which may change cellular function and further elucidate the function of the targeted gene. For example, a future study may remove a gene known to facilitate cilia growth in osteocytes. If osteocytes that lack cilia no longer respond to fluid shear, then perhaps the gene or the cilia are somehow involved in the process of fluid shear detection. Perhaps the addition of this knowledge will play a critical role in the development of new therapies for bone disorders.

1.12 Previous Work

Bone has been computationally modeled (Figure 1.11) in many studies; the models have grown in accuracy from early models based upon anatomical structure morphology. These prototypical models often relied upon stress, strain, or strain energy density as the sole entity responsible for stimulating bone remodeling. These models used varying mathematical approaches to gain understanding of the underlying processes responsible for bone remodeling and adaptation.

Figure 1.11 Iterative computational trabecular alignment. [47]

By producing models specifying different mathematical techniques and relationships, researchers were able to determine which models fit empirical in vivo bone performance the best. As more results expanded knowledge of bone function, the research evolved to include mechanistically driven computational models that utilized increasingly sophisticated finite element models.

Figure 1.12 Iterative apparent density predictions from a computational model of the femur. [12]

Huiskes et al. (1987) developed a computational finite element model based upon Wolff's Law of microstructure seeking alignment with principle stress directions, in an attempt to model the stress shielding phenomenon, where bone that is removed from stress will remodel to reduce local mass, leading to bone erosion and possible mechanical failure at the site of the implant. The model utilized a feedback control, employing a strain energy density variable, which altered according to correlating bone parameters, such as volumetric density. Emphatically, Huiskes et al. (1987) focused the model on mechanistic equations, instead of physiological agents. The group found that an equilibrium strain energy density existed where the bone resorption could occur on the outer periosteal surface as opposed to the cortical volume, resulting in decreased structural weakening due to stress shielding. This equilibrium was found to theoretically occur when implants were sufficiently flexible, which resulted in cortical bone adjacent to the implant receiving enough strain to prevent local bone resorption, which as a result prevented implant loosening.

Carter et al. (1989) used a two-dimensional finite element model to map transient apparent bone density under the application of varying loads. The finite element mesh was aligned in the principle stress directions described by Wolff's Law, but the team found that the calculated bone density map (Figure 1.12) related more closely to von Meyer's less accepted trabecular morphology, which begged further refinement of their mathematical platform. Force vectors were applied to different locations on the femoral head and the greater trochanter pursuant with normal loading due to walking, which was a departure from many previous models.

Harrigan and Hamilton (1994) used a finite element model to interweave two conceptual processes of bone function: the first process is oriented towards modifying bone macrostructure

to better support mechanical loading; the second process optimizes bone microstructure formation and removal to best support the loading while maintaining an efficient mass-performance ratio. Using strain energy as the central variable driving remodeling, the team utilized an “indicator function” which served to quantify the balance between strain energy experienced in a section of bone with the volumetric density of that section – chosen to relate to the Huiskes et al. (1987) equation for computing remodeling rate. The finite element model used a stiffness matrix based upon the elastic modulus of the bone tissue - which was assumed to be isotropic trabecular bone - at each finite element. This matrix relied upon the changing bone parameters within each element to calculate the overall matrix parameters. The result of the study showed that a single minimum indicator function magnitude existed, which suggested that the mathematical models used for the study had converged at a solution that pointed towards bone having a single optimal mass-performance structure for the calculated strain energies.

Turner et al. (1997) used finite element analysis to explore the creation of a model that could successfully predict structural modifications made by bone to adapt to specific loading conditions. The proximal femur model successfully showed density changes that concurred with empirical lab results. This demonstrated that it was feasible to predict trabecular alterations by utilizing mathematics aimed at minimizing strain gradients through the re-alignment of trabecular microstructure in a matrix of finite elements. The team used their results to try to gain insight into the fundamental mechanisms behind bone remodeling, hypothesizing that pressure gradient changes in trabecular bone could activate osteoregulatory cells through fluid flow modulation.

Hazelwood et al. (2001) created a mechanistic computational model of a representative volume of bone (Figure 1.13) for bone remodeling driven by the replacement and reinforcement

of damaged bone with fresh bone and the removal of under loaded bone to streamline the mass/performance relationship.

Figure 1.13 Representative volume of bone utilized for the Hazelwood et al. (2001) simulation. [28]

Their code was the first to incorporate an integrated disuse model by using common parameter variables of daily BMU activation frequency for both the damage and disuse removal portions of the code. Elastic modulus and porosity values are looped in a code circuit (Figure 1.14) that changes based upon the alteration of independent variables, such as the loading conditions or the number of walking cycles per day experienced. The mathematical model outputs the changes in bone parameters during the calculation interval, such as porosity, activation frequency and mechanical stimulus for a representative volume of bone. The research found that damped oscillations occurred with each of the remodeling parameters, which concurs with the physiological process of bone remodeling to find an optimal equilibrium value for each parameter. The Hazelwood et al. (2001) code is easily portable, resulting in its use in finite element models in various bones, animals and loading conditions.

Figure 1.14 Bone remodeling code flowchart. [28]

Nyman et al. (2004) adopted the original Hazelwood et al. (2001) computational model and modified it into a platform for the exploration of bisphosphonate –impacted remodeling processes using finite element modeling. This platform was an ideal candidate code upon which to base my thesis because of its ability to be modified by incorporating new parameters for osteocyte apoptosis. To my knowledge at the time of this writing, no research other than the current thesis has been performed that has developed a computational model of bone remodeling that is driven by the apoptosis of osteocytes in the bone matrix, used to predict both damage and disuse induced bone optimization.

1.13 Study Goals

The research and creation of a computational model using a foundational program modified to represent osteocyte mediated bone remodeling is important because it may further the understanding of the role of the osteocyte and may assist in paving the way to creating new

therapies for osteoporosis that utilize or target the osteocyte or its functional pathways. The research contained in this thesis serves three main purposes. The first is to validate a model against empirical data to demonstrate that a relationship can be drawn between osteocyte functionality and bone remodeling. Second, if the two can be validated, then it will have been demonstrated that it is feasible to predict remodeling parameters through an osteocyte-centric computational model. The third purpose is to show that future models can be used to further elucidate osteocyte function by validating empirical evidence in support of novel therapeutic endeavors with regard to the role of osteocyte apoptosis in osteoporosis.

As this thesis progresses, I will first state the research I build upon, explain the reasoning behind the modifications, then report the results of the research and discuss the outcome in the subsequent section.

CHAPTER 2: METHODS

2.1 Overview: Code Sections

The code modifications performed for this thesis were based upon the foundational code created by Hazelwood et al. (2001), and translated from Fortran to Matlab programming language by Nyman et al. (2004) and modified for a three-dimensional finite element of bone. The code is comprised of three discrete sections.

The first section is responsible for defining the parameter baseline values (Table 2.1), such as the time intervals required for the bone to undergo removal of bone, reversal, and addition of new bone. Anatomical dimensions, such as cement line radius and Haversian canal radius, are also defined in this section as well. This area is also used as a storage place for variables used within the mechanics of the code to process the inputs and formulate the outputs; loading rates, code run duration and time step size are defined here as well.

The second acts as a variable assignment zone, called the “Prior Conditions” and uses the first remodeling period (45 days in this simulation) of the code to generate these initial variables. This is required due to the physiological behavior of the basic multicellular unit. The BMU employs osteoclasts located at the frontal cutting cone to first remove bone for the removal time, T_r . Separating the cutting cone from the osteoblasts in the middle and rearward portions of the BMU is a “resting area” where the BMU reverses from removing bone to adding bone. This resting interval is called the “reversal interval”, T_v . After the T_v interval concludes, the osteoblasts at the middle and rearward positions of the BMU reach the bone area that was previously removed by the osteocytes. The osteoblasts add bone over a period of bone formation, T_f . Thus for the first remodeling period $= T_r + T_v + T_f = 45$ days, the code is cycling initial

parameters day by day in order to set up the initial placement and function of the BMU. At day 46, the code makes its first true prediction, based upon the elapsed variables initialized in the Prior Conditions.

2.2 Prior Conditions

Based upon equilibrium values, the Prior Conditions initialize the early iterations of the program (Appendix B.2) by defining initial variables set to match empirical data for a caged New Zealand White rabbit tibial equilibrium.

Table 2.1 Remodeling Parameters used in the Prior Conditions and Simulation.

Variable (units)	Description	Value	Source
R_c (mm)	Cement Line Radius	0.07159	Hadi (2007)
R_h (mm)	Haversian Canal Radius	0.02163	Hadi (2007)
T_r (days)	Resorption Period	9	Hedgecock et al. (2007)
T_v (days)	Reversal Period	10	Scaled From Nyman et al. (2004)
T_f (days)	Formation Period	26	Scaled From Nyman et al. (2004)
Φ_0	Equilibrium Stimulus	$0.53 \cdot 10^{-10}$	Iterative Testing
por_0 (void space/area)	Initial Cortical Porosity	0.0322	Hedgecock et al. (2007)
$Acfmax_1$ (#BMU/mm ² /day)	Max Damage Ac.f	1	Nyman et al. (2004)
$Acfmax_2$ (#BMU/mm ² /day)	Max Disuse Ac.f	0.5	Nyman et al. (2004)
$Acfdam_0$ (#BMU/mm ² /day)	Equilibrium Damage Ac.f	0.0094	Iterative Testing
$Acfdis_0$ (#BMU/mm ² /day)	Equilibrium Disuse Ac.f	0	Nyman et al. (2004)
d_0 (crack length/area, mm/mm ²)	Equilibrium Damage	0.03662944	Hazelwood et al. (2001)
R_L (cycles/day)	Loading Rate	3000	Qin and Ruben (1998)
sa_{max} (mm ²)	SA Normalization Constant	4.1905	Nyman et al. (2004)

2.3 Sources of Data

The code relies upon several constant values obtained from empirical data (Appendix B.1). The cement line radius was calculated from the relationship:

$$R_c = W.Wi + R_h \quad (1)$$

where $W.W_i$ is the wall width, and was determined empirically by Hadi (2007). The Haversian canal radius R_h was also determined by Hadi (2007). The formation time interval, T_f , was determined by Hedgecock et al. (2007), and the T_r and T_v values were determined by:

$$\frac{T_r}{N_r} = \frac{T_f}{N_f} \quad (2)$$

$$\text{so, } T_r = \frac{N_r * T_f}{N_f} \quad (3)$$

where N_r and N_f are the number of resorbing and reforming BMUs respectively. T_v was determined by scaling the original value in Nyman et al. (2004) with accordance to T_r (Equation 3), then iteratively adjusted until the output matched empirical values. The initial porosity for cortical bone, por_0 , was used from New Zealand white rabbit empirical data from Hedgecock et al. (2007).

The last section is responsible for running the actual simulation, beginning with day 46 and continuing until the last day of the code, adopts the variable values assigned in the Prior Conditions, and uses them to predict changes in porosity, osteocytes apoptosis, and other supporting variables. This section requires a more detailed discussion, as follows.

2.4 Simulation

The simulation (Appendix B.3 – B.7) occurs entirely within a Matlab “for” loop, used to cycle all of the relationships and variables from one day and iteratively carrying their values into the next day. The simulation flowchart (Figure 2.1) illustrates the processes that the code cycles through to deliver the porosity and remodeling parameters required to predict it. After the Prior Conditions have terminated and the simulation has begun, the first action within the iteration of

day 47 is to assign a value to the modulus of elasticity based upon the porosity of the last run of the Prior Conditions at day 46.

The relationship between elastic modulus, E , and the porosity is:

$$E = E_0 \times (1-\rho_{or})^b \quad (4)$$

as determined by Hazelwood et al. (2001). The cortical relationship specific to this thesis was determined by curve fitting $\ln(E)$ vs. $\ln(1-\rho_{or})$, where the data for elastic modulus and the porosity came from empirical data from Hedgecock et al. (2007). The resulting cortical relationship is:

$$E = 28969 \times (1-\rho_{or})^{5.24} \quad (5)$$

Trabecular values for E_0 and b were 14927 and 1.33, respectively, as determined by Hazelwood et al. (2001).

Figure 2.1 Flowchart of code mechanisms.

The force is constant for the duration of the Prior Conditions and the simulation. The cross sectional area of the representative bone volume used in the simulation is defined as a constant 100mm^2 in the parameter definition section. With the force and area known for the particular iteration, the stress is found using the equation, stress = force / area, and is in units of N/mm^2 . Using the Young's Modulus equation:

$$E = \frac{\sigma}{\varepsilon} \quad (6)$$

where σ is stress and ε is strain, strain was found from known values of E and σ . Strain is used by the code in units of “strain”, but is converted for output as “microstrain”, a term used because it is the standard “unit” used for bone microdamage expression.

The mechanical stimulus, a loading potential responsible for defining the magnitude of damage-induced remodeling, is defined by Nyman et al. (2004):

$$\Phi = R_L \times \varepsilon^q \quad (7)$$

where the daily occurrences of loading, R_L , is 3000 cycles per day as determined by strain values calculated from preliminary finite element modeling studies of the bone section and converted to daily cycles by using strain/loading rate relationships published by Qin et al. (1998). Also, $q = 4$, which is an exponent responsible for reconciling the balance between peak strain and R_L to the mechanical stimulus.

The mechanical stimulus (Φ) is responsible for translating the amount of strain experienced by the bone into the magnitude of the bone's reaction to that strain. This translation occurs in two separate relationships; the first occurs when the mechanical stimulus is greater in magnitude than the equilibrium mechanical stimulus and the second is when the stimulus is

lower in magnitude than the equilibrium value. The equilibrium mechanical stimulus occurs at a specific force where the amount of bone added and removed negate each other, resulting in a constant porosity over the interval of the prediction duration. Thus, when the mechanical stimulus is elevated over the equilibrium stimulus, the bone is in damage conditions and is experiencing microcrack damage from strain. In these conditions, the stimulus (Φ) defines the damage formation rate, D_f , which combines with the damage removal rate to form the total rate of damage accrual, $D(t)$. From there, $D(t)$ assigns a magnitude to the percent of the damage-induced osteocyte apoptosis. Likewise, when the mechanical stimulus is below the equilibrium stimulus, the bone is in disuse and is resorbing bone due to insufficient strain. The stimulus (Φ) then assigns a percent of osteocyte apoptosis caused by disuse.

2.5 Microdamage Creation and Removal

The rate at which microdamage accumulates in the bone matrix was determined to be related to a damage coefficient, k_D , and mechanical stimulus (Φ), caused by daily loading. The relationship was found by Nyman et al. (2004) to be:

$$\frac{d(D_f)}{dt} = k_D * \Phi, \quad (8)$$

$$\text{where } k_D = d_0 * Ac \cdot f_0 * \pi R_c^2 * \frac{F_s}{\Phi_0} \text{ for cortical bone, and} \quad (9)$$

$$\text{where } k_D = d_0 * Ac \cdot f_0 * 0.5 \pi R_c^2 * \frac{F_s}{\Phi_0} \text{ for trabecular bone,} \quad (10)$$

and where d_0 is the equilibrium damage, πR_c^2 is the cortical resorption area, $0.5\pi R_c^2$ is the trabecular resorption area (half of the area resorbed for cortical bone), and Φ_0 is the equilibrium mechanical stimulus. The equilibrium activation frequency relationship is:

$$Ac.f_0 = \frac{sa_0}{sa_{\max}} * (Ac.fdam_0 + Ac.fdis_0), \quad (11)$$

where $sa_0 = 0.9406 \text{ mm}^2$ is the surface area at equilibrium, $sa_{\max} = 4.1905$ is a constant used to normalize the surface area, $Ac.fdam_0$ is the damage-induced activation frequency at equilibrium, and $Ac.fdis_0$ is the disuse-induced activation frequency at equilibrium. For both cortical and trabecular damage formation rates, the damage repair factor, $F_s = 5$, is a specificity factor due to the fact that areas that undergo remodeling are linked to microdamage accumulation at a higher rate than stochastic remodeling can predict.

The rate at which microcrack damage is removed was borrowed from a previous model (Martin et al., 1998), and was defined as

$$\frac{d(D_R)}{dt} = D * Ac.f * Rs.Ar * F_s \quad (12)$$

where D is the previous iteration's accumulated damage, $Ac.f$ is the activation frequency, $Rs.Ar$ is the resorption area, and F_s is the specificity factor. $Rs.Ar$, the resorption area, has different values for cortical and trabecular bone; for cortical bone, $Rs.Ar = \pi R_c^2$, and for trabecular bone, $Rs.Ar = 0.5 \pi R_c^2$. The activation frequency, $Ac.f$, is an iterative variable calculated daily from the percent of osteocyte apoptosis as described in the following sections.

The sum of the damage formation rate and the damage removal rate is the damage update, which describes the daily amount of damage accumulation in terms of microcrack length/density in an element of bone. The overall daily damage rate (Appendix B.5) is

$$\frac{d(D(t))}{dt} = \frac{d(D(t-1))}{dt} + \left[\frac{d(D_F)}{dt} - \frac{d(D_R)}{dt} \right] dt \quad (13)$$

2.6 Simulation of Apoptosis - Preface

An explanation of general code mechanics is necessary before covering the specific methods used to obtain the relationships for apoptosis; while the main interest of the code is the effect on porosity of the number of osteocytes apoptosing, there was insufficient empirical data to use the number of osteocyte apoptosis occurrences. Instead, the percent change from the original magnitude of osteocyte apoptosis at the time interval of the first simulation cycle was a better unit to use, because it was a more universally used empirical data unit. While it is of course possible to calculate the number of osteocytes from a percentage, the overall numbers of osteocytes in bone were not judged under equivalent circumstances; the data for disuse conditions were harvested from a source (Aguirre et al., 2006) which used longitudinal vertebral cross sections of a murine model, while the data for damage conditions were sourced (Hedgecock et al., 2007) from a rabbit tibia cross section. Insufficient data exists to determine osteocyte numbers and apoptosis occurrence differences between the two animals of differing species, ages, activity levels, and bone anatomies, and with disagreeing data expressing the total number of osteocytes in the bone (Aguirre et al., 2006: 2974+/-176 total osteocytes “per animal”. Hedgecock et al., 2007: 545.6 osteocyte nuclei per mm² of cortical bone), it is necessary to rely chiefly upon percentage of osteocyte apoptosis to avoid additional loss of data resolution resulting from adding several routes of possible error. Since the number of osteocytes apoptosing

is of interest, a relationship was created to translate the percent of apoptosis to the number of osteocytes apoptosing. This relationship was created by assuming similar numbers of total osteocytes per mm^2 in each animal used for the harvest of empirical data. This allowed for the interpolation of the number of apoptosing osteocytes found from the Aguirre et al. data. Combining these data with the damage data from Hedgecock et al., and curve fitting the number of osteocyte apoptosis vs. the activation frequency ($\text{\#BMU}/\text{mm}^2/\text{day}$) allowed for the creation of a quadratic relationship (Appendix B.6), which offered the best curve fit to the data as determined by curve fitting at higher and lower polynomial degrees. Use of the quadratic formula and subsequent elimination of the unrealistic “twin” independent variable resulted in the following relationship:

$$\#ApoptoticOsteocytes/\text{mm}^2 = \frac{-b + \sqrt{b^2 - 4a(c - \text{AcfCondition})}}{2a} \quad (14)$$

where $a = 0.00005956430811$, $b = -0.002956855573126$, $c = 0.043179955361272$, and where *AcfCondition* differs in value depending upon the relative magnitudes of Φ and Φ_0 :

$$\text{AcfCondition} = \text{Acfdis} \text{ when } \Phi < \Phi_0, \text{ and} \quad (15)$$

$$\text{AcfCondition} = \text{Acfdam} \text{ when } \Phi_0 < \Phi. \quad (16)$$

Note that the “twin” independent solution utilizing:

$$\#ApoptoticOsteocytes/\text{mm}^2 = \frac{-b - \sqrt{b^2 - 4a(c - \text{AcfCondition})}}{2a} \quad (17)$$

was eliminated due to poor fit with empirical and expected results. In both Equations 14 and 17, *AcfCondition* is subtracted from c , because the original curve fit equation is:

$$AcfCondition = ax^2 + bx + c \quad (18)$$

where a , b , c hold values referenced in equation 14, and $x = \frac{\#ApoptoticOsteocytes}{mm^2}$. Using the quadratic equation requires $AcfCondition$ to be incorporated into c .

2.7 Simulation of Apoptosis – Damage Conditions

Damage-induced osteocyte apoptosis (Appendix B.5) is defined as the percentage of osteocytes undergoing apoptosis as a result of incurred microdamage in the bone per square millimeter, caused by the force exerted per number of cycles per day. This relationship, which was determined through best-fit curve fitting (Figure 2.2), is represented in the following equation:

$$Apopdam(t) = 11.123706479648 * D(t)^2 - 0.462061174425 * D(t) + 0.038583322125 \quad (19)$$

where $Apopdam(t)$ is the number of apoptotic osteocytes occurring in the bone concurrently with the day's damage, $D(t)$.

Figure 2.2 Blue circles represent data points of apoptotic osteocytes plotted against damage-contributing activation frequency. Red asterisks represent data points of curve-fit equation.

2.8 Simulation of Apoptosis – Disuse Conditions

Disuse conditions were defined as existing at mechanical stimulus values lower than the disuse threshold, Φ_0 . Apoptotic osteocytes provided the basis of the equations governing the disuse behavior of the code (Appendix B.4). Using empirical porosity values from Aguirre et al., (2006), mechanical stimulus during disuse was calculated from porosity values, and related to apoptotic osteocyte numbers. The resulting relationship was then converted into a composite of three linear equations, each applying to a consecutive mechanical stimulus range:

$$Apopdis(t) = 4.06666666666666 * 10^9 \Phi(t) - 0.000000000114717 * 10^9, \quad (20)$$

when $\Phi(t) < 3.16 * 10^{-11}$

$$Apopdis(t) = -2.967741935483874 * 10^8 \Phi(t) + 0.000000000231681 * 10^8, \quad (21)$$

when $3.16 * 10^{-11} \leq \Phi(t) < 3.47 * 10^{-11}$

$$Apopdis(t) = -1.294326241134754 * 10^7 \Phi(t) - 0.0000000001331913 * 10^7, \quad (22)$$

when $3.47 * 10^{-11} \leq \Phi(t) \leq \Phi_0$

Which is the numeric embodiment of the following (Figure 2.3):

Figure 2.3 Disuse apoptosis vs. Phi, Φ

Figure 2.3 was obtained from inputting the porosity values under disuse loading conditions from Aguirre et al. (2006) into the simulation (Appendix B.3 – B.4). These porosity values occurred at 1, 3, 7 and 18 days of elapsed unloaded conditions of murine tail bones. There were no pre-existing mention of mechanical stimulus (Φ , Φ) values corresponding to the Aguirre et al. (2006) data within that publication, and none were referenced from other sources therein. Therefore, the singular method of calculating the mechanical stimulus was by arranging the proper sets of equations with their applicable logic statements in the correct order, and inputting the Aguirre et al. (2006) unloaded porosity data into that computational arrangement. These data were placed in the simulation because the equations necessary to convert the Aguirre et al. (2006) unloaded porosity values into corresponding mechanical stimulus values were pre-existing in the code in the correct computational order. None of the equations contain modifications specific to this particular data source, though the algorithms that were specific to this study were the conversions from porosity to a cortical or trabecular Young's Modulus, which were customized to fit the tibial midshaft properties of New Zealand White rabbits used in this study. The Aguirre et al. (2006) team used different animals of different ages, possibly kept under different conditions than the rabbits used for this study; however, customizations particular to the Aguirre et al. (2006) porosity were not feasible due to the lack of relevant empirical data that would be vital to the calculation of these tailored Young's Modulus relationships. This is an acknowledged limitation, and the adoption of better set of modulus equations might further the accuracy of the current study.

The equations used to calculate the cortical disuse condition mechanical stimulus from the cortical disuse condition porosity from Aguirre et al. (2006) are, in order of operation:

$$Modulus(t) = 28969 * (1 - por(t - 1))^{5.24} \quad (23)$$

$$Stress(t) = \frac{Force(t)}{Area} \quad (24)$$

$$Strain(t) = \frac{Stress(t)}{Modulus(t)} \quad (25)$$

$$\Phi(t) = |Strain(t)|^q * R_L \quad (26)$$

where $Modulus(t)$ is the Young's Modulus of the current iteration, $por(t-1)$ is the porosity of the previous iteration, $\Phi(t)$ is the mechanical stimulus of the current iteration, q is the modifying exponent for the mechanical stimulus, and R_L is the loading rate in #cycles/day. In order to obtain an accurate computation of the mechanical stimulus from the input disuse condition cortical porosity values (Aguirre et al., 2006), the output of this chain of equations was extracted at day 1 of the simulation (day 46 of the over all code; the 45 day difference occurs during the Prior Conditions, before the simulation begins) – this allowed the correct value to be sampled before concurrent iterations modified the data for the simulation.

It is important to understand that there are no osteocytes that specifically apoptose from only damage or only disuse. Since the damage and disuse conditions do not occur simultaneously, the same population of osteocytes is at risk for apoptosis under both conditions. The total number of osteocytes apoptosing at any time is not the sum of the osteocytes apoptosing from disuse and damage; rather, either “Apopdis” or “Apopdam” will be nonzero, and the other will be zero because its condition of disuse or damage will not be applicable under the current condition at that particular day. The percent of damage-induced apoptotic osteocytes translates to the activation frequency contribution from damage. The relationship used to convert

the percentage of osteocyte apoptosis into the mechanical condition contribution to the activation frequency is determined by the same equation in both damage and disuse conditions:

$$Ac.fSubunit(t) = 17.739Apoptosis(t)^2 - 1.616Apoptosis(t) + 0.043 \quad (27)$$

where $Ac.fSubunit$ is the subunit of activation frequency occurring during disuse or damage (in units of #BMU/mm²/day) that is later used to calculate the overall activation frequency.

$SA(t) = \frac{sa(t)}{sa_{max}}$ is a unitless variable created from the normalization of the daily specific surface area, $sa(t)$ by the maximum specific surface area constant, sa_{max} . $Apoptosis(t)$ differs in value

depending upon the relative magnitudes of Φ and Φ_0 :

$$Apoptosis(t) = Apopdis(t), \text{ when } \Phi < \Phi_0 \quad (28)$$

$$Apoptosis(t) = Apopdam(t), \text{ when } \Phi_0 < \Phi \quad (29)$$

where $Apopdis(t)$ is the amount of disuse-induced osteocyte apoptosis and $Apopdam(t)$ is the amount of damage-induced osteocyte apoptosis. This equation was created by curve fitting (Figure 2.4) empirical data for the percent of apoptosis vs. activation frequency contribution for both damage (Hedgecock et al., 2007) and disuse (Aguirre et al., 2006). The Matlab code used to obtain the curve fit is exhibited in Appendix B.10.

Once the activation frequency contributions by damage and disuse are calculated, they combine to define the total activation frequency of BMUs for the current time interval, $Ac.f(t)$.

Figure 2.4 Curve fit of damage and disuse conditions for *Ac.f* contribution vs. percent apoptosis. Red points indicate the curve fit equation; blue points represent data points.

2.9 BMU Activation

BMU activity is governed by the amount of strain and the amount of microcracks in the bone element resulting from a combination of the magnitudes of the number of loading cycles and the forces on the bone element for the loading period. The activation frequency was borrowed from Nyman et al. (2004), who calculated the activation frequency based upon BMU activation curves that were formulated for the purpose of determining activation, given damage and disuse magnitudes. At equilibrium conditions, the mass of bone added or removed by rebuilding and resorbing BMUs, respectively, is balanced, with no overall gain or loss of porosity. This equilibrium stimulus (Φ_0), was set to 5.3×10^{-11} through iterative techniques used

to balance porosity. As the magnitude of activation frequency rises during disuse, defined as being below the disuse threshold, which is the equilibrium stimulus (Φ_o), the mass of bone declines in an inversely proportional manner. The total activation frequency is determined from both damage and disuse induced activation frequency subunits, $Acfdam(t)$ and $Acfdis(t)$, respectively. The equation for the total activation frequency in an element of bone on a particular day is:

$$Ac.f = SA(t) * (Acfdis(t) + Acfdam(t)) \quad (30)$$

where $Ac.f$ is the total activation frequency, and $SA(t) = \frac{sa(t)}{sa_{max}}$ is a unitless variable created from the normalization of the daily specific surface area, $sa(t)$ by the maximum specific surface area constant, sa_{max} . $Acfdis(t)$, the disuse-induced activation frequency subunit, and $Acfdam(t)$, the damage-induced activation frequency subunit, relate to $Apopdis(t)$ and $Apopdam(t)$ through Equation 27; these relations are then used to calculate the total activation frequency, $Ac.f$ in Equation 30.

Although histological studies clearly demonstrate that bone cavities from resorbing BMUs form into elongated, ellipses of irregular, decreasing diameter, for computational simplification, this code agrees with Nyman et al. (2004) in that it assumes that all resorbing BMUs create semicircular pathways through the longitudinal axis of bone, centered on the Haversian canals.

2.10 Change in Bone Porosity

The activation frequency is incorporated into two equations, which calculate the number of refilling and resorbing BMUs per mm^2 of bone (Appendix B.7):

For refilling BMUs:

$$NfBMU(t) = NfBMU(t-1) + [Ac.f(t - T_r - T_v) - Ac.f(t - T_r - T_v - T_f)] * dt \quad (31)$$

For resorbing BMUs:

$$NrBMU(t) = NrBMU(t-1) + [Ac.f(t) - Ac.f(t - T_r)] * dt \quad (32)$$

The number of refilling and resorbing BMUs is used to calculate the amounts of bone added and removed for the specific day are calculated from the following equations:

The amount of bone added for the day:

$$QfNf(t) = Qf * NfBMU(t) \quad (33)$$

The amount of bone removed for the day:

$$QrNr(t) = Qr * NrBMU(t) \quad (34)$$

$$\text{where } Qr = \frac{Rs.Ar}{T_r}, \text{ and } Qf = \frac{F.Ar}{T_f} \quad (35)$$

and where $Rs.Ar$ is the resorption area, and $F.Ar$ is the formation area. These two equations are used to find the total amount of bone added or removed for the particular day:

$$Qnet(t) = QfNf(t) - (1 - phc) * QrNr(t) \quad (36)$$

Where phc is a unitless constant equal to 0.0322, which is used to adjust the resorption rate to match the apposition rate for cortical bone. The porosity for the day is then determined from the change in bone mass:

$$por(t) = (por(t-1) - Qnet(t)) * dt \quad (37)$$

This porosity is then used to calculate the next day's modulus.

2.11 Validation

The results of the current computational model were compared to expected results and related research. The expected results came from the computational model developed by Hazelwood et al. (2001) to predict bone porosity changes from damage and disuse conditions; this code was used as a foundation upon which the current code was developed. Therefore, it was important to determine if the addition of osteocyte apoptosis as the main mechanism behind bone remodeling control caused differing results between the foundational Hazelwood et al. (2001) study and the current study. Additionally, independent one-sample t-tests were used to compare empirical porosity, apoptosis parameters and activation frequency data from by Aguirre et al. (2006) and Hedgecock et al. (2007) with corresponding results from the current simulation.

CHAPTER 3: RESULTS

3.1 Results Introduction: Three- and Two-Dimensional Output Plots

Three-dimensional output plots are useful for general, at-a-glance orientation with the results and trends of the current study. Located in Appendix A, they are plotted from 0N to 2000N for 2000 days, and results from 1600N to 2000N less accurately predict the outcomes due to limitations of the simulation in dealing with higher forces; however, they are included because they provide a valuable illustration of the general trends that occur during this high force interval.

Since two-dimensional plots more easily lend themselves to presentation and comparison of variables at specific forces, the following results sections will focus on two-dimensional outputs. In displaying the two-dimensional results, there are a collection of four forces of interest under which the changing morphologies of the outputs are best displayed; they are 0N, 445.8N, 891.6N, and 1600N. These points are important, both because they signify the maximums, minimums and central forces for disuse, equilibrium and damage, and because they were chosen by Hazelwood et al. (2001) for his assessment of the code. Since this thesis shares a foundation with the Hazelwood et al. (2001) code, both code revisions showcase important phenomena at similar forces.

Note that all reference to “the first day of the simulation” means from day 46 onward. The first 45 days are required by the code to set up the prior conditions on which to calculate the actual simulation. The last day of the simulation is referred to as day 2000, and the duration of the simulation, is 1955 days, excluding the 45 days required by the Prior Conditions section of the code. The results are plotted from day 46 to day 2000.

3.2 Output Battery at 0N

A force of 0N was chosen because it is under purely disuse conditions. However, since the code simulates a healthy, equilibrium-loaded New Zealand white rabbit abruptly placed in complete disuse (with appropriate acknowledgement that this is unlikely; complete unloading would include severing all tendon attachments to the tibia in zero gravity), there is residual damage still present in the rabbit's bones from the period of equilibrium loading. The rate of damage formation (Figure 3.3) is 0.0 mm/mm²/day for the duration of the simulation, and the damage removal rate reaches a peak at 79 days of 4.829×10^{-4} mm of cracks/mm²/day. The total residual damage is 0.0366 mm/mm² on the first day of the simulation, and it is completely reduced to 0.0 mm/mm² at 405 days. There is a moderate amount of damage-induced apoptosis for the first 405 days that corresponds to the residual damage from the equilibrium condition used to “prime” the remodeling parameters. After 405 days, the output and remodeling parameters reflect purely disuse conditions.

By 1000 days, the porosity (Figure 3.1) is 99%, which is the imposed upper bound for the porosity variable in the code, meaning that a maximum bone loss has occurred, after which no additional bone can be removed. The modulus is 0.0 GPa for the duration of the simulation; stress is also 0.0 GPa and strain is 0.0 microstrain. The peak activation frequency due to damage is 0.00708 BMU/mm²/day at day 190. After the damage is dissipated on day 405, there is no activation frequency contribution from damage since there is no apoptosis due to damage.

The activation frequency at 0N (Figure 3.4) is mostly governed by the disuse contribution to activation frequency, mediated only by disuse-induced osteocyte apoptosis. This disuse contribution expresses itself as a peak occurring at day 217 with the magnitude of 0.4618 BMU/mm²/day. The maximum number of osteocytes apoptosing at a force of 0N (Figure 3.2) is

112.32 osteocytes/mm². The mechanical stimulus (Φ) at 0N (Figure 3.5) is 0.0 for the duration of the simulation.

Figure 3.1 Porosity output at Force = 0N.

Figure 3.2 Total Osteocyte Apoptosis at Force = 0N.

Figure 3.3 Total damage at Force = 0N.

Figure 3.4 Activation frequency at Force = 0N.

Figure 3.5 Mechanical Stimulus (Φ) at Force = 0N.

3.3 Output Battery at 445.8N

The output occurring as a result of a force of 445.8N is important because this is an assessment point utilized by Hazelwood et al. (2001) to sample the morphology of the output battery during the middle of disuse conditions, as measured from a force of 0N to the equilibrium force, at 891.6N.

There are five major points of consideration for examining the porosity (Figure 3.6) at this force. The porosity increases from 3.24% to a value of 8.97% between days 46 and 109. An oscillation begins at day 229, and continues through to day 1033. The body of the oscillation is comprised of two distinct regions: the first region occurs between day 229 and day 760, the second region occurs between day 760 and day 1033. The first region has an approximate period of 15 days, with a peak-to-peak amplitude of approximately 0.29%. The second region has an approximate period of 28 days, within amplitude of approximately 0.30%. From day 1033 to the final day, at day 2000, the porosity increases from a value of 9.81% to a value of 11.70%.

There are four major points of consideration for examining the modulus at this force. At the first day of the simulation, the modulus value is 24.4 GPa, and by day 110, the value is 17.7 GPa. An oscillation extends from day 229 with a modulus value of 16.9 GPa, to day 990 with a modulus value of 13.0 GPa. The period of the oscillation is approximately 28 days, and the peak-to-peak amplitude is approximately 3.9 GPa. Stress maintains a constant value of .00445 GPa for the interval of the simulation.

Strain has four points of interest at this force. On the first day of the simulation, the strain is 182 microstrain. From here, it rises to a value of 263 microstrain at day 229. An oscillation occurs between days 229 and 990. The period of the oscillation is approximately 28

days, and the amplitude is approximately 39 microstrain. From day 990 to day 2000, the strain rises from a value of 343 microstrain to a value of 352 microstrain.

The mechanical stimulus (Φ) (Figure 3.12, Figure 3.13) has four points of interest at this force. The mechanical stimulus is 0.0334×10^{-10} . At day 229, an oscillation begins that spans until day 990. The period is approximately 10 days, and the amplitude is 0.125×10^{-10} . From day 990 to day 2000, the mechanical stimulus increases from a value of 0.411×10^{-10} , to a value of 0.462×10^{-10} .

There are three points of interest when considering the percent change in osteocyte apoptosis due to disuse. The percent increase in osteocyte apoptosis due to disuse is 10.1% on the first day of the simulation, and two regions of oscillation span days 229 to 990; the first is from day 229 until day 802, and the second is from 802 days until 990 days. The approximate period of the first region of oscillations is 20 days, and the approximate peak-to-peak amplitude is 6.96%. The approximate period of the second region is 28 days, with amplitudes identical to the first region of oscillation. From day 990 until the end of the simulation at 2000 days, there is a decrease in osteocyte apoptosis of 1.27%.

The disuse-induced activation frequency contribution holds several areas of interest. It is $0.0871 \text{ BMU/mm}^2/\text{day}$ on the first day of the simulation, and peaks to a value of $0.131 \text{ BMU/mm}^2/\text{day}$ on day 75. Two distinct regions of oscillations occur between days 229 and 990. The first region spans between day 229 and day 802, with a period of approximately 20 days, and peak-to-peak amplitude of approximately $0.0925 \text{ BMU/mm}^2/\text{day}$. The second oscillation region occurs between days 802, and 990; the period is approximately 25 days, with amplitudes

identical to the first region of oscillation. At day 990, the activation frequency contribution from disuse is 0.0142 BMU/mm²/day, and is 0.0142 on day 2000.

The activation frequency contribution from damage is initially 0.0017 BMU/mm²/day on the first day of the simulation, and rises to a value of 0.0046 BMU/mm²/day on day 106. From day 106 onward, the output maintains a generally level value, ending the simulation at 0.0047 BMU/mm²/day at day 2000.

The overall activation frequency (Figure 3.10, Figure 3.11) is 0.0888 BMU/mm²/day on the first day of the simulation and peaks to a value of 0.1353 BMU/mm²/day at day 75. Two distinct regions of oscillations also occur together spanning from day 229 to day 990. The first region spans from day 229 to day 802, with an approximate period of 15 days, and peak-to-peak amplitude of 0.0932 BMU/mm²/day. The second region spans from days 802 until the day 990, with an approximate period of 28 days, and similar amplitudes to the first oscillation region. At day 990, the value of the overall activation frequency it is 0.0182 BMU/mm²/day, which increases to a value of 0.0208 BMU/mm²/day at day 2000.

The rates of damage formation and damage removal both encounter the same connected regions of two distinct oscillations spanning days 229 to 990; these two variables combine to form an overall damage of 0.0366 mm/mm² on the first day of the simulation. Over the course of the simulation, the overall damage (Figure 3.9) decreases to 0.0029 mm/mm² at day 2000.

The total number of osteocytes apoptosing (Figure 3.7, Figure 3.8) from disuse per mm² of bone peaks on the first day of the simulation at 134.19 osteocytes/mm² and encounters two regions of oscillations spanning day 229 until day 990, of identical periods to the rates of damage formation, damage removal, activation frequency due to damage, and activation frequency due to

disuse. The approximate peak-to-peak amplitude of the oscillations is 38.01 apoptotic osteocytes/mm². The number of disuse-induced osteocytes apoptosing reaches a plateau of 71.11 osteocytes/mm² at day 990, which increases to a value of 71.48 osteocytes/mm² at day 2000.

Figure 3.6 Porosity at Force = 445.8N.

Figure 3.7 Total Osteocyte Apoptosis at Force = 445.8N.

Figure 3.8 Magnification of Figure 3.7.

Figure 3.9 Total damage at Force = 445.8N.

Figure 3.10 Activation frequency at Force = 445.8N.

Figure 3.11 Magnification of Figure 3.10.

Figure 3.12 Mechanical Stimulus (Φ) at Force = 445.8N.

Figure 3.13 Magnification of Figure 3.12.

3.4 Output Battery at 891.6N

A force of 891.6N was chosen because this is the force at which porosity is at an equilibrium in Hazelwood et al. (2001) and for comparison purposes this is the closest that the current code comes to equilibrium. At this force, the porosity (Figure 3.14) nearly reaches equilibrium as it does in Hazelwood et al. (2001). It starts from a first day porosity of 3.22% to a final porosity at 2000 days of 3.53%. Likewise, the Young's modulus makes a gradual change of magnitude from first day value of 24.4 GPa to a final value at 2000 days of 23.9 GPa.

Stress is defined as force, which is constant, divided by the area, which is also a constant; thus, the stress is constant throughout the simulation at a value of 0.00891 GPa. Strain is 365.4 microstrain on the first day of the simulation, and of 371.6 microstrain on the last day. The mechanical stimulus (Φ) (Figure 3.19) goes from an initial value of 0.5300×10^{-10} to 0.5721×10^{-10} at day 2000, reflecting a near-equilibrium condition. At this equilibrium force, disuse conditions do not occur; there is no osteocyte apoptosis due to disuse, and all apoptosing osteocytes are due to damage (Figure 3.16), which is 0.0366 mm/mm^2 on the first day of the simulation, and varies slightly over the course of the simulation due to the fluctuation in the mechanical stimulus. The total osteocyte apoptosis (Figure 3.15), defined solely by damage-induced apoptosis, is initially $29.3 \text{ osteocytes/mm}^2$, and declines to a value of $28.76 \text{ osteocytes/mm}^2$ at day 2000.

The rate of damage formation increases from $6.27 \times 10^{-6} \text{ mm/mm}^2/\text{day}$ on the first day of the simulation to a value of $6.71 \times 10^{-6} \text{ mm/mm}^2/\text{day}$ on the last day. The rate of damage removal decreases from a value of $6.22 \times 10^{-6} \text{ mm/mm}^2/\text{day}$ on the first day of the simulation to a value of $5.035 \times 10^{-6} \text{ mm/mm}^2/\text{day}$ on the 82nd day, reflecting a dissolution of the initial rate of damage removal. The final value of the damage removal rate, at day 2000, is $5.625 \times 10^{-6} \text{ mm/mm}^2/\text{day}$.

The activation frequency from the damage contribution is initially 0.0017 BMU/mm²/day on the first day of the simulation and 0.0018 BMU/mm²/day on the last. Disuse does not contribute to the activation frequency at or above forces of 891.6N. The total activation frequency (Figure 3.17, Figure 3.18) is 0.0017 BMU/mm²/day on the first day of the simulation, and 0.0018 BMU/mm²/day on the last, solely defined by the damage contribution.

Figure 3.14 Porosity at Force = 891.6N.

Figure 3.15 Total Osteocyte Apoptosis at Force = 891.6N.

Figure 3.16 Damage at Force = 891.6N.

Figure 3.17 Activation frequency at Force = 891.6N.

Figure 3.18 Magnification of Figure 3.17.

Figure 3.19 Mechanical Stimulus (Φ) at Force = 891.6N.

3.5 Output Battery at 1600N

The highest force of interest is at 1600N; at all forces in excess of this, the code encounters discontinuities, which Hazelwood et al. (2001) attributes to the occurrence of a stress fracture. At this force, the porosity (Figure 3.20) gradually curves upwards from an initial value of 3.22% to a final value of 4.89%. The Young's modulus decreases from an initial value at day 0 of 24.4 GPa to a value of 22.2 GPa at day 2000. The stress maintains a constant value of 0.016 GPa through days 46 to 2000. Strain begins the simulation at a value of 655.6 microstrain. Over the course of the simulation, the strain gradually increases to a final value of 718.2 microstrain at day 2000. The mechanical stimulus (Φ) (Figure 3.26) is 5.54×10^{-10} on the first day of the simulation and gradually increases to a final value of 7.98×10^{-10} . As with other forces at or above the equilibrium value, at this force, there is no percent change in osteocyte apoptosis due

to disuse, no osteocytes undergo apoptosis as from disuse, and there is no activation frequency contribution from disuse.

The rate of damage formation is $0.650 \cdot 10^{-4}$ mm/mm²/day on the first day of the simulation and increases to a value of $0.937 \cdot 10^{-4}$ mm/mm²/day at day 2000. The damage removal rate is initially $0.050 \cdot 10^{-4}$ mm/mm²/day and increases to $0.929 \cdot 10^{-4}$ mm/mm²/day at day 2000. The overall damage (Figure 3.22) is initially 0.036 mm/mm²/day, and rises to 0.091 mm/mm²/day on day 2000.

The damage-induced contribution to activation frequency is initially $1.70 \cdot 10^{-3}$ BMU/mm²/day and it experiences a low of $1.395 \cdot 10^{-3}$ BMU/mm²/day at day 325; from there, it increases to a final value of $12.7 \cdot 10^{-3}$ BMU/mm²/day. The total activation frequency (Figure 3.23, Figure 3.24, Figure 3.25) solely determined by the damage contribution, is identical.

The number of osteocytes apoptosing (Figure 3.21) is defined solely by damage-induced apoptosis, and begins at a value of 29.2 apoptotic osteocytes/mm² on the first day of the simulation and it reaches a maximum of 48.2 apoptotic osteocytes/mm² on day 2000.

Figure 3.20 Porosity at Force = 1600N.

Figure 3.21 Total Osteocyte Apoptosis at Force = 1600N.

Figure 3.22 Damage at Force = 1600N.

Figure 3.23 Activation frequency at Force = 1600N.

Figure 3.24 Magnification of Figure 3.23.

Figure 3.25 Magnification of Figure 3.23.

Figure 3.26 Mechanical Stimulus (Φ) at Force = 1600N.

CHAPTER 4: DISCUSSION

4.1 Research Summary

The current study adapted a bone remodeling simulation to employ osteocyte apoptosis centric relationships to predict changes in remodeling parameters and bone porosity. The computational model utilized iterations of one day over the elapsed time of 2000 days, and from forces of 0N to 1600N at 3000 cycles per day, which constitute normal motile behavior of the New Zealand white rabbit. This empirical data was used as the source of the osteocyte apoptosis relationships between activation frequency, mechanical stimulus, and damage creation. This study found that, when compared to the original bone remodeling simulation, remodeling parameters and corresponding bone porosity changes were favorably similar. This strengthens the hypothesis that relationships between osteocytes and bone remodeling parameters can be used to predict bone remodeling behaviors, lending support to the possibility that bone remodeling is inextricably linked to osteocyte apoptosis in both spatial and temporal circumstances. This result is important because it does not detract from the probability of creating treatments for osteoporosis that, by controlling bone remodeling through modulating osteocyte apoptosis occurrences, may provide better protection against fracture frequency and severity in patients who suffer osteoporosis, especially postmenopausal women.

4.2 On the Nomenclature of Disuse and Damage:

Disuse and damage are labels to describe conditions present below and above the equilibrium force, for disuse and damage respectively. However, the definitions of these words are not mirror images of each other, in that while disuse does not occur at over the equilibrium force, damage initially occurs during the Prior Conditions, when the bone parameters are being

initialized. For the first few days of the simulation, damage used in the Prior Conditions to initialize the bone is removed as the simulation proceeds. A reminder must be made that at equilibrium there is not an absence of disuse and damage; rather, the porosity is as close to a plateau as possible.

4.3 On the Conversion From Percent Apoptosis to the Number of Apoptotic Osteocytes

Two central articles were employed for the implementation of damage and disuse in this code; Aguirre et al. (2006) and Hedgecock et al. (2007). As mentioned in the methods section, there is a unit mismatch between the two articles. Aguirre et al. (2006) uses percent apoptosis as their primary unit, while Hedgecock et al. (2007) use the density of apoptotic osteocytes as their primary unit. Aguirre et al. (2006) does not state the methods they used to obtain the number of apoptotic osteocytes per unit area, however their magnitude of the total number of apoptotic osteocytes differs by an order of magnitude from the number in Hedgecock et al. (2007). However, Hedgecock et al. (2007) well documented the density of apoptotic osteocytes, as well as the methods for obtaining this number. This allowed me to convert their density of osteocytes apoptosing to a percent apoptosis, for use as a common unit between the two articles. Thus, while the density of apoptotic osteocytes would have been a more intuitive mechanism for calculating the change in porosity, it was not feasible due to the aforementioned limitations.

Since it is more intuitive to use the density of apoptotic osteocytes for an at - a - glance output, a method was developed to convert the intrinsic mechanism in the code - percent apoptosis - to a number of apoptotic osteocytes. There are accuracy constraints created by this conversion process, which uses a curve fit relationship between the activation frequency and osteocyte apoptosis percentage data in Aguirre et al. (2006) and Hedgecock et al. (2007). The conversion process from apoptosis percentage to the number of apoptosing osteocytes is

inherently inaccurate because of the occurrence of "tolerance stack ups" that occur from the compilation of imperfect data. For example, while twenty data points from Hedgecock et al. (2007) were useable, only two data points from Aguirre et al. (2006) were useable. This results in apoptotic number outputs that, while roughly within the expected range, cannot be interpreted as a direct, or accurate, prediction. In fact, by incorporating a post-simulation "module" to translate percent to number, a singularity is created that spans the interface between disuse and equilibrium conditions, further reducing the accuracy of the predictions.

4.4 Apoptosis Issue at the Interface Between Disuse and the Equilibrium Conditions

There exists a singularity at a force of 891.6N, when the simulation is at equilibrium because the porosity after 46 days does not experience considerable change; this is evidenced by a slight change of approximately 0.31% between days 46 and 2000. This singularity appears as a near-instantaneous change in osteocyte apoptosis from disuse conditions, to equilibrium conditions; the specific area of interest spans from 870.0N to 891.6N. At 870N, there are 42.75 apoptotic osteocytes/mm², which is in line with my expectations for this point in disuse, and corroborates with the published data in Hedgecock et al. (2007). However, osteocytes apoptosing from damage that have been growing through the latter part of the disuse area do not reconcile with the disuse osteocytes at 891.6N. At this point, the number of apoptotic osteocytes changes from being defined by disuse conditions to being defined by damage conditions, resulting in an instantaneous drop at 891.6N from 42.75 apoptotic osteocytes/mm² to 28.76 apoptotic osteocytes/mm². This flawed handoff between disuse and damage conditions occurs due to the aforementioned tolerance stack ups in the conversion module. It must be emphasized that the number of apoptotic osteocytes/mm² (as opposed to the apoptosis percentage) is more of a "curiosity", in that it is not intended, nor used, as an actual mechanism in the code. The

apoptosis percentage, which is the crucial mechanism, does indeed transfer from disuse, to equilibrium, to damage conditions correctly.

4.5 The Simulation of Porosity

The element of bone experiences the most noticeable gains in porosity while at disuse conditions, below the force of 891.6N. From the equilibrium force, to a force of 1600N, there is minimal change in porosity, owing to a suitable supply of strain on the bone as a result of adequate force and an adequate number of daily loading cycles. Forces above 1600N result in the distortion of remodeling parameters that Hazelwood et al. (2001) suggest may occur due to stress fractures. The most extreme increases in porosity can be seen at points below 445.8N, which is the midpoint of the disuse conditions. Below this midpoint, porosity swiftly rises to achieve a high equilibrium at days ranging from 500 to 1000, depending upon the exerted force. Under the absence of any force, a complete void occurs, with no bone present in the element, at a force of 0N; this change occurs within 1000 days. The magnitude and severity of the increase in porosity is intrinsically related to the experienced strain on the element; in the regional and temporal zones where the porosity is the highest, the strain is the lowest. As a result of the low strain, there is a correspondingly high value of apoptotic osteocytes owing their presence to disuse conditions; at a force of 0N, the total density of apoptotic osteocytes, generated wholly by disuse-induced apoptosis, begins at approximately 38 osteocytes per mm^2 , and swiftly rises to approximately 117 apoptotic osteocytes per mm^2 within 500 days. This phenomenon is theorized to occur (Gross et al., 2001) because at low strains, insufficient fluid transport within the canaliculi occurs. This lack of transport results in hypoxia and the buildup of wastes within the osteocyte, initiating the apoptosis process; at higher strains, there is enough within the bone matrix to facilitate fluid transport, preventing the initiation of apoptosis within the cell (Dodd et

al., 1999).

4.6 On the Apparent Imbalance Between Refilling and Resorbing BMUs.

There is an apparent imbalance between refilling and resorbing basic multicellular units. For example, at forces below 445.8N, which is the most distinct and concentrated region of disuse-induced bone density loss, there is a peak of approximately five resorbing BMUs per mm^2 . However, at the same point there is a peak of approximately twelve refilling BMUs per mm^2 . Despite the higher concentration of refilling in a force region that encourages a decrease in bone mineral density, this is not the error that it appears to be. The presence of a higher number of refilling BMUs vs. a lower number of resorbing BMUs is plausible and accurate because of the anatomical structure of the BMU. The BMU has three distinct regions, two of which are the cutting cone, where resorption occurs, and the refilling region, where osteoblasts deposit fresh bone. These anatomical regions in the BMU have different concentrations of resorbing osteoclasts and refilling osteoblasts, resulting in different time intervals required to remove and add bone. The statement that a BMU is refilling means that it has previously resorbed bone, and that the region of the BMU responsible for adding new bone is now exposed to the cavity formed from the cutting cone. Thus, the number of refilling BMUs, related to the number of resorbing BMUs, is not a good indication of the total amount of bone being added or removed.

4.7 On the Creation of Damage with Regard to Exercise.

There is a negligible amount of damage at forces below 445.8N; slightly above the equilibrium point, damage formation increasingly exceeds the rate of damage removal, resulting

in an overall increase of damage, where higher magnitudes of damage correlate both with later temporal points and the simulation, as well as higher forces. At forces in excess of 1400N, damage does reach a plateau at approximately 1600 days. However, this damage buildup does not result in a significant amount of osteocyte apoptosis; a damage level of 0.1 (mm crack length/ bone area), does not constitute a significant magnitude, and thus does not degrade bone quality. The maximum damage experienced by the bones in the simulation reflects high amounts of daily exercise and overloading, thus explaining the retention of bone porosity to a healthy porosity level of approximately 3.6% after 1450 days of loading at a force of 1400N 3000 times a day. A loading rate of 3000 times a day, with an average stride length of 2.5 feet for an adult human male, equates to 1.42 miles walked a day. Since the code utilizes rabbit data, using an approximate stride length of 7.5 inches equates to 0.35 miles traveled per day at a walking pace to a slow “gallop”, which is the phrase for “running” in reference to rabbits.

4.8 Reoccurring Patterns and Limitations Therein

In nearly all output plots, there are transient responses associated with the dissolution of initial equilibrium variable magnitudes used to set up the calculations necessary for the operation of the simulation. These regions last for approximately the first 200 days of the simulation, and are best exhibited by the "pseudo peak" seen in the three-dimensional output from Figure A.13, the total damage vs. time vs. force. Once the code has dealt with the balancing of these inner mechanism initializations, the simulation can be compared and contrasted to previously published data, such as Hazelwood et al. (2001), and Hedgecock et al. (2007).

At 445.8N, the force midpoint for disuse, oscillations span nearly all outputs; this phenomenon is indicative of the juxtaposition of mechanical stimulus vs. osteocyte apoptosis due to disuse, as defined by a composite of equations constructed from the compilation of three linear

equations (Equations 20, 21 and 22), each representing a different region of interest along an axis as defined by the mechanical stimulus. This construct serves to translate the magnitude of mechanical stimulus to the percent of osteocyte apoptosis that is induced by disuse conditions. The oscillations are not ideal because they instantaneously vary between two plateaus of differing magnitudes without a realistic transition; in the span of 25 days, as seen with the activation frequency output at a 445.8N, the magnitude of the activation frequency can drop by 80%. These oscillations should be interpreted as an undesired effect caused by an inadequate interface between the mechanical stimulus, and disuse-induced osteocyte apoptosis conversion factor as a result of inadequate empirical data points from the reference articles, which serve to supply the current code with the necessary relationships to determine the variable definitions. This tangible limitation might be solved in the future by a higher data resolution of empirical data cataloging the relationship between the mechanical stimulus and osteocyte apoptosis.

4.9 Additional Limitations

A reoccurring limitation inherent to this computational model is that it is based upon few empirical data sources (Aguirre et al., 2006; Hedgecock et al., 2007), and an increase in empirical data resolution would greatly benefit the output of the current study:

The equations used to calculate the mechanical stimulus from input porosity values were tailored by changing the exponent and coefficient to adjust the relationship for the presence of voids and soft tissues interspersed in the cortical and trabecular regions. The foundational code (Nyman et al., 2004) customized both cortical and trabecular elastic modulus equations to represent human variables; the current study used cortical New Zealand white rabbit data from Hadi et al. (2007) to customize the elastic modulus equation requiring inputs in terms of cortical porosity. However, trabecular data for the New Zealand white rabbits were unavailable. The

tailored cortical elastic modulus equation for the New Zealand white rabbit was successfully calculated and was found to differ very slightly from the human counterpart cortical elastic modulus equation. Thus, in light of the lack of proper New Zealand white rabbit trabecular data, and the discovery that the cortical elastic modulus values compared well to the preexisting human values, the trabecular elastic modulus was maintained to the original human trabecular elastic modulus relationship. There is room for error caused by the possible difference of trabecular modulus calculation between rat and human trabecular porosities.

The use of elastic modulus equations customized for New Zealand White rabbit and humans values for the calculation of disuse-induced porosity values (Aguirre et al. 2006) for rats may be responsible for a decrease in general accuracy. There were no available data for the customization of the elastic modulus equations, and this lack of data likely introduced error because the disuse porosity data (Aguirre et al. 2006) were translated to mechanical stimulus through the use of an equation that uses a modulus equation adjusted to reflect the occurrence of soft tissues in New Zealand White rabbits.

The reversal period was scaled down to New Zealand white rabbit data from the human data used in the foundational code (Nyman et al., 2004). However, the use of this scaled-down rabbit equivalent reversal period value resulted in the appearance of a morphological anomaly in the first 100 days of the porosity output at forces below 200N. Since no empirical data shows that the scaled value – 3 days for the reversal interval – has physiological validity, the value was modified to 10 days to eliminate the anomaly. This final value for the reversal period also lacks empirical support; it is possible that the addition of empirical data for the interval of the reversal period in New Zealand white rabbits to the current code will improve the accuracy of the current outputs.

The values of the equilibrium mechanical stimulus, the equilibrium damage, and the equilibrium activation frequency were obtained by iteratively modifying them to match expected values based upon previous research (Hazelwood et al., 2001; Nyman et al. 2004) to fit New Zealand white rabbit data as closely as possible. While that previous research was based upon empirical data for humans, no data existed during the development of this code to empirically support the variable assignments. Thus, the values of the equilibrium mechanical stimulus, the equilibrium damage, and the equilibrium activation frequency were iteratively modified to estimate New Zealand white rabbit variables from the original human values. Given that empirical data for disuse conditions (Aguirre et al., 2006) is from murine data, and that no empirical rabbit data existed for the equilibrium mechanical stimulus, damage and activation frequencies, it is likely that the estimation of these variables during the Prior Conditions section of the code (Appendix B.2) is not ideal. The use of empirically based equilibrium values for New Zealand white rabbits would likely improve the accuracy of the current study's output.

The conversion of mechanical stimulus values to percent of disuse-induced apoptosis relies upon four empirical data points (Aguirre et al., 2006). The resulting relationship does not readily lend itself to curve fitting, and the conjugation of three linear relationships was required. However, the sudden transitions between linear equations results in the amplification of oscillatory behavior integral to the foundational model (Nyman et al., 2004) for forces originating in the mid-disuse region of approximately 445.8N +/- 100N. Increasing the resolution of the empirical disuse condition data would reduce the amplification of these oscillations, resulting in a more accurate porosity prediction for force values of 445.8N +/- 100N. Additionally, the simulation becomes inaccurate when predicting variable outcomes at forces

above 1600N. This was posited by Hazelwood et al. (2001) to occur due to the creation of a stress fracture in the bone, resulting in unstable remodeling parameters.

The Hedgecock et al. (2007) data was used to derive the relationships for damage loading; however, the rabbits used were not specifically placed in specific loading conditions, rather were allowed free movement within the confines of their cages. This is a large limitation as it prevents the current simulation from accurately predicting remodeling in damage loading conditions. However, no alternative damage loading data existed at the time of the development of the simulation. The goal of using the Hedgecock et al. (2007) data was to provide a non-disuse contrast with the Aguirre et al. (2006) data that drives the disuse portion of the code.

4.10 Comparisons With Previous Work.

There are three main articles with which to compare the current code output; Hazelwood et al. (2001), for the comparison to porosity and activation frequency, Hedgecock et al. (2007), for the comparison of activation frequency vs. osteocyte apoptosis, and Aguirre et al. (2006), for the comparison of percent apoptosis, disuse porosity, and disuse activation frequency.

The porosity outputs of the current code (Figure 4.2) compare favorably with the porosity results from Hazelwood et al. (2001) (Figure 4.1). At a force of 10N, (not shown in the current study) the current code plateaus to a porosity of 94.28%, as compared to a plateau of 94.2% porosity from Hazelwood et al. (2001). At a force of 445.8N, the midpoint of disuse conditions, the current code returned a porosity value of 11.7% at 2000 days, as compared to a porosity of 31.1% at 2000 days from the Hazelwood et al. (2001) results. This difference in porosity is attributable to two main categories; the first category is that the current code utilizes different constants for the formation, resorbing, and reversal periods, as well as different values for some of the parameters, such as equilibrium disuse activation frequency, and the initial Phi value, ϕ_{i0}

(Φ_0). The second category of possible causes for this divergence between porosity values is the addition of relationships for apoptosis from the articles by Hedgecock et al. (2007) and Aguirre et al. (2006). At an equilibrium force of 891.6N, the porosity of the current code at 2000 days is 3.53%; the Hazelwood et al. (2001) porosity output at 2000 days for the same equilibrium force is 4.43%. It is important to note that at this equilibrium force, the Hazelwood et al. (2001) porosity is at a complete plateau, with no movement whatsoever. In contrast, the current code sees a slight increase in porosity of 0.31% for the duration of the simulation. Thus, the use of the term "equilibrium force" is, for this code, a slight misnomer. Attempts to tailor an equilibrium force to this code have been unsuccessful; this is perhaps explainable by the inclusion of the apoptosis equations, some of which encourage minute gains of porosity for the duration of the simulation.

Figure 4.1 Porosity responses over time for Hazelwood et al. (2001) in disuse situations. [28]

Figure 4.2 Predicted porosities under disuse conditions.

The activation frequency outputs of the current code (Figure 4.4, Figure 4.5) compare favorably with the results from Hazelwood et al. (2001) (Figure 4.3) at forces of 10.0N and 891.6N; at 445.6N, several differences appear. At 10.0N, the current code reaches a peak of 0.46 BMUs/mm²/day at 216 days, whereas the output from Hazelwood et al. (2001) reaches approximately 0.5 BMUs/mm²/day at 80 days. In the cases of both codes, the activation

frequency reaches a plateau at approximately $0.005 \text{ BMUs/mm}^2/\text{day}$. It is necessary to note that the temporal discrepancy between the two peaks is a product of the differing physiological intervals required for BMU-based resorption, reversal, and reformation. At forces of 445.8N , the Hazelwood et al. (2001) activation frequency contains four discrete peaks followed by oscillations; the oscillations span days 315 to 1500. In order of the day of appearance, the values of the peaks are $0.34 \text{ BMUs/mm}^2/\text{day}$, $0.18 \text{ BMUs/mm}^2/\text{day}$, $0.11 \text{ BMUs/mm}^2/\text{day}$, and approximately $0.07 \text{ BMUs/mm}^2/\text{day}$. The maximum reached oscillation point is approximately $0.12 \text{ BMUs/mm}^2/\text{day}$, which occurs at approximately 525 days. The current code differs significantly from the Hazelwood et al. (2001) output at a force of 445.8N in terms of morphology, however the magnitudes between the two results are similar. The current code experiences only one peak that occurs at day 75, and holds a value of $0.135 \text{ BMUs/mm}^2/\text{day}$. Similar to the Hazelwood et al. (2001) results, the results of the current code experience oscillations. However, these oscillations start earlier at approximately 230 days, and span the interval until day 990. Again, temporal discrepancies between the two oscillation intervals are explainable by the differing physiological parameters required by the rabbit, as opposed to the human data used in the Hazelwood et al. (2001) code. At forces of 891.6N , the current code is corroborated by the results of Hazelwood et al. (2001); both experience plateaus at points comparably close to $0 \text{ BMUs/mm}^2/\text{day}$.

Figure 4.3 Activation frequency responses over time from Hazelwood et al. (2001) in disuse situations. [28]

Figure 4.4 Activation frequency responses over time for the model in disuse situations, current results.

Figure 4.5 Magnification of Oscillations at 445.8N in Figure 4.4.

Since the relationship in Hedgecock et al. (2007) between activation frequency and apoptosis for damage conditions was used to define relationships used in the current code, activation frequency vs. apoptosis outputs for the current code match the results of Hedgecock et al. (2001). However, there is a margin of error caused by the ability of the current code to select different forces at which to sample the relationship between the activation frequency and the apoptosis for damage conditions. Sampling at all forces in excess of the equilibrium force, 891.6N, and below 1940.0N, produced outputs comparable to the Hedgecock et al. (2007) results; an exception being that the linear relationship published in their results was translated to a more accurate curve representation through curve fitting prior to implementation in the current code. Thus, the current code outputs diverge from theirs in this slight morphological improvement.

The steady state results of the simulation compared well with the experimental results from Hedgecock et al. (2007) for damage loading conditions. The simulation predicted a porosity

of 3.8%, which was statistically similar to the experimental porosity of $3.2 \pm 0.9\%$ (Figure 4.6). The osteocyte apoptosis percentage was also statistically similar; the predicted osteocyte apoptosis rate of 8.9% compared favorably with the experimental rate of $9.1 \pm 8.3\%$ (Figure 4.7). The predicted activation frequency of 0.0066 BMUs/ mm^2/day was statistically similar to the experimental value of 0.032 ± 0.038 BMUs/ mm^2/day (Figure 4.8).

Under disuse loading conditions, the simulation was statistically similar to the experimental results (Aguirre et al., 2006) at 18 days (5.3% predicted, $4.9 \pm 1.2\%$ experimental. Figure 4.6); however, the simulation overestimated the rate of osteocyte apoptosis. The activation frequency of the BMUs under disuse conditions did not favorably compare between the predicted and experimental values (Figure 4.8).

Figure 4.6 Predicted and Experimental Porosity Comparison. Error bars indicate one standard deviation.

Figure 4.7 Predicted and Experimental Apoptosis Comparison. Error bars indicate one standard deviation.

Figure 4.8 Predicted and Experimental Ac.f Comparison. Error bars indicate one standard deviation.

The results of the code are compatible with the initial declared goal, which is demonstrating that it is possible and feasible to predict bone porosity parameter changes based upon changes in osteocyte apoptosis magnitude and frequency. By utilizing published data

relating osteocyte apoptosis to activation frequency, the porosity results reasonably compare to non-osteocyte apoptosis driven simulations. This implies that there is an inextricable relationship between osteocyte apoptosis and porosity parameters, thus adding another stone to the path towards assessing the viability of developing osteocyte-based therapies for the treatment of bone porosity losses, as seen in postmenopausal patients. It is important to recognize that the magnitude of importance of the results are somewhat reduced due to the small sample of articles and data used to formulate the simulation and create the results. An expansion of empirical data is crucial to increasing the relative importance of the contribution of the current code; since three primary sources were utilized, it is possible that the favorable comparisons between the current results and previously published data are to a certain extent an insufficient supply of sample points, thus not allowing sufficient drift to a true empirical correlation as seen with a larger population sample size.

4.11 Future Work

The current computational model of a representative volume of bone may be incorporated into a finite element model for the prediction of bone remodeling parameter and porosity outcomes under varying strains in a long bone solid model. The purpose of the future study may be to predict the temporal and spatial migration and occurrence of osteocyte apoptosis and consequential BMU appearances resulting from regional strain distributions, for comparison with empirical data to determine the efficacy of the osteocyte-driven simulation for predicting these phenomena.

CHAPTER 5: SUMMARY OF CONCLUSIONS.

- The code demonstrated that it is feasible to predict the porosity based upon osteocyte apoptosis; bone density changes, activation frequency changes, and the elastic modulus predictions all correlated favorably with previous simulations and empirical data.
- An increase in osteocyte apoptosis correlates with an increase in porosity. At disuse forces, below the equilibrium force of 891.6N, there is a marked increase in osteocyte apoptosis; this increase in apoptosis spurs the increased initiation of BMUs that are responsible for resorbing bone, and results in a loss of bone density, thus increasing the porosity. This is consistent with the theorized activity and purpose of osteocyte apoptosis with regard to signaling bone remodeling.
- The most significant magnitudes of change in both apoptosis rates and porosity increases occurred earlier in the simulation and under conditions significantly different from those initiated in the Prior Conditions section. The simulation predicts that the most deleterious forces with regard to loss in bone density occur between forces of 0.0N and 445.8N; disuse conditions are comparatively more severe in their ability to increase the risk of osteoporosis than damage conditions.
- Most changes in bone porosity occur within the first 1000 days of the simulation; this implies that bone accomplishes the vast majority of its remodeling in response to a new loading magnitude in less than three years for severe changes, and in one year for moderate loading changes.

CHAPTER 6: REFERENCES

- [1] Aguirre JI, Plotkin, LI, Stewart SA, Weinstein RS, Parfitt, AM, Manolagas SC, Bellido T. Osteocyte apoptosis Is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. *J Bone and Mineral Research* 2006; 21:605-615.
- [2] Ahuja SS, Zhao S, Bellido T, Plotkin LI, Jimenez F, Bonewald LF. CD40 ligand blocks apoptosis induced by tumor necrosis factor alpha, glucocorticoids, and etoposide in osteoblasts and the osteocyte-like cell line murine long bone osteocyte-Y\$. *Endocrinology* 2003; 144:1761-1769.
- [3] Araujo AB, Travison TG, Harris SS, Holick MF, Turner AK, McKinlay JB. Race/ethnic differences in bone mineral density in men. *Osteoporosis International* 2007; 18(7): 943-53.
- [4] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998; 281:1305-1308.
- [5] Atkins GJ, Welldon KJ, Halbout P, Findlay DM. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. *Osteoporosis International* 2008; 20:653-664.
- [6] Bentolila V, Boyce TM, Fyhrie DP, Drumb R, Skerry TM, Schaffler MB. Intracortical remodeling in adult rat long bones after fatigue loading. *Bone* 1998; 23:275-281.
- [7] Bertram JE, Biewener AA. Bone curvature: Sacrificing strength for load predictability? *J Theoretical Bio* 1988; 131:75-92.

- [8] Bone mineral density between osteoporosis and nominal health. 2009. Mayo Clinic. 6/16/2009. <http://www.mayoclinic.com/images/image_popup/w7_osteoporosis.jpg>
- [9] Bonewald LF. Osteocyte biology: Its implications for osteoporosis. *J Musculoskeletal Neuron Interact* 2004; 4:101-104.
- [10] Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women: interaction of mechanical, hormonal and dietary factors. *Sports Medicine (New Zealand)* 2005; 35(9): 779-830.
- [11] Bringhurst FR. PTH receptors and apoptosis in osteocytes. *J Musculoskeletal Neuronal Interact* 2002; 2:245-251.
- [12] Carter DR, Orr TE, Fyhrie DP. Relationships between loading history and femoral cancellous bone architecture. *Journal of Biomechanics* 1989; 22: 231-244.
- [13] Chen H, Blackburn WR, Wertelecki W. Fetal akinesia and multiple perinatal fractures. *American Journal of Medical Genetics* 1995; 55:472-477.
- [14] Demir B, Haberal A, Geyik P, Baskan B, Ozturkoglu E, Karacay O, Deveci S. Identification of the risk factors for osteoporosis among postmenopausal women. *Maturitas* 2008.
- [15] Divieti P, Inomata N, Chapin K, Singh R, Juppner H, Bringhurst FR. Receptors for the carboxyl-terminal region of pth(1-84) are highly expressed in osteocytic cells. *Endocrinology* 2001; 142:916-925.
- [16] Divieti PP. PTH and osteocytes. *J Musculoskeletal Neuronal Interaction*. 2005; 5(4):328-330.

- [17] Dodd JS, Ralieggh JA, Gross TS. Osteocyte hypoxia: a novel mechanotransduction pathway. *Am J Physiol Cell Physiol* 1999; 277:598-602.
- [18] Dunstan CR, Somers NM, Evans RA. Osteocyte death and hip fracture. *Calcif Tissue Int* 1993; 53 (Suppl.1): S113-S116.
- [19] Frost HM. In vivo osteocyte death. *J Bone Joint Surg Am* 1960; 42-A:138-143.
- [20] Garcia-Cabezas MA, Garcia-Alix A, Martin Y, Gutierrez M, Hernandez C, Rodriguez JJ, Morales C. Neonatal spinal muscular atrophy with multiple contractures, bone fractures, respiratory insufficiency and 5q13 deletion. *Acta Neuropathologica* 2004; 107:475-478.
- [21] Goldstein MF, Fallon JJ, Harning R. Chronic glucocorticoid therapy-induced osteoporosis in patients with obstructive lung disease. *Chest* 1999; 116: 1733-1749.
- [22] Gross TS, Akeno N, Clemens TL, Jomarova S, Srinivasan S, Weimer DA, Mayorov S. Physiological and Genomic Consequences of Intermittent Hypoxia Selected Contribution: Osteocytes up regulate HIF-1a in response to acute disuse and oxygen deprivation. *J Appl Physiol* 2001; 90:2514-2519.
- [23] Ground Bone. 2003. Southern Illinois University, School of Medicine. 9/17/2008. <<http://www.siumed.edu/~dking2/ssb/NM036b.htm>>
- [24] Gu G, Mulari M, Peng Z, Hentunen TA, Vaananen HK. Death of osteocytes turns off the inhibition of osteoclasts and triggers local bone resorption. *Biochem Biophys Res Commun* 2005; 335:1095-1101.

- [25] Hadi T. Bone turnover, osteocyte apoptosis, and the basic multicellular unit: a quantitative study of the rabbit tibial mid-diaphysis. Biomedical Engineering Dept Thesis, University of California, Davis. 2007.
- [26] Hannan EL, Magaziner J, Wang JJ. Mortality and locomotion 6 months after hospitalization for hip fracture: risk factors and risk-adjustment hospital outcomes. *Journal of the American Medical Association* 2001; 285 (21) 2736-42.
- [27] Harrigan TP, Hamilton JJ. Bone remodeling and structural optimization. *Journal of Biomechanics* 1994; 27:323-328.
- [28] Hazelwood SJ, Martin RB, Rashid MM, Rodrigo JJ. A mechanistic model for internal bone remodeling exhibits different dynamic response in disuse and overload. *J Biomechanics* 2001; 34:299-308.
- [29] Healthy trabecular structure and osteoporotic trabecular structure. 2005. International Osteoporosis Foundation. 9/15/2008. <www.osteofound.org/press_centre/visuals.html>
- [30] Hedgecock NL, Hadi T, Chen AA, Curtiss SB, Martin RB, Hazelwood SJ. Quantitative regional associations between remodeling, modeling, and osteocyte apoptosis and density in rabbit tibial midshafts. *Bone* 2007; 40: 627-637.
- [31] Hert J. New attempt at the interpretation of the functional architecture of the cancellous bone. *J Biomechanics* 1994; 27:239-242.
- [32] Hillam RA, Skerry TM. Inhibition of bone resorption and stimulation of formation by mechanical loading of the modeling rat ulna in vivo. *J Bone and Mineral Research* 1995; 10:S443.

- [33] Huiskes R, Weinans H, Grootenboer HJ, Dalstra M, Fudala B, Slooff TJ. Adaptive bone-remodeling theory applied to prosthetic-design analysis. *J Biomechanics* 1987; 20:1135-1150.
- [34] Israeli RS, Ryan CW, Jung LL. Managing bone loss in men with locally advanced prostate cancer receiving androgen deprivation therapy. *Journal of Urology* 2008; 179(2): 414-23.
- [35] Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporosis International* 2006; 17:1726-33.
- [36] Karasik D, Ferrari SL. Contribution of gender-specific genetic factors to osteoporosis risk. *Annals of Human Genetics* 2008; 72:696-714.
- [37] Krum SA, Miranda-Carboni GA, Hauschka PV et al. Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. *Journal of the European Molecular Biology Organization* 2008; 27(3): 535-45.
- [38] Kudachadkar R, O'Regan RM. Aromatase inhibitors as adjuvant therapy for postmenopausal patients with early stage breast cancer. *CA: The Cancer Journal for Clinicians* 2005; 55(3): 145-63.
- [39] Lam S, Zouzias K. Strontium ranelate for the management of osteoporosis. *Journal of the American Society of Consultant Pharmacists* 2008; 23 (7): 531-7.
- [40] Lerner UH. Bone Remodeling in Post-menopausal Osteoporosis. *J Dent Res* 2006; 85:584-595.

- [41] Liang XH, Yu J, Brown K, Levine B. Beclin 1 contains a leucine-rich nuclear export signal that is required for its autophagy and tumor suppressor function. *Cancer Research* 2001; 61:3443-3449.
- [42] Luthi AU, Martin SJ. The CASBAH: a searchable database of caspase substrates. *Cell Death and Differentiation, Nature* 2007; 14:641-650.
- [43] Malone AD, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proceedings of the National Academy of Sciences* 2008; 104: 13325-13330.
- [44] Martin RB, Burr DB, Sharkey NA. *Skeletal Tissue Mechanics*. New York: Springer; 1998.
- [45] Menopause and Osteoporosis. 2008. Australian Bone Density Testing Centre. 9/17/2008. <http://www.bonedensitytesting.com.au/pages/default.cfm?page_id=19695>
- [46] Miller ME. The bone disease of preterm birth: A biomechanical perspective. *Pediatric Research* 2003; 53:10-15.
- [47] Mullender MG, Huiskes R, Weinans H. A physiological approach to the simulation of bone remodeling as a self-organizational control process. *J Biomechanics* 1994; 27:1389-1394.
- [48] Nakamura T, Imai Y, Matsumoto, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, Yamamoto Y. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell* 2007; 135:811-23.

- [49] Navarro MC, Sosa M, Saavedra P, Lainez P, Marrero M, Torres M, Medina CD. Poverty is a risk factor for osteoporotic fractures. *Osteoporosis International* 2009; 20:393-8.
- [50] Ng MY, Sham PC, Paterson AD, Chan V, Kung AW. Effect of environmental factors and gender on the heritability of bone mineral density and bone size. *Annals of Human Genetics* 2006; 70(Pt 4): 428-38.
- [51] Noble BS, Peet N, Stevens HY, Brabbs A, Mosley JR, Reilly GC, Reeve J, Skerry TM, Lanyon LE. Mechanical loading: biphasic osteocyte survival and targeting of osteoclasts for bone destruction in rat cortical bone. *Am J Physiol Cell Physiol* 2003; 284:C934-C943.
- [52] Nyman JS, Oscar CY, Hazelwood SJ, Martin, RB. A theoretical analysis of long-term bisphosphonate effects on trabecular bone volume and microdamage. *Bone* 2004; 35:296-305.
- [53] Osteocyte and processes. 2009. University of Washington. 1/01/2009. <
<http://depts.washington.edu/bonebio/ASBMRed/cells/ocyte.jpg>>
- [54] Petrtyl M, He J, Fiala P. Spatial organization of the Haversian bone in man. *J Biomechanics* 1996; 29:161-167.
- [55] Phases of fracture repair. 2009. University of Glasglow. 2/15/2009.
<<http://www.gla.ac.uk/ibls/US/fab/images/generic/bofractu.gif>>
- [56] Physiological Mechanisms Behind Apoptosis. Genentech Biooncology. 2009. 1/01/2009.
<www.biooncology.com/bioonc/images/apoptosis-image-lg.jpg>

- [57] Qin Y, Rubin CT, McLeod KJ. Nonlinear dependence of loading intensity and cycle number in the maintenance of bone mass and morphology. *J Bone Joint Surgery* 1998; 16:482-489.
- [58] Qiu SJ, Hoshaw SJ, Gibson GJ, Lundin-Cannon KD, Schaffler MB. Osteocyte apoptosis in reaction to matrix damage in compact bone. *Trans Orthop Res Soc* 1997; 22:89.
- [59] Raisz L. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *J Clinical Investigation* 2005; 115: 3318-25.
- [60] Rubin J, Rubin C, Jacobs CR. Molecular pathways mediating mechanical signaling in bone. *Gene* 2006; 367:1-16.
- [61] Seeman E. The dilemma of osteoporosis in men. *Am J Med* 1995; 98(2A): 76S-88S.
- [62] Skerry TM. The response of bone to mechanical loading and disuse: fundamental principles and influences on osteoblast/osteocyte homeostasis. *Archives of Biochemistry and Biophysics* 2008; 473:117-123.
- [63] Smit TH, Burger EH, Huyghe JM. A case for strain-induced fluid flow as a regulator of BMU-coupling and osteonal alignment. *J Bone Mineral Res* 2002; 17:2021-2029.
- [64] Stevens HY, Meaysa DR, Frangos JA. Pressure gradients and transport in the murine femur upon hind limb suspension. *Bone* 2006; 39:565-572.
- [65] Structure of bone tissue. 2007. US National Cancer Institute. 9/15/2008. <
http://training.seer.cancer.gov/module_anatomy/images/illu_compact_spongy_bone.jpg>

- [66] Structure of cortical and trabecular bone regions. 2008. Am Med Assoc. 7/13/2009.
<http://www.ama-cmeonline.com/osteo_mgmt/module03/images/m3_02path_02.jpg>
- [67] Tami AE, Nasser P, Verborgt O, Schaffler MB, Knothe Tate ML. The role of interstitial fluid flow in the remodeling response to fatigue loading. J Bone Min Res 2002; 17:2030-2037.
- [68] Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267:1456-1462.
- [69] Tracy JK, Meyer WA, Flores RH, Wilson PD, Hochberg MC. Racial differences in rate of decline in bone mass in older men; the Baltimore men's osteoporosis study. J Bone Min Res 2005; 20(7): 1228-34.
- [70] Turner CH, Robling AG, Duncan RL, Burr DB. Do bone cells behave like a neuronal network? Calcif Tissue Int 2002; 70:435-442.
- [71] Turner CH, Vital A, Ramana M, V Pidaparti. A uniform strain criterion for trabecular bone adaptation: Do continuum-level strain gradients drive adaptation? J Biomechanics 1997; 30:555-563.
- [72] U.S. Department of Health and Human Services. Bone Health and Osteoporosis: A Report of the Surgeon General. Rockville, MD: U.S. Department of Health and Human Services, Office of the Surgeon General, 2004.
- [73] Verborgt O, Gibson GJ, Schaffler MB. Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. J Bone Miner Res 2000; 15:60-67.

- [74] Wimalawansa SJ. Prevention and treatment of osteoporosis: efficacy of combination of hormone therapy with other antiresorptive agents. *J Clinical Densitometry* 2000; 3(2): 187-201.
- [75] Wu CY, Yu TJ, Chen MJ. Age related testosterone level changes and male andropause syndrome. *Chang Gung Medical Journal* 2000; 23(6): 348-53.
- [76] Xie LQ, Jacobson JM, Choi ES, Busa B, Donahue LR, Miller LM, Rubin CT, Judex S. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. *Bone* 2006; 39:1059-1066.

APPENDIX A: 3D OUTPUT OF PARAMETERS VS. FORCE AND TIME

Figure A.1 Porosity surface plot.

Figure A.2 Daily bone formation surface plot.

Figure A.3 Daily bone resorption surface plot.

Figure A.4 Net daily change in bone surface plot.

Figure A.5 Modulus surface plot.

Figure A.6 Strain surface plot.

Figure A.7 Damage contribution to activation frequency surface plot.

Figure A.8 Disuse contribution to activation frequency surface plot.

Figure A.9 Total activation frequency surface plot.

Figure A.10 Damage formation rate surface plot.

Figure A.11 Damage removal rate surface plot.

Figure A.12 Total damage surface plot.

Figure A.13 Number of refilling BMUs surface plot.

Figure A.14 Number of resorbing BMUs surface plot.

Figure A.15 Number of disuse-induced apoptotic osteocytes surface plot.

APPENDIX B: MATLAB CODE

```
%{
Apoptosis-Induced Bone Remodeling Simulation For the Prediction of Remodeling
Parameters Through the Use of Apoptotic Osteocytes.
Michael Alexander 13July2009
%}

close all
clear
clc

%Loop for changing force by intervals of 1N for generation of 3D surfaces.
%istart=1; iend=2000;
iforce=2000; % Manual force setting shortcut
%for iforce=istart:iend%iforce is the changing force
```

B.1: Remodeling Parameter Assignments

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Remodeling parameters
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Rabbit parameters
Rc = 0.07159; % Cement line radius
Rh = 0.02163; % Haversian canal radius
Tr = 9; % Resorption period
Tv = 10; % Reversal period
Tf = 26; % Formation period
trab1 = 0.2; % Porosity partition for cortical to
trabecular bone
trab2 = 0.097267787; % Porosity partition for cortical to
trabecular bone
phi0 = .53*(10^-10); % Equilibrium stimulus determined by
changing for best damage fit
%por0 = 0.2; % Initial trabecular porosity
por0 = 0.0322; % Initial cortical porosity
sa0 = (((28.8*por0-101)*por0+134)*por0-93.9)*por0+32.1)*por0; % Initial
surface area
phc = 0.0322; % Adjusts resorption rate to match
apposition rate for cortical bone

% Activation frequency conditions.
Acfmax1 = 1.0; % Maximum Ac.f due to damage
(BMU/mm2/day)
Acfmax2 = 0.5; % Maximum Ac.f due to disuse
(BMU/mm2/day)
samax = 4.1905; % Normalizes specific area
Acfdis0 = 0.0; % Equilibrium Ac.f for disuse
(BMU/mm2/day)
Acfdam0 = 0.0094; % Equilibrium Ac.f for damage
(BMU/mm2/day)
Acf0 = (Acfdam0 + Acfdis0)*sa0/samax; % Equilibrium Ac.f (BMU/mm2/day)
Acfbaseline = .01479; % Rabbit value from Hedgecock.
Baseline (5.4 BMU/mm2/year = .01479 BMU/mm2/day ) when Apop(t)<=Apop0
kb = 65000000000;
kc = phi0/2;
```

```

% Apoptosis Conditions
Apop0 = 45.0; % (45 Ap.Ot/mm2) Below this number of
Ot's, there's an Acfbaseline

% Damage conditions
fs = 5; % Damage repair factor
d0 = 0.03662944; % Equilibrium damage
if por0 <= trabl
    kd = (d0*Acf0*(pi*Rc^2)*fs/phi0); % Equilibrium damage constant
else
    kd = 0.5*d0*Acf0*(pi*Rc^2)*fs/phi0;
end
kr = -1.6;

% Mechanical conditions
Area = 100; % Cross-sectional area of bone
rll = 3000; % Frequency of loading in no. of
cycles per day
q = 4; % Exponent of mechanical stimulus
change = 0; % Percent change in force
chgper = 1; % time period of force change
days = 2000; % Time length of simulation
dt = 1; % Time step; One day

```

B.2: Prior Conditions

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Prior conditions before start of simulation
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

for t=1:(Tr+Tv+Tf) % Prior Conditions set up
    variables for actual simulation

        por(t) = por0; % Porosity
        if por(t) <= trab2
            modulus(t) = 28969*(1-por(t))^5.24; % Stiffness of bone if
cortical
        else
            modulus(t) = 14927*(1-por(t))^1.33; % Stiffness of bone if
trabecular
        end

        Phi(t) = phi0; % Mechanical stimulus
        strain(t) = (phi0/rll)^0.25; % Principal strain
        stress(t) = modulus(t)*strain(t); % Principal stress
        Force(t) = iforce; % Force on bone (Newtons).
        SA(t) = sa0/samax; % Normalizes Ac.f by available

    surface area
        Df(t) = kd*phi0; % Damage formation rate
        Dr(t) = d0*Acf0*(pi*Rc^2)*fs; % Damage removal rate
        if por(t) > trabl
            Dr(t) = 0.5*Dr(t);
        end
        D(t) = d0; % Damage
        NfBMU(t) = Acf0*Tf; % No. of refilling BMUs
        NrBMU(t) = Acf0*Tr; % No. of resorbing BMUs
        if por(t) <= trabl
            Qf(t) = pi*(Rc^2-Rh^2)/Tf; % Mineral apposition rate
            Qr(t) = pi*Rc^2/Tr; % Resorption rate
        end
    end
end

```

```

        QrNr(t) = (1-phc)*Qr(t)*NrBMU(t);           % Amount bone removed
        QfNf(t) = Qf(t)*NfBMU(t);                 % Amount of bone added
    else
        Qf(t) = 0.5*pi*Rc^2/Tf;
        Qr(t) = 0.5*pi*Rc^2/Tr;
        QrNr(t) = Qr(t)*NrBMU(t);
        QfNf(t) = Qf(t)*NfBMU(t);
    end
    Qnet(t) = QfNf(t)-QrNr(t);                     % Difference between mineral
added and mineral removed
    Acfdam(t) = Acfdam0;                           % Ac.f due to damage
    Acfdis(t) = Acfdis0;                           % Ac.f due to disuse
    Acf(t) = (Acfdam(t) + Acfdis(t))*SA(t);        % Total Ac.f
    TIME(t) = t;
end

```

B.3: Simulation: Mechanical Conditions

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Simulation
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

for t=(Tr+Tv+Tf+1):days
    TIME(t) = t;

    % Mechanical conditions update
    if por(t-1) <= trab2
        modulus(t) = 28969*(1-por(t-1))^5.24; % Stiffness of bone if cortical
(NZWR value)
    else
        modulus(t) = 14927*(1-por(t-1))^1.33; % Stiffness of bone if trabecular
(Hazel said cortical close; use default)
    end
    %3Dmodulus(t,iforce)=modulus(t);                % 3D surface plotting

    if t < (chgper+Tr+Tv+Tf+1)
        Force(t) = Force(Tr+Tv+Tf)*(1 + change/100*(t-Tr-Tv-Tf)/chgper);
    else
        Force(t) = Force(t-1);
    end

    stress(t) = Force(t)/Area;                       % Stress on bone
    %3Dstress(t,iforce)=stress(t);                   % 3D surface plotting
    strain(t) = stress(t)/modulus(t);                % Strain on bone
    microstrain(t)=(10^6)*strain(t);                 % Conversion to Microstrain
    %3Dstrain(t,iforce)=(10^6)*strain(t);            % 3D surface plotting

    Phi(t) = (abs(strain(t))^q)*r11;                 % Calculate mechanical stimulus
    %3DPhi(t,iforce)=Phi(t);                         % 3D surface plotting

```

B.4: Simulation: Disuse Portion

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Disuse
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Units: Apopdis is in decimals of Apop. Ex: is in ".013",

    if Phi(t) < 3.16*(10^-11)                       % From curve-fitting: Por(t)-
>Phi(t)->Apopdis(t). Por(t) & Apopdis(t) from Aguirre DIS data.

```

```

        Apopdis(t)= 4.066666666666666*(109)*Phi(t)-0.000000000114717*(109);
elseif Phi(t) >= 3.16*(10-11) && Phi(t) < 3.47*(10-11)
        Apopdis(t)=-2.967741935483874*(108)*Phi(t)+0.000000000231681*(108);
elseif Phi(t) >= 3.47*(10-11)
        Apopdis(t)=-1.294326241134754*(107)*Phi(t)+0.000000001331913*(107);
else
end

Acfdis(t) = 0; % No demand for additional
remodeling if not in disuse

if Phi(t) < phi0 % If Phi<phi0, then in Disuse
        Acfdis(t)=17.739*(Apopdis(t)2)-1.616*Apopdis(t)+0.043;% Combined eqn
converts Apop to Acf for both Damage and Disuse
        if Acfdis(t) > Acfmax2
                Acfdis(t) = Acfmax2;
        end
        %NOTE: The following eqn is from solving the quadratic for X and
        %rejecting the "-b-sqrt(b2-4ac)" because its value was not in
        agreement with the expected #apop.
        %The "-b+sqrt(b2-4ac)" is used below.
        nOtApopdis(t)=(-(-0.002956855573126)+sqrt((-0.002956855573126)2-4*(
0.000059506430811)*(0.043179955361272-Acfdis(t))))/(2*( 0.000059506430811 ));
        else
                Apopdis(t)=0;
                nOtApopdis(t)=0;
        end
        DisplayApopdis(t)=Apopdis(t)*-1; % Used inverted Apopdis
magnitude; this inverts for display sense. Code uses inverted due to
interacting code fit.
        %3DnOtApopdis(t,iforce)=nOtApopdis(t); % 3D surface
        %3DAcfdis(t,iforce)=Acfdis(t); % 3D surface plotting

if por(t-1) <= 0.2
        area = pi*Rc2; % Resorption area if cortical
else
        area = 0.5*pi*Rc2; % Resorption area if trabecular
end

```

B.5: Simulation: Damage Portion

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Damage
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calculate change in damage level and update
Df(t) = kd*Phi(t); % Damage formation rate
%3DDf(t,iforce)=Df(t); % 3D surface plotting
Dr(t) = D(t-1)*Acf(t-1)*area*fs; % Damage removal rate
%3DDr(t,iforce)=Dr(t); % 3D surface plotting
D(t) = D(t-1) + (Df(t) - Dr(t))*dt; % Damage update
%dD(t,iforce)=D(t); % 3D surface
Apopdam(t) = 11.123706479648998*D(t)2 -0.462061174425421*D(t)
+0.038583322125937;% percent of apop ot dam
Acfdam(t) = 17.739*(Apopdam(t)2) -1.616*Apopdam(t) +0.043;% wholistic eqn
for DAM & DIS acf(/day).apop(%)

%{
% Baseline Acfdam below Apop0 causes DAM to be lower than it should be;
% Apopdam(#) is ALWAYS lower than the baseline of #45ot.apop.dam so

```

```

% Acfdam would always be = Acfdam baseline.
if Apopdam(t) <= Apop0
    Acfdam(t)= Acfbaseline;
end
%}

```

B.6: Simulation: Translation from % to # of Apoptotic Osteocytes

```

%3DAcfdam(t,iforce)=Acfdam(t); % 3D surface plotting
nOtApopdam(t)=((-0.002956855573126)+sqrt((-0.002956855573126)^2-4*(
0.000059506430811)*(0.043179955361272-Acfdam(t))))/(2*( 0.000059506430811 ));

if Phi(t)<phi0 % If in Disuse, damage parameters = 0
    % Apopdam(t)=0;
    % Acfdam(t)=0;
    % nOtApopdam(t)=0;
end
%3DnOtApopdam(t,iforce)= nOtApopdam(t); % 3D surface plotting
totlnOtApop(t)=nOtApopdam(t)+nOtApopdis(t);
%3DtotlnOtApop(t,iforce)=totlnOtApop(t); % 3D surface plotting

sa = (((28.8*por(t-1)-101)*por(t-1)+134)*por(t-1)-93.9)*por(t-
1)+32.1)*por(t-1); %Surface area

SA(t)=sa/samax;
Acf(t) = (Acfdam(t) + Acfdis(t))*SA(t); % Update Ac.f
%3DAcf(t,iforce)=Acf(t); % 3D surface plotting

% Calculate daily amount of bone removed per resorbing BMU
ac = area; % Resorption area
if por(t-1) <= 0.20
    ab = pi*(Rc2-Rh2); % Formation area of cortical bone (Cement line to
Haversian canal)
else
    ab = 0.5*pi*Rc2; % Formation area of trabecular bone (half of osteon
area w/o pore)
end
Qf(t) = ab/Tf;
Qr(t) = ac/Tr;

if por(t-1) > trabl
    if Phi(t) < phi0
        Qf(t) = (0.5 + 0.5*Phi(t)/phi0)*Qf(t); % For trabecular bone,
formation rate decreases in disuse
    end
end
end

```

B.7: Simulation: Bone Mass Calculation

```

% Calculate number of refilling/resorbing BMUs for current day
NfBMU(t) = NfBMU(t-1) + (Acf(t-Tr-Tv) - Acf(t-Tr-Tv-Tf))*dt;
%3DNfBMU(t,iforce)=NfBMU(t); % 3D surface plotting
NrBMU(t) = NrBMU(t-1) + (Acf(t) - Acf(t-Tr))*dt;
%3DNrBMU(t,iforce)=NrBMU(t); % 3D surface plotting

% Calculate net amount of bone added per day
QfNf(t) = NfBMU(t-1)*Qf(t-1) + (Acf(t-Tr-Tv)*Qf(t-Tr-Tv) - Acf(t-Tr-Tv-

```

```

Tf)*Qf(t-Tr-Tv-Tf))*dt;
%3DQfNf(t,iforce)=QfNf(t); % 3D surface plotting
QrNr(t) = NrBMU(t-1)*Qr(t-1) + (Acf(t)*Qr(t) - Acf(t-Tr)*Qr(t-Tr))*dt;
%3DQrNr(t,iforce)=QrNr(t); % 3D surface plotting
Qnet(t) = QfNf(t)-QrNr(t);
if por(t-1) <= 0.20
    Qnet(t) = QfNf(t) - (1-phc)*QrNr(t);
end
%3DQnet(t,iforce)=Qnet(t); % 3D surface plotting

% Calculate change in porosity
por(t) = (por(t-1) - Qnet(t))*dt;

uplim = 0.99;
if por(t) >= uplim;
    por(t) = uplim;
end
%3Dpor(iforce,t)=por(t); % 3D surface plotting
end

```

B.8: Output: 3D Surface Plot Generator

```

% 3D Surface Plot
t=1:100:days;
iforce=istart:100:iend;
[X,Y]=meshgrid(t,iforce);
Z = nOtApopdis3D (iforce,t); %must be 'por3D', not '3Dpor'
figure(1);
surf(X,Y,Z)
%axes1 = axes('Parent',figure1,'YTick',[0 500 1000 1500 2000],'XTick',[0 500
1000 1500 2000],'FontSize',16);
xlabel('Force (N)','FontSize',16);
ylabel('Time (days)','FontSize',16);
zlabel('Disuse-Induced Apoptosis (# apoptotic
osteocytes/mm^2)','FontSize',16);
title('Disuse-Induced Apoptosis','FontSize',20);
set(gca,'ydir','reverse');
saveas(gcf,'QfNf3D.jpg')

fh=figure(1);
set(fh,'color','white'); % sets the color to white
%}

```

B.9: Output: General Plots

```

%%{
% General Output
figure(1);
plot(TIME,Force);
title('Force');
xlabel('Time (days)');
ylabel('Force (N)');
saveas(gcf,'Force.jpg')
figure(2);
plot(TIME,por);
title('Porosity');
xlabel('Time (days)');
ylabel('Void space/Total area');
saveas(gcf,'Porosity.jpg')

```



```

figure(3);
plot(TIME,Qnet);
title('Change in Bone Density at Force=0N');
xlabel('Time (days)');
ylabel('delta Porosity');
saveas(gcf,'Qnet.jpg')
figure(4);
plot(TIME,modulus);
title('Modulus');
xlabel('Time (days)');
ylabel('Modulus');
saveas(gcf,'Modulus.jpg')
figure(5);
plot(TIME,stress);
title('stress');
xlabel('Time (days)');
ylabel('Stress');
saveas(gcf,'Stress.jpg')
figure(6);
plot(TIME,microstrain);
title('Strain');
xlabel('Time (days)');
ylabel('Microstrains');
saveas(gcf,'Microstrain.jpg')
figure(7);
plot(TIME,Phi);
title('Phi');
xlabel('Time (days)');
ylabel('Mechanical Stimulus');
saveas(gcf,'Phi.jpg')
figure(8);
plot(TIME,Apopdam);
title('% Change in Ot.apop.dam');
xlabel('Time (days)');
ylabel('Change in Apoptosis (%)');
saveas(gcf,'Apopdam.jpg')
figure(9);
plot(TIME,DisplayApopdis); %Inverts Apopdis for display because
Acfdis accepts -Apopdis.
title('% Change in Ot.apop.dis');
xlabel('Time (days)');
ylabel('Change in Apoptosis (%)');
saveas(gcf,'DisplayApopdis.jpg')
figure(10);
plot(TIME,Acfdis);
title('Activation Frequency from Disuse');
xlabel('Time (days)');
ylabel('Acf.dam (#BMU/mm2/day)');
saveas(gcf,'Acfdis.jpg')
figure(11);
plot(TIME,Acfdam);
title('Activation Frequency from Damage');
xlabel('Time (days)');
ylabel('Acf.dam (#BMU/mm2/day)');
saveas(gcf,'Acfdam.jpg')
figure(12);
plot(TIME,Acf);
title('Activation Frequency');
xlabel('Time (days)');
ylabel('Activation Frequency (# BMU/day/mm2)');
saveas(gcf,'Acf.jpg')

```

```

figure(13);
plot(TIME,Df);
title('Rate of Damage Formation');
xlabel('Time (days)');
ylabel('Crack length density/day');
saveas(gcf,'Df.jpg')
figure(14);
plot(TIME,Dr);
title('Damage Removal Rate');
xlabel('Time (days)');
ylabel('Crack length density/day');
saveas(gcf,'Dr.jpg')
figure(15);
plot(TIME,D);
title('Damage');
xlabel('Time (days)');
ylabel('Totl Crack Length/section area (mm/mm2)');
saveas(gcf,'D.jpg')
figure(16);
plot(TIME,nOtApopdis);
title('# Osteocytes Apoptosing from Disuse');
xlabel('Time (days)');
ylabel('# Apoptotic Osteocytes');
saveas(gcf,'nOtApopdis.jpg')
figure(17);
plot(TIME,nOtApopdam);
title('Osteocytes Apoptosing from Damage');
xlabel('Time (days)');
ylabel('# Apoptotic Osteocytes');
saveas(gcf,'nOtApopdam.jpg')
figure(18);
plot(TIME,totlnOtApop);
title('Total # Osteocytes Apoptosing');
xlabel('Time (days)');
ylabel('# Apoptotic Osteocytes');
saveas(gcf,'totlnOtApop.jpg')
%}
for fhvar=1:18
    fh=figure(fhvar);
    %hl=fh;
    set(fh, 'color', 'white'); % sets the output frame to white
end

%tilefigs                                % Tiles figure

```

B.10: Utility Code: Curve-Fitting

```

%{
CREATED BY MICHAEL ALEXANDER ON 16OCT2008
PURPOSE 1: Fit a curve to the Apoptosis and Damage chart.
PURPOSE 2: Determine how many polynomials to use in the curve equation.
%}
close all %Clear variable slots for run
clc
format long %Enables increased output decimal resolution

load data.dat; %Input damage and disuse data
apop = data(:,2); %Assign data to variables
D = data(:,1);
%{

```

```

a=polyfit(D,apop,2)
m = polyval(a,D);
aCorrel = corrcoef(apop, m)
%}
b=polyfit(D,apop,2) %Output equation for best fit at 2nd order
n = polyval(b,D);
bCorrel = corrcoef(apop, n) %Output how well the equation fits the data

figure(2)
plot(D,apop, 'bo', D, n,'r*')%,D, m,'b*') %Data with curve fit overlay
xlabel('Apoptosis, decimal %')
ylabel('Acf (BMU/mm2/day')
title('Curve Fit Wholistic Dam & Dis Eqn')

```