Modeling the Role of TGF-β in Bone Turnover

By

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I Hafsa Alyas **CIIT/FA16-RMT-018/LHR** hereby declare that I have produced the work presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except referred to wherever due, that amount of plagiarism is within acceptable range. If a violation of HEC rules on research has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of the HEC.

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It is certified that Hafsa Alyas, CIIT/FA16-RMT-018/LHR has carried out all the work related to this thesis under my supervision at the Department of Mathematics, COMSATS University Islamabad, Lahore Campus and the work fulfills the requirement for award of MS degree.

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DEDICATION

To My Family and Teachers

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ABSTRACT

Modeling the Role of TGF-β in Bone Turnover

Bone plays a very important role in our life. The process of deposition of old bones and the formation of new bones is known as bone remodeling. In bone remodeling mainly two cellular factors called osteoclasts (bone removing cells) and osteoblasts (bone building cells) and a signaling pathway RANK/RANKL/OPG are involved. Mathematical modeling of bone remodeling may provide a deep insight to understand the deep mechanism involved in bone remodeling. Several attempts have been made to study the dynamics of this complex cellular and molecular framework. Our methodology is founded on a discrete method formalized on the interaction among the components involved in bone remodeling process in terms of logical parameters, variables, and functions. The stability of the presented model has been checked through the loops (either +ve or -ve) involved in the mechanism of bone turn over. One of our models takes into account the feedback mechanism involved in coupling between osteoclasts and osteoblasts. Another discrete framework is established to discuss the RANK/RANKL/OPG signaling pathway. The results are varied through the numerical simulations. This thesis is divided into -ve sections. First chapter is dedicated to the introduction of bone biology. Second chapter is comprised of the previous work on mathematical modeling of bone remodeling. Chapter 3, 4, 5 are based on our main results, results and discussions along with some proposed open problems and references, respectively.

TABLE OF CONTENTS

Chapter 1

1 Introduction

Bone plays a very important role in human life, and it has several functions for instance protection, storage (calcium, phosphate) and most importantly the blood fabrication. It helps in the maintenance of PH level. Bones are the living tissues in our body and contain blood vessels which help in repairing and growth of bones. Protein, minerals and vitamins are also the important constraints which help in bone making. At the time of the birth child body contains about 300 soft bones which later on reduces to 206 bones. Bones are composed of mainly two types of tissues cortical and trabecular. Cortical is the outer most layer of the bone which is hard, strong and tough. Trabecular is the inner layer of the bone which is spongy and soft as compared to the cortical as shown in Fig (1). Cortical bones are also known as compact bones while trabecular bones are also known as cancellous bones. Bones performs multiple functions in the body of the organisms such as support, movement, blood cell production, energy storage and protection. Bone gives support to our body by providing a framework for the attachment of tissues and muscles. Bones help in the movement of the body by acting as a liver. Moreover bones help in the storage of lipids, minerals etc and works as an energy reservoir. Bones give us protection in a way that they produce several types of the blood cells which protect the body against infections. Amulgation of all these functions helps in making of 206 bones of human body which are necessary for our existence.

1.1 Bone Cells

Different types of cells that are involved in bone remodeling are osteoclasts, osteoblasts, osteocytes, immune cells, T-cells and B-cells, megakaryocytes and osteomacs. Osteoclasts are the cells which are involved in breaking or resorption of bones. Osteoclasts are multinucleated (having more than one nucleus) cells and they are present in pits in bone surface named as resorption bays (Howships lacunae). The life cycle of osteoclasts is about 10 days. The cell that helps in

bone making is known as osteoblast. Osteoblasts are the cells with mononucleus, a single cell cannot form the bone alone so the cells works as a network in which different cells are connected together to remodel the bone. Osteoblasts have the life time of about 15 days after that they die or convert into osteocytes, which are star like shaped cells and usually found in mature bones and have life equal to the life of the organisms. The number of osteocytes in adult body is about 42 billion and help in strengthen the bones. Immune cells give us protection against foreign particles. Combine working network frame of osteoblasts and osteoclasts is usually known as 'basic multicellular units' (BMUs). Activation (A), restoration(R) and formation (F) are three stages in BMU's. Bones are composed of three different special types of cells mainly osteoclasts, osteoblasts, osteocytes. Osteoclasts are the bone destroying cells which play major role in bone depositions and resorption. Osteoclasts cells are usually found attached to the bone surface from where they help in the differentiations of the minerals. Osteoclasts resorb bone matrix, and as a result bone mass reduces. Osteoblasts are the cells that help in the formation of bones. Osteoblasts produces bone matrix, and as a result bone mass increases. "Osteoid" are the newly formed bones. Matured osteoblasts cells are called osteocytes. Osteocytes are the cells that are found within the fully formed bones. Osteocytes help in the continuous formation of the bones. Osteocytes help in the maintenance of the bones, above this they give strength to the bones.

Osteoclasts, osteoblasts and osteocytes are the three most important types of bones cells. An occurrence of centimbalance between the activities of osteoclasts and osteoblasts gives rise to several diseases like osteoporosis, osteomyelitis and metastasis.

In recent past, it has become increasingly crystal clear that osteoclasts are not only the bone resorbing cells simply, but in addition they also promote the functioning of osteoblasts both negatively and positively, and resolve the passage of

the hematopoietic stems from the marrow into the blood, and plays role as immunomodulators in pathologic case.

1.2 Bone Turnover

Bone turnover plays the role of back bone in the life of living organisms. Shape and structure of the bones continuously change in the living tissues for the maintenance of the structure of bone framework. Skeleton provides support and protection to organs and muscles. Bone is the important part of defending cells. The process of resorption of old bones and the formation of new bones is known as bone turnover (bone metabolism). Bone remodeling is a continuous phenomena which occurs throughout the life of an organism from its birth till death. But its percentage varies at different stages for example at the very early stage of the birth, like in the first year, it changes cent percent but in adults remodeling percentage, it is ten percent per year. Bone turnover (formation and resorbtion) do not take place simultaneously, while it is a continuous phenomenon, as child born the mass of bone is less but with the passage of time bone becomes thicker and this process continue till adulthood . Bone remodeling comprises of five stages including activation, resorption, reversal, formation, and quiescence. In very first stage that is activation, preosteoclasts changes into mature active osteoclasts under the presence of growth factor and cytokines. In the second stage that is resorption, osteoclasts absorb the old bones. In the third stage that is reversal stage phenomena of bone resorption ended. In the fourth stage that is formation stage, osteoblasts form new bones. In the last quiescence stage osteoblasts stay on the newly bone surface as a lining cells. There are two types of bones woven bones and lamellar bones. The bones which are mechanically weak are called woven bones. On the other hand the bones which are mechanically stronger are called lamellar bones. When the production of osteoid increases rapidly by osteoblastic cells, woven bones are produced. This

process occurs at the initial stage, after that woven bone changes by remodeling. In the case of fracture repairing, woven bone are produced due to rapid process of bone formation. In the case of the fracture lamellar bones are deposited and woven bones are remodeled. In the case of fracture repairing, woven bone are produced due to rapid process of bone formation. In mature healthy person all bones are lamellar bones. Bone tissue formation (osteogenesis) occurs by two processes namely intramembranous ossification and endochondral ossification. In intramembranous ossification connective tissue membrane is replaced with the bone tissues, resultantly they form flat bones such as skull bone. While in endochondral ossification hyaline cartilage model is replaced with the bone tissues such as tibia bone and femur bone. Growth of the bones is a continuous phenomenon; bones become longer thicker with the passage of time from childhood to adolescence. This countionous change in the bone mass is due to the endohondral bone formation. By the process of bone remodeling, bones in matured skeleton deforms (renewed) continuously in regards to the variety of stimuli. In bone remodeling tranches are removed from the area of the cortical and trabecular bones by the osteoclasts. These trenches are then filled by osteoblasts. The mechanisms that governs bone remodeling are yet not fully explored, but they take into account the breakage of the bones parts in return to the normal consumption. Changes in the shape and weight of the body is due to the change in mechanical force, where local discharge of growth factor or cytokines is due to the changes in heights of systematic hormones. It is a solidly organized phenomena, formation proceeds resorption in not a specific order and there is approximately one million of thes negligible locus of remodeling in matured skeleton at any time.

Aims of Bone Turnover

Bone turnover is essential to achieve three basic aims:

(1) It helps in the regulation of balancing the important minerals into the human body by altering the serum concentration.

(2) It empowers the skeleton to fit to its environment that is mechanical which, consequently reduces the fracture risk.

(3) For the repairing of the damage bones, it provides structure.

By site independent first aim of remodeling of bone is carried out, in this process the bone remodeling location is not that much valuable, integrity of bone provision is not challenged and there is a proper maintenance of the minerals. On the other hand site dependent remodeling is needed for the second and third aim. To enhance turnover rating all over the skeleton at the time when there is only damaged at the single location, for the remodeling system it is not that much important.

1.3 Transforming Growth Factor β

The role of TGF-β is very crucial in bone remodeling. It has positive and negative feedbacks on bone remodeling. The effect of TGF-β depends on the maturation stage of osteoclasts. On one hand, TGF-β has the potential to stimulate osteoblast recruitment, migration and proliferation of osteoblast precursors (ROBs). Thus the number of ROBs increases under the influence of TGF- β. When TGF-β vanishes or becomes inactivated, the increased pool of ROBs undergoes differentiation, thus increasing the number of active osteoblasts (AOBs). The inhibitory effect of TGF-β is mediated by core binding factor, which is a potent osteoblast differentiation factor. TGF-β is also known to induce osteoclast apoptosis [39]. Bone consists of numbers of growth factors for instance, transforming growth factor beta (TGF-β). And its content is around about thousand folds more than the levels needed for stimulation of osteoblastic cells in the dried bones powder

[27]. The growth factors that releases at the time of the resorbtion of the bone may affect the osteoblastic cells. The TGF-β effect is bi-directional on the lineage of the osteoblastic and it depends upon the osteoblastic states. TGF-β has the power to enhances the osteoblasts enrollment, it also helps in the generation and propagation of the osteoblastic precursors. On the other side TGF-β reduces the differentiation of osteoblastic precursor's cells into the active osteoblasts cells [2]. Hence, TGF-β suppress active osteoblasts population. Transformation Growth factor beta is a key factor of bone remodeling; it enhances the protein synthesis and has significant effect on bone cells which are responsible for bone formation and resorption. These cells are present in larger amount in bone cells. It is acclaimed that the bone matrix provides a large supply for stockpile of growth factor such as TGF-β. When resorption of bones takes place by active osteoclasts TGF-β discharges into microenvironment of BMUs and serve as robotic agents on bone cells. TGF-β quickens the separation of uninvolved osteoblast progenitors (OBu), but it hinders the separation of osteoblast precursor cells (OBp). In this way the effect of TGF-β on osteoblastic cells gives rise to larger number of osteoblast precursor cells (OBp). From the inactivation of TGF-β osteoblastic precursor cells can separate and to turn into active osteoblastic cells (OBa). Furthermore, TGF-β has been found to encourage osteoclast apoptosis. Transformation Growth Factor beta (TGF-β) is a stiff growth factor. Transformation growth factor beta (TGF-β) has four types TGF-β1,TGF$-\beta$2,TGF-β3 and TGF-β4. 390 amino acids are found in TGF-β1 and 412 amino acids are present in TGF-β2 and TGF-β3. Modern knowledge propose that partially in return to mechanical forces, osteocytes control the functioning of osteoclasts at the position of resorption of bone by generating the statement of RANKL in the local micro environment by osteoblastic cells. Instead of this advancement, the specific molecular system that checks beginning, progression and recess of bone remodeling at any given spot is poorly

accepted. Osteocytes are eventually separated osteoblasts which entangled in the osteoid matrix, they were setting up. They connect with each other and with osteoblastic cells on the top of overlying bone, and through them with the cells of osteoblasts in the bone pith hole through which they balance RANKL statement by these terminal cells, even if they do not come out to show RANKL themselves. The number of the remodeling sites and the rate of bone remodeling are expended in a range of pathologic circumstances and influences the skeleton, along with post-menopausal osteoporosis, rheumatoid arthritis and hyperparathyroidism in which local or/and systematic changes in the levels of pro inflammatory cytokines or hormones enhances the bone resorption. Mostly these elements activate bone resorption mainly by a complicated system that includes the up regulation of the statement of RANKL and M-CSF by other cells and osteoblastic cells.

1.4 RANK-RANKL-OPG Signalling Pathway

The RANK-RANKL-OPG pathway contains three components. (1) receptor activator of nuclear factor kappa beta ligand (RANKL), a protein primarily produced by osteoblastic precursors as a soluble form (2) receptor activator of nuclear factor kappa beta, shown on hematopoietic precursor cells surface (3) osteoprotegrin (OPG), a "decoy receptor" primarily released by mature osteoblasts [27]. RANK and RANKL interacts with each other and enhances the activity of the formation of osteoclasts. OPG binds with the RANKL and reduces the interaction of RANK-RANKL. This signaling pathway is known as the RANK–RANKL–OPG pathway, and plays a critical role in the coupling of resorbtion of bone through osteoclasts and the formation of the bone through osteoblasts. It had been deliberated for many years that the activity of osteoclasts is controlled by osteoblastics cells. The interaction of RANK-RANKL on the surface of precursor cells increases osteoclast formation. The cooperation of RANK-RANKL is controlled by the soluble sub-

stance namely, osteoprotegrin (OPG), also monitored by osteoblastic cells. OPG attach to RANKL to stop RANKL binding to RANK, a receptor expressed by osteoclasts. This signaling pathway is called as RANK-RANKL-OPG pathway. RANK-RANKL-OPG system was discovered in the middle of $1990s$. From the mature osteoblast also known as active osteoblast OPG are produced. It prevents RANKL by developing RANK-RANKL complexes. Bone resorption will be greater if the ratio of RANK-RANKL is high. OPG helps to reduce bone resorption by making its binding with RANKL. The number of the remodeling sites and the rate of bone remodeling are expended in a range of pathologic circumstances and influence the skeleton, along with postmenopausal osteoporosis, rheumatoid arthritis and hyperparathyroidism in which local or/and systematic changes in the levels of pro inflammatory cytokines or hormones which enhances bone resorption. Mostly these elements activate bone resorption mainly by a complicated system that includes the up regulation of the statement of RANKL and M-CSF by other cells and osteoblastic cells. In recent past, it has become increasingly crystal clear that osteoclasts are not only the bone resorbing cells simply, but in addition they also promote the functioning of osteoblasts both negatively and positively, and resolve the passage of the hematopoietic stems from the marrow into the blood, and plays role as immunomodulators in pathologic case.

1.5 Basic Multicellular Unit

A Basic Multicellular Unit is a materialistic corporation of union of cells that brings about 1 quantum of turnover of the bone that is removal of the older bones with the new bones. The birth of Basic Multicellular Unit takes place within the bone and its progression takes place across the bone surface of trabecular bone at the time period of their time cycle.

It can be seen in the schematic diagram that the life period of the Basic Multi-

cellular Units comprising of 6 stages defined as following (1) resting (2) activation (3) resorption (4) reversal/(coupling) (5) formation (6) mineralization and back to resting.

1.5.1 Resting

The surfaces of the bones are not active in the stage of resting w.r.t the remodeling of bone. At any time, approximately eighty percent of cortical and trabecular bone surfaces are in the resting phase [16]. Bone lining cells cover these resting surfaces, later these bone lining cells act as endosteal membrane (EM) and osteogenic precursor cells (OPC).

1.5.2 Activation

In the phase of activation resting bone surface cells changes into resorption. This to factor of activation is still not fully understood. Despite it is believed that biomechanical concerns and local structures are involved [16].

1.5.3 Resorption

Resorption starts after the arrival of osteoclasts and it makes connection with the surface of bone. The formation of Howship's lacunaein is trabecular bone and Haversian canal in cortical bone occurs at the time when osteoclasts resorb the bone.

1.5.4 Reversal (coupling)

The reversal stage starts right after the resorption depth, that is maximum resorption has been attained through the osteoclasts. The total time duration between the completion of resorption of bone and the formation of the new bone is about one to two weeks. From the histology point of view, reversal stage is the cycle

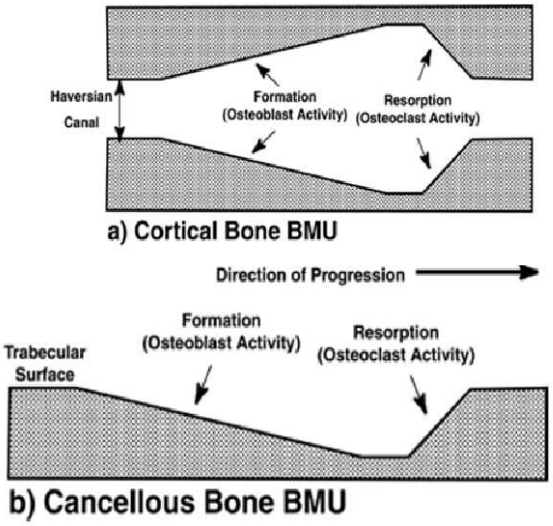

Figure 1: Cortical and Cancellous Bone BMU.

in which osteoclasts are absent in the Haversian canals and in Howship's lacunae. Osteoclasts go through apoptosis during the time period of reversal stage, although macrophage cell can be seen on the surface of the bone. These cells release factors that reduces the activity of osteoclasts and enhances the activity of osteoblasts.

1.5.5 Formation of Mineralization

Formation of the bones includes synthesis of the bone matrix that is followed by the extracellular mineralization. Osteoid seam is a lyer of the matrix which is built by osteoblasts. After five to ten days osteoid seam accomplish approximately seventy percent of its final mineralization. In cancellous and cortical bones complete mineralization takes almost three to six months. Fig (2) depicts different phases of the remodeling of the bone cycle.

1.6 Bone Disorders

Disorderness in bone turnover gives rise to several diseases such as osteoporosis, Paget's disease, primary hyperparathyroidism, hypothyroidism and multiple myeloma etc.

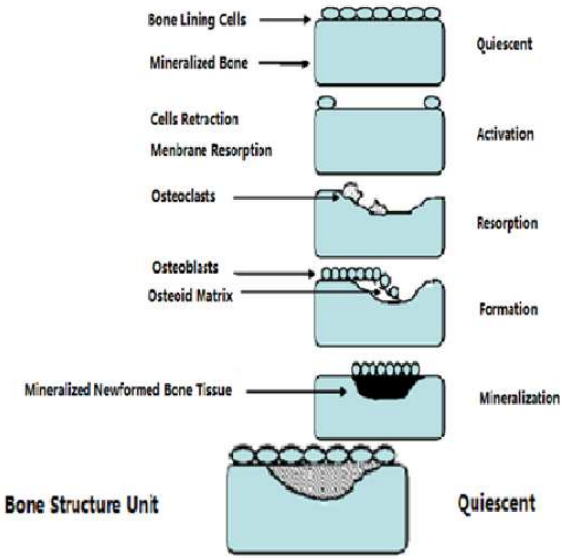

Figure 2: Phases of Bone Turnover Period.

1.6.1 Osteoporosis

Osteoporosis is the disease of bone which is related to the age. Due to this disease wrist, hip and spine fractures occur, and it is defined by less bone mass [15]. Loss of the bone is an imminent condition related to the age and can be found out by the bone remodeling rate and of the negative balancing of the bone between the resorbtion of bone and of formation of bone. It seems to be started between the ages of 18-30 years. In teenage this mechanism is very slow due to the slow remodeling rate. This disease of the osteoporosis point out a condition when the mass of the bone reduces to a critical level, below this the rate of the fracture risk becomes very greater [8]. Millions of the people are affected by this disease world-wide [30]. Osteoporosis can be classified into two classes, primary osteoporosis and secondary osteoporosis. Osteoporosis can be further classified into involutional osteoporosis and of juvenile osteoporosis. Senile osteoporosis and of postmenopausal osteoporosis are also included in this. Riggs in the year 1982 described two different disorders of the involutional osteoporosis that are high turnover and low turnover. High turnover osteoporosis found in postmenopausal women at the age

of 50-65 years and is pathogenetically is related to deficiency of the estrogen. On the other hand low turnover osteoporosis found in both men and women at the age of 75 and influences the cortical and trabecular bones. It is due to the age related downturn in the functioning of the osteoblasts and may causes vertebral and hip fractures [17]. Under some circumstances, the processes of loss of bones includes disorderness in both process of bone resorption and formation [17].

1.6.2 Paget's Disease

Paget's disease is due to the much more resorbtion of the osteoclastic cells that are situated in the single region of the skeleton for instance pelvis, skull and on the long bones ending points. The tissues that are influenced by the Paget's disease osteoclasts found abundantly and process almost hundred nuclei per cell. Although, the factors due to which the osteoclasts activity enhances and population becomes high have not been recognized. Pain, sensitivity to bone abnormality is some consequences of the disease.

1.6.3 Primary Hyperparathyroidism

Primary hyperparathyroidism occurs due to overproduction of the parathyroid hormones,which are produced by the parathyroid gland [3]. Primary hyperparathyroidism disease is a common disease. This disease can attack in any age but in the time period of age over 60, chances of attacking this disease becomes higher most dominantly in women [3]. Kidney and bones are the two most important organs that are affected by this disease. In the patient suffering from this disease of Primary hyperparathyroidism, the working of osteoclasts and the osteoblasts diminished, and there is approximately zero balance among the resorption and formation of bone when remodeling of the bone cycle ends. Although the patient that is suffering from Primary hyperparathyroidism disease the remodeling of bone

rate at a particular point on the trabecular surface is higher as compared to that of the healthier person. The kidney of the patient with Primary hyperparathyroidism disease may undergo renal stone disease.

1.6.4 Multiple Myeloma

Bone can avoid the offensive of most of the cancer cells due to its peculiar characteristics. Although multiple myeloma cells having the ability to live and progress inside the bone microenvironment may change their properties for the facilitation of their survival and development. Multiple myeloma is the second most persistent hematological malignancy and it is associated with very high rates of morbidity and after its diagnosis they have very short life cycle [13]. American Cancer Society investigated that about twenty thousand fresh cases of multiple myeloma disease were examined and ten thousand eight hundred among them died every year in US only. Due to multiple myeloma disease negative bone balancing enhances and increases the rate of bone resorption and reduces the bone formation rate [33].

1.7 Feedback Loops

Regulations can might be explained as constraints that adjusts the production rates of elements of a system with the system state and with the variables that are relevant environmental. Feedback loops is basically the main operator for all these adjustments. Closed pathway of any length is termed as circuit. Some mathematician reserve the loop word for one element circuit, while some use it for the circuits in which atleast three elements are present. Generally for any length of circuit biologist use the term "feedback loops".

(a) Every element effects all the other loop elements including itself. Positive loops can be defined as those loops in which every element having +ve effect on its own further production. Negative loops can be defined as the loops in which every

element has -ve effect on its own further production. It does not matter whether the loop is +ve or –ve, it only depends upon the number of –ve loop interactions. We deals with the +ve or –ve loops by keeping in mind whether the numbers of –ve interactions is odd or even.

(b) Main role of the feedback loops is to establish homeostasis (if –ve).

(c) Main role of the feedback loops is to establish multistationary (if +ve). Loop is called functional if it fulfils the two rules. defined above were the existance of loops may define the stability of the system in a complete manner. Several biological phenomena can be considered as networks.

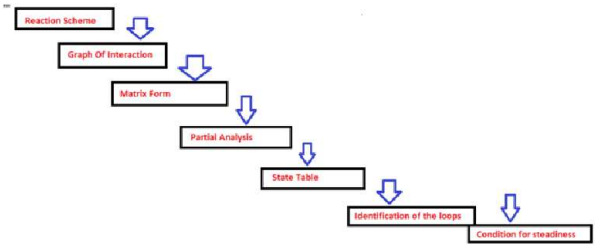

Figure 3: Methodology Flow Chart

When all possible states of the system place in a set then its called state space. Every coordinate is state variable all state variable values explains the state of system completely. State table is the sequential circuit representation consisting of three sections namely present, next and output states. Present state nominates the flip flop state before the clock pulse occurrence. Next state represents the flip flop states after clock pulse, and that of the output section lists the output variable values at the time of the present state. Regulations can might be explained as constraints that adjusts the production rates of elements of a system with the system state and with the variables that are relevant environmental. Feedback loops is basically the main operator for all these adjustments. Closed pathway of any length is termed as circuit. Some mathematician reserve the loop word for one element circuit. While some use the word "loop" for those circuits in which there are present at least three elements. Generally for any length of circuit biologist use the term "feedback loops".

Example 1

This biological example demonstrates the application of the concept of loop characteristic state to a specific biological system. Gene3 catalyzes the gene1 last step of reaction. Gene1 and gene2 combines together and reduces the synthesis rate of itself and that of the gene3. However, additionally due to auto controlled promoter and of its negativity for gene2 coding has 2nd promoter that is responsible for the small basic expression. This explanation is described by the reaction

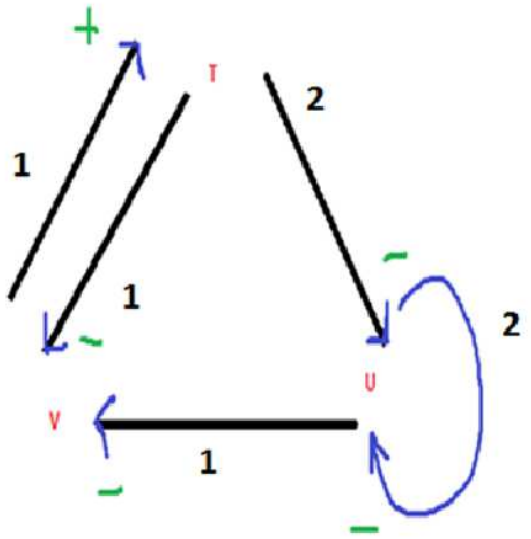

Figure 4: Graph of interaction

scheme as shown in the Fig (4) (reaction scheme diagram).

Simple arrows represent the regulatory interactions. Double arrow shows the metabolic interactions. In the above model T and U both are having different actions so, with these variables two thresholds are associated and three logical levels are associated that are 0,1 and 2. Contrarily V only acts upon the T, resultantly there are two logical values that are 0 and 1 and only one threshold associated with it.

The graph of interactions and that of the matrix of interactions includes more assumptions concerning to the thresholds order. Gene1 and gene2 both in this scheme act at two levels, having two threshold values that are s^1 and s^2 (0, 1 and 2 are the three logical values consequently), are referred to both of the variables.

The positive and negative signs correspond to the signs of interactions s^is representing the thresholds. From the above graph it is very easy to find feedback loops of the system they are three here(u, tv are two negative loops, tuv one +ve

loop) and given below is its matrix of interaction

$$M = \begin{bmatrix} 0 & 0 & 1 \\ -2 & -2 & 0 \\ -1 & -1 & 0 \end{bmatrix}$$

Matrix of interaction can be completed in four manners which depends upon the thresholds order. Which choice is most suitable it is not clear. Although many theoretical and experimental controversies may be use to differentiate among them. Especially, one can favour the concept that the gene3 synthesis repression needs the repressor which are lower level (lesse rs^1) than that of the auto repression of the synthesis of the gene2 (lesser s^2).

Main focus of this example is on the threshold combinations (one of the four combinations) shown in the graph of interaction and in matrix of interaction.

Above structure have three feedback loops.

(a) Between T and V a –ve loop, that can be symbolized by $t^{-1}v^1$, these explains the mutual regulation between gene1 and gene3.

(b) Loop on U that is a negative loop symbolized byu^{-2}, it explains the gene2 auto regulation.

(c) A +ve loop $t^{-2}u^{-1}v^1$, its existence is not clear.

Additionally, the disjoint loops union $t^{-1}v^1$ and u^{-2} (which are symbolized by the $t^{-1}v^1 + u^{-2}$ necessarily considered.

The +ve feedback loop presence propose that under suitable conditions system may display multi stationarity. This means for appropriate parametric values there are present various regulation states of the synthesis of gene3 and gene2, depends upon the historical detail not only on presently environmental conditions. Given below

is its partial analysis.

T	U	V
0	0	1
-2	-2	0
-1	-1	0

Partial Analysis

State table example for three variables. $(t\ u\ v)$ for every value of state vector table gives image vector values in logical parameters terms. M_i corresponding to element i expression when activator of it is absent, but its inhibitor is present; $M_{i.j}$ corresponds to the element i expression in the presence of j element if its an activator of i element, or in the non presence of j if its an inhibitor. $M_{i.jk}$ shows +ve contribution of both the elements j and k on the i element.

State Table					
t	u	v	T	U	V
0	0	0	M_1	$M_{2.12}$	$M_{3.12}$
0	0	1	$M_{1.3}$	$M_{2.12}$	$M_{3.12}$
0	1	0	M_1	$M_{2.12}$	$M_{3.1}$
0	1	1	$M_{1.3}$	$M_{2.12}$	$M_{3.1}$
0	2	0	M_1	$M_{2.1}$	$M_{3.1}$
0	2	1	$M_{1.3}$	$M_{2.1}$	$M_{3.1}$
1	0	0	M_1	$M_{2.12}$	$M_{3.2}$
1	0	1	$M_{1.3}$	$M_{2.12}$	$M_{3.2}$
1	1	0	M_1	$M_{2.12}$	M_3
1	1	1	$M_{1.3}$	$M_{2.12}$	M_3
1	2	0	M_1	$M_{2.1}$	M_3
1	2	1	$M_{1.3}$	$M_{2.1}$	$M_{3.2}$
2	0	0	M_1	$M_{2.2}$	$M_{3.2}$
2	0	1	$M_{1.3}$	$M_{2.2}$	$M_{3.2}$
2	1	0	M_1	$M_{2.2}$	M_3
2	1	1	$M_{1.3}$	$M_{2.2}$	M_3
2	2	0	M_1	M_2	M_3
2	2	1	$M_{1.3}$	M_2	M_3

Partial analysis is summarized above whatever the case is this can be easily read from the graph of interaction or from the interaction matrix that loop characteristics state of union of –ve loops is $[s^1s^2s^1]$ and the +ve loop characteristic state is $[s^2s^1s^1]$. Deep anaylisis of the parametric values that would give the +ve loop functional. For the combinations of the threshold the choice of the +ve loop can't be functional until and unless the parameter $M_{3.2}$ is nill. It is not legitimate, because it means that repressibility of protein only on depends upon gene1 and

gene2 presence. Although for other combinations of the thresholds, it is noted that realistic parametric values are consistent with the multiple steady states. So, with standard strains it doesn't expected multistationary. Although, mutants checking with changing threshold orders it would be valuable. By transient signal the state of +ve loop can be find out. In the case that is under consideration, obvious signal is extracellular concentration of gene1. This model gives the idea that for the appropriate parametric values the system state will strongly depends not only upon the present but also upon the previous extracellular concentration of gene1. No doubt, at that time in the model amino acid must then be introduced like an input variable.

Logical parameters explaining toughness of various interactions of system they are at least partly genetically find out. Coding portion of the gene2 that is effected by a mutation having the ability to change its affinity for promoters ($M_{2.2}$ and $M_{3.2}$ parameters) or its interaction with that of the gene1, so combine effect of it with gene1 upon its own synthesis and upon the gene3 synthesis ($M_{2.12}$ and $M_{3.12}$ parameters) etc. So, it can be expected that mutants will show all the values range of logical parameters. If the primitive type doesn't directed towards such an amazing behavior as multi-stationary constraints on the parameters can use to find out which mutants might.

From dynamical viewpoint the choice of parametric range will be richest (loops are functional). Although its interesting. Actually, it looks obvious that the realistic values will be different for some of the parameters.

Given below is the identifications of the loops (1) A +ve loop $t^{-2}u^{-1}v^1$ ($s^2s^1s^1$) is the characteristic state); (2) A –ve loop $t^{-1}v^1$ ($s^2s^1s^1$) is the characteristic state); (3) A –ve loop u^{-2} (ts^2v) is the characteristic state); Now the Conditions of the steadiness of $s^2s^1s^1$ and $s^1s^2s^1$, state of $s^2s^1s^1$ is present at junction between $2^3 = 8$ regular states. Pair of the adjacent states that are regular and their images are

under

State Table					
t	u	v	T	U	V
2	1	0	M_1	$M_{2.2}$	M_3
1	0	1	$M_{1.3}$	$M_{2.12}$	$M_{3.2}$

s_t^2, s_u^1, s_v^1, are be the thresholds conditions for the states to be steady, thresholds be included in the corresponding image interval that is $M_1 s_t^2 M_{1.3}$,$M_{2.2} s_u^1 M_{2.12}$,$M_3 s_v^1 M_{3.2}$, this conditions amount to $M_1 = 0$ or 1,$M_{1.3} = 2$,$M_{2.2} = 0$, $M_{2.12} = 1$,0r 2,$M_3 = 0$,$M_{3.2} = 1$ Likewise for the state $s^1 s^2 s^1$ the adjacent states(relevant regular) and their corresponding images are given below.

State Table					
t	u	v	T	U	V
1	2	0	M_1	$M_{2.1}$	M_3
0	1	1	$M_{1.3}$	$M_{2.12}$	$M_{3.1}$

So, steady state $[s^1 s^2 s^1]$ will be there if $M_1 = 0$,$M_{1.3} = 1$ or 2,$M_{2.1} = 0$ or 1, $M_{2.12} = 2$, $M_3 = 0$, $M_{3.1} = 1$

Its noticeable that $[s^1 s^2 s^1]$ and $[s^2 s^1 s^1]$ are not conflicting. Efficiently, if

$M_{2.2} = 0$,$M_{2.1} = 0$ or 1,$M_3 = 0$,$M_{3.1} = 1$,$M_{3.2} = 1$,$M_{1.3} = 2$, $M_{2.12} = 2$,

Both of the characteristic states are steady and they must at least be multi stationary simultaneously (in the variable space portion at least) of all the three variables a homeostatic behavior. For the conditions the state table is given below. It is noticeable that [2 0 1] is the regular steady state. $s^2 s^1 s^1$ and $s^1 s^2 s^1$, these two states are also steady. And there is not found any other steady state for the system.

Table three variables state table:

$M_1 = M_2 = M_3 = M_{2.2} = 0$, $M_{2.1} = 0$ or 1, $M3.1 = M_{3.2} = M_{3.12} = 1$, and $M_{1.3} = M_{2.12} = 2$

State Table					
t	u	v	T	U	V
0	0	0	0	2	1
0	0	1	0	2	1
0	1	0	2	1	1
0	1	1	0	2	1
0	2	0	0	0 or 1	1
0	2	1	2	0 or 1	1
1	0	0	0	2	1
1	0	1	2	2	1
1	1	0	0	2	0
1	1	1	2	2	0
1	2	0	0	0 or 1	0
1	2	1	2	0 or 1	0
2	0	0	0	0	1
2	0	1	2	0	1
2	1	0	0	0	0
2	1	1	2	0	0
2	2	0	0	0	0
2	2	1	2	0	0

Example 2 *Boolean Formalization*

Main ambition of formalization is purely vived. From this point of view this expression of formalization is not needed to be deliberate more than the sentences but it should be deliberates in more compact manners and less uncertain way. Secondly, an official language if suitable, should give permission not only to explain but also handle easily for the complex cinereous. particularly, it should allow a quick scanning of all the states that are possible for the system explained through

model. Boolean formalization language is beneficial over all others languages because in all the other languages it is very much difficult to detect the error occurs at any stage in contrast by using this Boolean Formalization language it should very much easy to avoid those errors and got the pure outcomes.

Main purpose of this is to explain genetic controls in terms of Boolean. Gene expression is binary function of the binary variables. If the gene is expressed the functional value is 1 if not then it is 0. The value 0 or 1 shows the absence or presence of gene allele. value 1 or 0 does not literarily means the presence or absent of the gene but it means present at the sufficient concentration to exerts its effect sufficiently. The associations used are given below

"+" (logical sum, it means not include (inclusive) "OR")

"-" (logical compliments, it means "NOT")

"." (logical product, it means "AND")

"=" (it means that the value 0 or 1 of the expression is same on both sides.

In many ways the given function can be represented. The case where the function is the product of the sums. For example $A = x.(y + z)$ means the A function expressed if and only if both x and $(y + z) = 1$ OR x and $(y + z) = 1$ are the essential conditions for A. The A function may also be written as $xy + xz$, means that either of the two conditions xy OR xz satisfies A function or simply xy and xz are sufficient condition for A. Much famous de Morgan theorem relates in a way given below

$\underline{x + y}$=$\underline{x}$. $\underline{y}$ this expression meant that not(x or y) is equivalent to (not x) and (not y) means neither x nor y $\underline{x}$+ $\underline{y}$ =$\underline{x}$ $\underline{y}$ this expression meant that (not x) or (not y) is equivalent to not(x and y). If the terms differ by values of only one variable then they can be fussed into each other for example,xyz and $xy\underline{z}$ fuses into xy since $xyz + xy\underline{z} = xy(z + \underline{z})$ and $z + \underline{z}$ must be 1. Terms which can not be fuses into other terms in the expression known as prime implicants.

To represents Boolean functions as veitch matrices is very useful. For each variable state these matrices give the functional values. If the variables are m then there will be 2^m variable states and one has to use matrix with 2^m boxes. In each box digits tell whether or not function is satisfactory for the specific variable state. For example Table (Fig5) veitch matrix of four variables for a simple Boolean function $H = xyg + x\underline{y}z + yzg + x\underline{y}\underline{z}g + \underline{x}yz\underline{g}$. The digits at the top of the columns indicates the values of the variables x and y and the digits at the starting of the lines to the values of the variables z and g so, the intersection box of 01 column and 11 line indicates the system state for which $x = 0$, $y = 1$, $z = 1$, $g = 1$. Each box is filled with "1" or "0" in the matrix accordingly whether or not states of the variables satisfy the function G or not. For example, the presence of yzg term is equivalent to $(xyzg = \underline{x}yzg)$ in the expression of H results in digit "1" in the rectangular box $\underline{x}yzg$ and also in neighbouring box $xyzg$, etc. By the fusion of adjacent square matrix in proper way prime implicants can easily be identified on matrix. Florine in 1973 pointed out that the combination order on the top of the column is 00,01,11,10 for symmetry reason. And the groups of 2,4,8 ... adjacent boxes can be fused. Examplary, boxes $xy\underline{z}g$, $xyzg$, $x\underline{y}\underline{z}g$ and $x\underline{y}zg$, each contains 1 digit can be fused simply into xg algebraic expression of square. Prime implicants of function H which is circled on the matrix are xg,yzg (which is present already on the starting expression of H), $x\underline{y}z$ and $\underline{x}yz$.

Now function H can be expressed in much more simpler way then before. Function H reduces to $H = xg + x\underline{y}z + \underline{x}zy$. It is very easy to notice that on matrix all states which satisfies function H only those states are replaced by this new expression H. Its not necessary that all the prime implicants must be present yzg term dropped because it comprises two states one state is $xyzg$ which is already represented in xg term, and the other $\underline{x}yzg$, is represented in $\underline{x}yz$ term. For simplicity one can write $xy + x\underline{y}z\underline{g}$ instead of $ag + a\underline{g}z$. Infact first expression not only be the complicated

	00	01	11	10
00	0	0	0	0
01	0	0	1	1
11	0	1	1	1
10	0	1	0	1

Figure 5: Veitch Table 1.

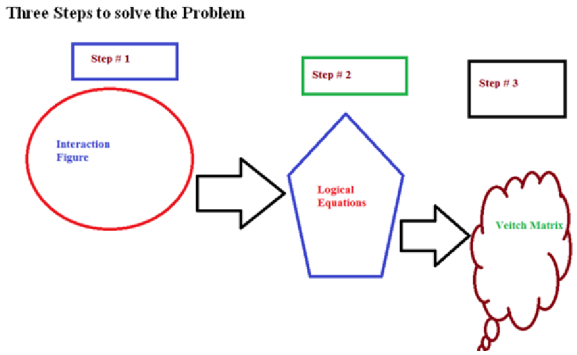

Figure 6: Steps of Boolean Formalization 1.

but also misleads because by looking at first time it might suggests that $x\underline{y}zg$ does not fit to the function. So, its best to use prime implicants in final function form.

Chapter 2

2 Review of Literature

The understanding of the bone remodeling mechanism at the Basic Multicellular Unit (BMU) level could guide us towards the bettered method for comprehending the remodeling cycle for the cure of bone diseases. For instance, to enhance BMU activation, the usage of therapeutic agents, conjointly with a diminution in the activity of osteoclast although conserving the activity of osteoblast, will give rise to built-up a new bone, likewise the hypothyroidism mechanism [32, 39]. Of course, above method has previously given rise to advancement of antiresorptive cures for post-menopausal loss of bone, which includes calcitonin, bisphosphonates, raloxifene and estrogen. Lately researches have exposed a greater number of factors that take part in the regulation of remodeling of bone [1]. They involved paracrine and autocrine signaling molecules, extracellular matrix components and systematic hormones, all of these having influence on cell-to-cell communication, proliferation, migration, differentiation and adhesion. Mostly these conclusions are retrieved from confined examinations via vivo experiments or in vitro studies, by considering genetically operated animals. The results depicted that the osteoblasts carry the ability of controlling osteoclast activities. Exemplary the RANKL interpretation by osteoblasts straightly interjects with the RANK on to the osteoclast progenitors (OCP) to drive osteoclastogenesis (OCG). The mechanism also lean to the level of OPG and may behave for RANKL as the soluble- decoy receptor (SDR) [7]. Despite of results obtained from these convey information in a limited way about comprehensive responses of aspects of these growth factors. The main purpose of this is the demand for the mechanisms which have the ability to combine such limited information into an organized sequence that defines the characteristics of such complicated system.

In comparison with, in vitro and in vivo, they ascribe to the biological analysis implemented outside as well as inside of the organisms that were alive, this analysis performed through computer simulations [25]. The main purpose of the

silico experiments is to check and understand the quantitative behavior of the biological systems, as computer simulation is much more beneficial than biological analysis [10]. Mainly, in vitro and in vivo experiments are costly and time taking. For instance, in an healthy singular one period of bone remodeling takes almost 7 months, and before the occurrence of the next remodeling period there is approximately 900 day passive cycle. Silico has advantage that, in silico simulations models the process at less rates and are much more frequent, plus applications of these experiments on human can cause several health issues. In few past years several mathematical models on bone remodeling have been developed and helped to cure different bone diseases. These models have determined tremendous potential to promote our knowledge for complicated biological mechanism, and discussed in this section.

2.1 Mathematical Models of Bone Remodeling

The theory of mechanostat by the Frost developed in year 1987 guided us towards the progress of great numbers of the models of that explained biochemical characteristics of the bones [11, 22, 38] mathematically. The summary of the mathematical models are given below:

2.1.1 Kroll and Rattanakul's Models

Net bone differentiation is due to parathyroid hormones (PTH) at the time when it is directed continuously, on the other hand when it is directed intermittently there is a net bone formation. In order to demonstrate such sophisticated behavior, a mathematical model by kroll was explained to connect preosteoblasts influential interactions.In [19], The basic purpose of this mathematical model is to treat the diseases related to bones, by providing a basis for parathyroid hormones based cure that are used for curing bone diseases.

The model presented by [19] determined that parathyroid hormones controls the

activity of osteoclasts through osteoblasts cells indirectly, the reason is that osteoclasts cells do not contain receptors for parathyroid hormones. The differentiation of osteoblastic precursors into pre-osteoblasts is regulated by paratheroid hormones (PTH), but it restrains the differentiation of pre-osteoblasts into osteoblasts, by combining with the receptors [19].

Model of kroll's contains four components (ODE) that explains ordinary change in quantity of osteoclasts, PTH, pre-osteoblasts(POB) and osteoblasts(OB). The total effect of the parathyroid hormone on formation of bone and resorption of bone is determined by the ratio of osteoblasts to osteoclasts. The output results after simulation shows that the periodic parathyroid hormone (PTH) administration enhances formation of bone, while regular parathyroid hormone (PTH) administration enhances loss of bones. For the facilitation of the construction of this model following assumptions were made. The setting of delay time one and two hours respectively, one hour is the differentiation time of progenitors pre-osteoblastic precursors(POBP) into the preosteoblasts (POB) and two hours is for the differentiation of pre-osteoblasts into osteoblasts. Plus, parathyroid hormone (PTH) proliferated effect on population of osteoclasts and osteoblasts depends upon temporary conditions. Kroll's model (2000) considered that PTH exerts a dynamic, steady impact upon the osteoclasts growth.

Kroll's model was extended by Rattanakul et al. (2003) by adding the estrogen simulation effect on the population on osteoclasts and on osteoblasts dynamics, and observed the working of parathyroid hormone (PTH) dealing with the process of bone remodeling. Rattanakul's model consists of 3 ordinary differential equations (ODEs), which explain the concentrations of osteoblasts, osteoclasts and flow of the parathyroid hormones (PTH) level. Rattanakul's model is based on clinical observations described within literature [6, 9, 36].

2.1.2 Komarova's Model

In bone remodeling regulation, interaction between osteoblastic and osteoclastic cells is clearly censorious. To simulate the paracrine and autocrine interaction among osteoblastic lineages and osteoclastic lineages, Komarova et al [18] developed a model based on the hypothesis that local affectors, released by osteoblasts and osteoclasts, can control their rate of formations. Komarova's model contains 3 ordinary differential equations (ODE), which explains the change in quantity of osteoblasts, osteoclasts and volume of bone with the time. Komarova's model was 1st model that explains physical fluctuations of remodeling of the bone period at a single Basic Multicellular Unit (BMU).

A very simple interaction between osteoblasts and osteoclasts was recommended by Komarova in [18], where precursors of matured cells and matured cells themselves are not considered as a single variable, and they can't be distinguished. The total production rates of every population of cell showed total conseuences of mature cell formation and precursors recruitment. Cells removal rate shows the death of the cells, and also the osteoblasts differentiation into bone lining cells and osteocytes. Resultantly, it was considered that cell interects with each other through effectors, which are activated or released by cells of bones and act in a way of paracrine and autocrine manner.

The Komarova's model was so efficient to simulate the variations which are temporal in bone mass and cell population at a time period of remodeling of bone at a distinct spot.

There are two stable modes in which the system exists;

(1) remodeling period which is a result of external stimulus,

(2) A number of internal initial periods of remodeling of bones, that correlates to the focused and arbitrary bone remodeling, respectively. Also the system having

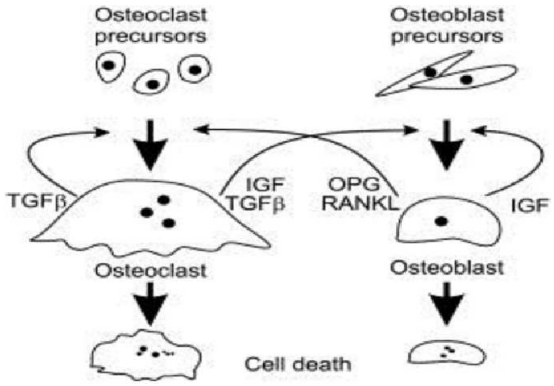

Figure 7: Interaction among Osteoclast and Osteoblast Lineage.

the unstability condition, symbolized by unsteady oscillatory difference in the bone mass and the number of cell with rising amplitude. This unstability mode behavior have resemblance with bone remodeling in paget's diseases in the individuals. The Komarov's model represents that the dynamical behavior of the system depends upon the autocrine regulation representation of the parameters of osteoclasts.
Necessarily, in Komarova's model there found few limitations. (1) Komarova's model considered only two types of cells.
(2) Autocrine and paracrine factors were considered which only regulates the osteoclasts and osteoblasts formation, on the other hand cellular death and cellular activity were considered to be the cell population proportionality.
(3) Effectiveness of paracrine regulation and autocrine regulation was described by the parameters, and multiple factors action.

2.1.3 Moroz's Model

The Komarovas's et al. model (2003) rebuilt the changes in osteobasts and osteoclasts population, and mass of the bone at the time of bone remodeling period at a seperate spot, their model anticipated the occurance of changing modes of dynamical behaviors of the Basic Multicellular Units (BMU) during the period of remodeling of bones. Nonetheless, the autocrine regulation loops and paracrine

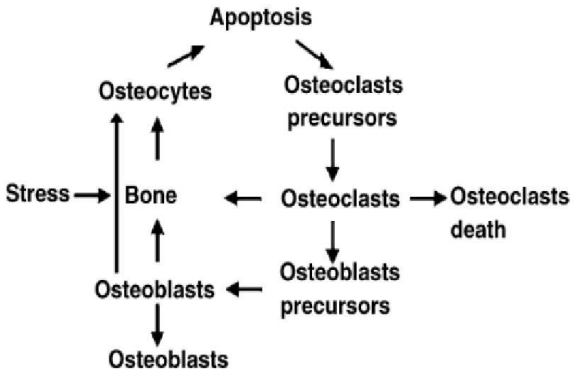

Figure 8: Interaction among Osteoclastic Lineage and Osteoblastic Lineage 1.

regulation loops were described by the parameters range which outstripped the biological potential. Additionally, osteocytes are missing in this model, infact they are of much importance in bone remodeling regulation [35].
Komarova's model was extended further by Moroz by describing the paracrine parameters and autocrine parameters within the biological capacity. And adding the osteocyte apoptosis role, mechanism of bone turnover [18]. The model representation is shown in Fig(8) Moroz's model comprises of 4 components (4-ODEs),and shows temporal changes in the osteoclast population, osteoblast population, and osteocytes population and volume of bone.

The model of Moroz determined the presence of steady states that is basic, with the occurrence of a 4 dimensional surface "bone-osteoblast- osteoclast- osteocytes" space. Moroz's model also explained the recovering potential existence, which is assisted against the biochemical bone damage and mechanical bone damage. By comparison with the normal remodeling of bone mechanism the Moroz's model was justified. Nonetheless, in order to examine the extensive range of contents more work is required.

2.1.4 Lemaire's Model

In the interaction between osteoclast and osteoblast the RANK-RANKL-OPG signaling pathway is a crucial factor, for the remodeling of bone regulation mechanism it behaves as a control network. [20, 23, 34]. Lemaire's model was the 1st try to integrate the RANK-RANKL-OPG signaling pathway into the mathematical modeling, and the main concept upon which it was based is of the relative proportions of the immature-osteoblasts (IOB) and that of the mature-osteoblasts (MOB) which controls the intensity of the activity of osteoclast, on the other hand osteoblast regulation by osteoclasts depends upon their differentiation phase.

On discordantly, remodeling of bone period simulation at distinct spots implemented stable states of many Basic Multicellular Units (BMU) over a finite bone volume and they were investigated by Lamaire's model. [18] as monitored states in the system of biology normally resembled with the system state which is stable. Further it also differentiates among the different osteoblastic lineage stage and osteoclastic leanage stage. Uncommitted progenitors (UP), osteoblasts-precursors (OBP), active-osteoblasts (AB) and osteocytes (O), bone lining-cells (BLC) and apoptotic osteoblasts (APB) are the 4 stages of the osteoblastic lineage. Osteoclast precursors (OBP), active osteoclasts (AOC) and apoptotic -osteoclasts (APC) are the 3 stages of the oesteoblast lineage which examined.

The schematic structure of Lmaire's model is shown Model considered 3 ordinary differential equations (ODE) and explains the change in the concentrations of the osteoblast precursors (OBP), osteoclasts (OC) and osteoblasts (OB). It stimulated the stiff bounding among osteoblasts (OB) and osteoclasts (OC), the catabolic consequences generated due to continuous control of parathyroid hormones (PTH) and the RANKL catabolic action. Additionally, Lemaire's model also simulated many diseases related to skeleton by imbeding dysfunctional contact in the coupling system, analyzed many diseases assumption and found power full remedial

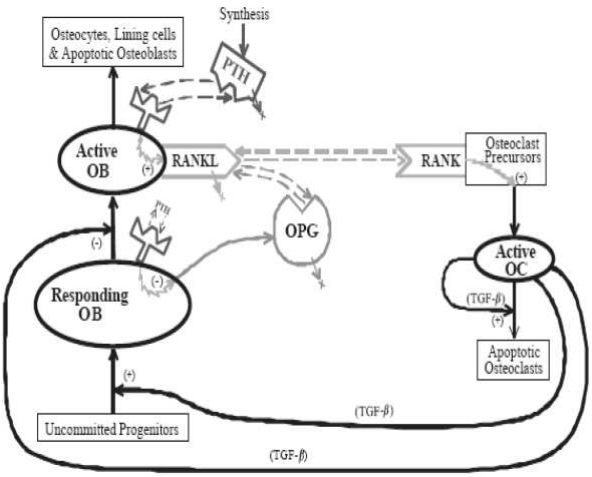

Figure 9: Schematic Diagram of Lemaire's model 1.

medication.

Lemaire work not only turned the researcher attentions from remodeling of bone period to the structural average of many Basic Multicellular Units (BMUs)but also assimilated more factors of biology into the modeling of mathematics. Despite in [20] another model showed that osteoprotegerin was released by the osteoblastic-precursors (OBP), while RANKL (L) was released by the matured osteoblasts (OB).

2.1.5 Pivonka's Model

OPG and RANKL are mainly secreted by matured osteoblasts and in osteoblastic precursors cells (OBPc), correspondingly [27]. Nonetheless, usefulness of this very important ligand-decoy-receptor(LDR) statement wasn't described earlier [27]. The understanding of the usefulness of significance of the very important RANKL-OPG statement on the bone volume is very important. Lemaire et al. (2004) work was further developed by Pivonka et al. (2008) and suggested a more comprehensive and extended dynamical model of the bone cell, which explains the functional behavior of Basic Multicellular Units.

Pivonka's model contains four components (ODE). These equations explain the

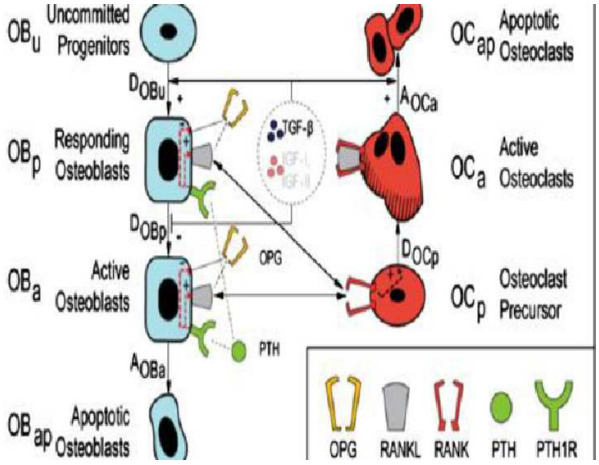

Figure 10: Basic Structure of Pivonka's Model 1.

temporal changes in osteoblasts precursor's concentrations, osteoclast's and osteoblast's concentrations along with the volume of bone. The Pivonka's model integrated many useful changes to the Lemaire model:

(1) addition of the differential equation which explains the temporal variation in the volume of bone.

(2) addition of differential equation which explains the significance of the Transformation Growth Factor Beta released from the bone matrix at the time of the resorption of bone.

(3) RANKL and OPG both were found as repressor or activator. Biological phenomena of [27] is illustrated in the schematic diagram.

Volume of bone was chosen as a criteria to analyze the practical utilization of ligand statement on special types of cell. Simulation outcomes show that there is a larger variation in the volume of bone that is produced by the BMUs which is a consequence of the variations in the differentiations rates. The authors also pointed out the combinations of the parameters of small numbers that corresponds to physiological responses, which gives us partial detail for the physiological action of Transformation Growth Factor Beta on bone.

By constructing another mathematical model Pivonka further developed their work

to explore the reaction of the RANK-RANKL-OPG signaling pathway on the remodeling of bone mechanism. The outcomes of the simulation showed that the diseases of the bones are the results from the irregularity in the RANK-RANKL-OPG signaling pathway. This agrees with" Hofbauer's convergence hypothesis", which theorized that the action of catabolic bone diseases are much more effective through the system of RANK-RANKL-OPG. Moreover, outcome of Pivonka's model explained that the secret of the bone diseases that are catabolic is proportional positively to the numbers of the components that are affected of this signaling pathway. Many successful cures for several different diseases explained, by using both single and dual therapies, were found out through theoretical model and optimization algorithms [28].

2.1.6 Ryser's Model

Remodeling of bone mechanism is affected by the structural arrangements of the Basic Multicellular units [31]. Ryser's et al established aspatio-physical model for investigating the structural properties of Basic Multicellular Units. Ryser's temporal model affects the dynamics cell populations of bone and OPG and RANKL at the cellular level for a trabecular Basic Multicellular units as well. In Ryser's model many hypothesis were made:

(1) cell populations were considered as a continuum to model the densities of the cells rather than the single cells.

(2)only osteoblasts osteoclasts and osteocytes were taken into account.

(3) The mechanical aspects that are responsible for the Basic Multicellular Units governing were separately modeled in the shape of distribution of RANKL in the starting field [31].

Ryser's model consists of five components (ODE) which are non-linear. Rayser's model pointed out the connection among the population dynamics and the bio-

chemical factors of the bone cells. They also checked out the biological experiments outcomes and gave new cure techniques. Ryser's model has been explained to successfully rebuild the dynamics of a Basic Multicellular Units. Natural elaborations of the conceptual models are the mathematical models and are capable to give quantitative, qualitative and dynamic explanations of the systems of the biology [26]. Mathematical models build the capability to affect the common behavior of a system, and also its inflection by dietetic and therapeutic mediation.

This whole chapter reviewed a number of the mathematical models on the modeling of the bone at the cellular level. These models give the general review on the development of the bone remodeling. The schematic structure given below shows the connection among the different mathematical models on the bone remodeling. Schematic diagram shows four subgroups. The first two that are the models of [19] , concentrate on describing the paradoxical influence parathyroid hormone on the remodeling of the bone. Third subgroup model developed from Komarova's model [18] model, provokes the high bounding between the osteoclastic and osteoblastic cell, and imitates the changes in the population of cells and mass of the bone during the period of remodeling of bone. The last subgroup containing 4 models developed from the Lemaire et al. (2004) model, which includes RANK-RANKL-OPG signaling pathway when reconstructing remodeling of bones periods. Previously proposed mathematical models were reviewed by the Zumsande's and construct a larger class of models by using the ideas of generalized modeling techniques.

All computational models explained in this chapter are comprised of the differential equations sets, which explain the rate of variations of different types of cells that participates in the remodeling of bone period. For the simulation of the complex bone remodeling mechanism through a differential equation set, simplifications are necessarily needed for the models construction. That's why, all the models that are discussed in this chapter have limitations of their own. Although,

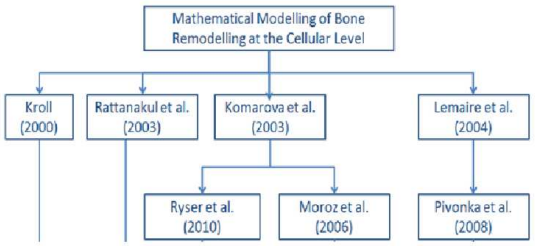

Figure 11: Mathematical Development Models of Bone Turnover at Cellular Level 1.

appreciatable outcomes achieved from these results have explained the tremendous potential of the mathematical modeling in progressing the understanding of remodeling of bone, altogether with the discrete experimental outcomes, and explaining and recommended expected therapies.

In this chapter the differential equations that explain the models were established based on parameteric values model and biological awareness related to signaling pathways and biochemical aspects. Although, above models of mathematics have still not rebuilt the remodeling of bone mechanism quantitatively. They are qualitative only and as an outcome, developing a quantitative comparison among the simulations of these models and of the experimental observations is impossible.

Chapter under consideration has shown a great need for the quantitative model development of the bone remodeling mechanism, and consecutive approval via contrast among theoretical indicators and the experimental observations. This yields practical method to find out the remodeling of bone mechanism and also the beneficial mediations.

Chapter 3

3 Our Results

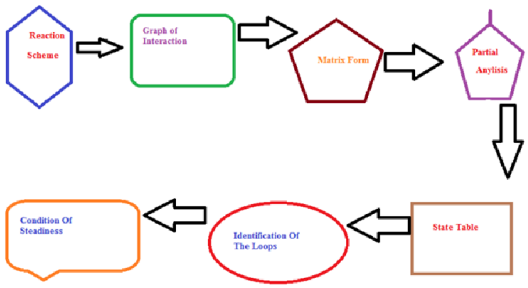

Figure 12: Methodology Flow Chart 1.

By applying above discussed methodology we solved different problems by considering different genes of bone regulatory networks. The number of results that we deduced are discussed as under

3.1 Analysis of Feedback Loops Involved in Bone Regulatory Networks

Flow Chart of the Steps involved Given above flow chart representing all the steps that are involved in our methodology. OPG combines with the RANKL-OPG which enhances the rate of RANKL-OPG. RANKL when combines with the RANKL-OPG it also increases the rate of RANKL-OPG. RANKL-OPG also having positive impact on OPG and RANKL both. This clear summary is shown through reaction mechanism. The interacting graph and that of interacting matrix

Reaction Scheme

Reaction Scheme Representations $E = OPG, F = RANKL - OPG, G = RANKL$

Two thresholds' are associated with this model. The signs positive and negative corresponding to the sign of interactions.

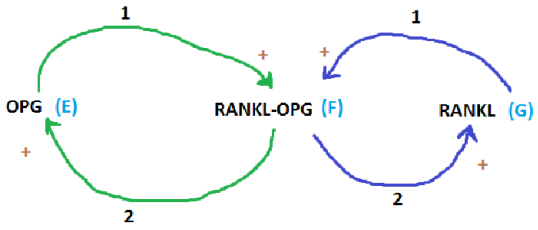

Figure 13: reaction Mechanism 1.

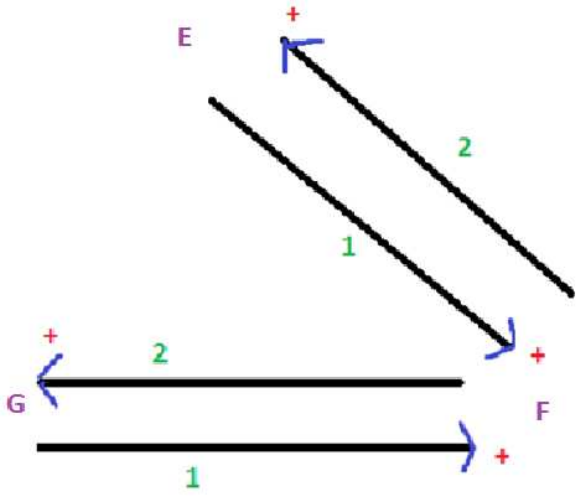

Figure 14: Graph of interaction

Graph of Interaction

Graph of interaction represents the total numbers of interactions takes place between OPG RANKL-OPG and RANKL.

Logical Equations

$$OPG = E = d_e(h_1 + h_{1.1}e^0 + h_{1.2}f^1 + h_{1.3}g^0)$$

$$RANKL - OPG = F = d_f(h_2 + h_{2.1}e^2 + h_{2.2}f^0 + h_{2.3}g^2)$$

$$RANKL = G = d_g(h_3 + h_{3.1}e^0 + h_{3.2}f^1 + h_{3.3}g^0)$$

Matrix Form

$$M = \begin{bmatrix} 0 & 2 & 0 \\ 1 & 0 & 1 \\ 0 & 2 & 0 \end{bmatrix}$$

There are 3 logical levels (0 1 2). First line of the above matrix representing that E (OPG) is under positive control of F product. First threshhold of it is acting above. F (RANKL-OPG) is under positive control of E product (OPG) and under positive control of product F(RANL-OPG) represented by the second line. Acting above is its second threshold. Third line shows that G (RANKL) is under positive control of F product acting above is its third threshold.

Partial Analysis of The System

E	F	G
0	2	0
1	0	1
0	2	0

Boolean Equations

$E = e^0 + f^2 + g^0$, $F = e^1 + f^0 + g^1$, $G = e^0 + f^2 + g^0$

Loops

Two feedback loops are shown in the above figure(13)

$e^1 f^2, f^2 g^1$.

Identification of Loops and Characteristic States

There are two feedback loops:

A positive loop that exists between E and F symbolized by e^1f^2(Characteristic state $s^1s^2g^1$)

Symbolization of the +ve loop between F and g is f^2g^1(Characteristic state$e^1s^2s^1$)

Loop recommends system might show multi stationary under suitable circumstances. It means for suitable parametric value there could be different states.

State Table

H_i= absence of its activators (all)

OR H_i= presence of its inhibitors (all)

$H_{i.j}$= presence of j if j positively activates i OR $H_{i.j}$= absence of j if j inhibits i

$H_{i.jk}$= positive contribution of both j and k on i [37]

State Table					
e	f	g	E	F	G
0	0	0	H_1	H_2	H_3
0	0	1	H_1	$H_{2.3}$	H_3
0	1	0	$H_{1.2}$	H_2	$H_{3.2}$
0	1	1	$H_{1.2}$	$H_{2.3}$	$H_{3.2}$
0	2	0	$H_{1.2}$	H_2	$H_{3.2}$
0	2	1	$H_{1.2}$	$H_{2.3}$	$H_{3.2}$
1	0	0	H_1	$H_{2.1}$	H_3
1	0	1	H_1	$H_{2.13}$	H_3
1	1	0	$H_{1.2}$	$H_{2.1}$	$H_{3.2}$
1	1	1	$H_{1.2}$	$H_{2.13}$	$H_{3.2}$
1	2	0	$H_{1.2}$	$H_{2.1}$	$H_{3.2}$
1	2	1	$H_{1.2}$	$H_{2.13}$	$H_{3.2}$
2	0	0	H_1	$H_{2.1}$	H_3
2	0	1	H_1	$H_{2.13}$	H_3
2	1	0	$H_{1.2}$	$H_{2.1}$	$H_{3.2}$
2	1	1	$H_{1.2}$	$H_{2.13}$	$H_{3.2}$
2	2	0	$H_{1.2}$	$H_{2.1}$	$H_{3.2}$
2	2	1	$H_{1.2}$	$H_{2.13}$	$H_{3.2}$

State Table					
e	f	g	E	F	G
0	2	0	$H_{1.2}$	H_2	$H_{3.2}$
0	1	1	$H_{1.2}$	$H_{2.3}$	$H_{3.2}$

State Table					
e	f	g	E	F	G
1	0	1	H_1	$H_{2.13}$	H_3
1	0	0	H_1	$H_{2.1}$	H_3

so, the corresponding values are $H_1 = 0$,$H_2 = 0, 1$,$H_3 = 1$,$H_{2.3} = 2$,$H_{1.2} = 1$,$H_{3.2} = 0$,$H_{2.1} = 2$,$H_{2.13} = 0, 1$,

State Table					
e	f	g	E	F	G
0	0	0	0	0,1	1
0	0	1	0	2	1
0	1	0	1	0,1	0
0	1	1	1	2	0
0	2	0	1	0,1	0
0	2	1	1	2	0
1	0	0	0	2	1
1	0	1	0	0,1	1
1	1	0	1	2	0
1	1	1	1	0,1	0
1	2	0	1	2	0
1	2	1	1	0,1	0
2	0	0	0	2	1
2	0	1	0	0,1	1
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2	1	1	1	0,1	0
2	2	0	1	2	0
2	2	1	1	0,1	0

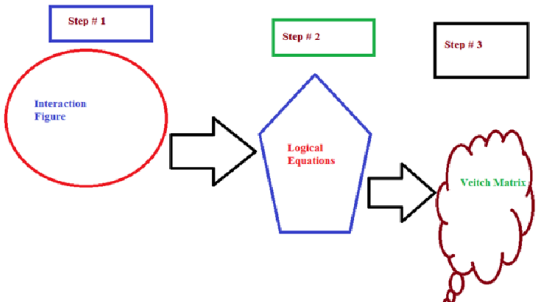

Figure 15: Boolean Steps.

its very much clear from the above table that [1 2 0] is the steady state and system will remains stuck in this state.

3.2 Boolean Formalization of Genetic Control Circuits

Three Steps to solve the Problem in considered sequential problem the values " 0" or "1" of the output functions, not only on depended upon the current values of input variables but, its also dependent upon the previous input system states. People are interested in assuming logical machines which shows this feedback of the last state by introducing "internal variables" used for memorization. Every additional variable (x_1,x_2,...) is a linked with a function (X_1,X_2,...) in a manner that internal variable having the value same as of the corresponding function in stable states, but at the point where there is the change in the functional value, for some period of time variable keeps the last value before switching to new value; thus the internal variable acts as a memory of the last state of system.

By introducing the appropriate internal functions and also the variables transforms the study and composition of sequential structures into the combinatory ones.

At the time of this work it was realized that there found similarity in internal variables pairs and functions in the problems of genetics; exemplary, as shown under, RANKL(L) expression and the presence of the RANK(K) this codes for, same correspondence of the relation is found in function X and memory variable

associated with it that is x.

Let us move towards the main problem RANKL (gene) and the expression of RANKL is blocked by reactions outcomes and it is catalyzed through RANK (enzyme) for which it codes as shown in the figure of interaction. Model formalization becomes easy if one clearly distinguished l the gene expression L and k the enzyme presence coded for RANKL gene "l" means gene L is active while "$\underline{l}$" means that it is not operating "k" means that the presence of RANK (enzyme) coded for by the RANKL (gene) "$\underline{k}$" means absent. The word absent means here that concentration does not meets the threshold above which the product works. In the same manners one must differentiate "o" which gives information whether the reaction B→O is occurring from O which give information even if the presence of the substance O at such an concentration that it accurately block gene expression L.

Figure of Interaction

FIG RANKL (gene) codes for the RANK (enzyme), RANKL catalyses reaction B→O. when presence of a substance above some threshold, blocks RANKL (gene) expression (so a may acts as a co repressor for some time).

When its steady state, l and k(or a and o) having matching values. For the some time when the RANKL (gene) is active, presence of the RANK (enzyme) for which it codes for($l = 1$,$k = 1$) at the time when the RANKL(gene) is not active (off) for the longer period, not any RANK(enzyme) present ($l = 0$,$k = 0$). Values of s and k change at the same time but with the delay of some time. More clearly, when the values of both l and k are 0 and l active (switch on), it will take some time before the 1st molecule of the RANK (enzyme) synthesized an h k reach at the proper concentration. And there occurs thus a short living unstable period at

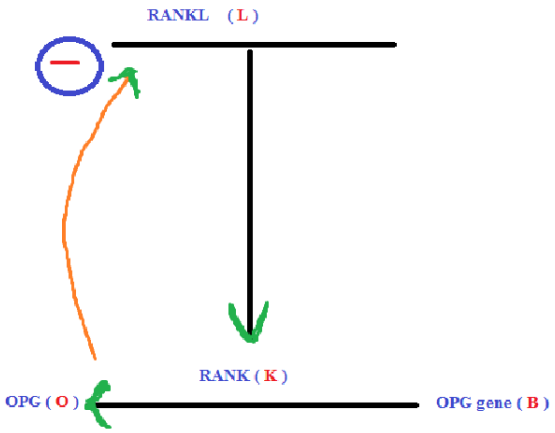

Figure 16: Boolean Figure of Interaction.

which l is already at 1 but is still at 0 , at that time then k also active(switch on). Alike, when l inactive (switch off) , it will take some time before the k which was present is reduced by the system growth, or consumed, we are dealing here with unstable state in this l is already at 0 but k is 1 transiently.

Noticeably the relationship among l and k (or among a and o) is necessarily same as among mentioned before internal function X and the variable x, thus l acts as an internal function and k acts as associated internal variable , value of whom acts as an memory of preceding l value and is used in feedback.

Formalization of Logical Equations

Now further logical equation formulation should obvious. Model illustrated in the fig conditions for the statement of function L are that the presence of the normal gene allele, and o be absent.

$l = L.\underline{O}$

The reaction conditions for B→O occurs at significant rate are the existing of B substance and enzyme both.

$a = B.k$

Veitch matrix of l function and a which is given by logical equations of these.

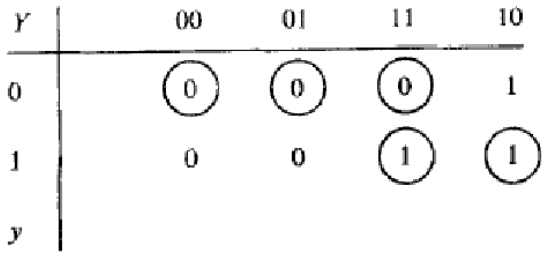

Figure 17: Boolean Figure of Interaction.

$Y = 0$ and $Y = 1$

veitch matrix				
Y	00	01	11	10
0	0	0	0	1
1	0	0	1	1
y				

Veitch matrix of the function $l = L.\underline{o}$ and $a = B.k$ which illustrate the model of the above figure.

Four columns in the veitch matrix corresponds to four values that are possible inputs variables, L and B, and also the four lines, to the four possible values of the variables that are internal variables, k and o. In every box of matrix the two digits corresponds to the l and a values, respectively, for the variables corresponding states. $l = 1$ in each of the 4 states for which $L = 1$ and $o = 0$ this is the state which is present at the intersection of the column 11 or column 10 and of the lines 00 or the line 10, and for all the other states $l = 0.a = 1$ in each of the 4 state for which $B = 1$ and $k = 1$ that is the intersection state of the columns 01 or 11 and of the lines 11 or 01, and in all other states $a = 0$

Stable states are the those states in which both l and a have the same values as those values they already had in preceding time period, that is the state for which

both l and a processes the same values as their memories, k and o respectively. On the veitch matrix these states are mentioned. For example in 10 column it can be seen that (K gene present, and the substance B is absent), there is present the stable state 10 which is circled shown in veitch matrix for $k = 1$ and $o = 0$ in this state $l = 1$ and $a = 0$. Clearly speaking, there is not B substance and resultantly the reaction B→O not be able to occur ($a = 0$), that is why there is no o substance and since, additionally, K gene is normal l function can be shown ($l = 1$). In the column 00 and 01 we found another stable state (not any normal allele of L gene is present, B absent or present). This 00 state is encircled in the veitch matrix, correctly speaking, there is not any gene, therefore L function cannot be expressed($l = 0$), thus there is no any k and resultantly the reaction B→O does not occur ($a = 0$).

Interesting circumstances are shown in column 11 (presence of L and B). There is not any stable state in this situation. Stating from the stable state mentioned in 10 column. By the addition of the substance B we can switch into 11 column, onward, system entangled in 11 column till the time when $B = 1$ is maintained. Following formally the predictions of the model on the matrix is very easy. The system states symbolized in this problem by k and o, followed after an underscore by those of l and a. By the addition of B this shifting occurs and it moves us toward the state which is unstable that is 10/11 in which the values of o and a are different (respectively, 0 and 1). Now o will shift to a value. And the system will move from the line 10 to the line 11. Again this is an unstable state that is 11/01 since the values of o and l are different (respectively 1 and 0). Since o must have to maintain the value of l, one shifts to 01 line. This leads us towards the state which is unstable 01/00and in this both o and a processes different values again, this times it's a which is switched off already on the other hand o is still active (on). Shifting to 00 line (here o is off), state 00/10 again l and o having different

values ($l = 0$ and $o = 1$)and system will again shifting to the 10 line that is the 10/11 state. It can be noticed that system moves periodically, l and a changing from 11→01→ 00→10→11... etc.

Clearly, in stable state of the 10 column, occurrence of o and the reason behind that a is not operating is the absence of B. As soon as the addition of B takes place, reaction a activated (switched on). Basically, o is still 0 (10 line) but later on it activated (switches on) and one moves to 11 line. As $o = 1$, RANKL (gene) quickly becomes inactive (switched off) still, RANK (enzyme) exists for short time period, for the continuity of a reaction thus the value of the function will 01. Finally, when o inactivates (switches off) RANK will deprave. And one will move to 01 line as, $l = 0$ the reaction B→O quickly inactive (switched off) thus the values 00 of functions, yet there is the presence of the o substance temporarily. After its decaying one switches to 00 line, as $o = 0$, so RANKL activates (switches on)($l = 1$) but, the value of will temporarily zero, that is why B→O reaction does not occurs (value of "a" is zero). As k activates one moves to 10 line, as $k = 1$ ($B = 1$), B→O reaction is taking place (value of "a" is one). O remains very much low for some period, so that RANKL operates continuously. At the time when o hits its proper concentration, one again shifts to 11 line, and it continue in the same manners. The system sustains the periodic variations indicated by the arrows in the veitch matrix.

Every time internal function values vary, corresponding internal variable value changes after some time (that is after delay). These delays may be different and they are very much different from each other sometimes. 'On' delay expected to be short in our case and the 'off' state period will be longer.

In the problem that is just mentioned above, although there is't any difficulty related to the order of the cases in the period, the reason is that internal variable values alters one at a time, every one leads to the further alteration. More critical

situation founds at the time when values are changing in more than 1 of the pairs of the functions and of the internal variables. For example, the state of the interaction of 10 column and 11 line, l and a both are off, on the other hand k and o are present still. Accordingly one should move from 11 line to 00 line, in this $k = 0$ and $o = 0$ same as l and a having in their preceding state. Although, it means that there is a simultaneous change in the values of the two internal variables. Practically, the value of one will alter first, and it will lead towards two different pathways.

11/00→01/00→00/10→10/10 OR 11/00→10/10

In logical circuits that are classical, pathways following depends upon the sophisticated and of the differences that are not predictable in characteristics of the memory organs, these circumstances are avoided. In our problem these situations are interesting and unavoidable both, the output rely only on the switching on (or switching off) timings of a gene and hits the gene concentration which is just above (or below) needed for the efficiency.

3.3 Model with Two Genes Cells

Model consists of the two most important cells that play vital role in bone remodeling called osteoclasts(OC) and osteoblasts(OB). The graph of interaction is shown in the Fig (18). OC prevents the differentiation of OB at the sufficient concentrations (above threshold one). OB represses its own differentiation (above threshold one). OC having positive effect on its own regulation (threshold 2). OB having positive effect on OC (above threshold 1). While OC having negative effect on the concentration of OB (above threshold 1). These thresholds have been decided by considering several growth factors involved in the coupling of osteoclasts and osteoblasts, the most important factor influencing this coupling is transforming

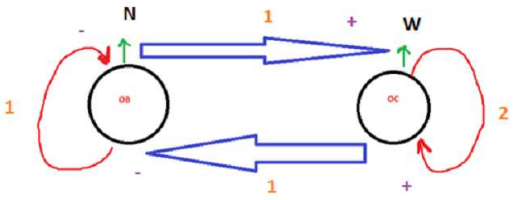

Figure 18: Boolean Figure of Interaction.

growth factor beta (TGF-β).

Graph of Interaction

Fig (18) represents graph of interaction of two genes model. Digits represents the threshold magnitude.

Representations: OB = Osteoblast = N, OC = Osteoclast = W

Logical Equations

$OB = N = d_n(k_1 + k_{1.1}n^{-1} + k_{1.2}w^1)$, $OC = W = d_w(k_2 + k_{2.1}n^{-1} + k_{2.2}w^2)$

Matrix Form

Matrix form is the compact way to represent the same interactions.

$$M = \begin{bmatrix} -1 & -1 \\ 1 & 2 \end{bmatrix}$$

In the matrix above 1st line shows that N (OB) is under the negative control of product n and under –ve control of product w, acting above is its 1st threshold. 2nd row shows that W (OC) is under the positive control of the n product and it

is under positive control of its own w product, acting above is its 2nd threshold.

Boolean Equations

$N = n^{-1} + w^{-1}$, $W = n^1 + w^2$

Loops

n^{-1},w^2,n^1w^{-1}

State Table

The state table can be completed in a way as under:

(1) If either or both conditions n^{-1},w^{-1},is attained then we write $N = 1$ otherwise $N = 0$.

(2) If either or both conditions n^1,w^2,is attained then we write $W = 1$ otherwise $W = 0$.

Withal we want more explanation and refinement and introduce the weighable parameter that are logical parameters these parameters give weight to the term logical expression. We associate

(1) With the term n^{-1}, parameter K_1,

(2) With the term w^{-1}, parameter K_2,

(3) With the term n^1, parameter K_3,

(4) With the term w^2, parameter K_4,

(5) With the logical sum of $n^1 + w^2$, parameter K_34.

Now the state table can be filled with the images values of N and W

(1) If the condition n^{-1} is attained that is $(n = 0)$ we write $W = K_1$ otherwise $N = 1$ or $N = 2$ we write $W = 0$.

(2) If the condition w^{-1} is attained that is $(w = 0)$ we write $N = K_2$ otherwise $W = 1$ or $W = 2$ we write $N = 0$.

(3) If the condition n^1 is attained that is $(n = 1)$ we write $W = K_3$.

(4) If the condition w^2 is attained that is $(w = 2)$ we write $W = K_4$.

(5) If condition n^1 and $w = 2$ both attained that is $(n = 1$ and $w = 2)$ we write $W = K_34$.

(6) If neither n^1 nor $W = 2$ is attained that is $(n = 0$ and $w = 0$ or $w = 1)$ we write $W = 0$.

Additionally, parameters and that of logical function can take the same values as that of the corresponding multiple level variables.

Table

n	w	N	W
0	0	K_2	$K_1, 0$
0	1	0	$K_1, 0$
0	2	0	K_1, K_4
1	0	K_2	$0, K_3$
1	1	0	$0,\ K_3$
1	2	0	$0,\ K_3, K_4, K_{34}$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$:

Possible Steady States

Possible steady states are as following (1) 02 will be the steady state if and only if $K_1 = 2$

(2) 01 will be the steady state if and only if $K_1 = 1$

(3) 10 will be the steady state if and only if $K_2 = 1$

(4) 00 will be the steady state if and only if $K_1 = 0$ and $K_2 = 0$

Cells and State Tables

State table when $K_1 = 1$,$K_2 = 1$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow$ 02/01	$\leftarrow$ 12/00$\downarrow$
01/01	$\leftarrow$ 11/00$\downarrow$
$\uparrow$00/11	10/10

State table when $K_1 = 2$,$K_2 = 1$ State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow$02/02	$\leftarrow$ 12/00$\downarrow$
$\downarrow$01/02	$\leftarrow$ 11/00$\downarrow$
00/12	l0/10

State table when $K_1 = 0$,$K_2 = 0$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow$ 02/00	$\leftarrow$ l2/00
$\downarrow$01/00	$\leftarrow$ l0/00
00/00	$\leftarrow$ l1/00

State table when $K_1 = 0$,$K_2 = 1$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow$02/00	$\leftarrow$ 12/00$\downarrow$
$\downarrow$01/00	$\leftarrow$ 11/00$\downarrow$
00/10	$\leftarrow$ l0/10

State table when $K_2 = 0$,$K_1 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow 02/02$	$\leftarrow 12/00\downarrow$
$\downarrow 01/02$	$\leftarrow 11/00\downarrow$
$00/12$	$10/00$

State table when $K_2 = 0$,$K_1 = 1$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$\downarrow 02/01$	$\leftarrow 12/00\downarrow$
$\downarrow 01/01$	$\leftarrow 11/00\downarrow$
$00/01$	$\leftarrow 10/00$

And State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

$02/0K_4$	$12/0K_3$
$01/00$	$11/0K_3$
$00/K_20$	$10/0K_2\ K_3$

Possible steady states are as following (1) 02 will be the steady state if and only if $K_4 = 2$

(2) 00 will be the steady state if and only if $K_2 = 0$

(3) 12 will be the steady state if and only if $K_3 = 2$

(4) 11 will be the steady state if and only if $K_3 = 1$

(5) 10 will be the steady state if and only if $K_2 = 1$ and $K_3 = 0$

State table when $K_2 = 0$,$K_3 = 2$,$K_4 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

02/02	$\leftarrow$ 12/02$\downarrow$
$\downarrow$01/00	$\leftarrow$ 11/02$\downarrow$
00/00	$\leftarrow$ 10/02

State table when $K_2 = 0$,$K_3 = 1$,$K_4 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

02/02	$\leftarrow$ l2/01$\downarrow$
$\downarrow$ 01/00	$\leftarrow$ l1/01$\downarrow$
00/00	$\leftarrow$ l0/01

State table when $K_2 = 1$,$K_3 = 0$,$K_4 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

02/02	$\leftarrow$ l2/00$\downarrow$
$\downarrow$01/00	$\leftarrow$ l1/00$\downarrow$
00/10	l0/10

State table when $K_2 = 1$,$K_3 = 1$,$K_4 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

02/02	$\leftarrow$ l2/01$\downarrow$
$\downarrow$01/00	$\leftarrow$ l1/01$\downarrow$
00/10	$\leftarrow$ l0/11

State table when $K_2 = 1$,$K_3 = 2$,$K_4 = 2$

State table for $N = 0$ and $N = 1$ AND for $W = 0$ for $W = 1$ for $W = 2$

02/02	$\leftarrow$ l2/02
$\downarrow$01/00	$\leftarrow$ l1/02$\uparrow$
00/10$\rightarrow$	l0/12$\uparrow$

According to the table given in appendix, there exists a cycle here.

Appendix

Chapter 4

4 Discussion

4.1 Discussion

Mathematical modeling and mechanistic insight of bone remodeling is really crucial since bone plays a very important role in human life, and it has several functions for instance protection, storage (calcium, phosphate) and most importantly the blood fabrication. The process of deposition of old bones and the formation of new bones is known as bone remodeling. A number of cellular and molecular components take part in the process of such practice. In bone remodeling mainly two cellular factors called osteoclasts (bone removing cells) and osteoblasts (bone building cells) and a signaling pathway RANK/RANKL/OPG are involved. Osteoblasts and osteoclasts work together in a network named as basic multicellular unit. Several attempts have been made to study the dynamics of this complex cellular and molecular framework. TGF-β also plays a very important role in the coupling of osteoclasts and osteoblasts. This growth factor plays an important role in formation of new bones so the feedbacks thresholds considered in the model of osteoclasts and osteoblasts have been decided by taking into account the effect of TGF-β in bone remodeling. Our methodology is founded on a discrete method formalized on the interaction among the components involved in bone remodeling process in terms of logical parameters, variables, and functions. The stability of the presented model has been checked through the loops (either +ve or –ve) involved in the mechanism of bone turn over. One of our models takes into account the feedback mechanism involved in coupling between osteoclasts and osteoblasts. Another discrete framework is established to discuss the RANK/RANKL/OPG signaling pathway. The results verifies the existing outcomes of biological studies available in the literature.

4.2 Open Problems

There are several genes which have not been considered yet like estrogen, calcitonin, so in future work might be conducted by considering the feedback loops involving such growth factors.

Chapter 5

5 References

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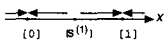
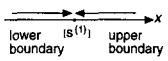
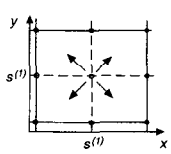
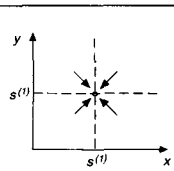
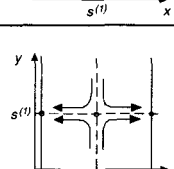
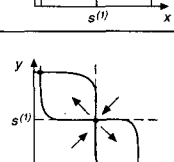
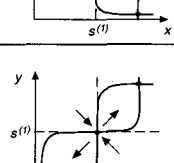
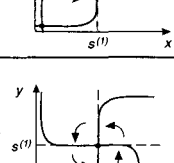
Table 1. Examples of feedback loops, with a description of their dynamical properties and characteristic states

loops and their matrix description	Typical pattern in the variable space	Logical description	Differential description	Roots of the characteristic equation	Remarks
One-element positive loop on x		Three steady states : [0] and [1] are attractive, [^{1}s] is repulsive	Three steady states: - 2 stable - 1 unstable	real: - +	
One-element negative loop on x		A single steady state : [^{1}s] is attractive	A single stable steady state: a stable node	real: -	
One-element positive loops on x and y		Nine steady states: [00], [01], [10] and [11] are attractive; [0 ^{1}s], [^{1}s0], [^{1}s1] and [1 ^{1}s] are repulsive along one direction; [^{1}s ^{1}s] is repulsive along both directions	3^2 steady states: - 4 stable nodes - 4 saddle points - 1 unstable node	real: -, - +, - +, +	[^{1}s ^{1}s] is the characteristic state of the union of the two loops
One-element negative loops on x and y		A single, stable state: [^{1}s ^{1}s] is attractive along both directions	A single stable steady state: a stable node	real: -, -	[^{1}s ^{1}s] is the characteristic state of the union of the two loops
One-element loops, a positive one on x, a negative one on y		Multistationarity on variable x, homeostasis on variable y; three steady states: [0 ^{1}s], [^{1}s ^{1}s] and [1 ^{1}s]; the loop-characteristic state [^{1}s ^{1}s] is repulsive in x, attractive in y	Three steady states: - 2 stable nodes - 1 saddle point	real: -, - +, -	[^{1}s ^{1}s] is the characteristic state of the union of the two loops
Two-element positive loop		Multistationarity on both variables; three steady states: [01] and [10] are attractive; [^{1}s ^{1}s] is repulsive in one direction, attractive in the orthogonal direction	Three steady states: - 2 stable nodes - 1 saddle point	real: -, - +, -	The elements are exclusive of each other; either x:[10], or y:[01] is present
Another two-element positive loop		Multistationarity on both variables. Three steady states: [00] and [11] are attractive; [^{1}s ^{1}s] is attractive in one direction, repulsive in the orthogonal direction	Three steady states: - 2 stable nodes - 1 saddle point	real: -, - +, -	The elements are interdependent; one has either both [11] or neither [00] element present
Two-element negative loop		A single steady state, [^{1}s ^{1}s], surrounded by a logical cycle: 	A single steady state: - a stable focus	complex, real part negative	

In the text of this table, we have used the notation ^{1}s instead of $s^{(1)}$ to indicate the order of the threshold for compactness.