BONE TISSUE RE MODELING – THE LOCAL AND SYSTEMIC CONTROL AND MATHEMATICAL MODELING

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Content

Summary

This chapter provides an insight into the different mathematical approaches employed in the field of bone remodeling. The mathematical modeling of bone tissue is an important aspect of mathematical physiology because bone remodeling is a highly organized physiological process that occurs in bone tissue and supports many other physiological processes in the body at different levels. There are many mathematical and statistical methods which can be applied to the modeling of the mechanical and biological properties of bone tissue. However, despite the considerable success in bone tissue modeling, many difficulties still remain. One critical factor is that bone tissue presents significant challenges with respect to experimental investigation. Several local and systemic levels of regulation control the bone remodeling processes and these in turn are influenced by environmental factors, such as mechanical stress, for example. All of these "loops of regulation" need to be taken into consideration in the mathematical model. This chapter has been undertaken in an attempt to illustrate the systemic and local regulation in bone and compare the real biological system with the mathematical models developed. The chapter ends with the conclusion that an integrated approach which is based on systems biology, cybernetic approach is required.

1. Introduction

Bone, together with cartilage, forms the skeleton, which is the hard structural system that supports body locomotion and protects internal organs. Damage to the human skeleton can create significant problems leading to pain, reduced mobility, morbidity and may even give rise to life threatening medical conditions. Bone is a metabolically active tissue which is under a steady process of development and renewal, called remodeling. Bone comprises of two types of tissue**;** cortical and trabecular bone. Cortical bone is very compact and provides 75% of the weight of the human skeleton,

whereas trabecular bone has an open porous structure of lattice-shaped spicules. Trabecular bone tissue provides three quarters of the bone surface that is involved in bone remodeling. It is highly metabolically active and therefore sensitive to metabolic disorders.

Bone consists of both organic and inorganic material. The main organic component of bone tissue is osteoid which contains collagen and non-collagen proteins. Collagen is the most significant part of structural osteoid and comprises about 95% of its volume. The inorganic part of bone tissue contains hydroxyapatite (HA), a microcrystalline mineral which is initially deposited in layers as calcium phosphate and is later transformed into apatite crystals. Many other ions and minerals (carbonates, magnesium, potassium, calcium, fluorine etc) are present in the inorganic part of bone tissue and have an important metabolic role and influence the mechanical properties of the bone. The mineral components of bone are stored in both the cortical and trabecular bone.

2. Bone Turnover

2.1. Bone Tissue Remodeling

Bone is a tissue with a unique mechanism of regeneration (commonly referred to as remodeling or turnover. It is one of the simplest examples of tissue regenerating processes in animals and so the study of it could help to form a foundation for understanding more sophisticated metabolic and cellular cycles in the body.

2.2. The Basic Multicellular Unit (BMU)

The BMU can be regarded as a team of cells which are active in bone tissue remodeling. The BMU is the active element of the bone tissue which over a period of time results in the local resorption and rebuilding of the bone tissue, which referred to as remodeling.

The generally accepted concept of the Basic Multicellular Unit (BMU) is that it is comprised of two cell types; osteoclasts and osteoblasts. In addition to these “active” cells BMU also contains the active mesenchymal cells and capillary loops. The size of a BMU is 0.05-0.1 mm^3 and an adult organism, under normal physiological conditions, 10^5-10^6 BMU function simultaneously.

2.3. Osteoclasts

Osteoclasts are multinucleous large bone tissue cells that are specialized microphages. Their main function is tissue resorption. Osteoclasts are only present in a small lacunes (cavities) on the surface of bone during the bone demineralization/resorption phase. Osteoclasts are formed by the fusion of several mononucleosis precursors of the monocytes family which originate from stem cells in the bone marrow. Osteoclasts work together with osteoblasts to resorb and remodel the bone in a controlled process of reconstruction. Bone remodeling is finely balanced process controlling skeletal growth and development to yield bone with the appropriate properties of stiffness and elasticity.

For a variety of reasons it is possible for resorption of the bone to become predominant and this can lead to many types of bone disorders.

The multistage process of osteoclast differentiation and maturation is triggered by integrin and is then controlled by many chemical signals, including receptor activator for nuclear factor κ B ligand (RANKL), and Osteoprotegerin (OPG). Stem cells and osteoblasts secrete RANKL and M-CSF. This process can be activated by Parathyroid hormone (PTH) and inhibited by OPG. RANKL and M-CSF interacts with the correspondent receptor on the membrane of precursor cells (a common precursor for both osteoclasts and monocytes-monofags) and triggers cell differentiation in favor of osteoclasts. This process can be inhibited by OPG.

Differentiated osteoclasts accumulate on the bone surface to form a cytoskeleton which enables a resorption cavity (microspace between the osteoclasts and bone tissue) to develop. This process involves a protein integrin avb3. The osteoclast layer within the cavity forms folds and as a result the resorption surface is significantly enlarged. The media inside of the resorption cavity is acidic due to the addition of protons. The intercellular pH of osteoclasts is sustained by HCO_3^-/Cl^- interchange across the antiresorptive membrane. The HCO_3^- ions move from the cell in extracellular space and ions Cl^- comes from extracellular space into the osteoclast cytoplasm. Cl- ions are secreted by means of ionic canals within the folded osteoclast membrane. Then pH of the resorptive cavity drops to about 4.5. This acidic environment creates conditions for resorption of the mineral part of the bone. Degradation of the organic component of bone occurs due to the presence of cathepsin A, an enzyme that is synthesizing and secreting in the resorption cavity by osteoclasts.

2.4. Osteoblasts

Osteoblasts are mononuclear cells which play a principal role in the process in bone remodeling by the formation of new bone following tissue resorption by osteoclast activity. Osteoblasts are located on internal and external bone surfaces in close proximity to intensive bone formation (2-8% of the total bone surface). Together with osteoclasts, they form the so-called Basic Multicellular Units (BMU). Active osteoblasts are cubic or cylindrical shaped cells having minute processes (typically 5nm wide). The main function of osteoblasts is the creation of the organic extracellular bone matrix, synthesis of extracellular bone material and participation in osteoid creation and its subsequent mineralization.

Osteoblasts originate from mesenchymal cells. The following stages of osteoblasts development are suggested: proto-osteoblast proliferation, maturation and differentiation. Osteoprogenitors (flat, plane cells) secrete growth transforming factors (transforming growth factor- β, TGF- β, TGF-beta). These factors trigger osteoblasts proliferation. Proliferation is accelerated by the osteoblastic protein osteopontin. Proliferating osteoblasts synthesize the main protein component of the extracellular matrix (collagen type I) and also proteins which stimulate cell proliferation (histons, c-fos-protooncogen, c-myc-protooncogen). Maturating osteoblasts adopt a cubic shape and secrete alkaline phosphotase, a protein that participates in osteoid mineralization. At the mineralization phase osteoblasts produce osteocalcin which is the second main

protein of bone tissue. The majority (~3 quarters) of osteoblasts die by programmed (preordained) apoptosis. The remaining cells transform into two other types of cells in bone tissue; lining cells which form the mono-layer of cells that line the external and internal bone surfaces and osteocytes which are the bone cells that form the 3D lattice in the bone tissue. Lining cells are also called as mesenchymal bone cells or osteogenic cells.

Differentiation and osteoblasts activity is controlled by a number of hormonal and chemical signals of an autocrine (endogenous) and paracrine (exogenous) nature. In order to recognize and respond to these signals osteoblasts have the number of receptors on their membrane. The binding of these signal ligands to the receptors activate the transmission of the signals which finally reach the cell nuclei. The osteoblasts nuclei consequently develop further regulatory signals for controlling the metabolic processes. Such hormonal signal messengers are, for example, estrogens and parathyroid hormone. Others local factors (for example growth factors, necrosis factors etc.) also activity participate in the regulation of osteoblasts activity. By coordination with the other cells, through many levels of regulation, osteoblasts provide the basic function of bone remodeling.

2.5. Osteocytes

The primary function of osteocytes is to provide a signal when bone becomes damaged, through for example injury. The branch like structure of osteocytes creates a functional syncytium (network) inside the bone tissue. Depending on the condition of the bone tissue locally (i.e. at rest or remodeling) the syncytium also includes a large number of lining cells. The precise mechanism of osteocyte operation is a subject of intense debate but it is clear that they provide a signal for osteoclast activity to resorb of the damaged areas of bone, followed by subsequent tissue rebuilding by osteoblasts to generate healthy remodeled bone.

2.6. Lining Cells

Lining cells are cells that transform into a layer of cells on the surface of bone. A lining cell is an inactive post-proliferated cell that covers the surfaces of the tissue which are not undergoing resorption or formation. Lining cells are results of the transformation of osteoblasts to form “flat” cells that cover about 70-80% of total bone surface in adult skeleton. These cells form a “hematocellular” barrier on the bone. Observations indicate that these cells can be reactivated into active osteoblastic cells when required, through signaling from the osteocytes. It is suggested that these cells participate in bone remodeling by synthesis and emission of cytokines and other intermediates which perform the signaling control and activate osteoclasts. Lining cells cooperate with osteocytes, which the syncytium produce, and perform control signals which are proportional to the mechanical loading.

2.7. Bone Remodeling Regulation from a Phenomenological Cellular Perspective

Regulation of bone tissue remodeling depends on local factors, growth factors, mechanical loading, nitric oxide and intercellular communication. BMU function is

remodeling of bone tissue by highly coordinated activity of osteoclasts and osteoblasts. This cellular construction activity takes place over a relatively long time period. The entire cycle of resorption and bone rebuilding may take several months to complete.

2.7.1. Osteoclasts

The large multi-nucleus cells, osteoclasts, are characterized by high levels of activity of tartrate-resistant alkaline phosphotase. In mature bone tissue about 0.1-1% of the surface area is populated by lacunes. 90-95% of these lacunes contain osteoclasts, engaged in active resorption; the remaining lacunes are empty. The area where the osteoclasts contact the bone tissue gives two regions which are differentiated morphologically; a ruffled border and a much lighter edge. Under the action of ferments and hydrogen ions secreted by osteoclasts the bone matrix is dissolved and disintegrates.

2.7.2. Osteoblasts

Active osteoblasts form osteoid plates, along newly formed bone tissue, by forming collagen fibers and proteoglicans (major component of the animal extracellular matrix) which the osteoblasts synthesize. In the formation zone there are about 300-400 osteoblasts. In a period of 8 to 9 days they form a layer of osteoid (non-mineralized matrix) about 12μm deep. After about 12 days of osteoid maturation the mineralization phase starts. Around 10% of osteoblasts transforms into osteocytes and are integrated into the mineralized matrix. The remaining osteoblasts become inactive and are left on the surface. The active life of osteoblasts is approximately 2 to 3 weeks.

2.7.3. Osteocytes

Osteocytes are localized in lacunes in the mineralized matrix of the bone. Every cell is in contact (in communication) with neighboring cells by means of a number of processes in the bone canals (canaliculus). Osteocytes in normal conditions provide inter-tissue transport of resources, minerals and products of metabolism and participate in coordination/control of the activity of all osteocells. Cell-cell communications between all three types of osteocells (osteoclasts, osteoblasts, osteocytes) plays an important role in the control of bone turnover. It is well-known that the gap junctions, transmembrane channels are important mechanisms of this communication.

2.7.4. Apoptosis

In the last few decades several authors have highlighted the importance of the level of osteocyte regulation, for example, the role of osteocyte apoptosis as a part of the mechano-transduction control mechanism.

2.8. Histological/Cellular Scheme of Remodeling

Micro-damage or aging of bone tissue causes the release of molecular messengers and BMU is activated (damage signaling phase). Histologically, the shape of lining cells

becomes cubic-like (originally flat). There are indications that osteocytes are also involved at the start of the remodeling process.

In response to the osteocyte signals the osteoclast recruitment phase commences. The osteoblast precursors start to synthesize messengers which interacts with the surface of osteoclasts precursors. As a result these differentiate into mature multinucleous osteoclasts which develop the "ruffled border" and start to resorb bone tissue.

During the resorption phase mature osteoclasts resorb bone within the resorption cavity. At every separate bone remodeling site resorption lasts about two weeks. At the end of this process the remaining osteoclasts die by means of apoptosis.

In the osteoblast recruitment phase osteoblasts differentiate from bone marrow stromal cells.

During the osteoid formation phase active osteoblasts fill the absorbed cavity forming the non-mineralized new tissue (osteoid).

When the osteoid reaches a depth of about 6-12μm the mineralization phase commences and the osteoblasts transform into new bone tissue. The remodeling process can be summarized by the following characteristics: life span of BMU – 6 to 9 months; bone volume replaced/formed by one BMU around 0.2 to 0.3 mm^3; life span of osteoclasts is about 12 to 15 days; life span of osteoblasts is around 10 weeks; the average interval between two episodes of remodeling in the same area of bone is typically 2 to 4 years; the average rate of bone tissue remodeling is approximately 10% per year (cortical bone ~ 5% a year, trabecular bone ~ 25% a year). These characteristics are very useful when considering/developing a model of bone remodeling.

3. Regulatory Factors of Bone Remodeling

3.1. Local Growth Factors

Growth factors (GF) and differentiation factors are polypeptides that exert multiple effects on target cells, including mitosis, gene expression, cell shape, polarization and secretion. These effects consequently depend on others factors of target cells such as cell-cell interaction, cell-matrix interaction and stage of maturation (differentiation). The growth factors that affect bone and bone cells are described in great detail in the literature, for example Rosen **(**2002). Particular attention is paid to growth factors which have shown similar effects both *in vitro* and *in vivo*.

Insulin-Like Growth Factors

IGF-1 and IGF-2 are one-chain polypeptides that have 70 and 67 amino acids respectively. The homology (similarity) of these two hormones is about 62% and they have about 50% identical amino acids to insulin. However, they have different antigens and are regulated in a different way. Each of these hormones has its own specific receptors (primarily IGF-IR) and binding proteins (IGFBPs). Research indicates that

IGF-1 stimulates replication by the cells of the osteoblast lineage, and new bone formation by osteoblasts.

Transforming/Tumor Growth Factors (TGFs)

TGFs are polypeptides containing about 400 amino acids. It is important type of growth factors in bone matrix. In the cell culture the TGF-β effects are sometimes are called "multifunctional" because of the number of cellular and intercellular responses that it causes, including up-regulation of other growth factors. TGF-β is a potent growth inhibitor for a vast variety of cells. In the majority of cells types it inhibits proliferation, however, in some osteocells it up-regulates the mitosis. In many cells, including osteoblasts, TGF-β increases collagen synthesis and the development of the intracellular matrix. The effects of transforming growth factor-β are also quite complex, although it is generally found to be a stimulatory influence on osteoclasts differentiation. Taking into account the different effects that TGF-β produces on bone tissue, is suggested that this growth factor plays a significant role in bone turnover and remodeling.

Bone Morphogenic Proteins

Bone morphogenic proteins (BMPs), with the exception of BMP1 are members of the TGF-β "superfamily" (30-40% homology). BMP1 is different, however, as this is a metalloprotease that metabolises procollagen I, II, and III. BMPs have been exploited (extracted and purified) due to their ability to induce ectopic osteogenesis after implantation into skin or into muscle. BMP is known as a dimeric polypeptide molecule, containing two chains that are held together by a single disulphide bond. They have a 40-50% similarity of primary structure to that of TGF- β. About 20 BMP "family" members have been identified and characterized. Like TGF-β, these proteins are expressed during the development of bone tissue. As opposed to TGF-β, BMPs are rarely expressed and in the last few decades a number of studies have been undertaken to understand their physiological role in bone remodeling. The differentiation of osteoblasts is strongly influenced by BMP from mesenchymal stem cells. BMP-2 is known as key mediator of osteoblast differentiation. In some animal models and clinical trials it has been indicated that BMPs enhance fracture healing. BMP-4 promotes the osteogenic phenotype *in vitro* and is expressed by differentiated osteocells at the site of fracture healing. Localized delivery of growth factors is important method for bone healing following fractures. Though BMPs belong to the TGF-β superfamily, the effects of TGF-β are often the opposite to BMPs.

Control of the BMP signaling pathway is carried out by a complex array of receptors (BMPR) with inherent serine/threonine kinase activity. BMPs affinity to receptors is significantly amplified when both receptors (type I and II) are located in close proximity to one another. In the process of signal transduction the type-I receptor initiates phosphorylation of specific intracellular proteins called Smads.

Fibroblast Growth Factors

Fibroblast growth factors (FGFs) encompass at least twenty three known homological polypeptides that contain 150-250 residuals which bind to and activate four

transmembrane tyrosine kinase receptors FGFRs 1–4). They are secreted peptides with a molecular size of approximately 20–35 kDa and are expressed in many different types of tissues at different stages of development.

In earlier research it has been revealed that FGFs have an important regulatory function in bone formation. Research in the last decade also indicate the involvement of FGFs in bone formation; formation of mesoderm, its expression in bone tissue at development, ossification, angiogenesis (new blood vessel growth from existing vessels), production of endothelial cells, macrophages and osteocells (Rennel et al., 2003).

There are several other growth factors that affect osteoblasts proliferation in-vitro, for example epidermal growth factor (EGF). EGF affects both osteoclastic bone resorption and osteoblastic bone formation. In the last few decades a number of other factors which have been studied less intensively have been found to play a role in bone remodeling, for example heparin–binding, EGF and salivary epidermal growth factor sEGF.

Osteocells also produce colony stimulation factors (CSFs), also referred to as hematopoietic growth factors, which regulate bone marrow production of cells of hematopoietic lines. CSF-1 (monocyte/macrophage CSF) plays a role in osteoblasts/osteoclasts interactions during the process of osteoclasts differentiation. Multipotential colony-stimulating factor or IL-3 can also play role in osteoclasts genesis and interactions between bone tissue and bone marrow.

To conclude, many growth factors affect osteoblastic cell lineage *in vitro* and some stimulate bone formation *in vivo* (FGF, CSF). Some of the factors are consecutively involved in fracture healing (trombocyte growth factor, FGF, TGF). None of them is unique to bone tissue. A selective factor that can stimulate bone formation at the process of bone remodeling, in the same way as erythropoetin stimulates erythropoiesis, has not been identified as yet.

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Bibliography

Davidson P. L., Milburn P. D. and Wilson B. D., (2004). Biological adaptive control model: a mechanical analogue of multi-factorial bone density adaptation. *J.Theor Biol*, 227(2) 187-195. [A mechanical model of multi-factorial bone density adaptation has been described].

Doblare, M., Garcıa, J.M., (2002). Anisotropic bone remodeling model based on a continuum damage-repair theory. *J Biomechanics.* 35, 1–17. [A concept of remodeling tensor, analogous to the standard damage tensor was proposed in this study].

Doblare M., Garcia J. M., (2001). Application of an anisotropic bone-remodeling model based on a damage-repair theory to the analysis of the proximal femur before and after total hip replacement. *J Biomechanics*, 34(9)1157-1170. [An interesting FE framework for bone remodeling was developed].

Frost, H. M. (1964). *Mathematical Elements of Lamellar Bone Remodeling*. Charles C. Thomas Publisher. [This presents concept of a mechanostat and adaptive idea of the bone tissue remodeling].

Garcia J. M., Doblare M. and Cegonino J., (2002). Bone remodeling simulation: a tool for implant design. *Comp Materials Sci*, 25(1-2), 100-114. [The special behavior of adaptive bone remodeling has also been investigated in this paper].

Garcia-Aznar J.M., J.H. Kuiper, M.J. Gómez-Benito, M. Doblaré and J.B. Richardson, (2007). Computational simulation of fracture healing: Influence of interfragmentary movement on the callus growth. *J Biomechanics*, 40(7), 1467-1476. [The influence of inter fragmentary movement on callus growth using a computational simulation of fracture healing has been studied].

Geris L., A. Gerisch, J. V. Sloten, R. Weiner and H. Van Oosterwyck, (2008). Angiogenesis in bone fracture healing: A bioregulatory model. *J Theor Biol*, 251(1) 137-158. [Interesting bioregulatory model has been described].

Huiskes R., R. Boeklagen **(**1989). Mathematical shape optimization of hip prosthesis design. *J Biomech*, 22, Issues 8-9, 793-799. [The FE framework was applied to simulate the bone remodeling].

Komarova S.V., Robert J. Smith, S. Jeffrey Dixon, Stephen M. Sims and Wahl L.M., (2003). Mathematical model predicts a critical role for osteoclast autocrine regulation in the control of bone remodeling. *Bone*, 33(2), 206-215. [A phenomenological cellular model of bone remodeling based on the ODE technique described].

Lemaire V., Tobin F. L., Greller L. D., Cho C. R. and Suva L. J., (2004). Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. *J Theor Biol*, 229(3) 293-309. [The model considered detailed role of PTH in bone remodeling control].

Moroz A, Wimpenny D I. (2007). Bone Turnover Cycle Model with a Torus-like Steady State". In "*Mathematical Modeling of Biological Systems*. Vol 1." A Deutsch, L. Brusch, H. Byrne, G de Vries and H-P Hercel (eds). Birchauser, Boston, 261-270. [At first time the equation for the osteocyte dynamics has been included].

Pena E., Calvo B., Martinez M.A., Palanca D., Doblare M., (2005). Finite element analysis of the effect of meniscal tears and meniscectomies on human knee biomechanics *Clinic Biomechanics*, 20(5) 498-507. [The 3D FE modeling has been employed to study the effect of graft stiffness and graft tensioning].

Rattanakul C., Lenbury Y., Krishnamara N. and Wollkind D. J., (2003). Modeling of bone formation and resorption mediated by parathyroid hormone: response to estrogen/PTH therapy. *Biosystems*, 70(1), 55-72. [Article simulated the response of tissue remodeling to estrogen and PTH therapy].

Ruimerman R., P. Hilbers, B. van Rietbergen and R. Huiskes, (2005), A theoretical framework for strain-related trabecular bone maintenance and adaptation. *J Biomechanics*, 38(4) 931-941. [The application of bone remodeling parameters within the finite element algorithm, which was already employed in scaffold design, used as an intermediate stage, has been described].

Wolff J. (1986), *The law of bone remodeling* (Das Gesetz der Transformation der Knochen, Hirschwald, 1892) [Maquet P, Furlong R, Trans.]. Springer, Berlin. [A formal link between bone tissue structure and applied to bone loads was described].

Biographical Sketches

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