# Systems analysis of Bone Mechanotransduction at Cellular level

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***Abstract*—A 'systems-level' computational modeling approach is implemented to study the mechano-regulation of bone at cellular level. Issues addressed using this approach include - determining the intra-cellular response of bone cells to mechanical stimulus, bone response to different mechanical loading conditions, the role of intra-cellular feedback regulation in bone remodeling, and the link between reduced mechanical loading and decreased bone mass. An inter-connected network of signal transduction pathways in osteoblasts and osteoclasts is considered for modeling. The salient features of this modeling technique are systems biology based network modeling to simulate the temporal dynamics of the signaling proteins, parameter estimation based on evolutionary computing, and control systems theory to model feedback in the signaling network. The results indicate that signaling networks respond uniquely to different mechanical stimuli, the stimulus signal is gradually attenuated in the signaling cascade, and the disruption of intra-cellular feedback regulation leads to decreased bone formation in osteoblasts and increased bone resorption in osteoclasts. This results in low bone mass, a phenomenon generally observed in reduced loading conditions. It is deduced that reduced mechanical loading leads to disruption in the feedback to result in low bone mass. The results of these simulation studies are expected to serve as useful guidelines for planning relevant experimental work to study the effect of mechanical loading on bone at cellular level.**

## I. Introduction

THE process of converting physical forces into biochemical signals and integrating these signals into physiological responses is referred to as mechanotransduction [1]. In bone, mechanical forces regulate the rate of bone formation and resorption. Increased loading enhances bone formation, while decreased loading increases bone resorption. In vitro and in vivo studies [2] – [6] conclude that the bone mechanotransduction response involves changes in cellular physiology, like cell proliferation, gene expression, protein synthesis, apoptosis, or cell differentiation. This is because a number of secondary messenger pathways and local mediators generated in response to secondary messenger activation, like *cAMP, cGMP, PI3K, intracellular* $Ca^{2+}$*, Adenylate cyclase, ERK, PKA, JNK, PKC, Akt,* and *NF-κB* are affected in the load transduction of osteoblasts and osteoclasts.

Current studies on this transduction mechanism preclude investigations on the dynamics of these signaling molecules. Understanding the signaling dynamics is expected to explain how different loading conditions regulate bone mass, how bone responds to different types of forces like fluid shear stress or pulsatile fluid flow, the role of feedback regulation in the intra-cellular signal transduction network, and the link between reduced mechanical loading and decreased bone mass.

In this paper, we present a 'systems-level' computational modeling approach to address the above issues on intra-cellular signaling dynamics. An inter-connected network of eight major signaling pathways in osteoblasts and seven in osteoclasts, which are initiated as part of the intra-cellular response of bone to mechanical stimulus, are modeled as a system of differential equations based on Michaelis-Menten enzyme kinetics to simulate the temporal dynamics of the signaling proteins in these two networks. Parameter estimation, based on evolutionary computing, is used to estimate the rate constants of the kinetic models of the networks. Control systems theory is used to model feedback in the signaling networks. This computational modeling approach is implemented in MATLAB®'s SIMULINK® environment. As the network behavior is simulated and analyzed at the intra-cellular systems level and not at the individual signaling pathway level, the models are referred to as systems-level computational models.

## II. METHODOLOGY

This section describes the four steps used in developing the systems-level computational models of the osteoblast and the osteoclast intra-cellular signal transdudction pathways.

### *A. Determining the signaling networks*

Literature [7] - [18] indicates the activation of eight major signaling cascades in osteoblasts when bone is subjected to mechanical stimuli. These signal transduction pathways (JNK, Akt, ERK, NFkB, CREB, PKC, PKA, and Wnt), as shown in Fig. 1, induce the osteoblast response to stimuli. This response includes gene expression, cell proliferation, or differentiation. Studies show that inhibition of even a single component in any of these pathways can alter the mechanotransduction response [3], indicating inter-connectedness among these signal transduction pathways.

Manuscript submitted on July 4, 2008.

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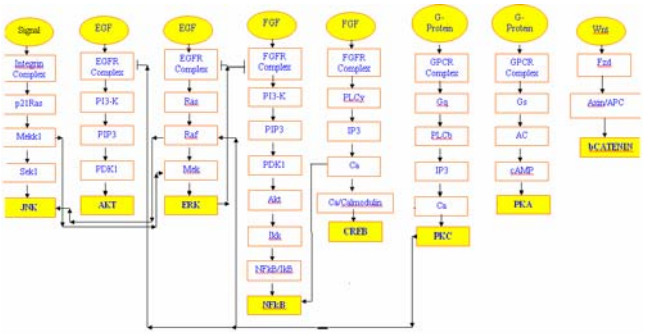


Fig. 1. Block diagram representation of the osteoblast signaling network

Osteoclasts respond to mechanical stimulus either directly or through osteoblasts. Osteoblasts release RANKL and/or OPG seconday messengers on applied stress. Receptor activator of NF-kB Ligand (RANKL) binds to RANK (its receptor) to drive osteoclast development from haematopoietic progenitor cells as well as to activate mature osteoclasts. Osteoprotegerin (OPG) negatively regulates RANKL binding to RANK and therefore inhibits bone turnover by osteoclasts [19] - [20]. Recent studies [21] - [24] have shown that RANKL-RANK induced intracellular signaling cascades engage in multiple cross-talk to effect osteoclastogenesis, osteoclast activation and proliferation. The corresponding signal transduction pathways (Akt, PKC, JNK, p38MAPK, ERK, NFAT, and NFkB) are mapped out in Fig. 2. Some pathways, like JNK, ERK, NFkB and Akt, are common in both osteoblast and osteoclast networks as they are essential for cell proliferation and apoptosis.

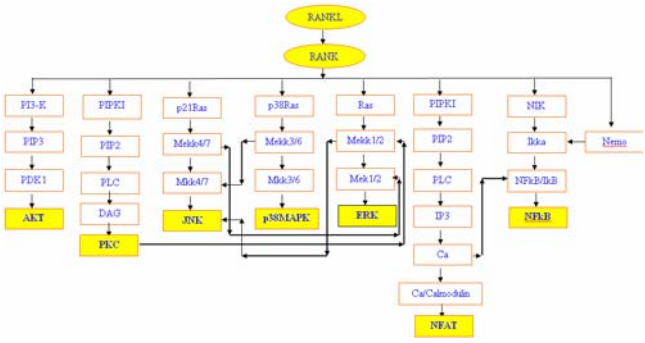


Fig. 2. Block diagram representation of the osteoclast signaling network

### *B. Michaelis-Menten Kinetic Modeling*

Figures 1 and 2 are the block-diagram representations of osteoblast and osteoclast signaling networks respectively. Investigating the temporal dynamics of signaling pathway involves determining unknown variables like the concentration, expression level, amplitude, and duration of activation of each of the signaling components at a given time. Hence, the interactions between the components are modeled as a system of differential equations, as such equations describe the rate of change of a variable over time.

In this work, Michaelis-Menten kinetics is used as the basis for differential equations [25] – [27] because activation of signaling components parallels closely with such enzyme kinetics. The enzyme kinetics model expresses rate of change of substrate and product over time, the kinetics follows the conservation law and assumes quasi-steady state conditions to derive the reaction rate, and the reaction rates are scalable to include competitive inhibition and uncompetitive upregulation.

A system of Michaelis-Menten based rate equations is developed for the signaling networks of osteoblasts and osteoclasts. One such rate equation is reproduced below for the ERK pathway (no feedback considered). EGF protein is taken as the stimulus –

EGF → EGFR complex → Ras → Raf → MEK → ERK

$$\frac{dEGFR}{dt} = \frac{k_1 EGF(\overline{EGFR} - EGFR(t))}{Km1 + (\overline{EGFR} - EGFR(t))} - \frac{k_2 EGFR(t)}{Km2 + EGFR(t)}$$

$$\frac{dRas}{dt} = \frac{k_3 EGFR(t)(\overline{Ras} - Ras(t))}{Km3 + (\overline{Ras} - Ras(t))} - \frac{k_4 Ras(t)}{Km4 + Ras(t)}$$

$$\frac{dRaf}{dt} = \frac{k_5 Ras(t)(\overline{Raf} - Raf(t))}{Km5 + (\overline{Raf} - Raf(t))} - \frac{k_6 Raf(t)}{Km6 + Raf(t)}$$

$$\frac{dMEK}{dt} = \frac{k_7 Raf(t)(\overline{MEK} - MEK(t))}{Km7 + (\overline{MEK} - MEK(t))} - \frac{k_8 MEK(t)}{Km8 + MEK(t)}$$

$$\frac{dERK}{dt} = \frac{k_9 MEK(t)(\overline{ERK} - ERK(t))}{Km9 + (\overline{ERK} - ERK(t))} - \frac{k_{10} ERK(t)}{Km10 + ERK(t)}$$

In the above equation, $\overline{EGFR}$ refers to the total EGFR present in the system (both activated and deactivated).

When feedback is considered, the rate equations of the affected variables are modified to reflect the positive or negative feedback. The equations for the unaffected variables remain the same. For example, in the ERK pathway (Fig.1), EGFR complex is negatively regulated by ERK and Raf is positively upregulated by PKC. Hence, the corresponding rate equations are –

$$\frac{dEGFR}{dt} = \frac{k_1 EGF[1/(1+[\frac{ERK(t)}{K_N}])](\overline{EGFR} - EGFR(t))}{K_{m1} + (\overline{EGFR} - EGFR(t))} - \frac{k_2 EGFR(t)}{K_{m2} + EGFR(t)}$$

$$\frac{dRaf}{dt} = \frac{k_5 Ras(t)[1/(1+[\frac{\overline{PKC} - PKC(t))}{K_P}])](\overline{Raf} - Raf(t))}{Km5 + (\overline{Raf} - Raf(t))} - \frac{k_6 Raf(t)}{Km6 + Raf(t)}$$

Here, $K_P$ and $K_N$ are constants that define the strength of the positive feedback and negative feedback respectively. The above two equations are framed using the concepts of control theory, as described in [25]-[27].

### C. Parameter estimation using evolutionary computing

In the rate equations, there are a total of 292 rate constants (considering both osteoblast and osteoclast networks). Literature on enzyme kinetic experiments was surveyed to derive the relevant rate constant values. But sufficient data was not available from the studies conducted till date, most likely due to the limited experiments taken up in this direction. Hence, in this work, parameter estimation was implemented to overcome these limitations to determine the rate constants. Parameter estimation has been successfully applied in many domains, like Bayesian model learning, geometric curve fitting, and control of large dynamical systems [28].

The estimation is based on evolutionary computing, and is implemented as follows –

(i) With a total of N proteins in the signaling pathway, the total number of parameters to be estimated is 4N: 2N rate constants ($k$) and 2N Michaelis-Menten constants ($K_m$).

(ii) The minimum and maximum values of the parameters considered are as shown in Table I. Protein concentration is considered in percentage values as the actual estimates are not known.

(iii) Mp sets of parent parameters [$k_1$, $Km_1$, $k_2$, $Km_2$… $k_N$, $Km_N$] are initialized. The values of these parameters should fall within the boundaries decided in step (ii).

(iv) Each set of parent parameters is substituted into the mathematical model and solved.

(v) The cost function for each set of parent parameters is computed. Cost function is a measure of the goodness of the fit of the model. Denoting the simulated protein concentrations as $SP_1(t)$-$SP_N(t)$ and the experimental results as $EP_1(t)$-$EP_N(t)$, the cost function J can be written as -

$$J = \sum_{t=0}^{T} \sum_{i=1}^{N} (EP_i(t) - SP_i(t))^2$$

(vi) Two parents from the Mp sets of parent parameters are randomly selected for recombination to form a set of Mc child parameters.

(vii) In the next step, known as mutation step, the value of each parameter is adjusted by an amount equal to a Gaussian random variable multiplied by its variation.

(viii) Mc sets of child parameters are substituted into the mathematical model and solved. Cost function is computed to give a total of Mc cost values

(ix) With Mp sets of parent parameters and Mc sets of child parameters, there would be a total of Mp + Mc cost values and Mp + Mc sets of parameters. From these, Mp sets of parameters with the lowest cost values are selected to be the parent parameters.

(x) Steps (iv) to (ix) are repeated till the RMS error value of the successive parameters equals zero.

TABLE I
CONSTANTS USED FOR THE PARAMETER ESTIMATION ALGORITHM

| Constants | Values |
|---|---|
| Total number of proteins, N | Depends on Equations |
| Minimum value of rate constant, $k_{min}$ | 0.1 $s^{-1}$ [27] |
| Maximum value of rate constant, $k_{max}$ | 3.0 $s^{-1}$ [27] |
| Minimum value of Michaelis-Menten constant, $Km_{min}$ | 0.1 nM [27] |
| Maximum value of Michaelis-Menten constant, $Km_{max}$ | 20.0 nM [27] |
| Variation of rate constant, $k$ | 0.001 $s^{-1}$ [27] |
| Variation of Michaelis-Menten constant, $Km$ | 1 nM [27] |
| Number of sets of parent parameters, Mp | 30.0 |
| Number of sets of child parameters, Mc | 20.0 |
| Number of generations | 100.0 |
| Value of feedback constant, $K_P = K_N$ | 9 nM [27] |
| Minimum value of activated protein concentration (at time 0 unit) | 0% |
| Maximum value of activated protein concentration (at time 200 units) | 100% |

Based on the parameter estimation algorithm, we derived the rate constants for osteoblast network and osteoclast network respectively.

### D. Simulations

Two states of intra-cellular bone response are assumed for the simulation studies – a steady state, where feedback is present in the signaling network, and an unsteady state in which feedback is not present in the signaling network. These assumptions are made to verify the role of feedback. Three different types of mechanical stimuli are modeled to perturb the network to investigate the network behavior in each case - fluid shear stress as a step signal (of step time 1), oscillatory fluid flow as a sine wave signal (amplitude -1, frequency – 1Hz), and periodic substrate stretch as a pulse generator signal (amplitude -1, period – 2s). These stimuli

are considered based on the literature [29] - [33]. Simulations are generated in SIMULINK® [34]. The main reason for using this environment to simulate systems level models is due to its support of modular and scalable architecture for systems robustness and sensitivity.

## III. RESULTS

The simulation results shown in this section illustrate the dynamic concentration profiles of signaling components of ERK and PKA pathways in the osteoblast network. These profiles allow us to determine the rate and duration of activation of the components for the given stimuli.

### *A. Response to mechanical stimuli*

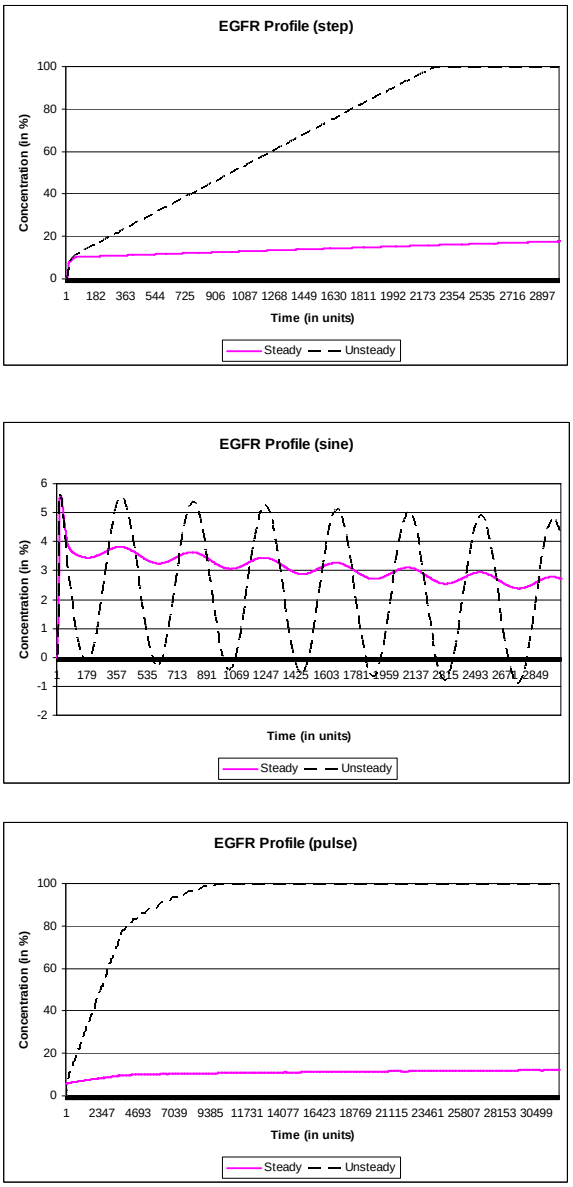


Fig. 3. Activation profile of EGFR complex in ERK pathway

Fig. 3 indicates the activation profile of the EGFR complex of ERK pathway in osteoblast network over time. We can observe dissimilar activation rates to different stimuli, thus indicating unique cell response to loading conditions. As EGFR complex in negatively feedback regulated, its steady and unsteady states show different profiles.

### *B. Feedback regulation*

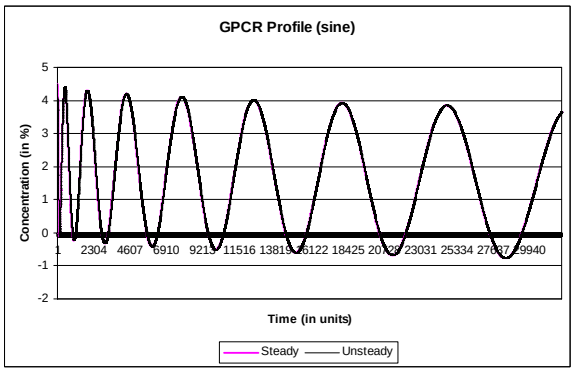


Fig. 4. Activation profile of GPCR complex in PKA pathway

Fig. 4 indicates the activation profile of the GPCR complex of PKA in osteoblast network over time. In contrast to EGFR (in Fig. 3), the steady and unsteady profiles share similar response to the input stimulus. This is because GPCR is not regulated by feedback, whereas EGFR is negatively feedback regulated by ERK.

### *C. Attenuation of stimulus signal*

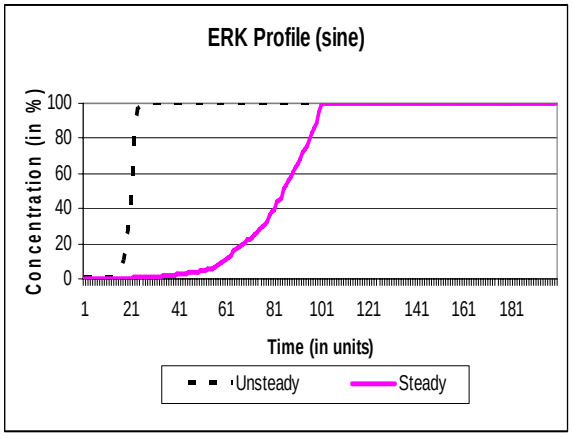


Fig. 5. Activation profile of ERK in ERK pathway

In contrast to the oscillatory behavior of the EGFR complex to the sinusoidal input stimulus, as shown in Fig. 3, ERK shows a smooth activation profile in both steady and unsteady states (Fig. 5). EGFR complex and ERK are the first and last signaling components of the pathway. This indicates that the stimulus signal is attenuated as it propagates along the pathway.

## IV. DISCUSSION

In the context of mechano-regulation of bone, a few salient inferences are derived from these simulations –

### *A. Network sensitivity*

Dissimilar activation profiles to the three input stimuli - step signal, sine wave signal, and pulse generator signal, are observed in the results. This indicates that bone cell responds uniquely to different input stimulus. A likely inference for such observation is that the cell responds dynamically to the input stimulus and relays activation signal downstream to its signaling network. The network uniquely adapts to this signal and hence results in a unique physiological response.

### *B. Role of feedback*

Different activation profiles are observed in steady and unsteady states for NFkB, ERK and JNK signaling factors. This indicates higher activation rate of factors in the unsteady state compared to the steady state. NFkB, ERK or JNK cause cell proliferation and matrix synthesis in osteoblasts, and are responsible for osteoclastogenesis in osteoclasts. Hence, these factors are regulated by feedback to check for unwanted proliferation.

As described in the methodology section, feedback regulation is assumed to be disrupted in the unsteady state, while it is kept intact in the steady state. This implies that higher activation rate is caused due to disruption in the feedback regulation. Higher activation rate in the profiles indicates increased proliferation rate of osteoblasts and osteoclasts. In osteoblasts, unchecked proliferation ultimately leads to maturation of osteoblasts into osteocytes, eventually resulting in decreased bone formation. In osteoclasts, it causes extended family of osteoclasts. Maturation of osteoclasts transforms them to macrophages, which engage in bone resorption. More osteoclasts at a given stage indicate increased bone resorption. Hence, it is inferred that disruption of the feedback causes decreased bone formation in osteoblasts and increased bone resorption in osteoclasts.

### *C. Link between reduced loading and low bone mass*

Low bone mass is a phenomenon observed during decreased mechanical loading conditions. The simulation studies indicate that low bone mass is a possible result of disruption of feedback in intra-cellular signaling network. From this, it can be deduced that reduced loading conditions and feedback disruption might be related. Reduced loading conditions imply weaker input stimulus sensed by the mechanotransduction sensing factors in the cells. This implies weaker signal propagation inside the cell. Weak signal strength inside the cell may imply that the signaling proteins might not be fully active to carry out the dual functions of activating downstream signaling cascade and feedback regulation on proteins in other pathways. This means that one of these two regulatory functions might be compromised during reduced loading conditions. When the feedback regulation is not carried out, cell proliferation becomes unchecked. This might lead to decreased bone formation and increased bone resorption, i.e. reduced bone mass. Hence, it is deduced that decreased mechanical stimulus causes disruption in the feedback regulation to result in low bone mass. Illustrating this deduction -

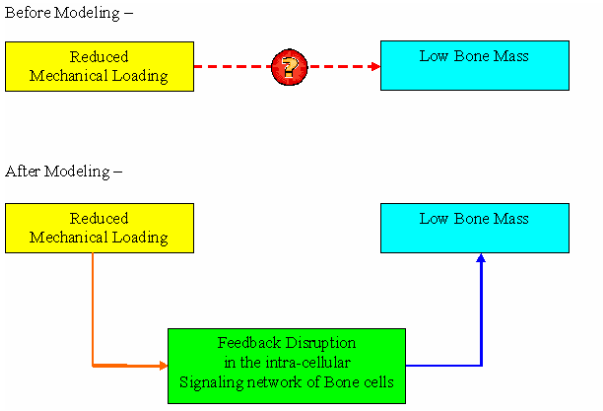


Fig. 6. The link between reduced loading and low bone mass

## V. CONCLUSION

In this paper, we implemented a systems-level computational modeling approach to address specific issues in the mechano-regulation of bone. We determined the intra-cellular response of bone cells to mechanical stimulus, simulated the response to different loading conditions, investigated the role of feedback regulation in bone remodeling, and inferred a link between reduced mechanical loading and decreased bone mass.

The major contribution of this work is the systems level approach to simulate the signaling dynamics of bone cell response and the implementation of parameter estimation to estimate the Michaelis-Menten rate constants of the kinetic models of the networks.

The results of these simulation studies can serve as useful guidelines for planning relevant experimental work to study how reduced loading conditions can lead to low bone mass and to investigate the dynamic affect of the mechanical environment on bone cells in vivo. Potential areas of application include tissue engineering and degenerative disease (like Osteoporosis) therapeutics.

## ACKNOWLEDGMENT

The authors acknowledge Ms Ling Wen Wan and Mr Geoffrey Koh for their contribution to the parameter estimation section.

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