MECHANICALLY INDUCED TRABECULAR BONE REMODELING INCLUDING CELLULAR ACCOMMODATION EFFECT: A COMPUTER SIMULATION

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ABSTRACT

Trabecular bone's capability to adapt its architecture to the surrounding environment has been a subject of research for more than a century. The purpose of the present work is to simulate and improve a semi-mechanistic bone remodeling theory. In doing so, a computer code was developed using finite element method, also a cellular accommodation effect was incorporated in the model. Using this novel approach, trabecular-like structures for different loading conditions and directionalities similar to actual human bone have resulted for a square plate. Furthermore, it was shown that the model is sensitive loading time history. In summary, results of our research showed that a semi-mechanistic model including cellular accommodation effect is scientifically valid, and is able to predict more realistic morphologies in time-dependent simulations.

REMODELAGE DE L'OS TRABECULAIRE INDUIT PAR DES EFFORTS MECANIQUES : UNE SIMULATION INFORMATIQUE INCLUANT LES EFFECTS DE L'ADAPTATION TEMPOREL DES CELLULES.

RESUME

Depuis plus d'un siècle la recherche a mis en évidence que l'os trabéculaire possède la capacité d'adapter son architecture à son l'environnement. L'objectif du travail présenté ici, est d'améliorer la simulation théorique semi-mécanique du remodelage osseux. Pour ce faire, un model s'appuyant sur la méthode des éléments finis a été développé en intégrant les effets de l'adaptation temporel des cellules (méthode cellulaire). Cette nouvelle approche à permis d'étudier la réponse des structures de l'os trabéculaire, via l'étude d'une plaque carrée, pour différents états de charge. De plus, il a été démontré que le modèle est bien sensible aux effets temporels de chargement. En conclusion, les résultats de nos recherches ont montré que le modèle semi-mécanique qui inclus les effets de l'adaptation temporel des cellules est scientifiquement valable et est en mesure de prévoir des morphologies plus réalistes dans des simulations temps-dépendant.

INTRODUCTION

Bone's capability to adapt its internal architecture and external shape to the surrounding environment has been a subject of research for more than a century [1]. Based on a trial and error procedure and by closely following the latest developments in bone physiology, many researchers have tried to develop mathematical models for the bone adaptation process. The common motivating factors for the theoretical and computational modeling efforts were, first, to understand and quantitatively describe the functional adaptation of bone, then, to simulate and predict bone adaptation, and finally, to simulate the effects of manipulations to processes, in the hope of testing ideas that may be therapeutically beneficial [1]. Wolff [2] is usually given credit for proposing that bone adapts to mechanical loading during its growth and development. He hypothesized that "Every change in the form and function of bones is followed by certain definite changes in their internal architecture and equally definite alteration in their external conformation in accordance with mathematical laws" [3].

For many years after Wolff, there was little progress towards a complete mathematical formulation of bone remodeling. In 1964, Frost [3] introduced the first mathematical description of trabecular bone remodeling. He also later introduced the mechanostat theory that predicts when adaptive response of bone will be triggered [4]. Frost suggested that there is a constant equilibrium range of strain values (threshold stimulus) which will evoke no response in bone remodeling process; strains above this range will increase bone mass and strains below that will cause bone loss [4]. More than three decades later, Turner challenged Frost's mechanostat theory by introducing the principle of cellular accommodation based on an ever changing threshold stimulus [5]. Turner hypothesized that the transient nature of many cellular biochemical responses to mechanical loading is a result of adaptation in cellular mechanical behavior, whereby the cell becomes mechanically tuned to its mechanical environment [5]. Principal of cellular accommodation was based on experimental results suggesting that when a strain threshold is surpassed the sensor cells will gradually accommodate to the new state (e.g. by cytoskeletal reorganization). Recent experimental findings support Turner's cellular accommodation theory. For instance, Saez et al. [6] showed that an epithelial cell can adapt its stiffness to maintain a specific range of habitual cellular deformations. Also, latest findings of Jaasma et al. [7] support the hypothesis that osteoblasts become mechanically adapted to their surrounding environment via cytoskeletal modifications and, accordingly, regulate the magnitudes of mechanical stimuli that initiate mechanotransduction signaling.

The first rigorous mathematical model of bone remodeling was developed by Cowin and Hegedus [8, 9]. In their model, bone is defined as a porous medium with two phases; an elastic structure, and an extracellular fluid. According to this model, adaptation is caused by chemical reactions and is controlled by strain [8]. Following adaptive elasticity theory, many other theories have been developed by others. In 1986, Huiskes et al. [10] suggested that in order to overcome the parameter identification problem in Cowin and Hegedus model [8 ,9], a scalar quantity, strain energy density, can be used as a suitable mechanical stimulus for bone remodeling instead of strain which is a tensor quantity. Weinans et al. [11] applied a time-dependent version of the Huiskes et al.'s theory [10] to a two dimensional plate model of bone. Bone was represented as a continuum, capable of adapting its apparent density due to mechanical stimulation. The convergence results for this plate produced a trabecular-like structure where most of the elements had either maximum or minimum density values [11]. In many cases, the "black and white" structures obtained with this method formed checkerboard patterns. Several studies were prompted to examine the stability of the resulting structures in more detail [12, 13]. It was earlier believed that checkerboards represent an optimal microstructure, but later studies showed that checkerboard patterns are due to numerical problems in the model [14]. This phenomenon was the result of dividing domain of interest into finite elements. The finite elements formed virtual boundaries in the domain and limited mechanotransduction signaling of bone cells [14]. Mullender and Huiskes [15] proposed an innovative approach that eliminated checkerboard patterns. They assumed that sensor cells (i.e. osteocytes) are uniformly distributed over the volume of bone and each sensor measures a mechanical signal, the strain energy density. The signal sent to actor cells by an osteocyte was assumed to decay exponentially with increasing distance [15], thus finite elements would not interfere with mechanotransduction signaling anymore.

In another attempt, Rouhi et al. [16] proposed a modification on the adaptive elasticity theory. Considering that all resorption and formation occurs on the interface of bone matrix and voids, they hypothesized that volume fraction should be replaced by a new parameter, so called free surface density. Free surface density is defined as total area of interface between bone matrix (solid phase) and fluid phase divided by total volume of the bone [16]. Their first model shows that surface remodeling equation can be concluded using remodeling equation for a hollow compact cylindrical bone. Furthermore, their model emphasizes on the effect of free surface density on the rate of remodeling,

and development of high porosity bone in osteoporotic cases. Rouhi et al. [17] in another attempt added a microcrack factor to their first modification [16], and showed that not only mechanical stimuli, but their rates and histories are all effective in the rate of remodeling. They showed that by introducing microcracks factor in their first model, the remodeling equation will be much more complex than previously proposed models, including their first model. The high level of complexity in the aforementioned model is not, of course, desirable, but it seems unavoidable because of an intricate interaction between bone sensor and actor cells in the bone remodeling process. Using a bi-phasic mixture theory with chemical reactions, Rouhi et al. [18] modeled only the first phase of bone remodeling, i.e. bone resorption process. Their model shows that not only mechanical factors, but also chemical and biological factors are at play in the rate of bone resorption, and consequently, in the rate of bone remodeling [18]. Also, their bi-phasic mixture model shows that strain energy density and hydrostatic pressure are effective mechanical stimuli in the bone resorption, and consequently in bone remodeling processes [18]. Recently, Rouhi [19] used a tri-phasic model consisting of bone resorption cells, bone matrix, and bone fluid to study bone resorption process. In their tri-phasic bone resorption model, it is concluded that rate of bone resorption is a function of apparent density of bone matrix and bone fluid, fluid velocity, momentum supply to the fluid phase, and internal energy densities of different constituents.

In the beginning of the 21st century, Huiskes and co-workers [20] developed a semi-mechanistic model including latest experimental findings in bone physiology and cellular biology such as a separate description of osteoclastic resorption and osteoblastic formation [21], a biological osteocyte mechanosensory system [22], and role of microdamage [23]. The purpose of the proposed research is to incorporate Turner's cellular accommodation principle [5] in the Huiskes et al.'s [20] model, and to evaluate the possible resulting changes in the behavior of trabecular bone remodeling. A second goal of the proposed research is to investigate the scientific merit of Huiskes et al.'s [20] model.

METHODS

Recently, in a computational model presented by Huiskes et al. [20], it is assumed that bone contains n osteocytes per cubic millimeters located in the mineralized matrix with a total of N in the domain of interest. Also, it is assumed that osteocytes are sensitive to the maximal rate of the strain energy density (SED) in a recent loading history. Each osteocyte i measures a mechanical signal $R_i(t)$, the rate of strain energy density, in its location. Then, the osteocytes recruit osteoblasts to form new bone depending on the difference between the measured signal and a reference formation threshold, k. Furthermore, it is assumed that the influence of an osteocyte on its environment decays exponentially with increasing distance from the osteoblasts. Thus, the influence of osteocyte i on the osteoblast at location x is described as follows [15]:

(1)

where $d_i(x)$ is the distance between osteocyte i and location x. The parameter D represents the distance from an osteocyte at which location its effect has reduced to e^{-1}. The spatial influence function, Eq. (1), is purely hypothetical and there is a great need to evaluate its validity and merit using experimental techniques in mechanotransduction of cells [1].

The osteoblast recruitment stimulus is given by $P(x,t)$ to which all osteocytes contribute relative to their distance from an osteoblast, x, hence [20]:

$$P(x,t) = \sum_{1}^{N} f_i(x)\mu_i R_i(t) \tag{2}$$

where μ_i is mechanosensitivity of osteocyte i and $R_i(t)$ is the mechanical stimulus (SED-rate) measured by the same osteocyte. The model is formulated as a balance of bone mass M in trabecular bone volume considered. The relationship between M and local remodeling activity is expressed as [24]:

$$\frac{dM}{dt} = \int_{\Omega} \rho_{\max} \frac{dm}{dt} \delta ds \tag{3}$$

where M is the total mass of trabecular bone volume, $m(x,t)$ is the relative density (between 0 and 1) of a small volume of thickness δ under a surface patch ds of the trabecular surface Ω and $\rho_{\max}$ is the constant maximum tissue density.

The regulation of the relative density m in location x is governed by [20]:

$$\frac{dm}{dt} = \tau\{P(x,t) - k\} - r_{OC} \quad \text{for } P(x,t) > k_{tr}$$

$$\frac{dm}{dt} = -r_{OC} \quad \text{for} \quad P(x,t) \le k_{tr} \tag{4}$$

where τ is a constant regulating the rate of the process, k_{tr} is the constant threshold level for bone formation and r_{oc} is the relative amount of mineral resorbed by osteoclasts per day [20].

This model includes a probability L of osteoclast activation per surface site at any time. This probability is assumed to be regulated either by the presence of microcracks or by disuse. The probability of resorption by microcracks was considered spatially random and was expressed as [20]:

$$L(x,t) = \text{constant}, \tag{5}$$

where this constant was selected to be 10% [20].

When assuming osteoclastic activation by disuse, the probability of resorption is higher in areas of lower strain. This strain dependent probability was formulated as [20]:

$$L(x,t) = c\left[a - P(x,t)\right] \quad \text{if} \quad P < a$$

$$L(x,t) = 0 \quad \text{if} \quad P \ge a \tag{6}$$

where c and a were chosen as 12.5 and 1.6, respectively.

In the Huiskes and co-worker's model a constant threshold stimulus is assumed. Whereas, it is well known that numerous cellular biochemical responses to mechanical loading are transient, indicating a cell's ability to adapt its behavior to a habitual mechanical environment [25-27]. On the basis of Turner's cellular accommodation theory [5, 28], we hypothesized that k_{tr} in Eq. (4), is better and more realistic to be replaced by the following expression, which is called a relaxation function:

$$F(x,t) = k + (P(x,t) - k)(1 - e^{\frac{-t}{\tau_{CAE}}}) \tag{7}$$

where τ_{CAE} reflects the time needed for the cells to accommodate. This new mathematical form is suggested based on the exponential equation proposed by Turner [5].

After adding cellular accommodation effect into Eq.(4), regulation of bone density in location x is governed by:

$$\frac{dm}{dt} = \tau\{P(x,t) - F(x,t)\} - r_{OC} \quad \text{for } P(x,t) > F(x,t)$$

$$\frac{dm}{dt} = -r_{OC} \quad \text{for} \quad P(x,t) \le F(x,t) \tag{8}$$

In response to a change in environment, bone cells become mechanically tuned to their environment by changing their threshold level, $F(x,t)$, as was described by Eq.(7). Under steady state conditions (i.e. when $t \to \infty$),

$F(x,t) = P(x,t)$, thus $\frac{dm}{dt} = 0$. Turner [5], also, proposed that in the principle of cellular accommodation, the set point, k_{tr}, will vary from site to site within the skeleton depending upon the local strain environment to which the cells have accommodated. This implies that set point (k) will be higher in weight-bearing bones and lower in bones where mechanical strains are minimal (e.g. in the skull). Recently, Schriefer et al. [29] performed an experiment investigating the adaptation of bone to decreasing-, increasing-, and constant peak mechanical loads in a rat model. To avoid the added complexities of bone surface area changes and trabecular orientation, their experiment focused on cortical rather than trabecular bone adaptation. They compared the experimental adaptation response of bone to changing mechanical loads to the predicted responses of algorithms with and without cellular accommodation effect. Interestingly, bone formation results in the mechanical loading experiment on Sprague-Dawley rats closely resembled the predicted results of the algorithm with cellular accommodation effect [29]. Simulation of an algorithm without cellular accommodation effect predicted the same change in bone structure regardless of whether the loads were progressively increased or decreased. These results demonstrated that the temporal order in which loading is applied influences the final bone structure as was predicted by the algorithm with cellular accommodation effect. We will follow the same approach to demonstrate effect of cellular accommodation in our computer model.

Simulations were performed on a $2 \times 2\ mm^2$, two-dimensional bone finite element (FE) model consisting of 6400 elements. We created the two-dimensional model by implementing the above mathematical expressions in a MATLAB code. For the sake of simplicity, the bone tissue was assumed to be isotropic. The structure was loaded by a sinusoidal stress cycling between 0 and 2 MPa at $1Hz$ which are values in a normal physiological range for human trabecular bone. Osteocytes were assumed to be sensitive to the maximal SED-rate during one loading cycle. It has been shown that the maximal SED-rate is related to the SED value for some substitute static load and that it can be calculated by static finite element analysis [24]. Hence, in the FE model used here, the stress components, σ_i, and the strain components, ε_i, were calculated at the integration points of each element, and interpolated per element to give their values in the sensor points. The strain energy density was calculated from the tensor product [15]:

$$U_i = \frac{1}{2}\varepsilon_i \sigma_i \tag{9}$$

where i refers to the sensor number. The signal per sensor point $R_i(t)$ is then determined. The stimulus $P(x,t)$ is evaluated using equation (2) and a new density value m_j is calculated in element j, in accordance with equation (4), using:

$$m_j(x, t + \Delta t) = m_j(x, t) + \Delta t \times \frac{dm_j(x, t)}{dt} \tag{10}$$

where Δt is the time step in the iteration process [24]. The iteration is continued until no more significant changes in the density distribution occur.

The elastic modulus $E(x,t)$ at each location is calculated from density according to [30]:

$$E(x, t) = \rho_{\max} \times m(x, t)^{\gamma} \tag{11}$$

where $\rho_{\max} = 5\ GPa$ and $\gamma = 3$ [15].

RESULTS

In order to verify that the developed code in this research is capable of simulating Huiskes et al.'s model [20], six different simulations were performed. The first two simulations tested whether the theory can produce trabecular-like 2D configurations from conceptual initial architectures, representing bone in the post-mineralized fetal stage and from a uniform density (both isotropic) (Fig. 1). Simulations were prolonged until no more gross architectural

changes occurred, representing the homeostatic mature stage. In these two simulations, the structures remodeled toward similar homeostatic configurations in which trabecular-like structures were created and trabeculae were aligned to the loading direction (Fig. 1). In the third simulation, when the external load applied to the homeostatic architecture was rotated by 30°, the orientation of the trabeculae gradually reoriented as well, to align again with the external load (Fig. 2). Trabeculae alignment with loading direction is in accordance with the Wolf's law [2] and corresponds to its load-bearing role. In the fourth simulation, the regulatory mechanism was able to adapt the structure to alternative loading conditions. A twenty five percent increase in load magnitude produced an increase in trabecular thickness, also increased the bone mass by 43% (Fig. 3). This result is in agreement with several experiments demonstrating that exercise or stronger muscles will increase bone mass [31-33].

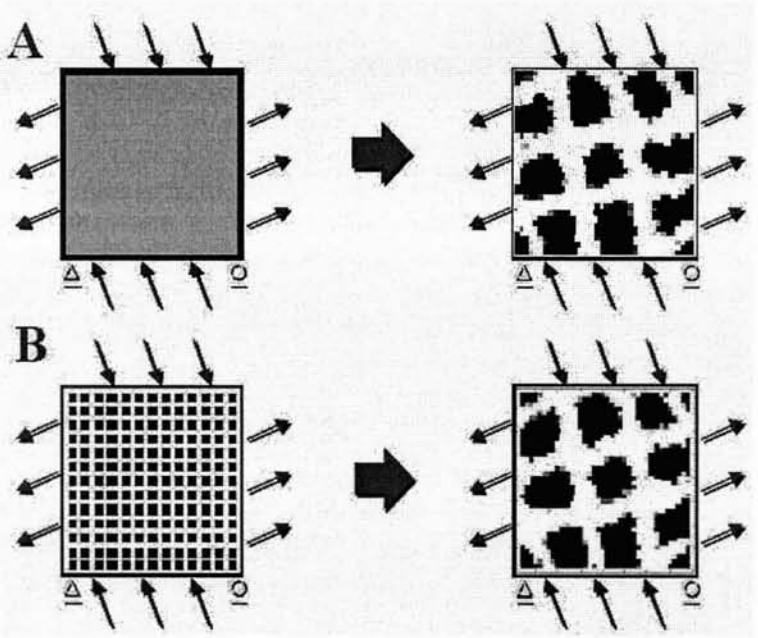

Figure 1. Transformation of morphology for (A) uniform density (B) post-mineralized fetal stage initial configurations. Structures are loaded at the edges by a sinusoidal stress, cycling between 0 and 2MPa, at 1 Hz, rotated by 30 degrees.

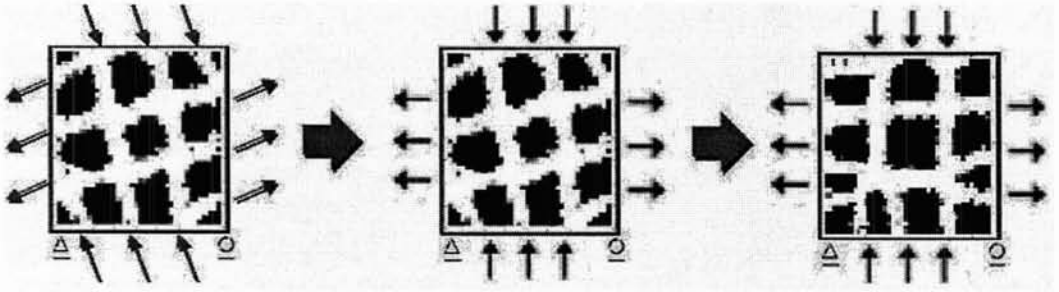

Figure 2. Orientation of the applied stresses was changed from 30° to 0° and the architecture adapted to align with the new stress orientation.

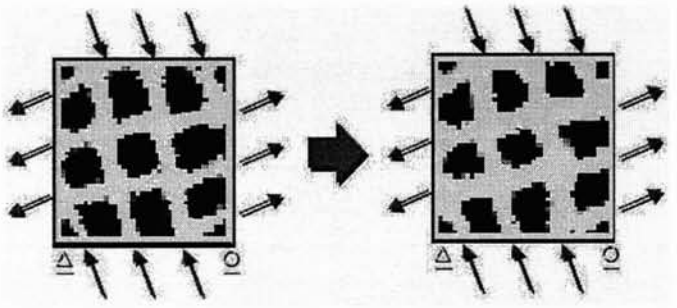

Figure 3. Effect of increasing loading magnitude by 25%; by increasing loading magnitude all trabeculae thicken.

The effect of overloading and unloading on trabecular adaptation was investigated in the fifth simulation where two trabeculae were artificially disconnected while the same externally applied load was maintained. The disconnected and therefore unloaded tarbeculae disappeared, while the neighboring overloaded trabeculae thickened (Fig. 4). This simulation can be considered as an extension of the previous simulation in which effect of both increased- and decreased loading have been taken into account. In our model, adaptive response of bone to increased- and decreased loading triggers bone formation and resorption, respectively, which is in agreement with numerous experimental results [e.g. 34-36]. In the sixth simulation, the remodeling theory used in this study was compared with a topology optimization model [37]. Similar final configurations have been resulted using our code, i.e. bone adaptation theory (Fig. 5), and a topology optimization code [37]. Although this simulation was performed for a simple loading condition (compressive ramp load), the similarity between the two final configurations seems interesting and encouraging.

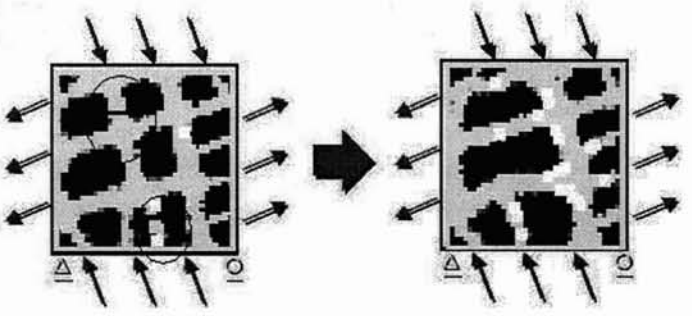

Figure 4. Two struts are artificially disconnected, while the external force is maintained. After adaptation, the existing architecture is again adapted to the applied stresses by removal of the unloaded trabeculae and thickening of the overloaded ones.

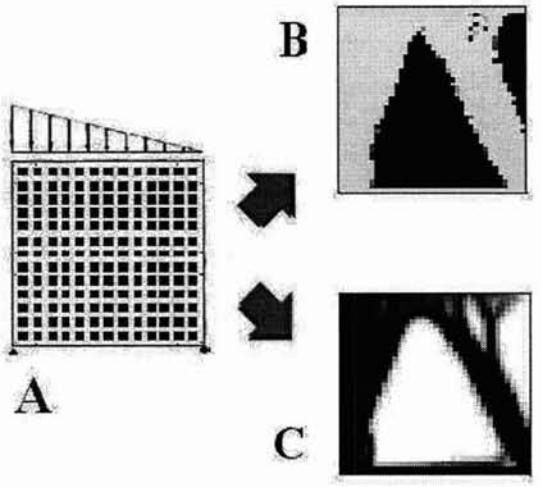

Figure 5. (A) Two-dimensional plate model of bone tissue subjected to a compressive load as indicated (B) Architecture after applying the bone adaptation theory (C) Architecture after applying the topology optimization code.

To demonstrate the effect of cellular accommodation in this research, three cases of decreasing-, increasing-, and constant peak load have been considered. Magnitude of the applied load in constant peak load case was the same as Huiskes et al.'s model [20]. For decreasing- and increasing peak load cases, loading magnitude was decreased and increased by fifteen percent progressively. Very similar to the results of Schriefer et al. for cortical bone remodeling [29], in the case that the largest force was applied at the start of the simulation (the decreasing peak load case), the largest overall changes were observed. The constant load case had the second largest response, since it had the second largest load applied at the start of the simulation. Final calculated densities of trabecular bone for these simulations are presented in Table 1.

Loading Case	Corresponding density after 300 iterations
	0.478
	0.455
	0.417

Table 1. Average density after 300 iterations for three cases of decreasing (), constant () and increasing () peak loads.

DISCUSSION AND CONCLUSION

We have investigated the dynamics of a bone remodeling model by incorporating the cellular accommodation effect into a semi-mechanistic bone remodeling theory. It combines concepts from Turner's principle of cellular accommodation [5] and Huiskes et al.'s model [20]. Although, we implemented the mathematical model in a computer code from scratch, we obtained final configurations very similar to the ones obtained by Huiskes and co-workers [20]. From our first five simulations, it was concluded that Huiskes and co-workers model with incorporating the cellular accommodation effect is able to relate trabecular bone remodeling caused by external loads.

Huiskes et al.'s model has the advantage of modeling the activity of bone resorbing and forming cells separately [20]. It is worth mentioning that osteoblasts and osteoclasts were often assumed to work together in bone, in most of the other models proposed for bone remodeling (e.g. 8-10, 16, 17). In a recent paper, Pogoda et al. discussed that bone resorption is independent of bone formation [38]. Studying transgenic mice, they induced a near-complete and reversible osteoblast ablation. In these animals, osteoblast ablation led to a complete arrest of bone formation accompanied by bone loss, thus illustrating that, in mice, the bone resorption function is independent of bone formation [38]. The most significant characteristic of Huiskes et al.'s model is taking into account this separate activity of actor cells in addition to an implicit coupling by mechanical factors.

There are certain characteristics of our algorithm that contains cellular accommodation (Eq. (8)) that clearly distinguishes it from the algorithms lacking it, e.g. Eq. (4). The response predicted by an algorithm incorporating cellular accommodation effect is path dependent. As Schriefer et al. [29] state, path dependence means that results produced by the algorithm are dependent upon the temporal sequence of the preceding mechanical loading events. Results of this study (Table 1) are also in agreement with studies of Kim et al. who found significant trabecular bone formation occurring in response to mechanical loading within the first week of loading with considerably less bone formation in the fourth week of loading [39]. On the basis of our simulations, we believe that including cellular accommodation effect is indispensable in any bone remodeling theory based on Frost's mechanostat concept [4]. Our results also imply that although Turner's proposal is phenomenological [5], it can be tailored for each new remodeling theory, and can be easily put into mathematical context.

Several limitations associated with this study may have influenced our results. In this analysis the bone tissue was modeled as linear elastic with isotropic material properties. In reality, trabecular bone tissue is an anisotropic material which consists of new and aged tissues at different stages of mineralization. The next step of this research can be including the more complex material properties and anisotropy in the model. Another limitation was that simulations were performed on a 2D model, and were, thus, an estimation of remodeling throughout 3D samples of trabecular bone. Currey's power law (Eq.(9)) for cortical bone was used to relate the change in density to the change in Young's modulus. Ideally, a similar relationship for trabecular bone tissue at microstructure level is required. But, in the absence of such a relationship for trabecular bone tissue, it was assumed that Currey's power law suffices due to the fact that the elastic properties of single trabeculae are very similar to the properties of nearby cortical tissue [40].

Understanding and modeling mechanical adaptation of bone need knowledge from different disciplines. Biomechanical engineers can provide vital contributions by elucidating mechanical rules behind this process, and also by the application of computer simulation. But, in order to have a more applicable bone remodeling theory, engineers' capabilities and potentials should be combined with experimental investigations and insights into different aspects of this complex and multidisciplinary problem. Considering recent advances on the experimental side of the process, e.g. mechanotransduction of bone cells, it is hoped that more useful and applicable theories of bone adaptation will soon emerge.

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REFERENCES

[1] Rouhi G. Theoretical aspects of bone remodeling and resorption processes. PhD Thesis, University of Calgary, 2006.

[2] Wolff J. The law of bone remodeling Das Gesetz der Transformation der Knochen, Kirschwald. (1892) Translated by Maquet P, Furlong R. Springer, Berlin, 1986.

[3] Frost HM, Mathematical elements of lamellar bone remodeling. Charles C. Thomas Publisher, 1964.

[4] Frost HM. A determinant of bone architecture: the minimum effective strain. Clinical Orthopaedics 1983, 175: 286–292.

[5] Turner CH. Toward a mathematical description of bone biology: the principle of cellular accommodation. Calcified Tissue International 1999, 65(6): 466–471.

[6] Saez A, Buguin A, Silberzan P, Ladoux B. Is the mechanical activity of epithelial cells controlled by

deformations or forces? Biophysical Journal 2005, 89: L52–L54.
[7] Jaasma MJ, Jackson WM, Tang RY, Keaveny TM. Adaptation of cellular mechanical behavior to mechanical loading for osteoblastic cells. Journal of Biomechanics. 2007, 40(9), 1938-1945.
[8] Cowin CS, Hegedus DH. Bone remodeling I: A theory of adaptive elasticity. Journal of Elasticity 1976, 6: 313–326.
[9] Hegedus DH, Cowin SC. Bone remodeling II: small strain adaptive elasticity. Journal of Elasticity 1976, 6: 337–352.
[10] Huiskes R, Weinans H, Grootenboer J, Dalstra M, Fudala M, Slooff TJ. Adaptive bone remodeling theory applied to prosthetic-design analysis. Journal of Biomechanics 1987, 20: 1135–1150.
[11] Weinans H, Huiskes R, Grootenboer HJ, The behavior of adaptive bone-remodeling simulation models. Journal of Biomechanics 1992, 25: 1425–1441.
[12] Harrigan TP, Hamilton JJ. An analytical and numerical study of the stability of bone remodeling theories: dependence on microstructural stimulus. Journal of Biomechanics 1992, 25(5): 477–488.
[13] Jacobs CR, Levenston ME, Beaupre GS, Simo JC, Carter DR. Numerical instabilities in bone remodeling simulations: the advantage of a node-based finite element approach. Journal of Biomechanics 1995, 28(4): 449–459.
[14] Mullender MG, Huiskes R, Weinans H. A physiological approach to the simulation of bone remodeling as a self organizational control process. Journal of Biomechanics 1994, 27: 1389–1394.
[15] Mullender MG, Huiskes R, Proposal for the regulatory mechanism of Wolff's law. Journal of Orthopaedic Research 1995, 13: 503–512.
[16] Rouhi G, Herzog W, Sudak L, Firoozbakhsh K, Epstein M. Free surface density instead of volume fraction in the bone remodeling equation: theoretical considerations. Forma 2004, 19 (3): 165–182.
[17] Rouhi G, Epstein M, Herzog W, Sudak L. Free surface density and microdamage in the bone remodeling equation: theoretical considerations. International Journal of Engineering Science 2006, 44(7): 456-469.
[18] Rouhi G, Epstein M, Sudak L, Herzog W. Modeling bone resorption using mixture theory with chemical reactions. Journal of Mechanics of Materials and Structures, 2007, 2(6), 1141-1156.
[19] Rouhi G, A preliminary study on bone resorption: a tri-phasic model, Journal of the Mechanical Behavior of Biomedical Materials (accepted subject to revision).
[20] Huiskes R, Ruimerman R, Van Lenthe GH, Janssen JD. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. Nature 2000, 405: 704–706.
[21] Burger EH, Klein-Nulend J, Mechanotransduction in bone—role of the lacunocanalicular network. The FASEB Journal 1999, 13: 101–112.
[22] Cowin SC, Moss-Salentijn L, Moss ML. Candidates for the Mechanosensry system in bone. Journal of Biomechanical Engineering 1991, 113: 191–197.
[23] Burr DB, Forwood M, Fyhrie DP, Martin RB, Turner CH. Bone microdamage and skeletal fragility in osteoporosis and stress fractures. Journal of Bone Mineral Research 1997, 12: 6–15.
[24] Ruimerman R, Huiskes R, van Lenthe GH, Janssen JD. A computer-simulation model relating bone-cell metabolism to mechanical adaptation of trabecular bone. Computer Methods in Biomechanics and Biomedical Engineering 2001, 4: 433-448.
[25] Chen NX, Ryder KD, Pavalko FM, Turner CH, Burr DB, Qiu J, Duncan RL. Ca (2+) regulates fluid shear-induced cytoskeletal reorganization and gene expression in osteoblasts. American Journal of Physiology and Cell Physiology 2000, 278: C989–C997.
[26] Kreke MR, Huckle WR, Goldstein AS. Fluid flow stimulates expression of osteopontin and bone sialoprotein by bone marrow stromal cells in a temporally dependent manner. Bone 2005, 36: 1047–1055.
[27] Nauman EA, Satcher RL, Keaveny TM, Halloran BP, Bikle DD. Osteoblasts respond to pulsatile fluid flow with short-term increases in $PGE_{(2)}$ but no change in mineralization. Journal of Applied Physiology 2001, 90: 1849–1854.
[28] Turner CH. Three rules for bone adaptation to mechanical stimuli. Bone 1998, 23: 399–407.
[29] Schriefer JL, Warden SJ, Saxon LK, Robling AG, Turner CH. Cellular accommodation and the response of bone to mechanical loading. Journal of Biomechanics 2005, 38: 1838–1845.
[30] Currey JD. The effect of porosity and mineral content on the Young's modulus of elasticity of compact bone. Journal of Biomechanics 1988, 21: 131–139.
[31] Vuori I. Peak bone mass and physical activity: a short review. Nutrition Reviews 1996, 54: 11–114.
[32] Turner C. Exercise as a therapy for osteoporosis: the drunk and the street lamp revisited. Bone 1998, 23: 83–85.

[33] Karlsson MK, Johnell O, Obrant KJ. Bone mineral density in weight lifters. Calcified Tissue International 1993, 52: 212–215.
[34] Alfredson H, Nordstrom P, Lorentzon R. Bone mass in female volleyball players: a comparison of total and regional bone mass in female volleyball players and nonactive females. Calcified Tissue International 1997, 60: 338–342.
[35] Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC. The effects of twelve weeks of bed rest on bone histology, biomechanical markers of bone turnover, and calcium homeostasis in eleven normal subjects. Journal of Bone Mineral Research 1998, 13: 1594–1601.
[36] Baldwin KM, White TP, Arnaud SB, Edgerton VR, Kraemer WJ, Kram R, Raab-Cullen D, Snow CM. Musculoskeletal adaptations to weightlessness and development of effective countermeasures. Medicine & Science in Sports & Exercise 1996, 28: 1247–1253.
[37] Sigmund O. A 99 line topology optimization code written in MATLAB. Structural and Multidisciplinary Optimization 2001, 21: 120–127.
[38] Pogoda P, Priemel M, Rueger JM, Amling M. Bone remodeling: new aspects of a key process that controls skeletal maintenance and repair, Osteoporosis International 2005, 16: S18–S24.
[39] Kim CH, Takai E, Zhou H, von Stechow D, Mueller R, Dempster DW, Guo XE. Trabecular bone response to mechanical and parathyroid hormone stimulation: the role of mechanical microenvironment. Journal of Bone and Mineral Research 2003, 18: 2116–2125.
[40] Rho JY, Roy M, Tsui TY, Pharr GM. Young's modulus and hardness of trabecular and cortical bone in various directions determined by nanoindentation. Transactions of the 43th Annual Meeting of the Orthopaedic Research Society, San Francisco, CA, 1997.