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Inevitable failures in geometric arrangement during bone remodeling determine irreversible bone loss: a study by Monte Carlo simulations

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ABSTRACT

Bone is a dynamic tissue that is constantly being remodeled by the action of osteoclasts (OC) and osteoblasts (OB), which are responsible for bone resorption and formation, respectively. The mechanism through which these cells help the bone tissue remain healthy is still unclear. Great efforts have been made toward the elucidation of bone loss, especially in the case of menopausal women, in order to lessen the effects of osteoporosis. It is known that protein and hormonal alterations may lead to an imbalance in the activities of OC and OB, thereby favoring the onset of bone disease. Other causes that are not associated with this imbalance are currently under investigation. We have carried out computer simulations in order to identify topological arrangements that could result in natural bone loss during the remodeling process. On the basis of a 2D model, which treats the OC and OB cells explicitly, bone remodeling was guided by an OB activation function of the $1/r^4$ type ($r =$ distance to the site resorbed by OC), whose value determines bone formation when a predefined Activation Threshold (AT) is exceeded. We have verified the existence of a critical AT that can significantly affect bone mass balance, thus producing failures in the geometric arrangement of the trabecula. These failures become inevitable and irreversible with age.

Keywords: Monte Carlo simulations, bone remodeling, osteoporosis.

1 INTRODUCTION

Bone remodeling is a dynamic process carried out by osteoclast (OC) and osteoblast (OB) cells, which work together as a basic multicellular unit (BMU). In this process, old bone is removed from the skeleton (bone resorption) and new bone is added to it (bone formation). An imbalance in the relationship between the activities of OC and OB leads to many metabolic bone diseases. The interactions between OB and OC, known as coupling, guarantee a proper balance between bone gain and loss [1].

Osteoporosis (OP) is a common disease of the skeletal system, characterized by reduction in the bone mass and degeneration of its microstructure. From a clinical viewpoint, this condition promotes high susceptibility to systemic fractures [2]. Osteoporosis has become a major health concern since there has been a sharp increase in the prevalence of OP among the elderly over the last decades [3].

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From a mechanical perspective, fractures in osteoporotic individuals result from alterations in the bone structure, which make it less resistant to mechanical stress. This may be due to either a decrease in bone mass or the daily load demand applied on the same bone region [2, 4, 5]. Such bone mass loss is a consequence of the imbalance in the dynamics of the BMU, which may lead to negative bone formation in relation to bone resorption.

This BMU imbalance may be caused by protein and hormonal changes, as well as other biochemical and physical factors that influence the processes of cell activation and differentiation. An imbalance in the production of cytokines affects the activities of OB and OC, thereby leading to common bone diseases. Osteoporosis is a very common example of a disease that may be caused by excess of the osteoprotegerin cytokine [16].

Bone inactivity or low mechanical stress may also affect osteoclast differentiation [17]. The bone structure generates mechanical signals at the cellular level upon mechanical stress, but their exact nature remains unknown [18, 19]. The regulation of bone remodeling is complex, and it involves many factors that can influence both bone formation and resorption [20].

During menopause, bone loss is often associated with the aging process responsible for decreased hormonal activity, as in the case of estrogen [22]. Lack of estrogen may raise the frequency of osteoclast activation and reduce its apoptosis [23]. However, it has recently been verified that rapid bone loss in osteoporosis can also be explained by mechanobiological processes [24]. It has been found that an increase in the resorption cavity leads to bone loss by continuous resorption of damaged tissue. In this same context, it has been shown that the rate of the local growth of tissue formed by osteoblasts is strongly influenced by the geometrical features of channels in an artificial tridimensional matrix [25]. The geometric arrangement of the local environment can be a critical factor in determining cell behavior and, consequently, the bone growth patterns.

Computer simulation model two-dimensional has allowed to explain adaptations observed in trabecular bone in a conceptual sense [26]. In this study we have employed a computer simulation of bone remodeling in order to obtain a more detailed description of this process. We have also investigated the possible effects of geometric arrangement failures on bone mass loss, particularly in menopausal women and in the elderly.

We introduce a 2D model where osteoblast and osteoclast cells are considered explicitly, so that fundamental aspects of the dynamics of these cells can be captured. Because osteoblast and osteoclast cells provide model parameters, such as their activi-ties, that can be varied independently through the osteoblast and osteoclast cell rates, we expect to distinguish the differences between the effects of such rates from those effectively generated from geometric arrangement during the remodeling process.

2   MATERIALS AND METHODS

2.1   System representation and initial configuration

A 2D-lattice consisting of n sites (50×50) was employed to represent the system. The sites were defined as being unoccupied or occupied by Trabecular Bone (TB), OB cells, or OC cells.

TB was generated in a regular arrangement in the initial system configuration, as in the procedure employed by Huiskes [26]. Figure 1 depicts the initial TB state, where each trabecula has a width equal to 2s ($s =$ site) in the initial configuration, with a 4s distance between them.
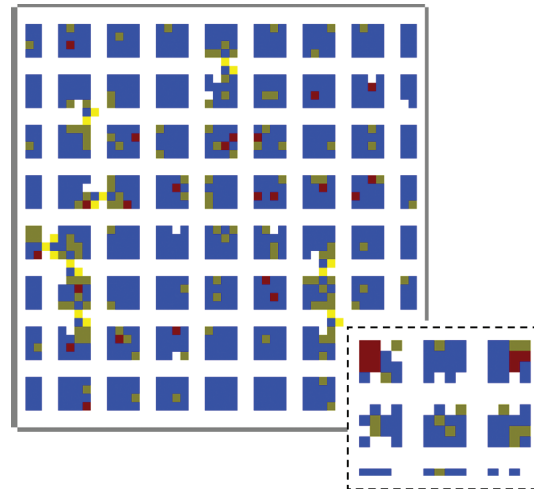

Figure 1 – Initial configuration: white sites correspond to the trabecular bone, where remodeling occurs; blue gaps are interstitial spaces; red and green cells represent osteoclasts and osteoblasts, respectively. The insert, enlarged from a part of the system, corresponds to the simulation that considers $\varepsilon_{OC-OC} = 2.5u$, where the formation of a cluster of OC cells mimicking multinucleated OC cells can be observed.

Initially, the OB and OC cells were randomly placed in the system. The densities of these cells were previously defined as a function of the ratio between the sites occupied by OB and OC and the total number of sites present in the lattice. In the initial configuration, no OC cell was initiated in its active state.

2.2   Resorption and deposition

The total energy involved in a particular system configuration is defined as,

$$E_{total} = E_{local} + E_S, \qquad (1)$$

where E_{local} is the local contact energy between neighbor cells and E_S is the surface energy generated from TB removal (which will be further described in Eq. 4). The term E_{local} measures the amount of energy between the neighbor cells by means of the parameter ε; that is,

$$E_{local} = \sum_{i,j} \varepsilon_{ij} \delta_{ij} \qquad (2)$$

In this equation, the δ_{ij} matrix identifies the sites that are closest to the network. Therefore, when an ith site is the closest neighbor to the jth site, it is said that $\delta_{ij} = 1$; otherwise $\delta_{ij} = 0$. ε_{ij} is the energy involved in the interaction between the i and j sites, whose values can be obtained from the type of cell present in i and j, and includes interactions between the OB-OB, OB-OC, OB-TB, OC-OC, and OC-TB cells.

The values of ε_{ij} in Eq. 2 are ascribed in such a way that the interactions are able to lead the system to typical states that can also be observed experimentally. For instance, a magnitude of $-2.5u$ for ε_{OC-OC} (u = arbitrary energy unit) and $\beta = 1$ results in a stable contact between OC cells, mimicking the conditions observed in the case of cells generated from the multinucleation of mononuclear osteoclasts (giant cells). On the other hand, a magnitude of $\varepsilon_{OC-TB} = -0.2u$ for may lead to a moderate stability for the contact between OC and TB, which is enough to favor a shift of OC beneath the trabecula at the same time it can freely move from one site to another.

2.3   Osteoclast and osteoblast activity

An OC cell located in a certain site i can only absorb TB from a neighbor site j when it is active. When OC is selected for displacement from one site to another, a new activation state will always be conferred to it by means of the activation probability Ω, given by the equation

$$\Omega_{ij} = \left\{ \begin{array}{ll} 1 & \text{if } rand \ \le p_0 \\ 0 & \text{if } rand \ > p_0 \end{array} \right\}, \qquad (3)$$

where $rand$ is a number between 0 and 1 that is randomly obtained, and p_0 is the expected TB activation probability in the absence of stress. In this way, when $\Omega_{ij} = 1$, the OC cell in i is active and capable of removing TB from the site j (Fig. 2a). When i has more than one neighbor (neighbors j, k, l, and m), the choice of which neighbor will be removed shall be random.
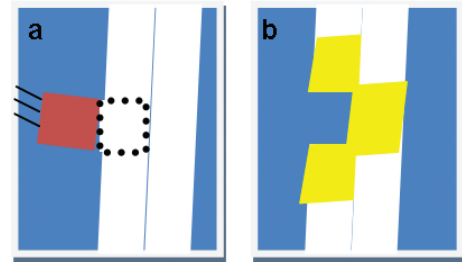

Figure 2 – Bone resorption. a) Osteoclast cell (red) approaching the surface of the trabecula for absorption (dotted line denotes the ith site involved in the removal). b) Sites next to i (yellow) activated after i removal.

When a TB cell is removed from a site by OC, all the neighboring TB sites are perturbed (Fig. 2b), and a surface energy responsible for the recruitment of OB cells comes into effect as

$$E_S = \sum_{i,j} \frac{\rho}{d_{ij}^4}, \qquad (4)$$

where d_{ij} is the distance between the ith osteoblast and the jth site activated by osteoclasts (yellow sites in Fig. 2b) and ρ is a positive constant.

Although the perturbed sites (PS) by osteoclasts can generate activated neighbors, thus resulting in the rapid approach of OB cells, deposition will only occur when the value of E_S (Eq. 4) is above the pre-defined Activation Threshold (AT). If this condition is satisfied, OB will be able to deposit bone in one of its neighboring sites if the following requirements are also met:

I. $E_S > $ AT, and

II. when its neighbor is next to TB (see Fig. 3, cases I and II).

If the aforementioned conditions are satisfied, this site will become a potential TB, here defined as deposition site (DS). If other DSs are present in the surroundings of OB, the choice of deposition site is random.

Still, if OB approaches DS, and if the latter is occupied by another OB (Fig. 3, case II), DS becomes a TB site independent of the presence of OB. This corresponds to the conversion of an osteoblast into an osteocyte when OB is captured by the bone matrix. Whenever an OB cell is converted into TB as pointed above, a new OB is randomly inserted into a site of the system.

Finally, when an OB cell close to DS has $E_S <$ AT, TB deposition does not take place, and an accumulation of OB cells can occur in the surroundings of DS (Fig. 3, case III). Following osteoblast deposition, the remodeling cycle in that site is halted, and remodeling can only happen again when activated OC cells establish a new contact with this site.
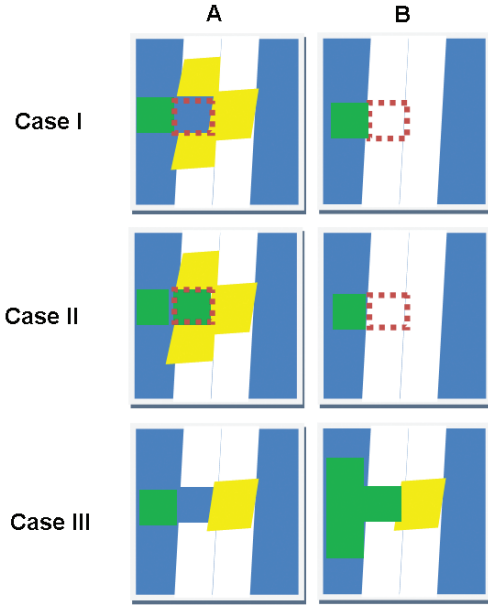
Figure 3 – Osteoblast action – Case I: A) OB (green) approach to DS surrounded by three active sites (yellow) and B) TB (white, dotted) just deposited by OB. Case II: A) OB approach to DS containing another OB cell and B) replacement of OB with TB in DS. Case III: A) OB approach to DS surrounded by 1 active site (yellow), the E_S energy of the approaching OB is lower than AT, and B) accumulation of OB cells in the surroundings of DS due to E_S.

When a TB cell is removed from an ith site of the network by OC, it is also necessary to assess the presence of bone fragments that are not associated with the network. This can be accomplished by verifying whether the TB neighbors of the site i can make contact with the system borders by means of consecutive sites of the TB type. This verification is carried out upon each removal with the aid of a recursive function that is able to identify, from i, whether there is percolation via sites of the TB type only. If this is not the case, the fragment is eliminated from the trabecular lattice, and the corresponding energy is subtracted from the total energy of the system (Fig. 4).

Figure 4 – Schematic representation of TB elimination. a) Osteoclast (red) acting on the bone surface (white); b) bone after removal of one TB site; c) removal of a TB fragment that is not in contact with the network borders.

2.3.1   Movements of OB and OC cells

A new system configuration is always attempted when a certain OB or OC cell is moved. Initially, an ij site containing either OB or

OC is randomly selected. Next, its position is modified to one of its first $nviz$ neighbors or it can remain in its own site. In other words, the movement can occur in the $[i \pm nviz, j \pm nviz]$ range.

The transition of a cell from the X state to a Y state follows the metropolis criteria [27], whose probability $p(X \rightarrow Y)$ is given by

$$p(X \rightarrow Y) = \min\left[1, \exp\left(-\beta \Delta E\right)\right], \qquad (5)$$

where β is a constant related to the system fluctuation and ΔE is the difference between the energies of the configurations Y and X; therefore,

$$\Delta E = E(Y) - E(X),$$

and E can be obtained by Equation 1.

2.4   Initial parameters

A total of 1.2×10^9 random configurations are produced for each simulation accomplished consecutively. A Monte Carlo Step (MCS) corresponds to 120 attempts to move or alter the state of a site/neighborhood. Therefore, each simulation comprises 10^7 MCSs.

The osteoclast and osteoblast densities were defined as 0.05 and 0.01, respectively. Although this difference results in five times more cells in the case of OC, osteoclast nucleation is driven by the contact energy ε (Eq. 2), so that OC cell clusters corresponding to multinucleate osteoclasts (MO) are formed. In this way, the number of MO is comparable to the total number of OB cells.

An $nviz$ value of 10 was chosen so that, according to the 2D model defined here, the OB and OC cells would be able to move from one trabecula to another with no ergodicity problems [28]. At the same time, nviz was minimized so as to maintain the locality features of the system, somehow enabling one to capture the cell dynamics. In a 3D model, $nviz$ could be minimized to 01 (one), since the interstitial fluid could be displaced from any point to any other point in the system without hindering the confinement of OB and OC cells, contrary to what occurs in 2D models.

Because of the OC and OB cell densities as well as the network topology, a value of $p_0 = 0.001$ was chosen, which corresponds to the order of magnitude established by Weinkamer [29]. The magnitude $\rho = 50$ was stipulated in order to ensure rapid attraction between OB and DS up to a certain distance. Therefore, for $d = 1$, the energy E_S between an OB cell in direct contact with a DS (closest neighbor) takes a value of $E_S = \rho$.
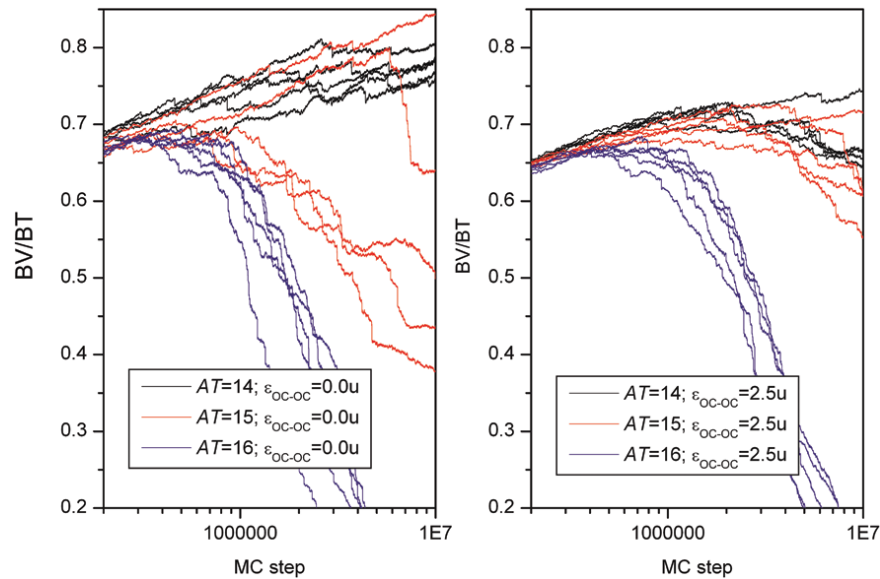
Figure 5 – Evolution of BV/BT following 10^7 MCSs. Five (05) independent simulations (random initial configuration for OB and OC) were carried out for each AT, combining (a) $\varepsilon_{OC-OC} = 0.0u$ and (b) $\varepsilon_{OC-OC} = 2.5u$. For each MCS, 120 configurations are generated. BV/BT values are calculated whenever an MCS is completed.

3   RESULTS

Firstly, we tried to identify the AT range for which a transition between TB loss and gain could be observed after the stipulated total number of MCSs. The behavior of the trabecular bone ratio BV/BT (number of TB cells in relation to the total number of sites in the lattice) was then accompanied for AT values of 14, 15, and 16. For each AT value, five (05) independent simulations (different initial states for OB and OC) were accomplished in the cases of $\varepsilon_{OC-OC} = 0.0u$ and $\varepsilon_{OC-OC} = 2.5u$ (Fig. 5 a-b). In both situations, we verified that transition occurs in the $14 < AT < 16$ range, since for $AT = 14u$ and $AT = 16u$ there is bone gain and loss, respectively, whereas the two processes take place when $AT = 15u$ (apparent instability). By comparing the differences between $\varepsilon_{OC-OC} = 0.0u$ and $\varepsilon_{OC-OC} = 2.5u$, it is possible to identify a larger BV/BT gain for $\varepsilon_{OC-OC} = 0.0u$, and the differences are more evident in the case of $AT = 14u$.

By increasing the AT transition zone and carrying out at least 05 (five) times more simulations for a smaller AT range, it was possible to detect the existence of a critical AT range around $AT = 15u$. In all the cases, there is an exponential bone mass gain until a critical time $t_c = 10^6$ MCS is reached. Thereafter, the behavior critically depends on the AT value for the onset of bone mass loss to take place. Stable BV/BT behavior does not occur in any of the cases, although it is steadier for $AT = 14.9u$. It is

noteworthy that outside the transition zone, the exponential gain observed for $\varepsilon_{OC-OC} = 0.0u$ and $\varepsilon_{OC-OC} = 2.5u$ (Fig. 5) implies different t_c values, and t_c is longer in the latter case.

In an attempt to understand the reason for the criticality caused by AT, we also followed the behavior of the number of Actived Cells (AC) as a function of AT and its influence on the parameter BV/BT (Figs. 6-7), since resorption and deposition directly depend on AC in our model. As verified in Figure 6, the behavior of AC changes when t_c is reached. An exponential increase in AC can be noted, with a brief equilibrium period around t_c. This behavior is similar for all the AT values. Nevertheless, for $AT < 16$, there is an inversion in the behavior of AC; a sharp reduction takes place after t_c, and it remains saturated for a longer period at $AC \sim 25$ when $\varepsilon_{OC-OC} = 2.5u$. This is more clearly seen in Figure 7, where saturation has a significantly larger BV/BT amplitude for $\varepsilon_{OC-OC} = 2.5u$. On the other hand, when $AT = 16$, the BV/BT behavior in both cases is similar, although there is a more pronounced decrease for $\varepsilon_{OC-OC} = 2.5u$ when $AC > 50$.

4   DISCUSSION

On the basis of our analyses, we suggest that there is an "intrinsic failure" in the dynamics of bone remodeling associated with the resorption and deposition process. In the long term, this failure will affect the topology of the trabecular bone through a mecha-
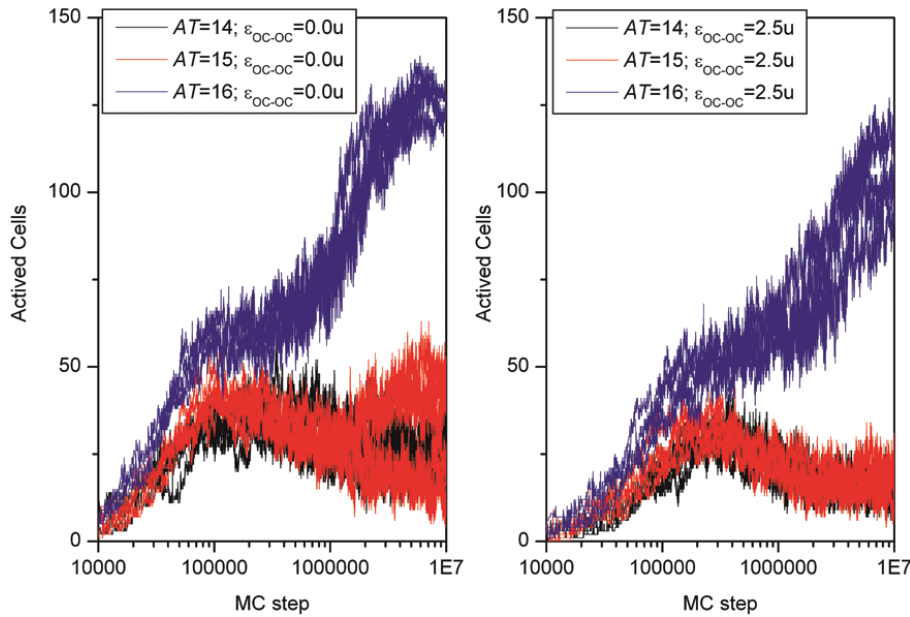
Figure 6 – Evolution of the number of cells activated by OC as a function of AT. Five (05) independent simulations are presented for each AT, shown in different colors.
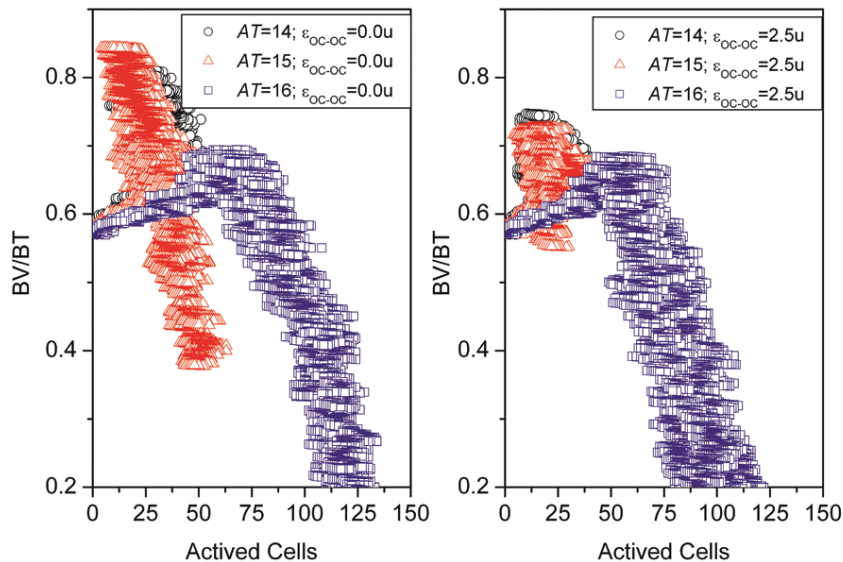

Figure 7 – BV/BT behavior as a function of the number of cells activated by OC at different ATs. Five (05) independent simulations were carried out for each AT, combining (a) $\varepsilon_{OC-OC} = 0.0u$ and (b) $\varepsilon_{OC-OC} = 2.5u$.

nism that does not depend on the balance between OC and OB, causing a reduction in bone mass (Fig. 5). Such alterations become critical when the architecture of the trabecula becomes topologically unfavorable to OB activation. Although this hypothesis is based on our phenomenological model, it is general and valid for any other model whose OC and OB cell dynamics is explicit and occurs by means of functions that depend on the inverse of the distance. Above all, we understand that the specificity imposed by Eq. 4 and by the two conditions involved in deposition are based on first principles essential to the model. Even if the behavior of E_S as a function of the distance were redefined ($1/r$, $1/r^2$ etc), the criticality in terms of AT observed here would still occur. This is clear from Figure 7, where it is possible to note that E_S may affect the initial topology and trigger a new TB struc-

Case A

Case B

$$E_s^i = E_{ij} + E_{ik} + E_{il}$$

$$E_s^i = -\frac{f_i}{(s\sqrt{2})^4} - \frac{f_i}{(2s)^4} - \frac{f_i}{(s\sqrt{2})^4}$$

$$E_s^i = -0.56\frac{f_i}{s^4}$$

$$E_s^i = E_{ij} + E_{ik} + E_{il}$$

$$E_s^i = -\frac{f_i}{\left(s\sqrt{5}\right)^4} - \frac{f_i}{\left(s\sqrt{5}\right)^4} - \frac{f_i}{s^4}$$
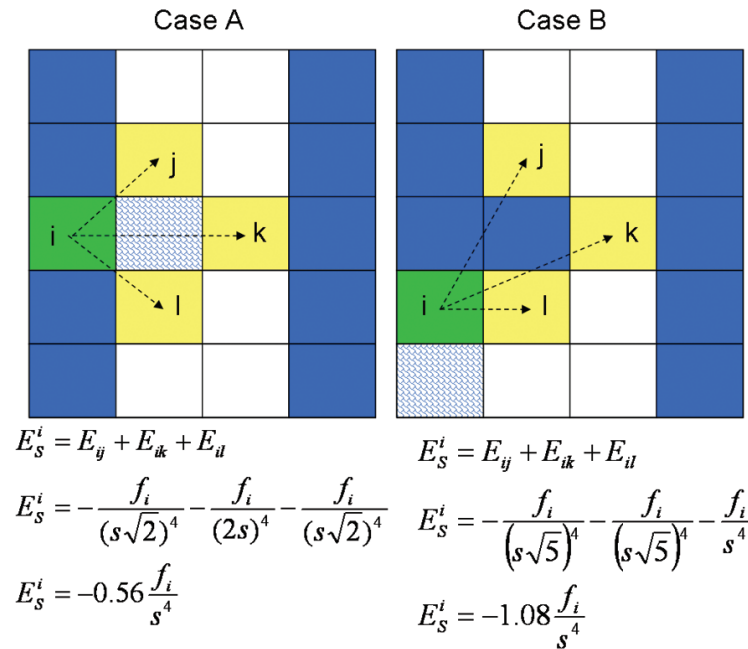
$$E_s^i = -1.08\frac{f_i}{s^4}$$

Figure 8 – Representation of deposition by OB generating topological damage. In case A, the OB cell (green) deposits bone on the site absorbed by OC (shaded site), and the structure of the trabecula is maintained. Although deposition is more favored in case B because E_S is lower than in A, deposition takes place in another site, not in the removed one.

ture that does not favor OB activation. It is noteworthy that the sharp decrease in BV/BT observed in Figure 7 for $AC > 50$ is an evidence of this hypothesis which, in this particular case, is critical from this AC value due to the size of the network under consideration. Therefore, in an environment unfavorable to OB activation, the system will trigger TB resorption only, thereby resulting in acceleration of irreversible bone mass loss. Moreover, if factors associated with menopause are also considered, such as the frequency of BMU activation [30], topological failures will be amplified, leading to more precocious bone loss.

As already mentioned, OC cells are known as giant cells [31]. They contain various nuclei and are present in larger quantities compared with OB cells, which in turn are smaller and mononucleated [32]. From a physical viewpoint, resorption taking place in a certain region leaves a gap that should be later filled with OB, thereby avoiding the loss of structural balance. Bearing in mind the size of an OC cell, the thickness of this gap should be much larger than the dimensions of an OB cell [33], thus favoring OB penetration and deposition. However, the thickness of this gap should decrease as it is filled with OB, until its dimension becomes the same as that of an osteoblast [34]. This is the moment when the situation in real bones resembles the network model studied here, once the size of the site removed by OC is always the

same as that occupied by OB. This means that it is not possible to avoid the topological damage to the trabecular structure highlighted previously because of the different dimensions of these cells, once at some moment these dimensions will be compared. In this way, it is expected that the same type of microdamage observed in Figure 8; that is, the one caused by E_S, should also be found when cells with distinct dimensions are treated, as in the case of the real system. It is true that other microdamage could also take place along the gap, which would worsen the damaging effects on the bone structure during deposition. This reinforces the importance of the fact that OC cells are multinucleated, since multinucleation provides this kind of system with balance [35].

Considering $\varepsilon_{OC-OC} = 2.5u$, OC cell clusters are generated in such a way that multinucleation is mimicked (Fig. 1, insert). Nevertheless, even though stability among the cells of this cluster exists, it does not mean that all the cells will be activated at the same time, since their activation occurs independently according to Eq. 3. Therefore, considering $\varepsilon_{OC-OC} = 2.5u$ influences "kinetics" much more than it affects the joint local action of the cells for the formation of larger cavities.

Figures 5-6 reveal there is a critical threshold for AT, which seems to be around $15u$. When the threshold is below this critical value, bone mass loss for $\varepsilon_{OC-OC} = 0.0u$ increases
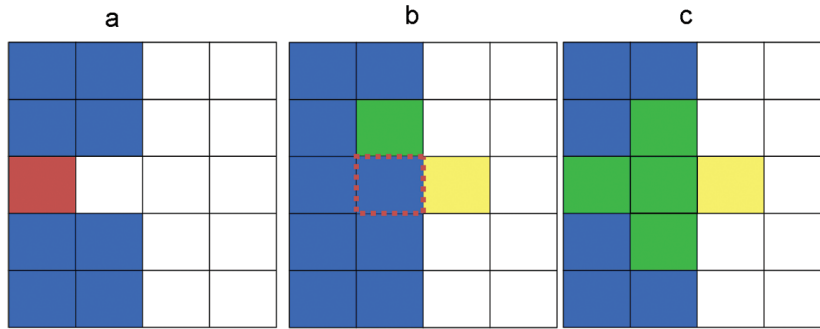
Figure 9 – Representation of the reduction in the number of activated cells (yellow) by OC (red) due to failure during deposition (Fig. 10, case B). (a) Approach of OC, to remove TB (white); (b) after TB removal, only one site is activated; (c) after TB removal, the energy is not sufficient for OB activation, leading to the accumulation of OB cells (green) in the surroundings of the activated cell.

exponentially with $AT = 14u$ (Fig. 5). At a first glance, this sounds contradictory because OC cells have much more freedom to move and absorb TB in different parts of the surface when $\varepsilon_{OC-OC} = 0.0u$. However, a larger number of AC cells accumulate in the network compared with $\varepsilon_{OC-OC} = 2.5u$. Consequently, when $AT < 15$, a higher proportion of sites involving AC cells results in DS. In this case, bone mass gain is expected to occur if the OB density is enough to deal with all the DS sites at the same time, thereby deactivating AC cells. On the other hand, when $AT > 15$, many of the sites surrounded by AC cells do not give rise to DS, leading to the subsequent accumulation of OB cells around AC cells because of the ES energy. In this way, if many of these sites do not result in $E_S > LA$, various OB cells will be retained in the surroundings of AC (see Fig. 9 for the representation), producing a delay in the displacement of OB to the other network sites (DS or not). If no deposition by OB takes place, bone mass is reduced because OC cells continue absorbing TB independent of OB.

Metabology studies have shown that bone density rises until 30 years of age, and the bone mass peak is reached between adolescence and 35 years of age, declining thereafter due to a complex combination of factors [36]. One way to delay the onset of osteoporosis is to increase bone mass during childhood and adolescence, especially through the practice of physical activities. In the elderly, physical activity placing mechanical stress on the bone has been shown to reduce osteoporosis [37].

Nevertheless, although remodeling responds to both an increase and reduction in mechanical demand, it is easier to lose bone mass through inactivity than via a rise in mechanical stress. A bedridden young adult may have a 1% spine density loss in one week, but it will take them almost a year to regain this same

mass [37]. Mechanical loads could favor a rise in E_S, thus increasing the probability of reduced bone mass loss even when $AT > 15$. However, because the area covered by topological damage becomes larger with time, there would be no correspondence between resorption and deposition after a certain period, even under the action of mechanical stress capable of favoring mass gain. In other words, no matter how beneficial and efficient the strain on the bone is, this stress would be abolished in the tips because of the pores generated from deposition failure. As a consequence, the sites of the AC type in these regions are more susceptible to deactivation by OB, giving rise to a more porous structure. In Figure 5, note that for $\varepsilon_{OC-OC} = 2.5u$ there is an initial bone mass gain followed by short stability in all cases. Even in the cases where the bone mass gain is larger, $AT < 15$, there is inevitable onset of mass loss some time later. This suggests that even under stimulation or the action of a compound capable of altering the balance between OB and OC, thereby minimizing loss, a reduction in bone mass is irreversible due to the inevitable damage to bone topology.

5 CONCLUSION

In this work, through a simple two-dimensional model, we reveal aspects of the kinetics of remodeling that indicate a phase transition of bone mass due to topological failures that accumulate during the deposition. Our hypothesis is that during the deposition of bone by OB, faults can occur in the geometrical arrangement the long term and create an topological condition energetically unfavorable to OB activation. This condition, regardless of the activation threshold or the proportion of these cells seems to be inevitable because of the nature of the interactions that occur between OB and TB.

However, we understand that in a real system where there is presence of mechanical forces that are essential for maintaining bone mass, the effects of these failures could be minimized or delayed, but long term they still affect the loss of bone mass. This can be confirmed in our future work to include stress in our model and assess the magnitude of these flaws in a three-dimensional model. This will validate the theory here proposed in a quantitative sense. For this, more sophisticated algorithms involving finite element analysis must be implemented in our model, which will require much more cpu time to perform our exhaustive analysis.

ACKNOWLEDGMENTS

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