Spatio-temporal structure of cell distribution in cortical Bone Multicellular Units: a mathematical model

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Abstract

Bone remodelling maintains the functionality of skeletal tissue by locally coordinating bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts) in the form of Bone Multicellular Units (BMUs). Understanding the emergence of such structured units out of the complex network of biochemical interactions between bone cells is essential to extend our fundamental knowledge of normal bone physiology and its disorders. To this end, we propose a spatio-temporal continuum model that integrates some of the most important interaction pathways currently known to exist between cells of the osteoblastic and osteoclastic lineage. This mathematical model allows us to test the significance and completeness of these pathways based on their ability to reproduce the spatio-temporal dynamics of individual BMUs. We show that under suitable conditions, the experimentally-observed structured cell distribution of cortical BMUs is retrieved. The proposed model admits travelling-wave-like solutions for the cell densities with tightly organised profiles, corresponding to the progression of a single remodelling BMU. The shapes of these spatial profiles within the travelling structure can be linked to the intrinsic parameters of the model such as differentiation and apoptosis rates for bone cells. In addition to the cell distribution, the spatial distribution of regulatory factors can also be calculated. This provides new insights on how different regulatory factors exert their action on bone cells leading to cellular spatial and temporal segregation, and functional coordination.

Keywords: bone cell interactions, cortical BMU, spatio-temporal bone remodelling, RANK–RANKL–OPG, mathematical modelling

1 Introduction

In human adults, between 5 and 30% of bone volume is replaced every year [1, 2] in a process referred to as remodelling. Bone replacement is accomplished by stand-alone groups of cells of the osteoclastic and osteoblastic lineage progressing through old bone over a period of several weeks. Such a group of cells is called a “Bone Multicellular Unit” (BMU) and can be viewed as the basic functional unit for bone remodelling [3, 4, 5, 6]. Tetracycline-based histomorphometry has considerably helped in the elucidation of the spatial organisation and kinetic properties of the different bone cells in cortical BMUs [7, 8, 9], which clearly indicates a spatial segregation of bone cell types depending on cell maturity. At the front of a BMU, in a region called the resorption zone (see Figure 1), active osteoclasts attach to the bone surface and dissolve bone by secreting a mixture of proteases that break down the collagenous matrix, and hydrogen ions that reduce the pH and dissolve the minerals into the micro-environment [10, 11]. Towards the back of the BMU, in the so-called formation zone, active osteoblasts refill the cavity by laying down a collagen-rich substance known as osteoid, which subsequently mineralises to form new bone over the following month or so (see [7, 1, 2]). The region between the resorption zone and the formation zone, referred to as the reversal zone, contains precursor cells of both lineages embedded in a loose connective tissue stroma [7]. New precursor cells and nutrients are supplied to the BMU by a small capillary that grows at the same rate as the BMU progresses into the bone. The net effect of the passage of a BMU at a specific location of bone is the local renewal of the bone matrix and the formation of a so-called “secondary osteon”, which includes a new Haversian canal.

The existence of such a functional remodelling unit (referred to by Frost as a “packet of turnover” [3]) suggests the presence of tight couplings between the various cell types composing BMUs. It has been hypothesised several decades ago that some combination of local and/or systemic signals structure this internal cellular organisation [3, 6]. In the mid 1990s, the discovery of the RANK–RANKL–OPG pathway explained many previous experimental observations.
This regulatory pathway can be expected to play a major role in BMU physiology. Many other potential regulatory molecules have been found by experimental biologists (including systemic hormones, nerve signals, vascular agents, growth factors, chemokines, etc; see [12, 13, 14]). However, it is yet to be proven that these local interactions are able to group several generations of osteoclasts and osteoblasts in the form of BMUS that present a clear spatial and temporal separation of these cellular activities. While the structure of BMUS is well understood at a descriptive level [7, 1, 2], how this structure is linked to the fundamental underlying cellular interaction mechanisms remains to be elucidated. The present work aims to address this question.

In this paper, we extend our previous temporal model of bone remodelling [14] into a one-dimensional spatio-temporal model. Using this model, we study how bone cells structure themselves into a cortical BMU under the action of intercellular signalling. This model is based on fundamental material-balance equations expressed as partial differential equations (PDEs). Non-conservative production or elimination of biochemical components in these general continuity equations are prescribed in accordance with the known biochemistry currently believed to play the most important role in bone remodelling. As such, the model explicitly includes transforming growth factor β (TGF-β), parathyroid hormone (PTH) and the receptor–activator nuclear factor κβ axis consisting of the receptor RANK, the ligand RANKL and the soluble decoy receptor osteoprotegerin (OPG). These regulatory factors couple two cell types of the osteoclastic lineage (a third one is introduced in Section 4) and three cell types of the osteoblastic lineage. Other components of the cellular communication system, known and unknown, are introduced implicitly through various model parameters and external model conditions. For example, the capillary assisting the progression of a cortical BMU is modelled as a localised supply of bone precursor cells around the capillary’s (growing) tip. Under these assumptions, we find that the model admits solutions for the cell distributions in the form of travelling waves that have profiles that match the observed internal spatial organisation of a cortical BMU.

In recent years, several teams of researchers have elaborated mathematical and computational models of bone remodelling, generally monitoring the evolution of the bone cells over time via ordinary differential equations (ODEs) [15, 13, 14]. Recently, Ryser et al. have included a spatial dimension in the model [15], addressing the important question of interaction between locally-expressed RANKL and soluble OPG for a trabecular BMU [16, 17]. In their model, BMUs are driven by a RANKL field in the surrounding bone matrix. Other researchers have developed cellular automata simulations to model resorption and formation on a per site basis [18].

To our knowledge, no group has yet addressed the issue of internal structuring of cortical BMUS. Our approach emphasises the detailed integration of the biochemical processes involving osteoclastic and osteoblastic cells at several maturation stages into a comprehensive partial differential model of the cortical BMU. Since it is based on a general formulation of the material-balance equation, the construction of the model is modular and extensible. New interaction pathways or cell types can be included as needed. The one-dimensional continuous formulation employed here enables us to investigate analytically how the various cell distributions making the internal structure of the BMU depend on the model assumptions.

The paper is organised as follows: the model formulation is described in Section 2. In Section 3, the system of coupled nonlinear PDEs is then solved nu-
merically for the various cell and regulatory factor distribution profiles along the BMU. Theoretical investigations of these profiles are performed, allowing us to map some of the profiles’ properties to parameters of the model. In Section 4, we investigate the effects of various model assumptions made in Section 2. Finally, we extend the initial model to include a new differentiation stage for osteoclasts, which is required to explain their observed spatial migration from the reversal zone to the resorption zone (see Figure 1). Concluding remarks are made in Section 5.

2 Mathematical model of cortical BMU remodelling

In the confined environment of a cortical BMU, the most important phenomena taking place are the biochemical interactions between the cells and their regulatory factors, as well as the directed or diffusive transport of these entities. These phenomena are described in general by the material-balance equations of the species considered [19, 20, 21]:

$$\frac{\partial}{\partial t} n_A(r, t) = \sigma_A(r, t) - \nabla \cdot J_A(r, t).$$  (1)

In Eq. (1), $A$ denotes any cell type or regulatory agent (such as hormones, growth factors, paracrine factors, etc.) explicitly accounted for in the model; $n_A(r, t)$ is the local density or concentration of $A$ (i.e., number of entities $A$ per unit volume) at point $r$ in space and at time $t$ ($r$ is a position vector); $\sigma_A(r, t)$ denotes local source/sink terms that account for non-conservative mechanisms, such as cellular proliferation, differentiation, apoptosis, or mass action kinetic rates of the regulatory factor binding reactions; $J_A(r, t)$ is the flux associated with transport properties of $A$, such as diffusion, advection, or resulting from inherent motility, e.g. chemotaxis. Due to the interactions between cells and regulatory factors, the material-balance equations (1) written for all $A$s are coupled. These couplings may arise both through the source/sink terms (e.g. hormonal up-regulation/down-regulation of a cellular response) and through the fluxes (e.g. chemotaxis). Note that since the fluxes are differential in space, they are expected to play an important role in the spatial organisation of the cells within the BMU.

In practice, the definition of local densities relies on a representative volume element large enough to contain many entities, yet small enough to remain local. While only few cells are present in a single BMU, continuous cellular densities can be defined in a statistical sense [20], i.e., by averaging histograms of cell counts over an ensemble of similar BMUs (see Figure 1).

Osteoclasts

Following the ODE model of bone remodelling proposed by Pivonka et al. [14], we consider two stages of osteoclast development: “precursor osteoclasts” (OCp) and “active osteoclasts” (OCa). Precursor osteoclasts are assumed to have derived from hematopoietic progenitor cells and to be delivered to the BMU cavity at the tip of the capillary (see Figure 1). In cortical BMUs, it takes 3 to 4 days for (single-nucleated) pre-osteoclasts to differentiate, migrate and join the dozen or so active multinucleated osteoclasts (each composed of around 10 nuclei) found at the front of the BMU. These individual nuclei in active osteoclasts are then degraded after around 12 days [9, 7, 22]. In the model, OCp represent single nucleated entities incorporated in a multinucleated active osteoclast, and OCa turn into OCa upon RANKL-mediated activation of their RANK receptor [11, 12]. Transforming growth factor $\beta$ is known to be a general inhibitor of osteoclast differentiation and activation [11]. For simplicity, here we only assume that OCa apoptosis is enhanced by the presence of TGF-$\beta$. Osteoclast maturation in the model can be summed up schematically as:

$$\text{OCp} \quad \xrightarrow{\text{RANKL}} \quad \text{OCa} \quad \xrightarrow{\text{TGF-}$\beta$} \quad \emptyset.$$  (2)

We translate this sequence of events into the following balance equation for OCa:

$$\frac{\partial}{\partial t} n_{\text{OCa}} = D_{\text{OCa}}(\text{RANKL}) n_{\text{OCp}} - $\alpha_{\text{OCa}}(\text{TGF-}$\beta$) n_{\text{OCa}} - \nabla \cdot J_{\text{OCa}},$$  (3)

where $D_{\text{OCa}}$ is the RANKL-dependent differentiation rate of OCp and $\alpha_{\text{OCa}}$ the TGF-$\beta$-dependent apoptosis rate of OCa. As in Ref. [14], the up-regulation and down-regulation of cellular responses by a ligand

To align with common practice, we shall use the terminology “density” for cells and “concentration” for regulatory factors even if the units are chosen the same.
are assumed proportional to the fraction of occupied receptors. Mass action kinetics of the binding reactions shows that this is equivalent to modulating the cellular responses by certain “activator” and “repressor” functions of the ligand concentration (see Refs. [13, 14, 23] for more details). With the dimensionless activator and repressor functions

\[ \pi^{\text{act}}(\xi) = \frac{\bar{\xi}}{1 + \bar{\xi}}, \quad \pi^{\text{rep}}(\xi) = 1 - \pi^{\text{act}}(\xi) = \frac{1}{1 + \bar{\xi}}, \]  

(4)

the functional forms of \( \mathcal{D}_{\text{oc}} \) and \( \mathcal{A}_{\text{oc}} \) can thus be written as

\[ \mathcal{D}_{\text{oc}}(\text{RANKL}) = D_{\text{oc}}^{\text{act}} \frac{n_{\text{RANKL}}}{k_{\text{RANKL}}}, \]  

(5)

\[ \mathcal{A}_{\text{oc}}(\text{TGF-\beta}) = A_{\text{oc}}^{\text{act}} \frac{n_{\text{TGF-\beta}}}{k_{\text{TGF-\beta}}} \]  

(6)

where \( k_{\text{RANKL}} \) and \( k_{\text{TGF-\beta}} \) are dissociation binding constants, and \( D_{\text{oc}} \) and \( A_{\text{oc}} \) are the maximal possible rates taken by \( \mathcal{D}_{\text{oc}} \) and \( \mathcal{A}_{\text{oc}} \).

In the confined space of a cortical BMU, cell diffusion is limited and we assume that directed motility dominates the movement of osteoclasts. The flux of \( \text{OC}_s \) can thus be written \( \mathbf{J}_{\text{oc}} = n_{\text{oc}} \mathbf{v}_{\text{oc}} \), where \( \mathbf{v}_{\text{oc}} \) is the velocity of \( \text{OC}_s \) cells with respect to the (fixed) bone matrix. The actual velocity of an active osteoclast is a combination of the dissolution process of the bone matrix, and of chemotactic and/or mechanotactic signals [10, 11, 24, 25, 26]. Precisely how this sensing by osteoclasts of their mechanochemical micro-environment occurs is still uncertain and not an issue for the purposes of this paper. For this reason, in our model, the rate of movement of \( \text{OC}_s \) is simply taken to be constant, matching the average velocity \( \mathbf{u} \) of the BMU’s progression through bone:

\[ \mathbf{J}_{\text{oc}} = n_{\text{oc}} \mathbf{u}. \]  

(7)

Note that typical cortical BMU velocities range from 20 to 40 \( \mu \text{m/day} \) [7, 1, 2].

**Osteoblasts**

Following the ODE model of bone remodelling proposed by Pivonka et al. [14], three stages of osteoblast maturation are considered. “Uncommitted progenitor osteoblasts” (\( \text{OB}_s \)) denote a pool of mesenchymal stem cells assumed to be provided around the tip of the capillary [8, 7, 1]. These cells are capable of committing to the osteoblastic lineage, becoming “pre-osteoblasts” (\( \text{OB}_{p} \)). This commitment is up-regulated by TGF-\( \beta \) [27, 28, 29]. Pre-osteoblasts further mature into “active osteoblasts” (\( \text{OB}_{a} \)), found in large numbers (1000–2000 cells) at the back of cortical BMUs (see Figure 1), actively laying down osteoid to refill the cavity opened by the osteoclasts [7]. Based on Pivonka et al. [14], osteoblast activation is assumed to be down-regulated by TGF-\( \beta \). The fate of active osteoblasts is either to be buried in osteoid and become osteocytes (approximately 95% of all bone cells are osteocytes), to undergo apoptosis, or to become so-called bone-lining cells covering the surface of the new Haversian canal [1]. Elimination of \( \text{OB}_{a} \) from the active pool is assumed here to include all three possibilities. Osteoblast development in the model can thus be depicted as the sequence

\[ \text{OB}_{a} \rightarrow \text{OB}_{p} \rightarrow \text{OB}_{u} \rightarrow \ldots, \]  

(8)

leading to the following balance equations for \( \text{OB}_{a}, \text{OB}_{p}, \text{OB}_{u} \):

\[ \frac{\partial}{\partial t} n_{\text{OB}_{a}} = \mathcal{D}_{\text{OB}_{a}}(\text{TGF-\beta}) n_{\text{OB}_{a}} - \mathcal{A}_{\text{OB}_{a}}(\text{TGF-\beta}) n_{\text{OB}_{a}} - \nabla \cdot \mathbf{J}_{\text{OB}_{a}}, \]  

(9)

\[ \frac{\partial}{\partial t} n_{\text{OB}_{p}} = \mathcal{D}_{\text{OB}_{p}}(\text{TGF-\beta}) n_{\text{OB}_{p}} - \mathcal{A}_{\text{OB}_{p}}(\text{TGF-\beta}) n_{\text{OB}_{p}} - \nabla \cdot \mathbf{J}_{\text{OB}_{p}}, \]  

(10)

where \( \mathcal{D}_{\text{OB}_{a}}, \mathcal{D}_{\text{OB}_{p}} \) and \( \mathcal{A}_{\text{OB}_{a}}, \mathcal{A}_{\text{OB}_{p}} \) are the \( \text{OB}_{a} \) differentiation rate, the \( \text{OB}_{p} \) differentiation rate and the \( \text{OB}_{u} \) elimination rate, respectively. Similarly to Eqs. (5)–(6), we set

\[ \mathcal{D}_{\text{OB}_{a}}(\text{TGF-\beta}) = D_{\text{OB}_{a}}^{\text{act}} \frac{n_{\text{TGF-\beta}}}{k_{\text{TGF-\beta}}}, \]  

(11)

\[ \mathcal{D}_{\text{OB}_{p}}(\text{TGF-\beta}) = D_{\text{OB}_{p}}^{\text{act}} \frac{n_{\text{TGF-\beta}}}{k_{\text{TGF-\beta}}}, \]  

(12)

with \( k_{\text{TGF-\beta}} \) denoting dissociation binding constants and \( D_{\text{OB}_{a}}, D_{\text{OB}_{p}} \) corresponding to the maximal possible rates taken by \( \mathcal{D}_{\text{OB}_{a}} \) and \( \mathcal{D}_{\text{OB}_{p}} \).

Active osteoblasts lay down osteoid in cortical BMUs mainly radially, from the circumference of the cavity towards the center [8, 7, 1]. As this process occurs on a time scale much larger than that of resorption, \( \text{OB}_{a} \)s remain essentially stationary with respect to bone along the BMU axis. Furthermore, it is observed that active osteoblasts, unlike osteoclasts, are
not dynamically replenished once they have started producing osteoid [7]. This suggests that the pre-osteoblasts they derive from are not moving longitudinally either (at least, not to a significant extent), and so we set $v\,_{\text{on}} = v\,_{\text{off}} = 0$, leading to

$$J\,_{\text{on}} = J\,_{\text{off}} = 0.$$  \hspace{1cm} (13)

As will be seen in Section 4, this hypothesis is crucial to explain the spatial segregation of active osteoblasts, pre-osteoblasts and uncommitted progenitors.

**Regulatory factors and binding reactions**

System-level coupling between the osteoclasts and osteoblasts occurs because the two direct regulatory factors in our model ($\text{TGF-}\beta$ and $\text{RANKL}$; see Eqs. (2) and (8)) are themselves driven by the cellular actions, both directly and indirectly via other interfering molecules.

$\text{TGF-}\beta$ is stored in high concentration in the bone matrix and released into the BMU environment in active form by the resorbing osteoclasts [11, 28, 29]. Assuming that $\text{TGF-}\beta$ degrades at a constant rate $D\,_{\text{r-c\beta}}$, we have:

$$\frac{\partial}{\partial t}n\,_{\text{r-c\beta}} = n\,_{\text{bone}}k\,_{\text{res}}n\,_{\text{OC}} - D\,_{\text{r-c\beta}}n\,_{\text{r-c\beta}} - \nabla \cdot J\,_{\text{r-c\beta}},$$  \hspace{1cm} (14)

where $k\,_{\text{res}}$ is the bone volume resorbed per unit time by a single osteoclast and $n\,_{\text{bone}}$ is the concentration of $\text{TGF-}\beta$ present in the bone matrix. Since $\text{TGF-}\beta$ is released in the environment in soluble form, its transport properties encoded in $J\,_{\text{r-c\beta}}$ are assumed to be independent of the cells. It is expected that high levels of $\text{TGF-}\beta$ are found up until the reversal zone where it promotes commitment and differentiation of mesenchymal cells to the osteoblastic lineage. For simplicity, we assume that $\text{TGF-}\beta$ has negligible diffusion, i.e., $J\,_{\text{r-c\beta}} \approx 0$. Nevertheless, the presence of $\text{TGF-}\beta$ in the reversal zone can be accounted for by assuming a weak degradation rate $D\,_{\text{r-c\beta}}$ (in a sense clarified below). Further comments on the effects of $\text{TGF-}\beta$ diffusion towards the back of the BMU are made in Section 4.

The local availability of $\text{RANKL}$, which is critical for the differentiation of $\text{OC}_{1,2}$ into $\text{OC}_{2}$, arises from the combination of several effects. $\text{RANKL}$ is a protein bound to the membrane of cells of the osteoblastic lineage. Its interaction with the $\text{RANK}$ receptor found on $\text{OC}_{1}$ is regulated by the presence of the soluble decoy receptor $\text{OPG}$, which is also expressed by osteoblastic cells. Furthermore, the relative expression of $\text{RANKL}$ vs. $\text{OPG}$ by osteoblasts is regulated by systemic $\text{PTH}$ concentrations. All these molecules and their competitive interactions are considered explicitly in our model. Here we only describe their main features, and refer the reader to Ref. [14] for further details. We will assume that $\text{RANKL}$ is only expressed by $\text{OB}_{3}$ and that $\text{OPG}$ is only expressed by $\text{OB}_{2}$ (corresponding to "Model Structure 2" of Ref. [14]). This choice of ligand expression is in agreement with experimental findings [30, 31] and the conclusions drawn in Ref. [14]. However, to reexamine this assumption in a spatio-temporal framework, we will study its influence in Section 4. While the flux of soluble $\text{OPG}$ is assumed independent of the cells (similarly to $\text{TGF-}\beta$), transport of membrane-bound $\text{RANKL}$ is tied to the cells expressing it: $J\,_{\text{r-c\beta}} = n\,_{\text{RANKL}}v\,_{\text{on}}$. However, osteoblasts are assumed to have negligible motility ($v\,_{\text{on}} \approx 0$), and so $J\,_{\text{r-c\beta}} \approx 0$.

A considerable simplification of the mass action kinetic equations considered for the competitive bindings between $\text{RANK}$, $\text{RANKL}$ and $\text{OPG}$ was obtained in Ref. [14] due to the separation of time scales between the fast reaction rates of ligands binding to their receptors on cells, and comparatively slow cell responses. We examine here the consequence of this separation of time scales in the presence of transport terms in Eq. (1). Let $r\,_{L}$ be the slowest reaction rate (e.g., in day$^{-1}$) to be found in the source/sink terms in $\sigma\,_{L}$ for the ligand $L$. Dividing Eq. (1) by $r\,_{L}$, one has

$$r\,_{L}^{-1}\frac{\partial}{\partial t}n\,_{L} = r\,_{L}^{-1}\sigma\,_{L} - r\,_{L}^{-1}\nabla \cdot J\,_{L}$$  \hspace{1cm} (15)

If reaction binding dominates transport, then $|r\,_{L}^{-1}\nabla \cdot J\,_{L}| \ll 1$ and $r\,_{L}^{-1}\sigma\,_{L} = O(1)$. Thus, changes in the local concentration of the free ligand occur on the short timescale $r\,_{L}^{-1}$ and only quasi-steady states need to be considered for the cellular dynamics, leading to

$$\sigma\,_{L} = O(1)$$  \hspace{1cm} (16)

This simplification is exactly of the same form as in the temporal model [14, Eqs. (16)–(20)]. We assume here that it holds for $\text{RANKL}$, $\text{OPG}$ and for $\text{PTH}$. As in Ref. [14], Eq. (16) is thus used to express the concentrations of these regulatory factors in terms of the
remaining unknowns of the system. This has been presented in detail in Ref. [14], so we only briefly mention the results here. The \( \text{PTH} \) endogenous production rate \( P_{\text{PTH}}(r,t) \) and degradation rate \( D_{\text{PTH}} \) are assumed to be given and not further regulated. Thus, Eq. (16) with \( \sigma_{\text{PTH}}(r,t) = P_{\text{PTH}} - D_{\text{PTH}}n_{\text{PTH}} \) determines the \( \text{PTH} \) concentration as

\[
n_{\text{PTH}} = \frac{P_{\text{PTH}}}{D_{\text{PTH}}} \tag{17}
\]

(see Eq. (25) of [14]). Production and elimination rates of \( \text{RANK} \) and \( \text{OPG} \) in Ref. [14] have a more complicated form owing to their regulation by \( \text{PTH} \), the interdependence between \( \text{RANK}, \text{RANKL} \) and \( \text{OPG} \), and an assumed saturation of the endogeneous production responses. With similar notations as in Ref. [14], Eqs. (30)–(36], the concentrations of \( \text{OPG} \) and \( \text{RANKL} \) can be rewritten with the help of the functions (4) as:

\[
n_{\text{OPG}} = \text{OPG}_{\text{max}} \pi_{\text{act}} \left( \frac{\beta_{1,\text{OPG}} n_{\text{OB}} + \beta_{2,\text{OPG}} n_{\text{OB}} }{\text{OPG}_{\text{max}} \text{D}_{\text{OPG}}} \right) \tag{18}
\]

\[
n_{\text{RANK}} = \frac{\beta_{\text{RANK}}}{\text{D}_{\text{RANK}}} \pi_{\text{rep}} \left( k_{\text{A1,RANK}} n_{\text{OC}} + k_{\text{A2,RANK}} n_{\text{RANK}} \right) \times \pi_{\text{act}} \left( \frac{\text{D}_{\text{RANK}}}{\text{D}_{\text{RANK}}} \left( R_{1}^{\text{RANK}} n_{\text{OB}} + R_{2}^{\text{RANK}} n_{\text{OB}} \right) \pi_{\text{PTH}} \right), \tag{19}
\]

In Ref. [14] and in the present model, the same constant number of \( \text{RANK} \) receptors \( N_{\text{OC}}^{\text{RANK}} \) is assumed to be expressed on each \( \text{OC} \) cell. However, while the density of \( \text{OC} \), was constant in Ref. [14], it is space and time dependent here. The constant \( \text{RANK} \) concentration occurring in the Eq. (36) of Ref. [14] has thus to be replaced in Eq. (19) above by the local, time-dependent concentration

\[
n_{\text{RANK}} = \frac{N_{\text{OC}}^{\text{RANK}} n_{\text{OC}}}{n_{\text{OB}}}. \tag{20}
\]

External conditions

Because all cells eventually differentiate further or undergo apoptosis, a continual supply of precursor cells is needed to reach nonzero cell populations over a period of time exceeding a couple of days. In cortical remodelling, this supply is local: it reaches the reversal zone of the \( \text{BMU} \) through an internal capillary that grows at the same rate as the \( \text{BMU} \) progresses (see Figure 1) [7]. We assume here that the replenishment of \( \text{OB} \) and \( \text{OC} \) cells occurs around the tip of the capillary in an unbounded and non-rate-limiting way. Under that assumption, the inhomogeneous densities \( n_{\text{OC}} \) and \( n_{\text{OB}} \) instantaneously reach a stationary distribution peaked around the tip of the growing capillary [7]. These densities become given external functions in Eqs. (3) and (9), of the form

\[
n_{\text{OC}}(r,t) = n_{\text{OC}}(r - ut), \quad n_{\text{OB}}(r,t) = n_{\text{OB}}(r - ut). \tag{21}
\]

We assume \( n_{\text{OC}}(r,t) \) and \( n_{\text{OB}}(r,t) \) to be Gaussian distributions centered around the capillary tip (see Figure 2). Finally, while \( \text{PTH} \) has been included into the model following Ref. [14], its spatial implications in the \( \text{BMU} \) will not be investigated for the purpose of the present study, and we assume that the concentration of \( \text{PTH} \) is constant and homogeneously distributed along the \( \text{BMU} \).

Solving the system of PDEs (3), (9), (10) and (14) requires appropriate initial and boundary conditions. In the following, these equations are solved in one spatial dimension with boundary conditions specified at the very front of the \( \text{BMU} \) and at its back.

3 Density profiles in the BMU

As spatial profiles in a BMU are predominantly structured along the longitudinal \( x \)-axis (see Figure 1), we restrict the mathematical model to this single spatial dimension, neglecting variations in transverse cross-sections: \( n_{j}(r,t) \approx n_{j}(x,t) \). As explained in Section 2, the fast binding approximation (16) allows to substitute \( n_{\text{PTH}}, n_{\text{RANK}}, n_{\text{RANKL}} \) and \( n_{\text{OPG}} \) with their expression (17)–(19) in the PDEs (3), (9), (10) and (14), which are then solved numerically (using Mathematica [32]) for the remaining unknown concentration profiles \( n_{\text{OC}}, n_{\text{OB}}, n_{\text{OB}} \) and \( n_{\text{TGF}-\beta} \). These PDEs are of the reaction-advection type...
and a boundary condition needs to be specified at a single point of the $x$ axis in each equation. Based on bone physiology, we prescribe both $n_{oc}$ and $n_{on}$, to be zero at the tip of the BMU cavity, and both $n_{ac}$ and $n_{oc-β}$ to be zero at the back of the BMU, where the new osteon cavity refills with osteoid up to the diameter of the Haversian canal. These requirements in turn specify a BMU spatial domain over which the PDEs are solved. This domain is set on either side of the capillary tip (which moves along $x$ at rate $u$) as follows. The tip of the BMU cavity is defined to be 350 μm ahead of the capillary tip while the back of the BMU is defined to be 4800 μm behind the capillary tip [1], thus allowing the BMU to spread over about 5 mm. To transform the moving-boundary conditions into time-independent conditions, the problem is solved in a reference frame co-moving with the BMU at rate $u$ along $x$ (see Ref. [33] for more details).

### 3.1 Numerical results and discussion

The evolution of the computed cell profiles is shown from the (static) bone frame in a series of temporal snapshots in Figure 2. These profiles define the shape of a multi-cellular wave front emerging and propagating into the bone at constant velocity $u = 40 \, \mu m/day$, corresponding to a remodelling BMU. Starting at $t = 0$ days from a tiny population of active osteoclasts present around the precursor cells (assumed to be recruited there during an “activation” phase of the BMU), the densities of $ob_p$ and $oc_a$ cells quickly increase to reach quasi-steady profiles at the front of the BMU over the next 20 days, progressing forward without changing shape. The tails of the $ob_p$ and (particularly) $ob_a$ profiles further at the back, however, take longer to develop. As a result of the differentiation sequences (2) and (8) the heights of the profiles automatically reach bounded values after an establishment phase and they are confined over a spatial range corresponding to the known dimensions of a BMU (of the order of a few millimetres). The development of a clearly structured travelling wave profile is predicted by the model, as is observed histologically [7, 1, 2]. Pre-osteoblasts and active osteoblasts are shifted towards the back of the BMU. The inver-

\[2\text{Note, however, that cell densities can be concentrated on a more restricted portion of this domain.}\]

\[3\text{The origin of the (static) x-axis is also chosen such that it coincides with the tip of the moving cavity at time } t = 100 \text{ days (see Figure 2).}\]

![Figure 2](image-url)  
**Figure 2** – Evolution of the cell profiles computed from the mathematical model. At $t = 0$ days, the initial conditions are shown: only precursor cells and a tiny population of $oc_{β}$ are present. At $t = 3$ days, these initial $oc_{β}$ have released enough $TGF-β$ in the environment to trigger differentiation of $ob_{β}$ into $ob_{p,β}$ which in turn have increased the $oc_{β}$ population by $RANKL$-binding. At $t = 20$ days, the profiles at the front of the BMU have already taken a constant shape. Active osteoblasts have started to emerge behind $ob_{p}$. At $t = 100$ days, all profiles have essentially reached a quasi-steady-state: they are moving forward into the bone matrix without changing shape. A sketch of a typical BMU cavity is aligned with these steady-state profiles for comparison. *Inset:* $ob_{p}$ and $ob_{β}$ profiles represented in logarithmic scale together with the asymptotic expressions given in Eqs. (28) ($a = 0.036$, $b = -0.0545$).
sion of the relative number of $OB_p$ vs $OB_u$ at around $-850 \mu m$ (at $t = 100$ days) in Figure 2 shows that the model captures the transition between the reversal zone and the formation zone along the longitudinal axis of the BMU (compare also with Figure 1).

On the other hand, it is apparent that the model does not capture the transition between the resorption zone and the reversal zone: the populations of $OC_u$ and $OC_p$ overlap everywhere at the front of the BMU. The bone remodelling biochemistry implicated in the model thus far, whilst reproducing bone-formation features of the BMU very well, is not satisfactory in explaining the spatial cell structure in the resorption zone, which suggests there are missing biochemical components not taken into account in this first model. In Section 4, we therefore supplement our model with a further cellular component to resolve this behaviour.

### 3.2 Theoretical analysis of the cell profiles

The simple wave-form Ansatz $n_s(x, t) \equiv A(x - ut)$ for the density profiles reduces the system of PDEs to a system of ordinary differential-algebraic equations (DAEs) to solve for the steady-state profiles $A(x)$, $A = OB_p, OB_u, OC_a, TGF-\beta$ (see also Ref. [33]).\footnote{The equation for $OC_a(x)$ becomes algebraical while the other equations keep a differential nature.}

For $OB_p$ and $OB_u$, these equations are

$$-u \frac{\partial}{\partial x} OB_p = D_{on_p} f(TGF-\beta(\text{x}^{*}_{on_p})), \quad \frac{\partial}{\partial x} OB_u = D_{on_u} g(TGF-\beta(\text{x}^{*}_{on_u})), \quad (23)$$

where

$$f(TGF-\beta) = \frac{\pi_{act}(TGF-\beta)}{\pi_{rep}(TGF-\beta)}.$$  \( \quad (24)$$

are monotonously increasing and decreasing functions of $TGF-\beta$, respectively. Due to the couplings existing between the various regulatory factors and the cells in the model, the $TGF-\beta$ concentration occurring in the right hand side of Eqs. (23) depends implicitly on all cell densities (and in particular on their ratios $OB_p/OB_u$, $OB_u/OB_p$), so Eqs. (23) do not express the cell density ratios in the left hand sides in closed form, and other dependences upon the fractions $D_{on_p}/D_{on_u}$ and $D_{on_u}/D_{on_p}$ exist in $f$ and $g$, respectively. Nevertheless, it can be checked numerically that the parameter fractions $D_{on_p}/D_{on_u}$ and $D_{on_u}/D_{on_p}$ are main regulators of the cell density ratios in the left hand side of Eqs. (23). In fact, it can be argued that the implicit dependence of $f$ and $g$ on these parameter fractions enhances the explicit linear dependences seen in Eqs. (23). Indeed, upon increasing $D_{on_u}/D_{on_p}$, $RANKL$ is increased over $OPG$, leading to an increase of $OC_a$, thus of $TGF-\beta$ and of $f$. On the other hand, upon increasing $D_{on_u}/D_{on_p}$, $OPG$ is increased over $RANKL$, leading to a decrease of $OC_a$ and of $TGF-\beta$, thus to an increase of $g$.

Multiplying the second equation in (22) by 1 or by $x$ and integrating it over the length of the steady-state BMU (from $-\infty$ to 0), one can use integration by parts, the boundary condition $OB_u(0) = 0$ and the fact that $OB_u(x)$ decays exponentially as $x \to -\infty$ (see Eq. (28)) to derive the following relations:

$$0 = \int_{-\infty}^{0} dx \ D_{on_u} OB_p - A_{on_u} \int_{-\infty}^{0} dx \ OB_u, \quad (25)$$

$$u \int_{-\infty}^{0} dx \ OB_u = \int_{-\infty}^{0} dx \ x \ D_{on_u} OB_p - A_{on_u} \int_{-\infty}^{0} dx \ x \ OB_u. \quad (26)$$

Under the assumption that the variation of $D_{on_u}$ along $x$ can be neglected for the values of the integrals,
and the identified model shortens essentially absent, so that $\mathcal{A}_{OB} \approx 0$, and $\mathcal{A}_{OB} \approx D_{OB}$ (see Eqs. (11), (12)). Eqs. (22) thus become linear and their solution can be calculated explicitly, leading to the asymptotic behaviours

$$\langle x_{OB} \rangle \approx \langle x_{OB} \rangle - \frac{u}{\mathcal{A}_{OB}},$$

(27)

meaning that $OB$s are shifted to the back of $OB$s by a length proportional to $u/D_{OB}$.

Finally, it is possible to give analytical expressions for the decays of the $OB$ and $OB$ profiles at the far back of the BMU. Indeed, in this region, TGF-β is essentially absent, so that $\mathcal{A}_{OB} \approx 0$, and $\mathcal{A}_{OB} \approx D_{OB}$ (see Eqs. (11), (12)). Eqs. (22) thus become linear and their solution can be calculated explicitly, leading to the asymptotic behaviours

$$ob_p(x) \sim a e^{-\frac{D_{OB}}{u} |x|},$$

(28)

$$ob_b(x) \sim b e^{-\frac{A_{OB}}{u} |x|} + \frac{a}{1 - \frac{A_{OB}}{D_{OB}}} \left[ e^{-\frac{A_{OB}}{u} |x|} - e^{-\frac{D_{OB}}{u} |x|} \right],$$

as $x \to -\infty$, where $a$ and $b$ are two integration constants. These asymptotic behaviours of the steady-state profiles are compared with the numerical profiles obtained by the temporal evolution in Figure 2 in logarithmic scale. While the slope of these curves is essentially determined by the ratios $D_{OB}/u$ and $A_{OB}/u$, the constants $a$ and $b$ shift the height of the curves in the logarithmic plot, and they have been fitted. The small discrepancy visible at the very back of the BMU is due to the fact that the numerical profiles have not yet reached a quasi-steady state there.

Equations (23), (26), (27) and (28) all relate kinetic properties of the cells (velocity, differentiation and apoptosis rates) to intrinsic features of spatial

Influence of cell motility

While the effects of many regulatory factors on cell commitment, proliferation, differentiation and apoptosis are well-established in the context of bone remodelling, less is known on the motile properties of the bone cells and the regulation thereof, al-
though recent progress has been made in this direction [29, 25, 26].

Here we show that these motile properties can influence dramatically the spatio-temporal coordination of the bone cells, and thus the functioning of bone remodelling. In Section 2, OBAPs and OBAs were assumed to stay stationary with respect to bone and we set their velocities to zero (see Eqs. (13)). The wave-like propagation of the density of OBAP and OBAs cells at rate $u$ observed in Figure 2 is entirely due to their creation upstream and elimination downstream. On the other hand, choosing OBAP and OBAs cell velocity to correspond to $u$, so that $v_{OBAP} = v_{OBAs} = v_{OC} = u$, still leads to a wave-like propagation of the cell densities at rate $u$. However, as can be seen from Figure 3, in this situation all the cell density profiles fall within the same region around the precursor cell source. Such cellular profiles are in clear mismatch with the experimentally-observed propagation of a structured cortical BMU with a separation in time and in space of the resorption and formation processes. Since all cells and regulatory factors overlap, their net interaction is modified and the cell density profiles also reach different heights, offsetting the bone balance. This result corroborates the experimental observation that osteoblasts do not move significantly after their commitment to the osteoblastic lineage [7] and emphasises the critical role that cell motility plays in BMU-based remodelling.

**Role of osteoblastic maturation stage for expression of RANKL and OPG**

Several experiments have characterised RANKL and OPG expression levels on maturing osteoblasts, concluding that RANKL expression dominates in immature osteoblasts while OPG expression dominates in more mature osteoblasts [30, 31]. In the purely temporal model of Pivonka et al. [14], various “model structures” of expression of RANKL and OPG by osteoblasts at different stages of maturation were tested, which supported these experimental findings. However, these model structures really need to be tested for their functional importance in the context of the spatio-temporal coordination of bone cells in a BMU. The density profiles predicted by our model when RANKL is only expressed on OBAPs and OPG is only expressed on OBAs (corresponding to “Model Structure 1” of Ref. [14]) are shown in Figure 4. Clearly, the lack of availability of RANKL in the reversal zone, due to its expression on OBAPs at the back of the BMU and its further screening by OPG expressed on OBAs, blunt activation of osteoclasts. Barely any OCs are found in the steady-state. Such a tiny population of OCs would not be able to create a BMU cavity as before.

**Figure 4** – Steady-state density profiles obtained when the expression of RANKL and OPG on precursor and mature osteoblasts is swapped, i.e., RANKL is expressed on OBAPs and OPG is expressed on OBAs. All parameters are otherwise taken as in Figure 2. The density of active osteoclasts has not grown past its small initial condition. Such a small population of OCs would not be able to create a BMU cavity as before.

**Role of TGF-β**

TGF-β is a multi-faceted regulatory factor serving several purposes in bone remodelling [28, 29]. In Ref. [14] and in our model, TGF-β up-regulates osteoblast commitment but down-regulates osteoblast activation (see Eq. (8)). Furthermore, it up-regulates active osteoclast apoptosis. These several roles find sense in the structured cell profiles of a cortical BMU. Physiologically, a negative feedback loop on osteoclasts is needed to keep resorption under control. Having TGF-β regulating this negative feedback is convenient, since it is then activated only once bone has started to be resorbed. On the other hand, the portion of bone just resorbed needs to be refilled with new bone. While TGF-β diffuses behind OCs in the cortical BMU, it reaches the reversal zone populated with OBAs. TGF-β commands their commitment to
the refilling task by up-regulating their development into OBₚ's. Finally, the down-regulation of activation of OBₚ into OBₚ by TGF-β helps preventing osteoid to be laid down too early, e.g. in the resorption zone. From our model, we observe that the presence of TGF-β behind OCₚ, acts to delay the onset of OBₚ and so to increase the spatial segregation between OCₚ and OBₚ.

**Model extension: including “mature osteoclasts”**

Precursor osteoclasts are known to be circulating cells [12, 11] and delivered to the reversal zone of cortical BMUS through the capillary (see Figure 1). Throughout the BMUS’s progression, the capillary tip is found at a distance of about 350 µm behind the resorbing front. This means osteoclasts need to travel this distance at a faster pace than the rate of progression u of the BMUS to reach the front [7]. On the other hand, activation of osteoclasts requires RANKL, which is expressed on the surface of pre-osteoblasts found around the capillary tip. In the model presented in Section 2, OCₚ are assumed to resorb the bone matrix, so while they have been activated by RANKL, they cannot move faster than u. For this reason, OCₚ are differentiating from OCₚ around the middle of the reversal zone and stay there as they have not been given regulatory mechanisms to distance themselves from their progenitors (such as chemotactic signals towards the bone surface [26]). This results in overlapping OCₚ and OCₚ density profiles in Figure 2.

To resolve this problem, we are led to introduce a new stage of osteoclast development in the model, that we call “mature osteoclasts” (OCₚ). Mature osteoclasts denote osteoclasts that have been activated by RANKL and that migrate towards the front at a velocity v_{OCₚ} > u until they reach the bone surface. Once at the bone surface, they join an active resorbing multinucleated osteoclast, hence becoming OCₚ progressing at rate u. The sequence of osteoclast maturation in Eq. (2) is thereby extended to

\[
OCₚ \xrightarrow{RANKL} OCₚ \xrightarrow{bone surface} OCₚ \xrightarrow{TGF-β} \emptyset. \tag{29}
\]

To model the transition from OCₚ to OCₚ at the bone surface of the genuinely three-dimensional BMUS cavity in our one-dimensional setup, we introduce a “probability of reaching bone” Φ(x, t), defined as

\[
Φ(x, t) = 1 - \frac{R^2(x, t)}{R^2_c}, \tag{30}
\]

where R(x, t) is the radius of the BMUS cavity depicted in Figures 2−5, and R_c = 100 µm is the maximal cavity radius (cement line radius). For simplicity, this cavity is assumed here to be given and to progress forward at rate u without changing shape (an assumption valid in the quasi-steady-state). According to Eq. (30), the function Φ(x, t) thus interpolates between one ahead of the (moving) cavity front (where the cavity radius R(x, t) is formally zero), and zero in the reversal zone (where R(x, t) reaches the cement line radius R_c). The function Φ(x, t) increases again to a positive value < 1 for x in the formation zone (where R(x, t) decreases towards the Haversian canal radius). With this definition, the sequence of

\[\text{Figure 5 – Cell density and regulatory concentration profiles in a cortical BMUS as given by the extended model (compare with Figure 2). Active osteoclasts are now clearly shifted towards the front of the BMUS compared to their progenitors. The overlap between OBₚ and OCₚ is practically inexistant. The osteoblastic profiles in the back are essentially unchanged except for their amplitude. The concentrations of OPG, RANKL and TGF-β are also shown. They have been scaled to correspond to OBₚ, OBₚ and OCₚ density levels, respectively, for comparison. Remarkably, OBₚ-bound free RANKL is not colinear with the presence of OBₚ: it is effectively shifted towards osteoclast precursor cells due to the presence of OPG. The concentration of TGF-β is also found behind the OCₚ peak.}\]
Osteoclast development in Eq. (29) can be expressed as:

\[
\frac{\partial}{\partial t} n_{\text{ocm}} = D_{\text{ocm}} (RANKL) n_{\text{ocp}} - D_{\text{ocm}} (\Phi) n_{\text{ocm}} - \nabla \cdot J_{\text{ocm}},
\]

\[
\frac{\partial}{\partial t} n_{\text{ocm}} = D_{\text{ocm}} (\Phi) n_{\text{ocm}} - \alpha_{\text{oc}} (TGF-\beta) n_{\text{ocm}} - \nabla \cdot J_{\text{ocm}},
\]

(31)

where \( D_{\text{ocm}}, \alpha_{\text{oc}}, \) and \( J_{\text{ocm}} \) are given by Eqs. (5), (6) and (7). The transition from \( \text{ocm} \) to \( \text{ocp} \) is assumed to take place at a rate \( D_{\text{ocm}} \), proportional to the probability of reaching bone:

\[
D_{\text{ocm}} (\Phi) = D_{\text{ocm}} \Phi,
\]

(32)

where \( D_{\text{ocm}} \) is the rate of osteoclast activation once at the bone surface. Finally, the flux of \( \text{ocm}s \) is taken to be

\[
J_{\text{ocm}} = n_{\text{ocm}} v_{\text{ocm}}.
\]

(33)

where the velocity is such that \( v_{\text{ocm}} > u \).

The simulation results of this extended model are presented in Figure 5. While the cell density profiles at the back of the BMU have not changed qualitatively, the front of the BMU now also exhibits structured profiles, clearly delineating a transition from the resorption zone (predominantly populated with \( \text{ocp}s \) and \( \text{ocm}s \)) to the reversal zone (predominantly populated with precursor cells). This structure reproduces the histologically expected cell profiles of a cortical BMU as schematically depicted in Figure 1.

The concentration profiles of OPG, RANKL and TGF-\( \beta \) are also plotted in Figure 5, with their heights scaled to correspond to the maximum of the \( \text{obp}, \text{obm}, \) and \( \text{oca} \) density profiles, respectively. One can see that TGF-\( \beta \) has slightly diffused to the back of \( \text{oca} \) and that its decline towards the back coincides with the onset of \( \text{obp}s \). As a result, there is no overlap of \( \text{obp}s \) with \( \text{oca}s \). Since transport of soluble OPG has been assumed slow compared to its reaction processes (see Eq. (16)), OPG is found mainly in the same location as \( \text{obp}s \) that express it. On the other hand, while RANKL is bound to the membrane of \( \text{obm}s \), the RANKL concentration profile is clearly shifted towards the front of the \( \text{obp} \) density profile, overlapping in particular with \( \text{ocp} \) and \( \text{ocm} \) cells. This is due to OPG inhibiting most of the available RANKL ligands at the back of the \( \text{obp} \) profile. With this observation, the role of OPG takes on a new fundamental meaning.

5 Concluding Remarks

In this paper, we have developed a spatio-temporal mathematical model of cortical BMU remodelling based on fundamental material balance equations expressed for different bone cell types. This model is an extension of the purely temporal model of Pivonka et al. [14]. TGF-\( \beta \), the RANK–RANKL–OPG pathway and PTH are explicitly taken into account in the model, and mass action kinetics is used to describe the reaction rates between ligands and their receptors. The resulting system of (nonlinear) PDEs is solved for the cell densities and regulatory factor concentrations in one dimension, corresponding to the longitudinal axis of a cortical BMU (see Figure 1).

We find that the cell interaction pathways in the model are able to explain the emergence of a multicellular travelling wave that develops structured profiles moving without changing shape. The spatial structure of these steady profiles corresponds to the known organisation of bone cells in a typical cortical BMU. It clearly delineates a resorption zone at the front, followed by a reversal zone, and a formation zone at the back. Several properties of the cell density profiles have been linked theoretically to kinetic properties of the cells in the model, such as differentiation and elimination rates. These rates may thus be directly inferred from the measurement of cell counts in serial histological sections taken at a particular time point.

It is experimentally known that several maturation stages of osteoclasts and osteoblasts have different expression patterns and responses in the TGF-\( \beta \) and RANK–RANKL–OPG pathways. Our model shows that this heterogeneity is essential to ensure a functional BMU-remodelling process with segregated but coordinated zones of resorption and formation, in particular:

- TGF-\( \beta \) plays a central role in modulating cell responses as soon as bone is resorbed. It moderates osteoclastic resorption and initiates osteoblastic commitment of mesenchymal cells once it has diffused from the resorption zone to the reversal zone. Furthermore, high concentration of TGF-\( \beta \) towards the front of the BMU helps prevent osteoblasts from becoming activated prematurely, or near active osteoclasts.

- The fact that RANKL is bound to the membrane of pre-osteoblasts helps ensure that osteoclasts...
do not initiate resorption without the presence of bone-refilling precursor cells. By shielding the availability of free RANKL on O_{2p}s, the expression of soluble OPG by active osteoblasts in the formation zone provides a mechanism to “shift” the peak of the concentration profile of free RANKL towards the front of the BMU, ahead of the peak of the O_{2p} population that expresses RANKL, and thus prevents activation of osteoclasts where new bone is being laid down. Our model shows that changing the RANKL and OPG expression pattern fails to coordinate O_{Ca} formation properly.

- The various motile properties of bone cells are absolutely crucial for the spatial organisation of the cells into a cortical BMU, both in the formation zone and in the resorption zone. In particular, our results suggest that osteoclasts migrate forward at various rates depending on their maturation, and corroborate the observation that osteoblasts are quasi-stationary with respect to bone. The importance of bone cell motile properties is expected to play a critical role in the balance between bone resorption and bone formation, both in health and disease.

While our one-dimensional model is capable of reproducing important features of cortical BMUs, there are a variety of possible improvements that could be addressed using the general formulation of the model presented in this paper. Solving the system of PDEs in higher spatial dimensions could be used to study how cells distribute themselves in transverse cross-sections of the BMU. Other specific cell interaction pathways could be included as needed and studied in detail on the basis of the present model. We note that investigating initiation and termination signalling mechanisms of cortical BMUs is very important to fully understand bone homeostasis during remodelling, and will be the subject of future work.

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