Growth of the flat bones of the membranous neurocranium: A computational model

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A B S T R A C T

This article assumes two stages in the formation of the bones in the calvaria, the first one takes into account the formation of the primary centers of ossification. This step counts on the differentiation from mesenchymal cells into osteoblasts. A molecular mechanism is used based on a system of reaction-diffusion between two antagonistic molecules, which are BMP2 and Noggin. To this effect we used equations whose behavior allows finding Turing patterns that determine the location of the primary centers. In the second step of the model we used a molecule that is expressed by osteoblasts, called Dlx5 and that is expressed from the osteoblasts of each flat bone. This molecule allows bone growth through its borders through cell differentiation adjacent to each bone of the skull. The model has been implemented numerically using the finite element method. The results allow us to observe a good approximation of the formation of flat bones of the membranous skull as well as the formation of fontanelles and sutures.

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1. Introduction

The membranous neurocranium (or calvaria) develops by intramembranous ossification. Initially, the mesenchyme covers the brain and experiences the gradual differentiation of mesenchymal cells into osteoblasts. In pursuing growth during fetal and postnatal life, these bones increase in size by apposition of layers on its outer surface and by osteoclasticresorption [1,2]. In the calvaria, upper part of the skull, the bones are not fully formed at birth and we can observe between the membranous flat bones the formation of sutures and fontanelles. At the time of birth, the bones of the skull are held by a membranous tissue with soft spots called fontanelles, which gradually are joining and sealing. This allows the brain to continue to grow, especially during the first two years of life, and in adulthood the bones of the skull are completely joined.

The bones of the top of the skull (frontal, parietal, temporal and occipital) ossify from centers of ossification that appear in the membrane of the connective tissue into the eighth and ninth week of gestation [3]. Thus, the frontal bone develops, in an intramembranous way, from two centers of ossification one on each side of the midline of the brain. These centers are above the orbital arc at the end of the eighth week of gestation (see Figs. 1 and 2). In week fourteen it heavily ossifies around these primary centers. In the eighteenth week it sets the margin of ossification between these two fronts, called sutures (metopic), which will disappears in the second year after birth [3]. Also, each parietal bone ossifies from a single center at the end of the eighth week [1–3]. Then, the
The origin of the intramembranous ossification of the calvaria may be due to genetic, biochemical (local and systemic) and mechanical effects. From the biochemical point of view Holleville et al. [5,6] conducted experiments where the Dlx5 gene was compared with other molecules involved in the process of ossification of the calvaria, especially bone morphogenetic protein 2 (BMP2), BMP4, BMP7, fibroblast growth factors (FGF) and its receptors FGFR1, FGFR2, FGFR4. Holleville et al. [5,6] take into account the effect of each molecule on the intramembranous ossification marker called osteopontin. In this work it was found that Dlx5 is in osteogenic fronts and in the mesenchymal sutures. Similarly, in cell cultures, it was found that Dlx5 is positively regulated by BMP and is inhibited by its antagonist, Noggin. It has also been found that FGFR1 helps cell differentiation into osteoblasts, FGFR2 promotes proliferation and FGFR3 is found, in a greater scale, in endochondral ossification. TGF1 and TGF2 allow the obliteration of the suture, while in TGF3 is expressed in sutures that have not been fused.

The aim of this paper is to model the development of the centers of ossification of the calvaria. Specifically, we model the appearance and development of the parietal, frontal and occipital bones. To verify the model we perform a computer simulation that takes into account: (1) The effect of molecular signals present in the intramembranous ossification and; (2) maturation cycles of mesenchymal cells in the cranium. The computational implementation is done using finite elements. This article will be of particular interest to researchers in the field of mechanobiology and biomechanics, as well as for physicians who need a starting point for the study of diseases of the formation of membranous neurocranium.

### 2. Materials and methods

#### 2.1. Hypotheses on the formation and growth of flat bones of the skull

This article assumes that the formation and growth of the flat bones of the membranous skull are due to two consecutive processes: formation of the primary centers of ossification and their growth. Formation (Stage I) is mainly due to the action of two molecules involved in the initial cellular differentiation of the primary centers, which are: BMP2 and Noggin. Between these two molecules there is a regulatory loop that develops spatial patterns where there are areas of higher concentration of either molecule. Then, thanks to the high concentration of BMP2, it will present a process of cell differentiation, which determines the location of the primary centers.

Later, in the growth stage (stage II), once the primary centers are developed, they grow through the action of the molecule Dlx5. This molecule is expressed on the fronts of ossification and allow growth of each of the primary centers, and therefore of flat bones. Additionally, each bone has encoded a morphogen, hypothetical and personal, allowing growth and prevents fusion with other membranous bones of the skull. The process is shown in Fig. 3.
3. Stage I: Appearance of the primary centers of ossification

3.1. Cell differentiation

By the action of the protein BMP2 [5], which is expressed by mesenchymal cells, generates the differentiation of these cells into osteoblasts. Additionally, in this article, we take into account the maturation of mesenchymal cells. This assumption is based on the theory of [7] who have postulated that only those cells that have completed a prescribed number of cell cycles can differentiate. This assumption was confirmed by Schmitt and Ruch [8]. Therefore, it is assumed that the position of the cells in the calvaria determines their differentiation due to cell cycles. Therefore, differentiation depends on the concentration of BMP2 that signals the process and cell maturation that depends on the spatial position, given by:

$$\frac{\partial c_o}{\partial t} = \eta \frac{S_{B2}^n}{S_{B2}^n + S_{B1}^n} \cdot \frac{T_{r_0}}{T_{r_0} + t'} f(x_{rel})$$  \(1\)

Eq. (1) represents the term of differentiation of mesenchymal cell into osteoblasts by the presence of BMP2 (where \(S_2\) is the concentration of BMP2), and cell maturation that depends on the spatial position \(f(x_{rel})\). In this equation \(\eta\) is a constant that regulates cell differentiation, \(S_{B2}\) represents the value of the concentration of BMP2 with which begins the process of differentiation. \(T_{r_0}\) is the time required to perform the differentiation and \(t'\) represents the time limit of action of the BMP2.

For its part, \(f(x_{rel})\) depends on the relative position \(x_{rel}\) that determines the distance between the rostral limit and any point of the calvaria. This assumption is based on that the rostral parts and the skull base differentiate more rapidly (seventh week) than the calvaria (eighth to ninth week) [9]. Therefore, the proximity to the face could determine cell maturation, which is given by:

$$f(x_{rel}) = \begin{cases} 1 & x_{rel} \leq r \\ 0 & \text{other case} \end{cases}$$  \(2\)

where \(r\) determines the threshold value from the rostral limit, to which the cells located below it, are mature enough to differentiate in the primary ossification center (Fig. 4).

3.2. BMP2-Noggin regulation

We assume the existence of a system of reaction–diffusion of two primary molecules, such as the BMP2 and Noggin [5]. The spatial distribution of these molecules produces stable patterns in time and unstable in space, similar to the patterns of differentiation of osteoblasts from mesenchymal cells.

The regulatory process that is proposed in this paper is outlined in Fig. 5, and is based on a reaction-diffusion system of the type activator-substrate (also called exhaustion model) (see, for example [10]). The process indicates that there is a control loop between BMP2 (activating factor) and Noggin (substrate), where the self-regulating BMP2 competes with the production of Noggin. The hypothesis is based on the results found by Zhu et al. [11], Walsh et al. [12] and Plikus et al. [13] whose findings relate the regulatory loop between BMP2 and Noggin. Thus, osteoblasts differentiate and develop highly
repeatable patterns, similar to those found in Turing patterns [14].

The definition of the relationships indicated in Fig. 5 can be quantified by the following equations:

\[
\frac{\partial S}{\partial t} = C(a_1 - \mu S_B + \gamma_0 S_B^2 S_N) + D_B V^2 S_B \quad (3a)
\]

\[
\frac{\partial S}{\partial t} = C(a_2 - \gamma_0 S_N^2 S_B) + D_N V^2 S_N \quad (3b)
\]

where C is the concentration of cells of the mesenchymal tissue that express the factors of BMP2 and Noggin; \( S_B \) and \( S_N \) represent the concentrations of BMP2 and Noggin, respectively. The remaining parameters of the model are: \( a_1 \) and \( a_2 \) are terms that quantify the production of each molecular factor by the epithelial and mesenchymal tissues, \( \mu \) is a constant that quantifies the inhibition in the production of BMP2 by its excess, \( \gamma_0 \) regulates the nonlinear interaction between the concentration of BMP2 - Noggin and quantifies the activation or inhibition of each molecular factor, \( D_B \) and \( D_N \) are the diffusion coefficients of BMP2 and Noggin, respectively.

In the biological interpretation of the above equations the term \( \gamma_0 S_N^2 S_B \) represents the nonlinear activation of \( S_N \) (BMP2 production by the presence of Noggin) and the nonlinear consumption of \( S_B \) (by the presence of BMP2).

4. Stage II: Growth of membranous flat bones

4.1. Dlx5-be regulation

The gene Dlx5-be (Dlx5 for each specific bone) is a morphogen expressed by osteoblasts present in each of the primary centers of ossification and membranous of the flat bones developing. This gene is expressed in each developing bone and diffuses to allow adjacent differentiation, to each flat bone, of mesenchymal cells into osteoblasts. This differentiation allows the gradual growth of each bone to allow development of the membranous skull. This hypothesis is based on the findings of Holleville et al. [5], who found that Dlx5 is in osteogenic front and in mesenchymal suture. Similarly, in cell cultures, it was found that Dlx5 is positively regulated by the BMP and is inhibited by its antagonist, the Noggin. This article assumes that the evolution of the concentration of Dlx5 is given by:

\[
\frac{\partial S_{D-i}}{\partial t} = \alpha C_D \cdot \frac{S^n_{D-i} - S^{T D-i}_{D-i}}{S^{T D-i}_{D-i}} - \beta \frac{\ln(2)}{r_D} S_{D-i} + D_{D-i} V^2 S_{D-i} \quad (4)
\]

where \( S_{D-i} \) is the concentration of Dlx5 that depends on each ith bone, being \( i = (1) \) left parietal, (2) right parietal, (3) left frontal, (4) right frontal and (5) occipital (derived from two bones which rapidly coalesce); \( \alpha \) is a constant which quantifies the amount of Dlx5 emission by osteoblasts (measured by the concentration \( C_D \)) present in each ith flat bone; \( S^n_{T D-i} \) is the saturation concentration of Dlx5, in which, after this value, osteoblasts do not release this molecule. \( n \) is a constant; \( \beta \) quantifies the degradation process of the molecule; \( r_D \) is the average time of degradation; and \( D_{D-i} \) is the diffusion coefficient of each ith bone.

4.2. Cell differentiation

This article assumes that the action of Dlx5, expressed by osteoblasts, allows cell differentiation of mesenchymal type to osteoblast in the adjacent limits to each bone. Therefore, cell differentiation is given by

\[
\frac{\partial c_{O-i}}{\partial t} = \lambda C_O \cdot \frac{S^n_{O-i} - S^{T O-i}_{O-i}}{S^{T O-i}_{O-i}} \cdot \prod_{j=1}^{5} \frac{S^n_{O-j} - S^{T O-j}_{O-j}}{S^{T O-j}_{O-j}} \quad (5)
\]

where \( C_O \) is the concentration of osteoblasts of the ith flat bone of the skull; \( S^{T O-j}_{O-j} \) is the threshold value of activation of cell differentiation due to the action of Dlx5, \( \lambda \) is a constant that quantifies the rate of differentiation and \( m \) is a value of the step function.

5. Numerical implementation

The set of Eqs. (1)–(5) were implemented and numerically solved using the finite element method with a Newton–Raphson scheme. For its solution we did a program in FORTRAN. The proposed examples were solved in a Laptop of 4096 MB and 800 MHz processor speed. Computer simulation was carried out in an incremental iterative scheme which allows solving, computationally, the evolution of both the concentration of molecular factors. In the first stage of appearance of the primary ossification centers, we obtain the evolution of the concentration of BMP2 and Noggin \( (S_B, S_N) \) as well as the appearance of osteoblasts. Initially, the mesenchymal tissue is assumed as a structural matrix with an initial concentration of mesenchymal cells (whose concentration is \( 4 \times 10^5 \) cell/mm³) [15–18]. The initial concentrations of BMP2 and Noggin are randomly distributed in the mesenchymal tissue, with a disturbance of 10% over the steady-state concentration given by \( (S^l_B, S^l_N) = (1.0, 10.9)[\text{ng/mm}^3] \) [19–21] (see Appendix). The selection of the random initial conditions around the steady state is similar to the event of the molecular expression by the mesenchymal cells in the area of differentiation to osteoblasts. The flow conditions, for each molecular factor in the boundary, are assumed null. This assumption is based on that, under the calvaria (in the condrocraneal region) endochondral ossification has already existed, and therefore decreases the permeability of forming a barrier between the condrocraneal and vicerocranial region and the membranous neurocranio region. In the second stage we take the conditions and values of ossification of the first stage, and assume a null concentration of each factor Dlx5.

Thus, we only take the upper portion of the skull also called calvaria (see Fig. 6), where it also shows the finite element mesh used to solve the problem. Similarly, in Fig. 7, show the geometric parameters used in the simulation.
6. Results

6.1. First stage: Appearance of the primary centers of ossification

Fig. 8 shows the results of the concentrations of BMP2 and Noggin in the calvaria. In this case the geometric parameters used are given by [22]: a = 32 mm, b = 48 mm (b/a = 1.5), c = 16 mm, d = 9.6 mm. Also, we have used r = 8 mm. The parameters and constants of the reactive and diffusive terms are shown in the appendix. It is noted that areas of higher concentrations of BMP2 (in black, Fig. 8a) there is a lower concentration of Noggin (Fig. 8b). We must remember that BMP2 signals the formation of the primary centers of ossification, therefore, in those places of greatest concentration, these centers will be located.

Note that the maximum concentration of BMP2 (1.9 ng/ml) is located in central zones of those areas that develop cell differentiation into osteoblasts. Similarly, at these areas, we get the lowest concentration of Noggin (0.55 ng/ml). The highest concentration of Noggin occurs at the interfaces of the future primary centers, with a value of 1.1 ng/ml.

By the differentiation mechanism described, we obtain the results from the location of osteoblasts. In Fig. 9 we can see the formation of two frontal centers of ossification, two parietals and two occipitals, one on each side of the midline of the brain. Notice that the formation of the primary centers, correspond to those areas of higher concentration of BMP2.

Fig. 7 – Geometric relationships used in the computational model.

Fig. 8 – Results of the reaction–diffusion system BMP2-Noggin. (a) Results for BMP2 (responsible of cell differentiation into osteoblasts), (b) results for Noggin (negative regulator of differentiation). Units in ng/mm³.
Fig. 9 – Results and location of the primary centers of ossification. (a) Frontal view, (b) sagittal view, (c) rear view, (d) abstraction of the results in the skull.

6.2. Stage two: Development of flat bones and fontanelles

The second stage of the model takes into account the development of each bone starting from its primary center of ossification. Fig. 10 shows the diffusion of the molecule Dxl5-5, corresponding to the expression that takes place from the occipital primary center. This molecule allows differentiation into adjacent osteoblasts to the primary ossification center, and thus, bones growth, in this case, the occipital (Fig. 10).

Note that the concentration increases to a value of 1 ng/ml, and is expressed by osteoblasts present in the bone.

In Fig. 11 we can observe the growth of each bone in the membranous skull. Note the radial growth of bones that follow the diffusion of the molecule Dxl5 specific of the bone. Furthermore, we can point out that at the time of birth, the bones leave wide spaces between them, called, fontanelles and sutures. In Fig. 11h we can see the pictorial representation of the membranous skull that is, particularly similar to the simulation, by comparing it, for example, with Fig. 11f.

Fig. 10 – Time evolution of the Dxl5-5 concentration for the occipital bone. Note that the initial area of release corresponds to the primary centers of ossification. (I): Intrauterine, (N): Postnatal.
Fig. 11 – Results of the time evolution of growth for each one of the flat bones in the membranous skull. (h) Coronal pictorial view of the real membranous skull. Note the formation of fontanelles at t = 11 months (postnatal). (I): Intrauterine, (N): Postnatal. These snapshots are in months (M).

7. Discussion and conclusions

This article has developed a model on the growth of the flat bones of membranous skull. To this effect we have assumed two stages of formation, the first one takes into account discus the formation of the primary centers of ossification. This stage takes into account the differentiation from mesenchymal cells into osteoblasts. Furthermore, uses a molecular mechanism based on a system of reaction-diffusion between two antagonistic molecules, which are BMP2 and Noggin. For this simulation we used equations whose behavior allows finding Turing patterns that determine the location of the primary centers. In the second step of the model we used a molecule that is expressed by osteoblasts, called Dlx5 and that is expressed from the osteoblasts of each flat bone. This molecule allows bone growth through its borders until it creates the fontanelles and sutures.

This paper develops a new model of growth of flat bones in the membranous neurocranium. To test this model we solved it using the finite element method, which has made it possible to find a good qualitative correlation between experimental evidence and the simulation results (see Figs. 8–11). In contrast different theories have been proposed. For example, Ref. [23] established the theory of sutural dominance. The article states that the growth of the cranial vault is caused by "intrinsic" and "proliferative" growth patterns. It further states that the responsibility of the craniofacial growth lies on the sutures, the cartilage and the periosteum, which are controlled genetically. Scott [24] assumes that the bones of the calvaria are separated in a transverse direction. On the other hand, Moss [25,26] states that the environment is who regulates skeletal growth, especially in relation to the expansive growth of the brain and the bones of the cranial vault. Similarly, Moss [26] corroborates its work using advances in biomedical sciences, bioengineering and computer, including two new topics: the mechanisms of mechanotransduction and the theory of biological network. This article states that there is an interaction between the epigenetic and genomic factors (intrinsic) that provides the necessary and sufficient causes for the morphogenesis. Van Limborgh [27], assumes that the postnatal craniofacial growth is controlled by a multifactorial system: intrinsic genetic factors, local epigenetic and general, as well as environmental factors. Limborgh [27] considers the importance of primary ossification centers in the cranial growth and assumes that their formation is also multifactorial. Thus, we can see that the accepted theories [25–27] assume that these centers are developed by multifactorial causes, being the most important, genetics. This article hypothesizes that the molecular mechanisms and local cellular are the most important in the development of the flat bones of the skull.

The formation of primary ossification centers has been achieved by a reaction diffusion model coupled to a differentiation one, so that we can obtain the following configuration: two parietal, two frontal and two occipital (one on each side of midline the skull). These results are consistent with medical experience [28]. For its part, the growth of these primary centers allows to obtain flat bones in a postnatal stage, where we can observe the formation of sutures and fontanelles.

However, during prenatal development there are additional factors such as mechanical [21], genetic [27] and environmental [25–27]. Therefore, this model is a simplification of reality,
for this reason it should be listed, as in any other mathematical model, its drawbacks and limitations.

The first limitation takes into account the number of stages in the formation of bones of the skull. Here we have assumed two stages, however, there may be a greater number of events that are unknown and that can lead to a complete formation of the membranous skull [2,9]. Moreover, the hypothesis presented assumes that the origin of osteoblast differentiation is internally controlled by mesenchymal cells through two biochemical signals: Noggin and BMP2, in the first stage [29], and Dlx5, in the second [5]. It is important to recognize that these molecular factors are not the only ones acting in this process [6]. We can count on other molecular factors, among which are FGF-8 and BMP4. However, it must be recognized, that these molecules may be the most important in shaping the bones of the calvaria.

Other limitation is the geometry used. It is noteworthy that the geometry is a simplification of the graphs presented by Sadler [2]. This article did not take into account the growth of the brain. However, the skull in formation also has a high expansion rate. This growth can modify the exact location of the centers of ossification and growth of the cranial bones.

The fourth limitation depends on the type of boundary conditions and the initial conditions. This article has assumed null flow in the domain of study. This assumption is based on that the membranous portion (calvaria) is made of, initially, mesenchymal cells. When it begins the formation of the primary centers, in the calvaria, the region of the chondrocranium takes a week or two of ossification [2]. Therefore, the boundary between these two regions (chondrocranium and calvaria) will have, on one hand, mesenchymal cells, and on the other, osteoblast that are already forming the bones of the face and skull base. Thus, in the step of molecular factors from and toward the calvaria may be negligible, because the diffusion coefficient of the ossified side is lower. This assumption should be studied in depth in future work in the area. In addition, the exact initial conditions are unknown, for this, we have chosen null initial conditions for Dlx-5.

In conclusion, this article makes evident, in a simplified way, the regulatory mechanisms of the formation of the membranous bones of the skull using a mathematical model. This work will serve researchers working in the pathology of cranial formation and may shed light on new lines of experimentation and new mathematical models that refine the one proposed here.

\[(\partial S_N/\partial t)(D_N = 0) = 0\]  (A1)

where \(S_N\) and \(S_B\) are the steady-state values for the concentration of Noggin and BMP2, respectively.

The linear analysis allows finding the range of parameters that ensure the emergence of such Turing patterns. Therefore, the solution can be expressed as

\[(S_N, S_B) = (u + S_N^*, v + S_B^*)\]

where \(u\) and \(v\) are small perturbations in each molecular factor, respectively. From Eqs. (1a) and (1b) and from the linear analysis (see details of the analysis in Ref. [20]) we find the geometric area where the parameters of the reaction-diffusion equation are found, in such a way, to develop Turing pattern, this is

\[C(2\gamma_S S_B S_N^* - \gamma_B S_B^* - \mu) < 0\]  (A2a)

\[C^2(\gamma_S S_B^* - 2\gamma_S S_B S_N^* + 2\gamma_B^2 S_B^2 S_N^*) > 0\]  (A2b)

\[C(2S_B S_B S_N^* - D_B \gamma_B (S_N^*)^2) > 0\]  (A2c)

\[C^2(D_N(2\gamma_S S_B S_N^* - \mu) - D_B \gamma_B (S_B^*)^2) - 4D_B D_N C^2(\gamma_S (S_B^*)^2(\mu - 2\gamma_S S_B S_N^* + 2\gamma_B^2 S_B^2 S_N^*)) > 0\]  (A2d)

If we express Eqs. (3a) and (3b) into a non-dimensional form (Schnakenberg equation [20]) and as a function of small perturbations of the molecular factor \((S_N, S_B)\), i.e. through \((u, v)\) we can obtain:

\[\frac{\partial u}{\partial t} = \gamma(a - u + u^2v) + \nabla^2 u\]  (A3)

\[\frac{\partial v}{\partial t} = \gamma(b - u^2v) + d \nabla^2 v\]

where we can identify the parameters that move from the non-dimensional model (or Schnakenberg) to the real model given in (1) (see [19]), i.e. we obtain the non-dimensionalization constants given by

\[a = \frac{\alpha_1}{\mu} \sqrt{\frac{\gamma_0}{\mu}}\]  (A4a)

\[b = \frac{\alpha_2}{\mu} \sqrt{\frac{\gamma_0}{\mu}}\]  (A4b)

\[d = \frac{D_B}{D_N}\]  (A4c)

\[T = \frac{L^2}{D_N}\]  (A4d)

\[\gamma = \frac{L^2}{D_N} \mu C\]  (A4e)

Appendix. Estimation of the values for the parameters

The set of equations (2) correspond to a coupled system of reaction–diffusion whose solution may have Turing patterns, when the parameters of the reactive term and diffusion constants satisfy certain restrictions. Therefore, for a non-null diffusion \((D_N, D_B \neq 0)\), the distribution pattern will appear to some combination of parameters \((D_N, D_B, C, \mu, \gamma_S, \alpha_1, \alpha_2)\) [20] that define the so-called Turing space.

To obtain a Turing space it requires a linear stability analysis of the reaction–diffusion system on the homogeneous solution [19], which is given by \((\partial S_B/\partial t)(D_B = 0) = 0\) and \((\partial S_N/\partial t)(D_N = 0) = 0\). This results in the stable state values given by:

\[(S_B^*, S_N^*) = \left(\frac{\alpha_1 + \alpha_2}{\mu}, \frac{\alpha_2 \mu^2}{\gamma_S (\alpha_1 + \alpha_2)^2}\right)\]

This analysis allows finding the range of parameters that ensure the emergence of such Turing patterns. Therefore, the solution can be expressed as

\[(S_N, S_B) = (u + S_N^*, v + S_B^*)\]

where \(u\) and \(v\) are small perturbations in each molecular factor, respectively. From Eqs. (1a) and (1b) and from the linear analysis (see details of the analysis in Ref. [20]) we find the geometric area where the parameters of the reaction-diffusion equation are found, in such a way, to develop Turing pattern, this is
where $T$ is the characteristic time of the biological process of differentiation of osteoblasts and $L$ is the characteristic length of the dimensional model where this biological process is presented. Therefore, defining $(\gamma, d, a, b)$ it is possible to obtain the eigenvalues and eigenvectors of the set of equations (Schnackenberg) and from them, the different spatial patterns corresponding to different wave numbers.

In the case of the proposed dimensional model it is necessary to define some parameters that are non-dimensional $(L, D_N, D_B, C, \mu, \gamma, a_1, a_2)$. To estimate these values, we must take into account some experimental evidence:

1. Zhao et al. [30] and Hoshino et al. [31] have reported a value of 26.018 Da for the molecular weight of BMP2. Moreover, Umulis et al. [32] have found typical concentrations of BMP2 in the range of 0 to 4.5 nM. Therefore, this article has used average concentrations of $S_B|_{\text{REF}} = S_N|_{\text{REF}} = S_{\text{REF}} = 1$ ng/ml.

2. The domain in study is a sphere of circumference 100 mm, then the characteristic radius is $L = r = 16$ mm, whose measure is obtained from the growth charts presented in [22] for a fetus in the fourteenth week of gestation.

3. Dennis et al. [15] have found an average concentration of $4 \times 10^6$ cells/ml of mesenchymal cells in the narrow cavity of a mouse.

4. Umulis et al. [32] have used a diffusion coefficient of $7.3 \times 10^{-7}$ cm$^2$/s for the BMP2.

5. To reproduce the patterns present in the architecture of the osteoblast cell differentiation, it is necessary that all parameters are in the Turing space and form the restrictions (A2). Thus, we must use the experimental values of $S_B|_{\text{REF}}, L$, $D_N$, $D_B$, and the set of values of the non-dimensional model, $\gamma$, $d$, $a$ and $b$ (see Table 1).

### Table 2 – Set of values for the process of ossification of the growth plate $a = 0.1$ and $b = 0.9$, $\gamma = 70.6$, $d = 11.5776$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</tr>
<tr>
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<td>$N$</td>
<td>8</td>
<td>Dimensionless</td>
</tr>
</tbody>
</table>

### Table 3 – Other parameters used in the numerical model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>0</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>$S^*_{\text{ND}}$</td>
<td>1.5</td>
<td>ng/ml</td>
</tr>
<tr>
<td>$n_D$</td>
<td>3600</td>
<td>s</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$1.7 \times 10^{-15}$</td>
<td>ng/(cell × s)</td>
</tr>
<tr>
<td>$D_N$</td>
<td>$8.66 \times 10^{-5}$</td>
<td>mm$^2$/s</td>
</tr>
<tr>
<td>$m$</td>
<td>10</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>$N$</td>
<td>10</td>
<td>Dimensionless</td>
</tr>
</tbody>
</table>

Furthermore, using the references (A4) through which we can go from the non-dimensional constants, of the Schnakenberg model, to the model described here, we can find all the parameters that represent the biological process. Table 2 shows these values used in Eq. (1).

Other parameters used can be seen in Table 3.

### REFERENCES


