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In Silico Modeling of a Bone Repair Strategy Combining an Osteoconductive Biomaterial with a Mechanical Stimulation Rehabilitation Program

Jean-Louis Milan[1, 2, 3, 4], **Nicolas Rousseau** [1, 2, 3, 4, 5] **and Martine Pithioux** [1, 2, 3, 4,*]

[1]Aix Marseille Univ, CNRS, ISM, Marseille, France

[2]Aix Marseille University, Marseille Public University Hospital System (APHM), French National Center for Scientific Research (CNRS), Institute of Movement Science (ISM), Sainte Marguerite Hospital, IML, Department of Orthopedics and Traumatology Marseille, France

[3]Aix Marseille University, Marseille Public University Hospital System (APHM), French National Center for Scientific Research (CNRS), Anatomic laboratory, Timone, Marseille, France

[4]Aix Marseille University, Mecabio Platform, French National Center for Scientific Research (CNRS), Marseille, France

[5]Selenium Medical, La Rochelle, France

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2. Key words

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1. Abstract

Bone repair in case of major defects remains a problem poorly solved by conventional techniques. Many researches are currently carried out in tissue engineering, using *in vivo* models on animals or in vitro on cell cultures, to understand and guide the mechanisms of bone repair and consolidation. New osteoconductive and osteoinductive biomaterials are developed to promote bone formation. Moreover, there is a lot of evidence on the importance of the mechanical stimulation of bone cells in the process of bone repair. Nevertheless, the mechanical environment proposed to cells within a porous biomaterial is difficult to estimate. And more importantly, in the follow-up of a patient treated for bone fracture, there is no precise management of mechanical stimulation during the rehabilitation phase with the setting up of an adapted program and the use of modern measuring tools. To study the influence of mechanical stimulation during rehabilitation and prior to complex *in vivo* experiments, the use of theoretical and numerical mechanobiological models of bone repair could be an alternative. Here tissue formation and differentiation were predicted in a porous poly-lactic acid biomaterial and a hydrogel membrane filling a large bone defect in a human tibial diaphysis. We identified optimal loading case promoting the differentiation of tissue into mature bone in the diaphysis defect. We indicated that the rehabilitation program should be adapted to reproduce this optimal mechanical stimulation. Taking advantage of the growth of the simulation means and by a greater synergy with the experimental models, the numerical modeling of the bone consolidation can constitute a complementary tool for the benefit of patients.

3. Introduction

Natural consolidation of bone is an original healing process leading to the complete reconstruction of the injured tissue. This ability to repair long bones is due to the presence of a highly vascularized envelope, the periosteum, which allows the immediate supply of mesenchymal cells that can differentiate into osteoblasts and synthesize bone matrix. Unfortunately, in case of large bone lesions of pathological (tumorous or infectious) or traumatic origin, the risk of imperfect bone reconstruction remains significant and unpredictable and may result in a pseudo arthrosis preventing any future consolidation (Rolland et al. 1995) [1]. Moreover, success in bone consolidation may depend on the location. Epiphyseal and metaphyseal fractures consolidate more rapidly than diaphyseal ones. The great majority of diaphyseal fractures are secondary to trauma, which can associate cutaneous and vascular lesions. The most frequent diaphyseal lesion in healthy humans is the fracture of the tibia. The loss of diaphyseal osseous substance is a difficult problem

***Corresponding Author (s):** Martine Pithioux, Aix Marseille University, Marseille Public University Hospital System (APHM), French National Center for Scientific Research (CNRS), Institute of Movement Science (ISM), Sainte Marguerite Hospital, IML, Department of Orthopedics and Traumatology Marseille, France, E-mail: martine.pithioux@univ-amu.fr*

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to be solved in traumatology due to lesions of the soft parts leading to a significant risk of infection (Masquelet et al. 2012) [2]. Bone fracture healing is influenced by many factors and among them the mechanical factor (Victoria et al. 2010) [3]. If the absence of immobilization prevents the consolidation, the strict immobilization is not essential. The existence of a mobility allowing mechanical stresses in compression and flexion is favorable to the development of the bone callus. On the other hand, the excessive mobility inducing high shear stresses remains detrimental to the consolidation (Palomares et al. 2010) [4]. To stabilize the fracture gap, the surgeon uses osteosynthesis plates but mainly external fixators. The advantages of the external fixator are the absence of material in the fracture gap reducing infectious risk and the protection of fracture gap from the mechanical stress which are stand by external fixator. The external fixator guides mechanical force away from the fracture gap. The bone anchorage is ensured by plugs or pins fixed in each of the fragments and the attachment of the system is located outside the body. However, it remains difficult to obtain anatomical reduction. There is an increased risk of delayed consolidation and infection on pin paths leading possibly to death occurring after two and three years of care and poor functional results responsible for a real handicap. Therefore, amputations in 1st intention or in case of functional failure remains a surgical solution (Masquelet et al. 2012) [2]. The main causes of this relative failure could be the lack of consideration of the initial state of the tissues undergoing repair, notably the role of periosteum, as well as poor vascularisation and innervation. To this regards, we previously demonstrated on animal that the preserved periosteum (without bone under), alone but still vascularized, can induce the complete regeneration of a large volume of bone 3 weeks later, in contrast to experimental controls without periosteum (Casanova et al. 2010)[5].

Immobilization and rehabilitation depend on the treatment of the fracture. While immobilization is required at the beginning, it is necessary to avoid the stiffening of the joints above and under the fracture, to maintain or to awaken the muscular atrophy. Support must be progressive from the complete discharge to the complete support. In the case of diaphyseal fractures of leg, the circular type external fixators most often allow earlysupport.

Biomaterials have been developed to fulfill the missing mechanical function of damaged bone in supporting compression. Their use in orthopedic surgery is expanding. Research focalizes on the improvement of their properties in conduction and induction of bone formation in attracting stem cell from bone marrow. To improve the effectiveness of bone regeneration, different authors in tissue engineering tend to develop biomaterials with osteoconductive functions and bone filling. For instance, Navarro et al. in 2006 developed a degradable and porous composite material made of poly lactic acid (PLA), with mechanical characteristics close to bone tissue and able to ensure an osteogenic filling function (Navarro et al. 2006) [6]. Harris et al. 2008 studied in vitro the proliferation and the differentiation of the cells within this bioactive ceramic (Harris et al. 2006) [7]. More recently, the use of hydrogel resorbable biological membranes made of porcine collagen, polycaprolactone or polyethylene glycol for instance (Wang et al. 2016) [8], is intended to promote the process of bone regeneration. Indeed, the physicochemical properties of the hydrogel matrix favor hemostasis and healing.

To quantify and predict the influence of mechanical stimulation on bone fracture healing, a numerical approach was developed being based on the mechanoregulation of cell differentiation (Lacroix et al. 2002) [9]. This approach was applied to the bone tissue formation within a porous biomaterial (Milan et al. in 2010) [10]. Then Checa et al. in 2010 studied the differentiation and the tissue repair of a diaphyseal single fracture by numerical modeling (Checa et al. 2010) [11] while Sandino et al. 2010 analyzed the formation of bone within a phosphor-calcium biomaterial by simulating angiogenesis (Sandino et al. 2010)[12].

We here propose a computational model of bone formation in the line of the previous works cited above to analyze the benefits of an osteoconductive biomaterial implantation combined with a rehabilitation program based on mechanical stimulation. Based on the recent development of hydrogel membranes (Coïc et al. 2010; Oliveira et al. 2010; Sheikh et al. 2015) [13-15], that could mimic some features of the periosteum which are crucial for bone repair (Casanova et al. 2010) [5], we analyzed the implantation of a hollow cylinder made of porous PLA wrapped with a hydrogel membrane. Tissue formation was predicted via a mechanoregulation algorithm. In identifying the appropriate mechanical stimulation program adapted to the biomaterial design, the present study provides additional knowledge that can optimize therapeutic management to enable faster and more complete functionalrecovery.

4. Methods

Bone formation and tissue differentiation were simulated according to a mechanoregulation algorithm that predicts the tissue phenotype and its mechanical properties depending on the local mechanical stimulation. The local mechanical stimuli resulting from overall loading on the tibia were computed via finite element method.

4.1. Finite Element Model of Damaged Tibia

In this study, a frozen cadaveric tibia specimen from an 85-year old female was obtained from our Department of Anatomy at the Aix-Marseille University. The specimen was scanned using a standardized CT scan protocol led by CERIMED, Marseille, France using Discovery 710 device from GE Medical Systems with the following acquisition parameters: 120 kV, 400 mA and 0.625-mm-thick slices. DICOM images were imported into Mimics software (Materialise®, Leuven, Belgium) to reconstruct the tibia numerically in 3D and create a finite element model taking into account the bone mass densities (Figure 1). To reproduce the gap of a long bone fracture, a diaphyseal bone deficit of 6 cm in length was created in the 3D reconstruction of the tibial bone. The proximal and distal part of the tibia is composed of 150,000 linear tetrahedra, type C3D4.

Figure 1: Finite element model of the tibia with a biomaterial colored in red implanted in a large diaphyseal lesion. Views of the model in front (a) and sagittal (b) plane.

Isotropic linear elastic material properties of were attributed to the finite elements composing the tibial bone depending on tomography Hounsfield units (HU). Bone mass densities (ρ) were derived from HU and Young's moduli (E) were derived from ρ. We used 2 different sets of laws dedicated respectively to the site of the tibia, one for the cortical (eq. 1, (Snyder & Schneider 1991) [17]):

$$\begin{cases} \rho = 1 * HU, \ \rho \ in \ kg/m^3 \\ E = 0.06456 * \rho^{0.74}, \ E \ in \ GPa \end{cases} \quad \text{Eq. 1}$$

the other for the trabeculae (Hobatho et al. 1997) [18]:

$$\begin{cases} \rho = 0.916 * HU + 114, \ \rho \ in \ kg/m^3 \\ E = 0.51 * \rho^{1.37}, \ E \ in \ GPa \end{cases} \quad \text{Eq. 2}$$

Poisson coefficient of tibia bone materials was set to 0.3.

To treat the bone gap defect, we considered a composite biomaterial made of a PLA scaffold shaped as a hollow cylinder wrapped by a hydrogel membrane. The PLA hollow cylinder was 60mm-high with a total diameter of 25mm, a center hole diameter of 15mm and a thickness of 5 mm. The finite element model of PLA scaffold was composed by 6,500 linear C3D8R hexahedra. The hydrogel membrane was 60mm-high and 5mm-thick and was composed by 36,000 linear C3D8R hexahedra.

The PLA scaffold and hydrogel membrane were considered as filled by biological fluids after their implantation. They were represented by poroelastic materials whose properties are reported in (Table 1).

Table 1: Poroelastic properties of the biomaterials implanted in tibial bone defect. Material properties of PLA scaffold and hydrogel membrane were reported from (Navarro et al. 2006; Charles-Harris et al. 2008) [6, 7] and (Hwang et al. 2010) [16], respectively.

	PLA scaffold	Hydrogel membrane
Young's modulus (kPa)	100	1
Poisson's coefficient	0.3	0.3
Permeability (m^4/Ns)	2 e^{-7}	1 e^{-10}
Porosity	0.95	0.8
Solid bulk modulus (MPa)	500	2300
Fluid bulk modulus (MPa)	2300	2300

4.2. Loading and Boundary Conditions

We simulated a rehabilitation program in which compression loading was applied from the proximal to the distal part of the tibia, cyclically every 1s and during 1-2 hours per day. We express the loadings in maximal compressive displacement amplitude per second (mm/s). We tested 4 compressive rates: 0.3, 0.6, 1.8 and 3 mm/s. Then the algorithm of mechanoregulation predicted tissue phenotype that would form within PLA scaffold and hydrogel membrane after 2 months of rehabilitation program. To obtain stable *in silico* prediction of tissue phenotype and to reach convergence, the mechanoregulation algorithm was performed iteratively for every loading case. The media of PLA scaffold and hydrogel membrane were totally permeable with zero pressure at their boundaries except at the external surface of the membrane. This side of the hydrogel membrane was set as impermeable with zero fluid velocity through it to simulate membrane features avoiding cell invasion from surrounding soft tissues.

4.3. Model of Mechanical Regulation

The diaphyseal lesion causes an influx of undifferentiated mesenchymal cells that colonize the porous filling biomaterial. This phase of cell migration and colonization of the biomaterial by stem cells is essential and can be a brake on osteosynthesis. However, to only discriminate the influence of mechanical stimulation on bone formation, we assumed that the biomaterials were completely colonized by the stem cells. Thus, during the simulation whatever the region of the biomaterial considered, the stem cells could differen-

tiate into fibroblasts, chondrocytes or osteoblasts according to the mechanical stimuli according to the algorithm shown in Figure 2. We did not consider any other biological factor. The procedure was iterated to achieve a possible convergence of the tissue phenotype under mechanical stimulation. The algorithm consisted in calculating, for each iteration, the mechanical stimuli in each element of the PLA scaffold and hydrogel membrane to identify the phenotype of the promoted tissue and to update the properties of the materials. For each iteration, finite element analysis was performed under the same boundary and initial conditions. The process of differentiation was governed by the biophysical stimulus $\boldsymbol{S}$ (Eq. 3) as a combination of tissue shear strain $\boldsymbol{\varepsilon}$ and interstitial fluid velocity $\boldsymbol{v}$ (Prendergast et al. 1997; Lacroix and Prendergast, 2002),

$$S = \varepsilon/a + v/b \qquad \text{(Eq. 2)}$$

wherein $\boldsymbol{a}$ and $\boldsymbol{b}$ are equal to 3.75% and 3mm.s^{-1} respectively

As shown in in Table 2, four $\boldsymbol{S}$ thresholds were used to determine whether the cell differentiates into fibroblasts, chondrocytes or osteoblasts, leading respectively to the formation of fibrous tissue, cartilage tissue or bone tissue. If $\boldsymbol{S}$ <0.01067, the cells are under stimulated, which leads to tissue resorption and replacement by the new granulation tissue.

No predetermined pathways led to tissue differentiation from one phenotype to another. The phenotype of the tissues at the iteration *i* did not depend on the phenotype at the iteration *i*-1 but only on $\boldsymbol{S}$ (*i*). The tissues were considered as poroelastic materials whose properties are given in Table 2. During the simulation, when the tissue formed inside the finite elements of the PLA scaffold and hydrogel membrane, those elements were viewed as composite elements made of biological tissue and PLA or hydrogel; their mechanical properties were homogenized. Over the time of 2 months of rehabilitation program we simulated in this study, we considered no degradation of PLA scaffold and hydrogel membrane; their material properties remained unchanged.

Table 2: Poroelastic properties of the specific tissue phenotypes promoted by biophysical stimuli, S given by Eq. 3 (Lacroix and Prendergast). The values are reported from (Checa et al. 2010) [11].

	Granulation tissue	Mature bone	Immature bone	Cartilage	Fi b rou s tissue
B i o p h y si c a l stimulus	S < 0.01067	0.01067 < S< 0.267	0.267 < S < 1	1 < S < 3	3 < S
Y o u n g ' s modulus (MPa)	0.2	6000	1000	10	2
P o i s s o n ' s coefficient	0,167	0,3	0,3	0,167	0,167
Pe rme a b i li t y (m⁴/Ns)	1 e^{-14}	3,7 e^{-13}	1 e^{-13}	5 e^{-15}	1 e^{-14}
Porosity	0,8	0,8	0,8	0,8	0,8
Solid bulk modulus (MPa)	2300	13920	13920	3400	2300
Fluid bulk modulus (MPa)	2300	2300	2300	2300	2300

At the beginning of the simulation, the biological material consists entirely of granulation tissue. We assume that the mechanical properties of the liquid phase in the biological material remain unchanged during the simulation of the differentiation.

The model does not account for biological time in the simulation of tissue differentiation. The application of mechanical loading here results in the formation of a differentiated tissue without considering the duration of the stimulation nor the time required for the cells to synthesize tissues. Thus, the model directly calculates the effect of stimulation on tissue differentiation since it must be applied throughout the biological process of formation and differentiation. In this study, we analyzed the cyclic compression effect applied dynamically, the displacement being imposed for a time of 1s with various compressive amplitudes. The overall procedure combining loading simulation and mechanoregulation algorithm (Figure 2) was iterated 50 times. The evolution of the distribution of the various phenotype would indicate if the simulation has converged.

4. Results

The 3D finite element model of tibial bone with a diaphyseal bone deficit of 6 cm in length was obtained by image segmentation and the isotropic linear elastic material properties were assigned depending on local HU values (Figure 3). We performed simulations of mechanoregulation algorithm and obtained tissue formation within the PLA scaffold and hydrogel membrane implanted in the diaphyseal bone defect. Final tissue phenotypes highly depend on the compressive rate applied on the tibial bone (Figure 4). In the loading cases of 0.5mm/s and above, the distributions of tissue phenotypes are heterogenous in the diaphyseal direction (Figure 5). The distributions of tissue phenotype are quite homogeneous in the radial direction without any great variation between PLA scaffold and hydrogel membrane.

The results indicate an optimal case of stimulation promoting the regeneration of mature bone tissue within the composite biomaterial. The loading condition of 0.1mm/s allows up to 97% regeneration in mature bone (Figure 4). For lower compressive amplitude, cell apoptosis and tissue resorption are predicted due to the lack of stimulation. For higher compressive amplitude, the stability of the bone tissue is not guaranteed, and cartilage and fibrous tissue formed.

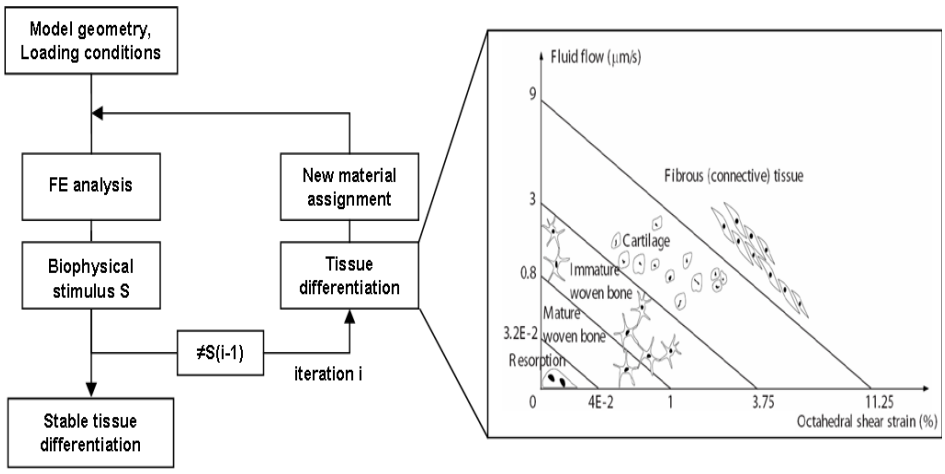

Figure 2: Mechanoregulation algorithm. Iterative procedure and diagram of tissue differentiation following mechanical stimulus thresholds (Prendergast et al. 1997; Lacroix and Prendergast 2002).

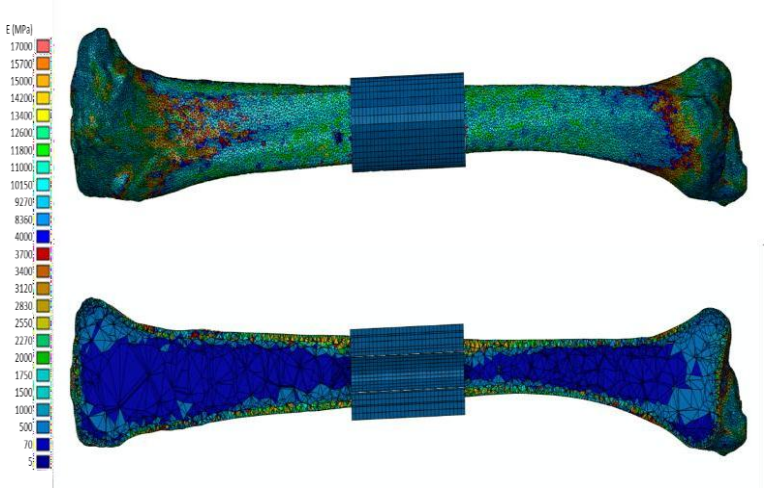

Figure 3: Distribution of material properties expressed in terms of Young's modulus, E (Mpa) in the finite element model of tibia with biomaterial implanted in the diaphyseal defect.

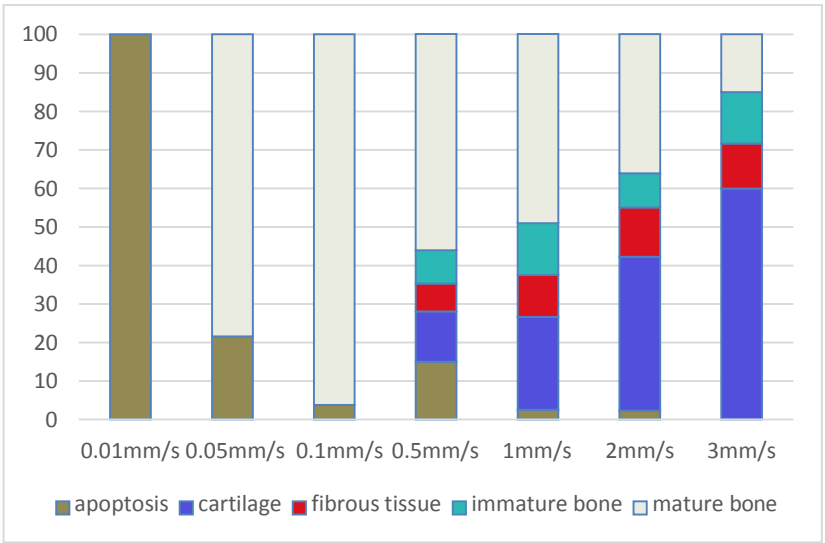

Figure 4: Proportions of the different tissue phenotypes that formed in PLA scaffold and hydrogel membrane at the end of simulation of tissue differentiation depending on the amplitude of mechanicalloading.

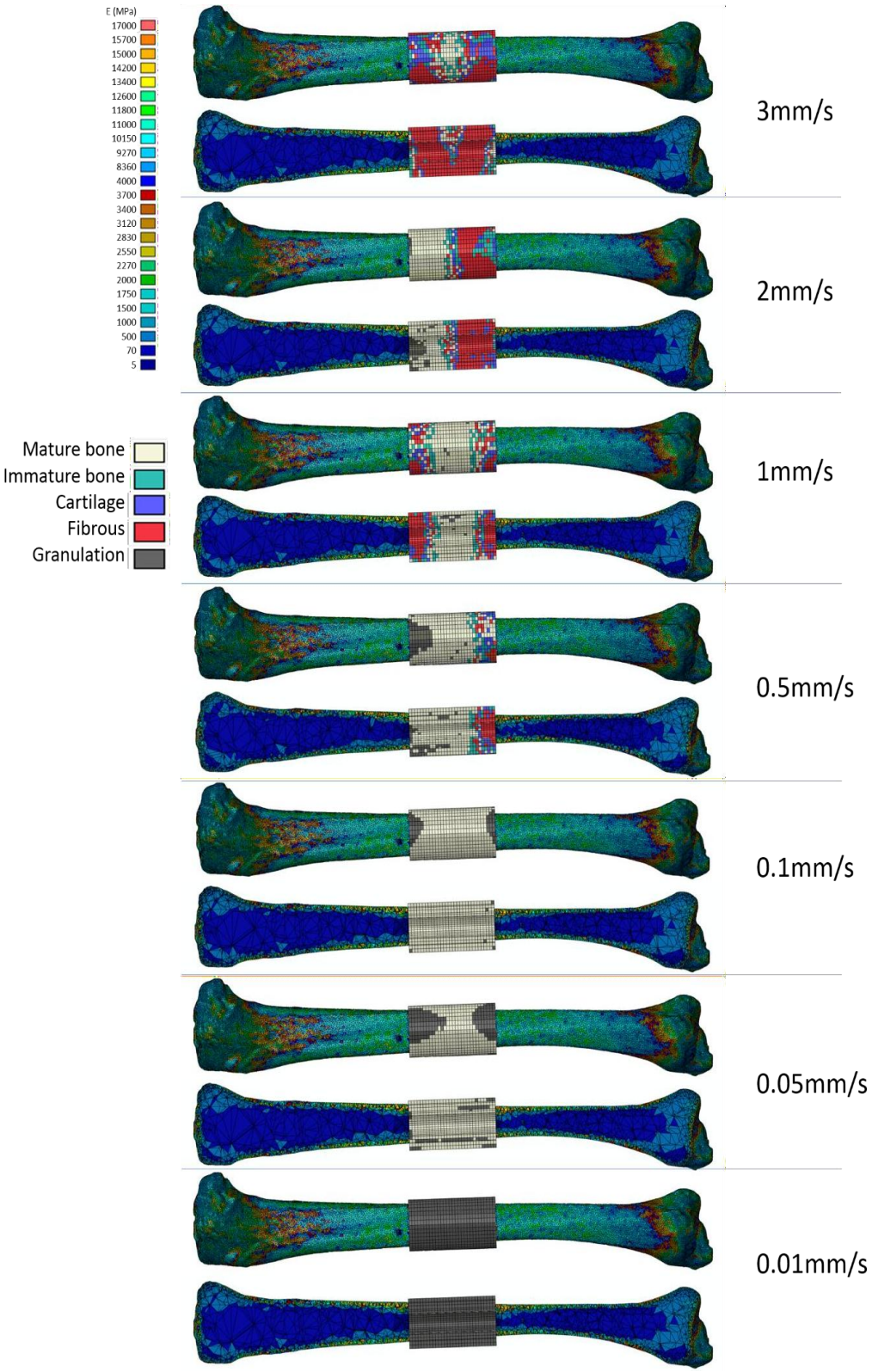

Figure 5: Distribution of tissue phenotypes formed within PLA scaffold and hydrogel membrane at the end of the simulation depending on the loading case. For every loading case, the tibial bone and biomaterial are showed in sagittal plane both in side-view and cut-view.

(Figure 6) showed the evolution of the proportion of mature bone during the iterative simulations for all loading cases while (Figure 7) shows the evolution of the proportion of all tissue phenotypes during optimal iterative simulation of 0.1mm/s. The loading case of 0.1mm/s predicts maximal and stable formation of mature bone since the first iteration.

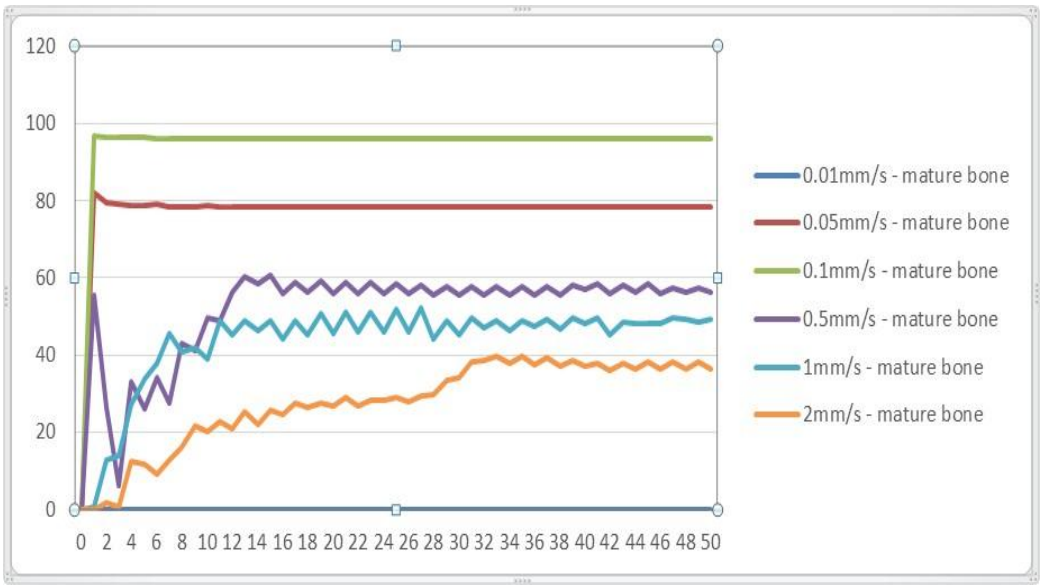

Figure 6: Proportion of mature bone formation in PLA scaffold and hydrogel membrane depending on the iteration of tissue differentiation simulation for all loading cases.

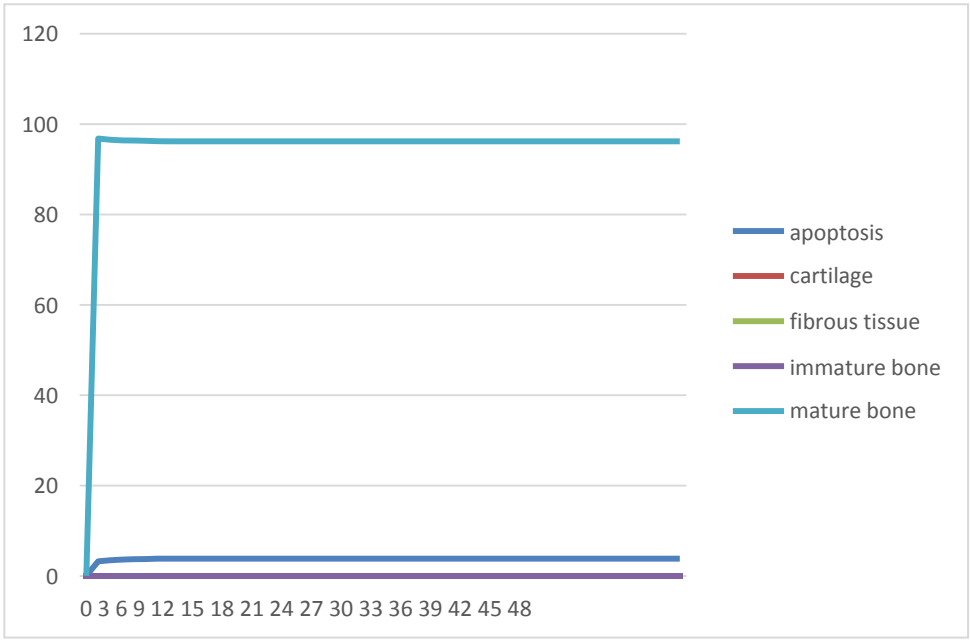

Figure 7: Proportion of tissue phenotype formation in PLA scaffold and hydrogel membrane depending on the iteration of tissue differentiation simulation for the loading case of 0.1 mm/s.

5. Discussion and Conclusion

The model directly calculates the effect of mechanical stimulation on tissue differentiation within an osteoconductive biomaterial composed by a PLA scaffold wrapped by a hydrogel membrane. If the first iteration gives us information about the formation of a phenotype specifically to the loading case, nonetheless, the results may show fluctuation in the proportions of tissue phenotype which are formed from one iteration to another. The aim of the method using the mechanoregulation algorithm is to obtain at the end of the stimulation a stable distribution of materials. This stability, which may not necessarily be achieved, results from the fact that under overall mechanical loading the local stress and strain within the materials are in the range of the stimuli which would lead to this same material. Indeed, depending on the stimuli at the iteration *i*, the material elected, because of its own properties in terms of stiffness, porosity, permeability may modify the distribution and values of stress and strain as well as fluid velocity under compression. The challenge in this study is to maintain, for the following

iterations, the mature bone which has been already formed and to obtain the differentiation of immature bone, cartilage or fibrous tissue into mature bone.

Previously study (Casanova et al. 2010) [5] have shown, *in vivo*, that the periosteum alone can regenerate a large volume of bone. In the case of major trauma, it is therefore necessary to find a biomaterial that serves as a stent and allows the regeneration of the periosteum. The hollow cylinder made of PLA foam acts as a stent and the collagen membrane will allow regeneration of the periosteum. In order to differentiate the cells into mature bone tissue, it is necessary to know precisely the mechanical conditions to be applied to the bone to be regenerated. The numerical model developed plays this role. Then, the objective of this study is to develop a numerical model which mimicking the periosteal role observed in *in vivo* model.

The numerical model of mechanoregulation developed by Prendergast and Lacroix has been validated on several clinical applications such as fracture repair, mandibular bone distraction and several in vitro experiments of bone formation in porous biomaterials. So, we used this model to answer the problems of our study: the influence of mechanical stimulation on bone regeneration at the level of a large diaphyseal defect. To help bone formation, we considered the presence of a guide, namely hollow cylinder of PLA scaffold wrapped with a hydrogel membrane. The design of hollow cylindric biomaterial tends to improve the entry of blood, bone marrow and stem cells while hydrogel membrane at the periphery promotes the local periosteal vascularization essential to the process of bone regeneration. The model of mechano-regulation applied to the solid biomaterial shows very good results in the regeneration of mature bone tissue and allows to refine the prediction of bone repair. The results of this model verify the working hypotheses. The mechanical quality of the bone tissue formed depends on the mechanical stresses which play a discriminating role depending on the properties of the scaffold which is implanted. This *in silico* modeling allowed to discriminate cases of mechanical loads favoring the formation of immature and mature bone which answers the original question of the study. Ultimately, the strategy to be adopted and the clinical response must involve the clinicians, the rehabilitation workers and the experts in biomechanics to optimize and integrate the patient-dependent specificities. Thus, this study brings new perspective and could be part of an overall approach to help bone repair and favor faster recovery.

6. Acknowledgements

The authors declare no conflict of interest.

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