



**HAL**  
open science

## **In silico modelling of long bone healing involving osteoconduction and mechanical stimulation**

Jean-Louis Milan, Ian Manificier, Nicolas Rousseau, Martine Pithioux

► **To cite this version:**

Jean-Louis Milan, Ian Manificier, Nicolas Rousseau, Martine Pithioux. In silico modelling of long bone healing involving osteoconduction and mechanical stimulation. *Computer Methods in Biomechanics and Biomedical Engineering*, 2023, pp.1-13. 10.1080/10255842.2022.2052051 . hal-03618025

**HAL Id: hal-03618025**

**<https://hal.science/hal-03618025>**

Submitted on 23 Mar 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **In silico modelling of long bone healing involving osteoconduction and mechanical stimulation.**

2 Jean-Louis Milan <sup>a,b,c,d</sup>, Ian Manificier <sup>a,b,c,d</sup>, Nicolas Rousseau <sup>a,b,c,d,e</sup>, Martine Pithieux <sup>a,b,c,d</sup>

3 <sup>a</sup> Aix Marseille Univ, CNRS, ISM, Marseille, France;

4 <sup>b</sup> Aix Marseille University, Marseille Public University Hospital System (APHM), French National Center for  
5 Scientific Research (CNRS), Institute of Movement Science (ISM), Sainte Marguerite Hospital, IML, Department  
6 of Orthopedics and Traumatology Marseille, France;

7 <sup>c</sup> Aix Marseille University, Marseille Public University Hospital System (APHM), French National Center for  
8 Scientific Research (CNRS), Anatomic laboratory, Timone, Marseille, France;

9 <sup>d</sup> Aix Marseille University, Mecabio Platform, French National Center for Scientific Research (CNRS), Marseille,  
10 France

11 <sup>e</sup> Selenium Medical, La Rochelle, France

12

13 **Abstract**

14 Large diaphyseal fractures is one of most challenging fractures to treat. More research is needed  
15 to reliably promote diaphyseal bone healing. Many research projects currently aim to understand and  
16 guide the long bone repair and consolidation. These projects typically involve in vivo or in vitro models.  
17 Moreover, new osteoconductive and osteoinductive biomaterials are developed to promote bone  
18 formation. In addition, a lot of evidence has shown the importance of stimulating cell mechanically  
19 during bone repair. However, the mechanical environment proposed to cells within a porous  
20 biomaterial is difficult to evaluate. More importantly, after a bone fracture, there is no precise  
21 management of mechanical stimulation during a patient's rehabilitation that involves an adapted  
22 therapy program guided by modern measuring tools. Instead, we believe that theoretical and  
23 numerical mechanobiological models of bone repair could become practical tools to narrow down key  
24 parameters before moving to in vivo experiments. It then seems logical that modeling can be useful to  
25 optimize mechanical stimulation during a patient's rehabilitation. In this study, we modeled the  
26 fracture healing of a large bone defect in tibial diaphysis. To fill the fracture gap, we considered the  
27 implantation of a porous osteoconductive biomaterial made of poly-lactic acid wrapped by a hydrogel  
28 membrane mimicking osteogenic properties of the periosteum. We identified the optimal loading case  
29 that best promotes the formation and differentiation into bone tissue within the diaphyseal bone  
30 defect. Our results support the idea that a patient's rehabilitation program should be adapted to  
31 reproduce optimal mechanical stimulations.

32

33

34 **1. Introduction**

35 In many instances natural bone healing and consolidation leads to the complete reconstruction  
36 of the injured tissue. The periosteum, a highly vascularized bone envelop, is a major contributor to the  
37 healing process. The periosteum acts as a clear boundary between the area where bone tissue must  
38 regrow and surrounding tissues. In addition, the periosteum favors the supply of mesenchymal cells  
39 that can differentiate into osteoblasts and synthesize bone matrix. Unfortunately, in the case of large  
40 bone lesions of either pathological (tumorous or infectious) or traumatic origin, the periosteum may  
41 be completely damaged. As a result, the risk of inadequate bone reconstruction remains significant  
42 and unpredictable. Such lesions may result in a pseudo arthrosis preventing any future consolidation  
43 [Rolland1995].

44 Moreover, the success of bone consolidation may depend on location. Epiphyseal and  
45 metaphyseal fractures consolidate more rapidly than diaphyseal ones. The great majority of diaphyseal  
46 fractures are secondary to trauma, which can be associated to cutaneous and vascular lesions. The  
47 most frequent diaphyseal lesion in healthy humans is the fracture of the tibia. The loss of diaphyseal  
48 osseous tissue is a difficult problem in traumatology due to soft tissues lesions that in turn lead to a  
49 significantly higher risks of infection [Masquelet2012].

50 Bone fracture healing is influenced by many factors and mechanics is an important one  
51 [Victoria2010]. Proper consolidation cannot occur without immobilization. However, strict  
52 immobilization is neither essential nor optimal. Instead, limited mobility enables mechanical strain and  
53 fluid flow induced shear stresses, both of which contributes to the proper development of a bone  
54 callus. However, excessive mobility leads to high fluid induced shear stresses that are in turn  
55 detrimental to consolidation [Palomares2010].

56 To stabilize the fracture gap, the surgeon uses osteosynthesis plates and external fixators.  
57 External fixators have the advantage of not interfering with the site of injury while shielding the  
58 fracture gap from excessive external mechanical forces. Nonetheless external fixators increase the risk  
59 of delayed consolidation. Serious infection can occur on pin paths and may lead to death a few years  
60 later. Therefore, amputations by primary intention or in case of functional failure remains a surgical  
61 solution [Masquelet2012]. Fracture healing failures highlight a poor understanding of the whole  
62 process and in particular the role of the periosteum. Indeed, on this last point, we have previously  
63 demonstrated in animal models that a well-preserved and well-vascularized periosteum can induce  
64 the complete regeneration of a large bone gap after 3 weeks [Casanova2010].

65 Although, it is necessary to limit the relative mobility between the proximal and distal tibial  
66 fragments, associated joint stiffening and muscular atrophies must also be avoided. Support must be

67 progressive from complete discharge to full weight loading. In the case of diaphyseal leg fractures,  
68 external fixators have the advantage of allowing patient to walk sooner. The present study analyzed  
69 the potential of scalable external fixators that could allow limited and controlled interfragmentary  
70 movement (IFM). Such a system is not yet widely used in orthopedics. Nevertheless, it could  
71 mechanically stimulate the bone callus and accelerate fracture healing.

72 In orthopedic surgery, the use of biomaterial meant to bear the mechanical load during the  
73 recovery process is expanding. Many research projects focalize on the improvement of the  
74 osteoconductive and osteoinductive properties of biomaterials and their ability to properly recruit  
75 osteopotent stem cells from bone marrow. For instance, Navarro et al. in 2006 developed a porous  
76 biodegradable material composite made of poly lactic acid (PLA). Its mechanical properties closely  
77 matched those of bone tissue and promote bone tissue formation [Navarro2006]. Harris et al. 2008  
78 studied in vitro cell proliferation and differentiation within this bioactive ceramic [Harris2006].  
79 Furthermore, biomaterials made of resorbable hydrogels are good candidates for tissue engineering  
80 [Yue2020]. They have properties similar to the native extracellular matrix and thus promote cell  
81 adhesion and proliferation. Hydrogel membranes were developed to perform the function of biological  
82 membranes promoting hemostasis and bone regeneration. These membranes consisted of porcine  
83 collagen, polycaprolactone, or polyethylene glycol [Wang2016]. Hydrogel membranes could be used  
84 to replicate the crucial role of the periosteum in the healing process of large bone defects [Coïc2010;  
85 Oliveira2010; Sheikh2015].

86 To quantify and predict the influence of mechanical stimulation on bone fracture healing,  
87 numerical approaches were developed. One of the most widely used is that based on  
88 mechanoregulation theory which predicts cell differentiation and tissue formation as a function of the  
89 range of fluid and solid mechanical stimuli [Prendergast1997; Lacroix2002]. We used this approach to  
90 model bone tissue formation in a porous biomaterial [Milan2010]. Checa et al. in 2010 also used a  
91 numerical model to study tissue differentiation and repair of a diaphyseal fracture [Checa2010]. On  
92 the same note, Sandino et al. 2010 analyzed the formation of bone within a phosphor-calcium  
93 biomaterial by simulating angiogenesis [Sandino2010].

94 In line with previous work, we propose here a in silico model of tibial bone healing. More precisely,  
95 this model applies to a large diaphyseal defect. With the model, we analyzed the benefits of implanting  
96 an osteoconductive biomaterial in conjunction with a mechanically oriented rehabilitation program.  
97 The biomaterial was shaped as a hollow cylinder made of porous PLA wrapped in a hydrogel  
98 membrane. Tissue formation was predicted by the mechanoregulation algorithm. As a result, we  
99 define the optimal mechanical stimulation to promote bone defect healing. Our results provide

100 additional knowledge to optimize therapeutic management in orthopedics and enable faster and more  
101 complete functional recovery.

102

## 103 2. Methods

104 Bone formation and tissue differentiation were simulated according to a mechanoregulation  
105 algorithm. The latter is used to predict tissue phenotypes and their mechanical properties depending  
106 on local mechanical stimuli, which resulted from imposed mechanical loading on the tibia. We used a  
107 finite element method to calculate these stimuli.

### 108 2.1 Finite element model of damaged tibia

109 A frozen cadaveric tibia specimen from an 85-year old female was obtained from our Department  
110 of Anatomy at the Aix-Marseille University. The specimen was scanned using a standardized CT scan  
111 protocol led by CERIMED, Marseille, France using Discovery 710 device from GE Medical Systems with  
112 the following acquisition parameters: 120 kV, 400 mA and 0.625-mm-thick slices. DICOM images were  
113 imported into Mimics software (Materialise®, Leuven, Belgium) to numerically reconstruct the tibia in  
114 3D and create, based on bone mass densities, a finite element model (Figure 1). To reproduce the gap  
115 of a long bone fracture, a diaphyseal bone deficit of 6 cm in length was created in the 3D reconstruction  
116 of the tibial bone. The proximal and distal part of the tibia is composed of 150,000 linear tetrahedra  
117 (type C3D4). The quality of the tibia and biomaterial meshes was validated by Abaqus mesh verification  
118 analysis, keeping the standard threshold values proposed by default and following the  
119 recommendations of Burkhart et al. 2013 [Burkhart2013] (Table 1).



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

<b>Meshed Part</b>	<b>Tibia</b>	<b>Biomaterial</b>
Number of elements	152 076	4 320
Shape of elements	Tetrahedral	Hexahedral
Type	Linear	Linear
Percentage of elements with Min angle on Triangle Faces < 5°	0%	0%
Average Min face corner angle	28.57°	84.61°
Worst Min face corner angle	6.61°	82.39°
Percentage of elements with Max face corner angle < 170°	100%	100%
<b>Percentage of elements with Max face corner angle &lt; 120°</b>	<b>96.28%</b>	<b>100%</b>
Average Max face corner angle	98.80°	95.43°
Worst Max face corner angle	163.33°	97.47°
Percentage of elements with Aspect ratio < 10	100%	100%
Average aspect ratio	2.22	1.39
Worst aspect ratio	7.89	1.85
Percentage of elements with Shape factor > 0.0001	100%	n/a
<b>Percentage of elements with Shape factor &gt; 3</b>	<b>90.65%</b>	<b>n/a</b>
Average shape factor	0.43	n/a
Worst shape factor	2.38e-04	n/a
Percentage of elements with Stable time increment > 0.0001	100%	n/a
Average stable time increment	0.0076	n/a
Smallest stable time increment	4.61e-04	n/a
Percentage of elements with Min edge length > 0.01mm	100%	100%
Average min edge length (mm)	1.44	1.74
Shortest edge (mm)	0.12	1.18
Percentage of elements with Max edge length > 1mm	99.67%	100%
Average max edge length (mm)	2.99	2.37
Longest edge (mm)	23.47	3.01
Percentage of elements with Analysis errors	0%	0%
Percentage of elements with Analysis warnings	1.40 %	0%

124 **Table 1** - Mesh quality analysis for the tibia and the biomaterial. In red are the checks of mesh quality  
125 following the recommendations of Burkhart et al. 2013 [Burkhart2013].

126

127 Each finite element of the tibial bone was defined as isotropic. However local linear elastic  
128 material properties were based on tomography Hounsfield units (HU). HU allowed us to derive local  
129 bone mass densities ( $\rho$ ), which in turn allowed us to calculate the corresponding Young's moduli (E).  
130 We used 2 different sets of laws dedicated respectively to the tibia, one for the cortical (eq. 1,  
131 [Snyder1991]):

$$132 \quad \begin{cases} \rho = 1 * HU, \rho \text{ in } kg/m^3 \\ E = 0.06456 * \rho^{0.74}, E \text{ in } GPa \end{cases} \quad \text{Eq. 1}$$

133 the other for the trabeculae [Hobatho1997]:

$$134 \quad \begin{cases} \rho = 0.916 * HU + 114, \rho \text{ in } kg/m^3 \\ E = 0.51 * \rho^{1.37}, E \text{ in } GPa \end{cases} \quad \text{Eq. 2}$$

135 Poisson coefficient of tibia bone materials was set to 0.3.

136 To treat the bone gap defect, we considered a composite biomaterial made of a PLA scaffold in  
 137 the shaped of a hollow cylinder and a hydrogel membrane wrapped around it. The PLA hollow cylinder  
 138 was 60 mm in length with an external diameter of 25mm, a center hole diameter of 15 mm and a  
 139 thickness of 5 mm. The PLA hollow cylinder approximated the diaphyseal hollow shape. The finite  
 140 element model of the PLA scaffold was made of 6,500 linear C3D8R hexahedra. The hydrogel  
 141 membrane was 60 mm in length and 5mm thick and was composed by 36,000 linear C3D8R hexahedra.

142 We considered that, after implantation, the PLA scaffold and hydrogel membrane became filled  
 143 with biological fluids. They were represented by poroelastic materials whose properties are reported  
 144 in Table 2.

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

145 **Table 2.** Material properties of the PLA scaffold [Navarro2006; Charles-Harris2008] and the  
 146 hydrogel membrane [Hwang2010].

147

## 148 2.2 Loading and boundary conditions

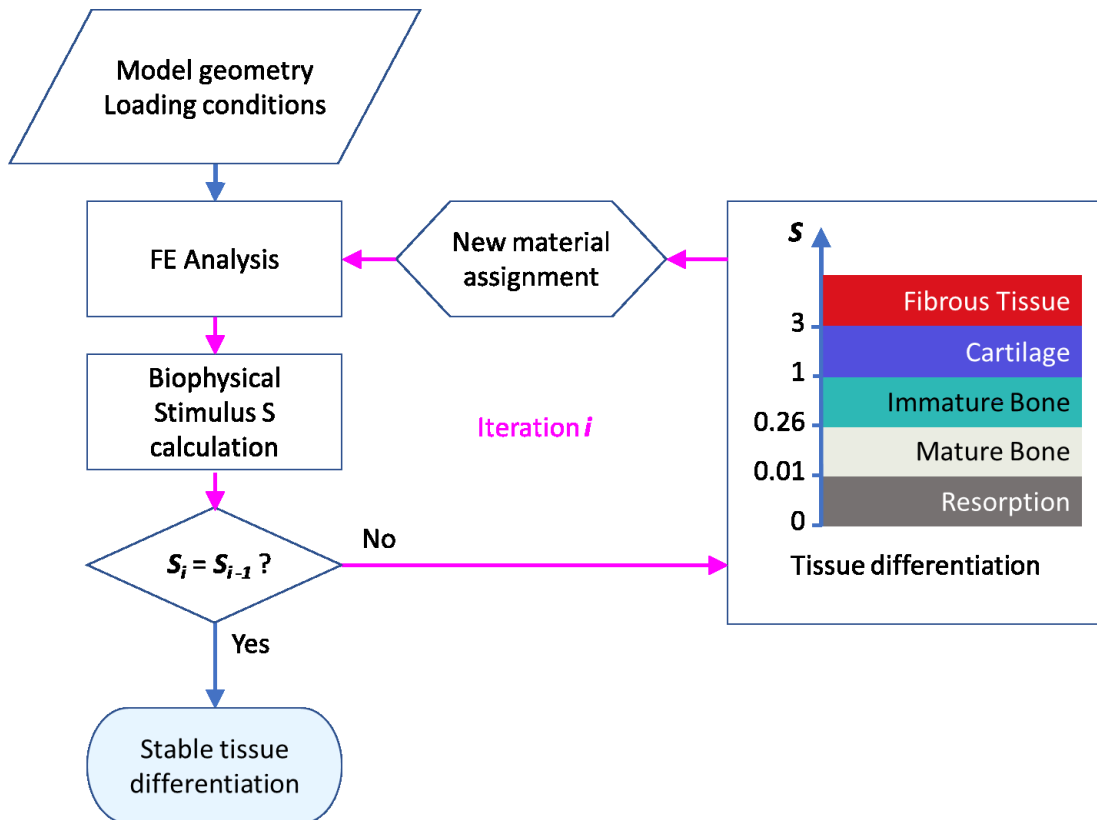
149 We simulated a rehabilitation program in which the distal part of the tibia is compressed, cyclically  
 150 every 1s and during 1-2 hours per day. We express each loading condition by the amplitude of  
 151 compressive distal displacement which was applied per second (mm/s) and induced IFM in the  
 152 diaphyseal bone defect. We tested 4 compressive rates: 0.3, 0.6, 1.8 and 3 mm/s. Then the  
 153 mechanoregulation algorithm predicted the local tissue phenotype that would form within the PLA  
 154 scaffold and hydrogel membrane after a 2-month long rehabilitation program. To obtain stable in silico  
 155 predictions of tissue phenotypes and to reach convergence, the mechanoregulation algorithm was  
 156 performed iteratively for every loading case. The media of the PLA scaffold and the hydrogel  
 157 membrane were permeable with zero pressure at their boundaries except on the outer side of the  
 158 membrane. A zero-velocity boundary condition was applied to the outer side the hydrogel membrane  
 159 to simulate its impermeability that prevents cell invasion by surrounding soft tissue.

160

161 **2.3 Mechanical Regulation Model**

162 The diaphyseal lesion causes an influx of undifferentiated mesenchymal cells that colonize the  
 163 porous biomaterial. It is essential that stem cells fully colonize the biomaterial. Otherwise,  
 164 osteosynthesis may be compromised. However, in this study, our intention was only to consider the  
 165 influence of mechanical stimulation on bone formation, we therefore assumed that the biomaterials  
 166 were completely colonized by stem cells at the beginning of the simulation. Thus, during the simulation  
 167 and throughout the porous biomaterial, stem cells were able to differentiate into fibroblasts,  
 168 chondrocytes or osteoblasts according to the mechanoregulation algorithm shown in Figure 2. No  
 169 other cell differentiation factor was considered. The procedure was iterated several times to achieve,  
 170 when possible, convergence of the tissue phenotype due to mechanical stimulation. For each iteration,  
 171 the mechanical stimulus  $S$  in each element of the PLA scaffold and hydrogel membrane was used to  
 172 identify the phenotype of the new tissue and to update the properties of the materials. For each  
 173 iteration, finite element analysis was performed under the same boundary and initial conditions via  
 174 the Standard Abaqus solver (Dassault Systems Simulia Corporation).

175



176

177 **Figure 2:** Mechanoregulation algorithm. Iterative procedure and diagram of tissue differentiation  
 178 following mechanical stimulus thresholds [Prendergast1997; Lacroix2002].



179 The differentiation process was governed by the biophysical stimulus  $S$  as a combination of the  
180 shear strain  $\epsilon$  in the tissue and the interstitial fluid velocity  $v$  (Eq. 3) [Prendergast1997; Lacroix2002],

$$181 \quad S = \epsilon / a + v / b \quad (\text{Equation 3})$$

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

183 As shown in in Table 3, we considered 4 different  $S$  thresholds to determine whether if the cells  
184 differentiate into fibroblasts, chondrocytes or osteoblasts, respectively leading to the formation of  
185 fibrous tissue, cartilage tissue or bone tissue. If cells were under stimulated ( $S < 0.01067$ ), the tissue  
186 was replaced by its granular counterpart.

187 No predetermined pathways led to tissue differentiation from one phenotype to another. The  
188 phenotype of the tissues at the iteration  $i$  did not depend on the phenotype at the iteration  $i-1$  but  
189 only on  $S(i)$ . The tissues were considered as poroelastic materials whose properties are given in Table  
190 3. During the simulation, when tissue either formed inside the scaffold and the membrane, the  
191 corresponding elements were viewed as homogenized composites made of biological tissue and PLA  
192 or hydrogel. Since, the time required to degrade the biomaterial is much longer than the 2-month  
193 duration of the rehabilitation program, we assumed that the implant did not degrade and that its  
194 material properties remained unchanged [Navarro2006]. At the beginning of the simulation, all tissue  
195 contained within the pores of the biomaterial started as granular. We assumed that the mechanical  
196 properties of the liquid phase in the biological material remained unchanged during the simulation.

197 Tissue differentiation and formation correspond to a gradual process spanning over the  
198 rehabilitation period. However, the computation predicted only the final tissue phenotypes after 2  
199 months of a daily stimulation rehabilitation program. Thus, an iteration of the algorithm did not  
200 correspond to a day of stimulation. Instead, it was a prediction of the final tissue phenotype after 2  
201 months of stimulation based on the last differentiation factor  $S$  to be calculated. As a result, an  
202 iteration should be interpreted as an optimization step necessary to converge towards a stable  
203 solution. We consequently ran each simulation with enough iteration to observe convergence.

204 In this study, we analyzed the effect of dynamic compression cycles, distal displacement being  
205 imposed for a time of 1s with various compressive amplitudes. The overall procedure which included  
206 both mechanical computations and the mechanoregulation algorithm (Figure 2) was iterated 50 times.  
207 If the phenotype remained constant in 95% of biomaterial elements we consequently assumed that  
208 the simulation had converged.

209

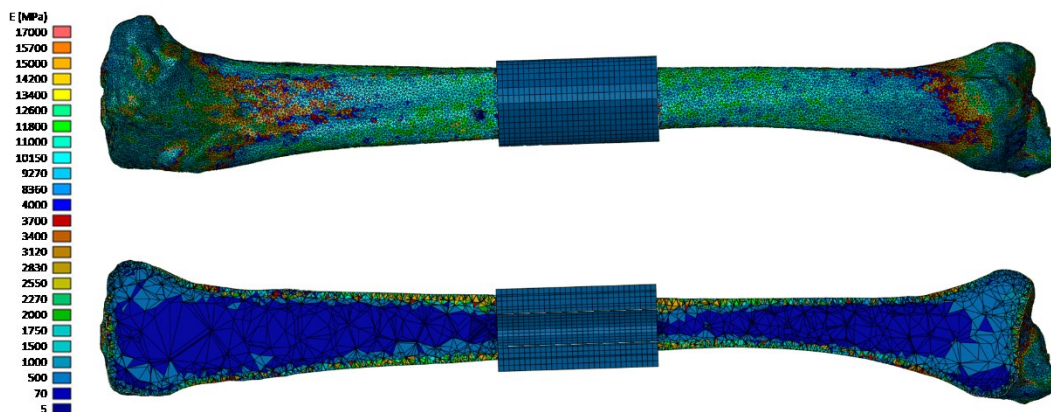
	Granulation tissue	Mature bone	Immature bone	Cartilage	Fibrous tissue
<b>Biophysical stimulus</b>	$S < 0.01067$	$0.01067 < S < 0.267$	$0.267 < S < 1$	$1 < S < 3$	$S < 3$
<b>Young's modulus (MPa)</b>	0.2	6000	1000	10	2
<b>Poisson's coefficient</b>	0,167	0,3	0,3	0,167	0,167
<b>Permeability (m<sup>4</sup>/Ns)</b>	$1 \text{ e}^{-14}$	$3,7 \text{ e}^{-13}$	$1 \text{ e}^{-13}$	$5 \text{ e}^{-15}$	$1 \text{ e}^{-14}$
<b>Porosity</b>	0,8	0,8	0,8	0,8	0,8
<b>Solid bulk modulus (MPa)</b>	2300	13920	13920	3400	2300
<b>Fluid bulk modulus (MPa)</b>	2300	2300	2300	2300	2300

210 **Table 3.** Poroelastic properties of the specific tissue phenotypes promoted by biophysical stimuli, *S*  
211 [Lacroix2002; Checa2010]

212

### 213 3. Results

214 The 3D finite element model of tibial bone with a diaphyseal bone deficit of 6 cm in length was  
215 obtained by image segmentation and the isotropic linear elastic material properties were assigned  
216 depending on local HU values (Figure 3).



217

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

220

221 After 50 optimization iterations, of the mechanoregulation algorithm, tissue had formed and  
222 differentiated within the implanted PLA scaffold and the hydrogel membrane (Figure 4). Results show  
223 that the final tissue phenotypes highly depend on the compressive rate applied on the tibial bone. In

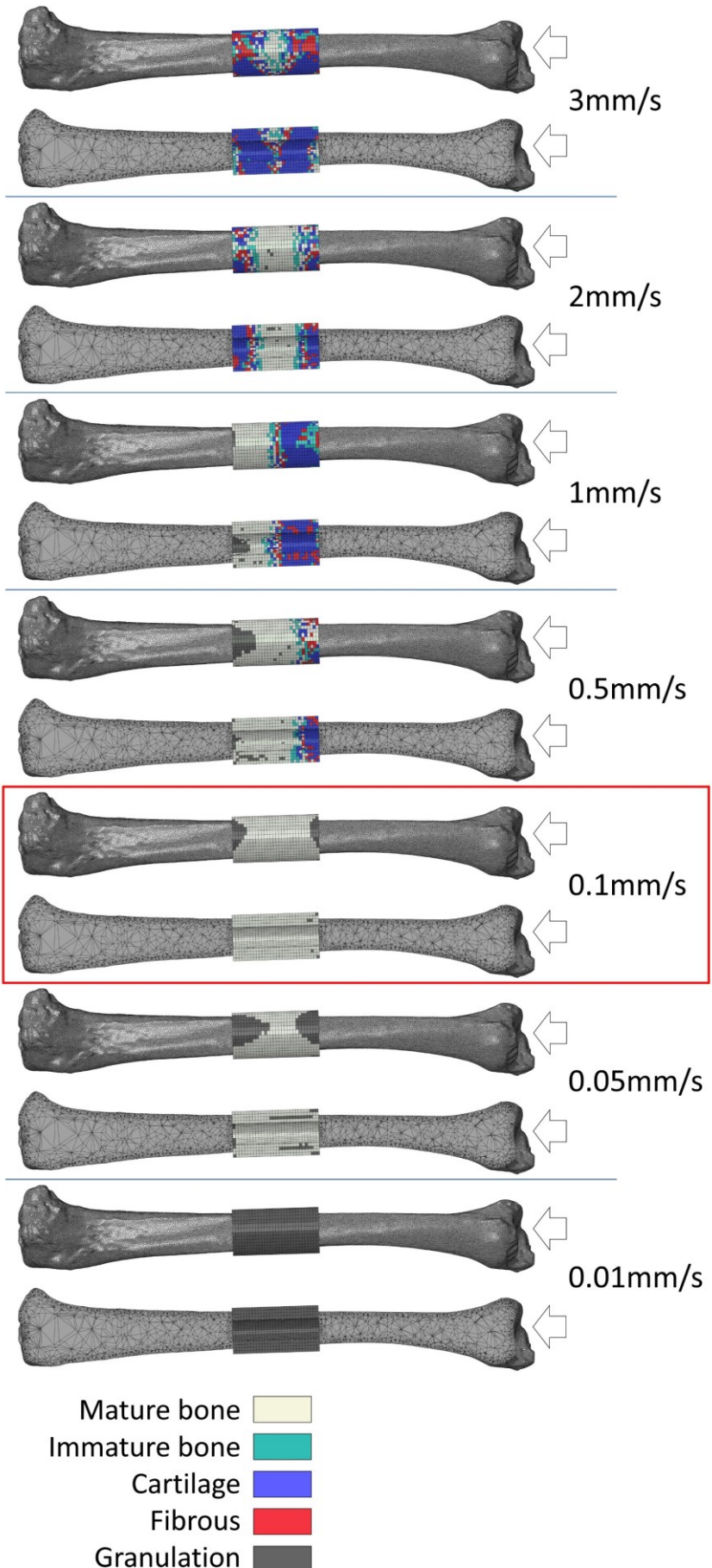
224 the loading cases of 0.5mm/s and above, the distributions of tissue phenotypes are heterogenous in  
225 the diaphyseal direction. The distributions of tissue phenotype are quite homogeneous in the radial  
226 direction without any great variation between PLA scaffold and hydrogel membrane.

227 Figure 5 shows the proportions of final tissue phenotypes within the biomaterial. The results  
228 indicate an optimal stimulation scenario promoting the regeneration of mature bone tissue within the  
229 composite biomaterial. The loading condition of 0.1mm/s allowed up to 97% regeneration in mature  
230 bone. For lower rates of compression, cell apoptosis and tissue resorption were predicted due to  
231 insufficient mechanical stimulation. For higher compressive amplitudes, stable differentiation into  
232 mature bone tissue was not guaranteed, instead the model predicted the formation of cartilage and  
233 fibrous tissue. The increase in distal displacement, and consequently in IFM, induces large formation  
234 of cartilage up to 60% (Figure 5). At this point, by reducing distal displacement to 0.1 mm/s in the  
235 following loading cycles, we observed that the large amounts of cartilage then differentiated into  
236 mature bone, giving the same distribution of mature bone as that produced by imposing 0.1mm/s from  
237 start to finish.

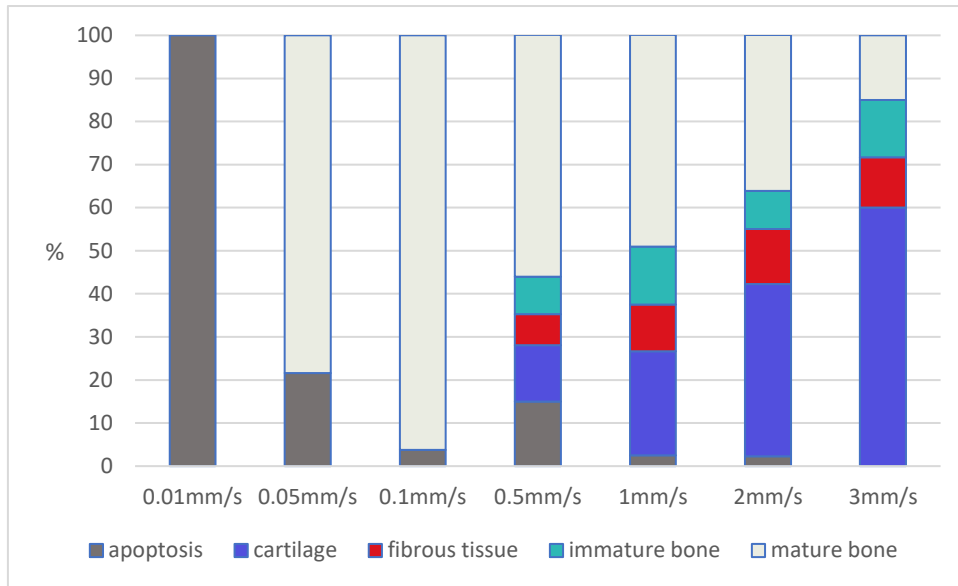
238 Figure 6 shows the evolution of the proportion of mature bone for all loading cases during the  
239 iterative simulations. Figure 7 shows the evolution of the proportion of all tissue phenotypes during  
240 the optimal loading scenario of 0.1mm/s. In this scenario, maximal and stable formation of mature  
241 bone was predicted since the first iteration.

242

243

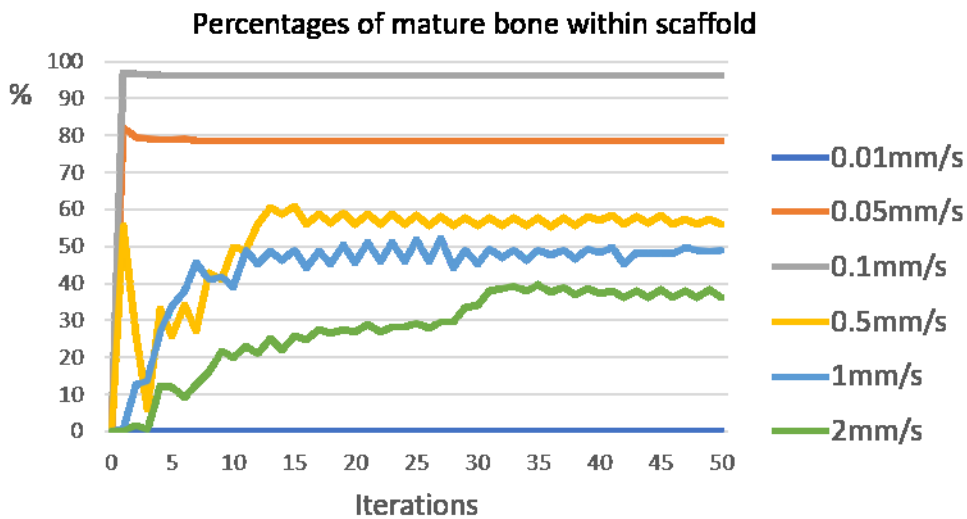


**Figure 4:** Distribution of tissue phenotypes formed within the PLA scaffold and the hydrogel membrane at the end of the simulation based on the loading scenario. For every loading case, the tibial bone and biomaterial are showed in the sagittal plane both in side-view and cut-view. Red box indicates the optimal condition of loading leading to mature bone differentiation.



273

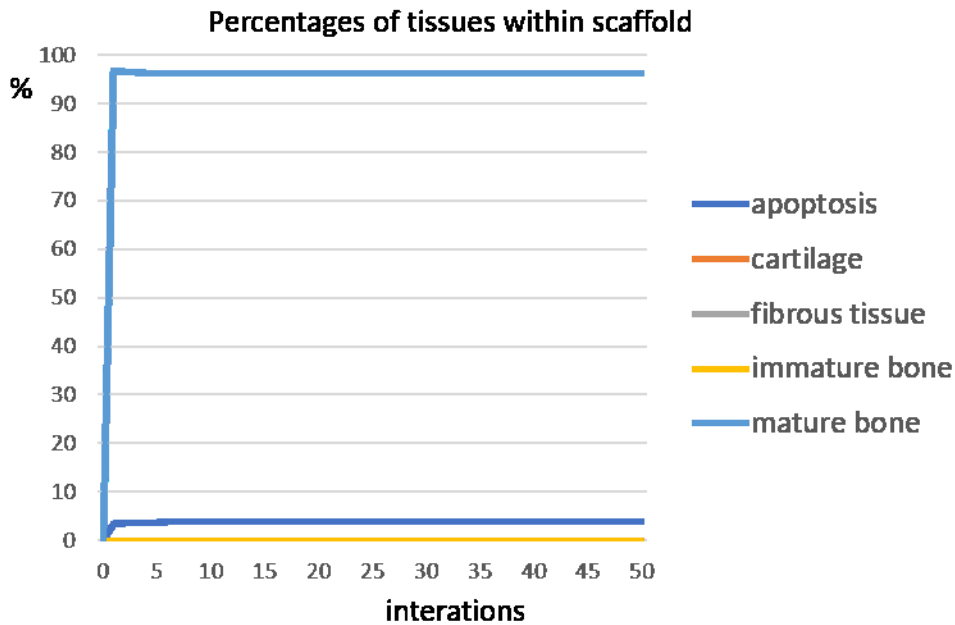
274 **Figure 5:** Final percentage of each type of tissue formed in the PLA scaffold and hydrogel membrane  
 275 based on mechanical loading conditions.



276

277 **Figure 6:** Percentage of mature bone formed in the PLA scaffold and hydrogel membrane depending  
 278 on the iteration of tissue differentiation simulation for all loading cases.

279



280

281 **Figure 7:** Percentage of tissues formed in PLA scaffold and hydrogel membrane depending on the  
 282 iteration of tissue differentiation simulation for the loading case of 0.1 mm/s.

283

284

#### 285 4. Discussion

286 The objective of this study is to find the appropriate mechanical loading to promote bone healing  
 287 of large tibial diaphyseal defect filled by a porous biomaterial. The biomaterial acting as a scaffold for  
 288 tissue growth, we used the model of mechanoregulation to predict the effect of mechanical  
 289 stimulation on the phenotype of the tissue that could form within the biomaterial.

#### 290 4.1 Biomaterial design

291 The biomaterial was a hollow cylinder composed of a PLA scaffold wrapped with a hydrogel  
 292 membrane. The hollow design is inspired by the geometry of the diaphysis. The hollow design allows  
 293 for internal blood flow, bone marrow penetration and internal stem cell colonization. On the other  
 294 hand, the hydrogel membrane that surrounds it, promotes the regeneration of the periosteum  
 295 providing local vascularization and recruitment of stem cells, which are essential for bone healing. The  
 296 semi-permeable hydrogel membrane protects the bone defect from invasion by peripheral soft tissue.

297 Casanova et al. 2010 have shown, *in vivo*, that the periosteum alone can regenerate a large bone  
 298 volume. In the case of a major trauma, it is therefore necessary to find a biomaterial that serves as an

299 envelope and allows the regeneration of the periosteum. The hollow cylinder made of PLA foam acts  
300 as a scaffold and the collagen membrane will allow regeneration of the periosteum.

#### 301 **4.2 Mechanoregulation model prediction**

302 To stimulate bone healing mechanically, it is necessary to precisely know the required mechanical  
303 conditions for bone regeneration. This consequently involves stem cells differentiating into active  
304 osteoblasts. The numerical model of mechanoregulation developed by Prendergast and Lacroix  
305 simulates the mechanobiological effect on bone regeneration and is able to identify the optimal  
306 loading scenario [Prendergast1997; Lacroix2002].

307 The model of mechanoregulation was validated on several clinical applications such as fracture  
308 repair, mandibular bone distraction and several in vitro experiments of bone formation in porous  
309 biomaterials [Isaksson2006; Boccaccio2008; Checa2010; Milan2010; Sandino2010]. We therefore used  
310 this model to determine the influence of mechanical stimulation on bone regeneration in a large  
311 diaphyseal defect.

312 After the first optimization step of the mechanoregulation algorithm, we may already have some  
313 indication of the expected outcome. However, in some instances the proportion attributed to each  
314 tissue phenotype fluctuates slightly between iterations.

315 The relevance of the prediction of the mechanoregulation algorithm relies primarily on the  
316 stability of local tissue phenotypes. This stability results from the fact that the local solid strain and  
317 fluid flow are within the stimuli domain that would lead to the same tissue phenotype.

318 This stability is not always achieved. Tissue differentiation is the result of a specific biophysical  
319 stimulus  $S$  value, calculated at one iteration based on local strain and fluid flow. As a consequence,  
320 tissue differentiation results due to the changes in local stiffness, porosity and permeability, which in  
321 turn, may profoundly modify the value of biophysical stimulus  $S$  that will be calculated in the next  
322 iteration. One of the challenges of this study is to find a solution that maintains the already formed  
323 mature bone, between iterations and also differentiates immature bone, cartilage and fibrous tissue  
324 into mature bone. Compared with the other scenarios, the 0.1 mm/s scenario led to a rapid  
325 convergence and a very stable mature bone distribution. Moreover, if we assume that convergence  
326 was achieved when less than 5% of the regenerated tissue volume experienced phenotype  
327 fluctuations, regardless of the predicted dominant phenotype, our results indicate that stability was  
328 achieved for all mechanical stimulation scenarios.

#### 329 **4.3 Imposing force or displacement on bone fracture callus?**

330 Mechanical stimulation of the bone should be included in the early phases of the healing process.  
331 In the case of the tibia this may be achieved by walking with crutches. Early weight-bearing is limited  
332 by the fixation device which provides most of the support, leaving a limited part of the load to be  
333 transmitted through the bone callus. Considering large long diaphyseal defects, the implantation of a  
334 scaffold and the application of mechanical loading allow bone tissue formation as observed in vivo in  
335 sheep by Pobloth et al. 2018 [Pobloth2018]. Diaphyseal defect was stabilized by a locking compressive  
336 plate. The mechanical stimulation was introduced by allowing the sheep to walk, the load applied to  
337 the scaffold being a portion of body weight. By implementing a mechanoregulation model, Perier-Metz  
338 et al. 2020 reproduced in silico the experimental conditions of Pobloth et al. 2018 and simulated the  
339 same results of bone healing [Perier-Metz2020]. Bone healing was the result of the mechanical loading  
340 of the tissue that grew within scaffold pores. Mechanical stimulus depended on the level of  
341 stabilization of locking plate and on the mechanical properties of the scaffold. However, it is hard to  
342 predict the mechanical environment of the growing tissue and the studies did not report the  
343 deformation and displacement that were generated globally and locally.

344 Most studies that analyzed mechanical stimulation of long bone healing did so through early  
345 loading, imposing a near-constant level of force on the fracture callus. In our opinion, this is where the  
346 problem lies. In fact, the growing tissue has mechanical properties that gradually evolve first from a  
347 very soft, non-differentiated and non-mineralized granulation tissue to a very rigid and mineralized  
348 mature bone. The tissue formed after a few days may be not able to withstand the same mechanical  
349 loading as a tissue that had 2 weeks to form without excessive deformation. Of course, mineralized  
350 mature bone does not appear immediately. It is the result of successive differentiations involving, in  
351 particular, the transformation of granulation tissue into a cartilaginous callus, which gradually calcifies.  
352 During the regeneration, the progressive stiffening of the callus induces a necessary decrease in strain  
353 amplitudes that in turn stimulate bone matrix synthesis. At first, early weight-bearing can lead to high  
354 strains stimulating the differentiation of stem cells into chondrocytes. Chondrocytes will synthesize  
355 cartilage, stiffer, capable of supporting the applied load and drastically reducing the strain level, thus  
356 promoting bone differentiation. However, producing local strain by imposing global force is very risky  
357 and can impair bone healing if local strain becomes too high. Since the level of local strain is what  
358 matters at the cellular level, it would be better to control the local and global strains rather than  
359 impose a global force. From our point of view, the complete and viable healing of a fracture or a bone  
360 defect requires strain stimulation. This is exactly what we propose in our study. We propose to impose  
361 limited distal displacements to better control local deformations. We believe that this approach may  
362 be more promising than the current force-dependent standard achieved by applying a fraction of the



363 patient's weight. Producing local strain by distal displacement is simple to monitor and is closer to what  
364 is needed to stimulate cells within growing tissue.

#### 365 **4.4 Level of interfragmentary strains**

366 Perren postulated in 2015 that for interfragmentary strains that exceed 2%, fibrocartilage or  
367 granulation tissue can form but no direct bone formation can occur [Perren2015]. On the contrary,  
368 Claes et al. in 1997 observed in an in vivo sheep model that larger interfragmentary strains at the very  
369 beginning shortened healing time of long hind leg bones fractures [Claes1997]. They showed that an  
370 initial interfragmentary strain of up to 30% still allowed bone fractures to heal. For large gaps, fast  
371 bone healing occurred with lower strain (7%). In 1997, Gardner et al. reported that the same positive  
372 influence of early high interfragmentary strains can occur in patients during early weight bearing.  
373 These results were obtained via instrumented external fixator on patients with tibial shaft fractures  
374 [Gardner1997]. In the very early healing phase, interfragmentary strains, ranging between 30% and  
375 100%, produced some stimulatory effects and did not obstruct the repair process. In 2017,  
376 Bartnikowski et al. observed faster bone healing under evolutive interfragmentary strains. Using a  
377 fixator with an adjustable stiffness, they introduced large deformations at the very beginning, then  
378 gradually reduced them as ossification occurred [Bartnikowski2017].

379 In a patient-specific case, Wehner et al. 2010 demonstrated bone healing of a large tibial fracture,  
380 approximately 2.5 times the diaphyseal diameter, using a flexible fixator and early loading  
381 [Wehner2010]. Experimentally, bone fracture healed and initial maximum IFM was 1.5mm. By using a  
382 mechanobiological model, the authors predicted faster bone healing with an optimal IFM of 0.5mm.

383 In accordance with the studies cited above, our present findings reiterate on the importance of  
384 the IFM. In our study, we found that, during the healing period, a distal displacement of 0.1 mm per 1s  
385 cycles led to complete and mature ossification of the diaphyseal defect. At the very beginning of the  
386 simulation, the biomaterial was very soft and the IFM was equal to 0.1mm per cycles with  
387 interfragmentary strain of 0.17%.

388 This level of stimulation is consistent with one we identified in a previous in silico study based on  
389 exactly the same mechanoregulation algorithm. At the time, we obtained mature bone formation  
390 within cylindrical PLA scaffold (height 10mm, diameter 6mm; porosity 95%; pore size ~100-500 $\mu$ m)  
391 under 0.5%-1% of compression applied in cycles of 2s [Milan2010]. This level of stimulation is  
392 consistent with the results we obtained via our previous in silico studies based on the same  
393 mechanoregulation algorithm. Zhao et al. 2018 reported that a strain of 0.5% induced bone formation  
394 within a soft porous scaffold (pore size 300 $\mu$ m; porosity 70%) made of hydrogel ( $E= 2.3$ GPa)  
395 [Zhao2018].

396 The literature indicates that large IFMs at the very beginning followed by a significant IFM  
397 reduction accelerate bone healing. In our present study, low IFMs ensure mature bone formation at  
398 the end, while higher IFM induces substantial cartilage formation, which can reach up to 60% of the  
399 overall tissue volume (Fig 4 & 5). We observed that the large amounts of cartilage that were produced  
400 by high IFM, then differentiated into mature bone if IFM was drastically reduced. The final distribution  
401 of mature bone was the same as that produced by a low IFM imposed from start to finish. Our results  
402 suggest that the positive consequence of a large early IFM is to promote the prior formation of cartilage  
403 as a transition tissue to mature bone, eventually accelerating bone healing.

#### 404 **4.5 Isotropic linear elastic material properties of the tibia bone**

405 We developed a finite element model of the tibia. Local Young's modulus values were based on  
406 the mineral density values acquired by a tibia tomography. This led to a heterogeneous model that  
407 represented the local material properties throughout the whole tibia bone, this method was  
408 consequently more accurate than in a homogeneous model [Taddei2006]. We also considered the  
409 common assumption of representing bone tissue as an isotropic elastic material. However, the  
410 material properties of long bones have been characterized as anisotropic, while the cortical is  
411 considered transversely isotropic. Anisotropic power-law relationships have been identified to  
412 determine the component of Young's modulus in the axial and transverse directions, based on bone  
413 density [Wirtz2000, Taylor2002].

414 Some studies compared isotropic and anisotropic models of femur based on Hounsfield unit  
415 values and power law [Peng2006, Yang2010, Ün2016, Hamandi2022]. Peng et al. 2006 reported that  
416 isotropic and anisotropic femur models give very similar results in loading conditions simulating  
417 standing. Stress and nodal displacement differ by only 0.6% and 1.2% respectively. Hamandi et al. 2022  
418 simulated gait loading on femur models and showed that the average and maximum stresses were  
419 only 1.80% and 3.9% higher, respectively, in the model with elastic properties compared with the  
420 anisotropic model [Hamandi2022]. By extrapolating these results to the tibia we can safely assume  
421 that an isotropic model is accurate enough. Ün et al. in 2016 showed that under axial compression,  
422 anisotropic and isotropic material models of tibia produced very similar von Mises stress distribution  
423 of over the length of the tibia [Ün2016]. Since material properties variations have a stronger effect on  
424 strain than on stress, Ün et al. noted that, under pure compression, the isotropic model overestimated  
425 the maximum von Mises strain in the middle of the diaphysis by 10% compared to the anisotropic  
426 model. However, the authors did not give the error on the axial diaphyseal strain which should be less  
427 than 10% and which is the one that impacts the biomaterial in our study. The authors concluded that

428 anisotropic properties should be preferred for studies focusing on local bone strength or damage  
429 accumulation.

430 Considering the anisotropy in tibia bone requires defining the orientation of major anisotropy  
431 axes, which is not necessarily the same throughout the tibia. For example, the trabecular bone in the  
432 epiphysis may have different anisotropy axis than the diaphyseal cortical bone. This highlights the  
433 difficulty of accurately defining the major anisotropy axes in trabecular bone. Ün et al. pointed out that  
434 the literature still lacks a realistic mechanical analysis of bone that correctly accounts for its anisotropy  
435 and heterogeneity. At this time, there is still no consensus on the choice of anisotropic power-law that  
436 could finally propose significantly superior anisotropic model than the isotropic approach [Ün2016].  
437 Recently, anisotropic power laws were identified for trabecular bone of the proximal tibia by Munford  
438 et al. in 2020 [Munford2020]. In the future, we expect that reliable standard methods fully characterize  
439 bone anisotropy as a function of density. Numerical models of bone based on tomography and  
440 segmentation will be able to provide more accurate simulations of the mechanical behavior of bone.

441 In our study, we did not focus on local bone strength or damage accumulation. Instead, we only  
442 on force transmission from the proximal and distal parts of the tibia on the biomaterial implanted in  
443 the bone defect. Moreover, we only considered axial compression of the tibia and were only interested  
444 in loading a diaphyseal biomaterial. The axial components of stress and strains in the diaphyseal  
445 biomaterial are the same whether the material assignment is isotropic or anisotropic, with the Young's  
446 modulus values in the longitudinal direction being the same in both formulations. We therefore  
447 considered that the isotropic elastic model used in this study was simpler and more appropriate choice,  
448 compared to a more complex anisotropic model, whose axes of anisotropic could not be defined  
449 rigorously.

#### 450 **4.6 Accuracy of finite element model mesh**

451 The quality of the tibia and biomaterial meshes was validated by an Abaqus mesh verification  
452 analysis keeping the standard threshold values proposed by default (Table 1). The mesh of the  
453 biomaterial is fully structured and homogeneous; it does not contain any defect. The mesh of the tibia  
454 is homogeneous on the surface but heterogeneous within. We have also checked mesh analysis with  
455 strict threshold values that should be used in a biomechanical finite element calculation as recommend  
456 by Burkhart et al. in 2013 [Burkhart2013]. According to the latter criteria, the mesh of the tibia is 91%  
457 accurate (Table 1), which is a very acceptable level following recommendation from Burkart et al. 2013.

#### 458 **4.7 Limits of the study**

459 As limits of our study, we can argue that the tibia specimen we modelled is from elderly individual  
460 and may be significantly weaker in terms of mechanical properties and biological activity than the bone  
461 of younger individual who could to benefit from the implantation of a biomaterial.

462 We should add that our study only addressed the influence of mechanical loading on tissue  
463 differentiation in the diaphyseal defect based on Prendergast's model of mechanoregulation. The  
464 tissue differentiation results we obtained within the porous biomaterial are based on its complete  
465 colonization by cells from the very start of the simulation. We did not consider the process of cell  
466 invasion, neither due to cell migration nor proliferation. This prevents us from discussing the influence  
467 of this process which is often a problem in tissue engineering, as cells often remain at the periphery of  
468 the scaffold. Although our scaffold is shaped as a porous and hollow tube to promote cell invasion both  
469 from the internal and external boundaries, it is still possible that in reality less tissue is formed with in  
470 the thickness of the scaffold due to limited cell colonization.

#### 471 **4.8 Application of in silico results to dynamic fixators**

472 The literature clearly establishes that the correction of a large diaphyseal bone defect in long bone  
473 depends primarily on its stabilization [Perren1977]. However, clinical and pre-clinical studies based on  
474 flexible fixators showed that limited inter-fragmental strains may accelerate bone healing [Augat2021].  
475 Dynamic orthopedic systems with controlled flexibility have been developed to allow limited  
476 mechanical strain on the healing zone. These innovative systems are dynamic external fixators  
477 [Borgiani2019] or dynamic intramedullary nails [Dailey2021]. Plates locked with distal cortical screws  
478 or dynamic screws can also be a dynamic osteosynthesis device [Richter 2015, Bottlang2010,  
479 Boerckel2012]. Such dynamic devices could be used to treat the study case we considered, a 60-mm-  
480 wide diaphyseal bone defect filled with a porous biomaterial. In this case, the dynamic device loading  
481 should target the 0.1 mm level of distal displacement that we identified in silico as the best mechanical  
482 stimulation that led to mature bone formation.

#### 483 **5. Conclusion**

484 By implementing Prendergast's and Lacroix's mechano-regulation model in a large defect in  
485 diaphyseal bone of the tibia, we identified the best mechanical stimuli for optimal bone healing. We  
486 considered that the bone defect was filled by a hollow porous PLA scaffold wrapped by a hydrogel  
487 membrane mimicking the periosteum. The results of our simulations verify our initial hypothesis. The  
488 mechanical quality of the bone tissue formed depends on local mechanical stresses. This in silico  
489 modeling approach allowed us to compare different mechanical load conditions based on the imposed  
490 relative displacement of both bone extremities. We, therefore, selected the solution that favored the  
491 formation mature bone. Ultimately, the strategy to be adopted and the clinical response must involve

492 the clinicians, the rehabilitation workers and the experts in biomechanics to optimize and integrate  
493 patient-dependent specificities. This study, thus offers a new perspective and could be part of an  
494 overall approach to better promote bone repair and faster recovery.

495

## 496 **6. Acknowledgements**

497 The authors declare no conflict of interest.

## 498 **6. Ethics approval and consent to participate**

499 Local ethics committee approval was not required to use a scan from an anonymized cadaveric sample.

500 The protocol of this study was approved by the scientific committee of our anatomical department.

## 501 **7. References**

502 [Anderson1967]. Anderson CB. Mechanics of fluids. In: Baumeister T (ed) Marks' saturated handbook  
503 of mechanical engineers, 1967. pp 3.48–3.76

504 [Augat2021] Augat P, Hollensteiner M, von Rüden C. The role of mechanical stimulation in the  
505 enhancement of bone healing. *Injury* 52S2 (2021) S78–S83.  
506 <https://doi.org/10.1016/j.injury.2020.10.009>

507 [Boccaccio2008] A. Boccaccio, P. Prendergast, C. Pappalettere, et al. 2008. Tissue differentiation and  
508 bone regeneration in an osteotomized mandible: a computational analysis of the latency period. *Med  
509 Biol Eng Comput* 46: 283–298.

510 [Boerckel2012] Joel D. Boerckel, Yash M. Kolambkar, Hazel Y. Stevens, Angela S.P. Lin, Kenneth M.  
511 Dupont, and Robert E. Guldberg. Effects of in vivo mechanical loading on large bone defect  
512 regeneration. *J Orthop Res.* 2012 July ; 30(7): 1067–1075. doi:10.1002/jor.22042.

513 [Bottlang2010] Bottlang M, Lesser M, Koerber J, et al. Far cortical locking can improve healing of  
514 fractures stabilized with locking plates. *J Bone Joint Surg Am.* 2010;92(7):1652-1660.  
515 doi:10.2106/JBJS.I.01111

516 [Burkhart2013] Burkhart TA, Andrews DM, Dunning CE. 2013. Finite element modeling mesh quality,  
517 energy balance and validation methods: a review with recommendations associated with the modeling  
518 of bone tissue. *J Biomech.* 46(9):1477–1488.

519 [Casanova2010] R. Casanova, D. Moukoko, M. Pithioux, C. Paillet-Mattéi, H. Zahouani, P. Chabrand.  
520 Temporal evolution of skeletal regenerated tissue: what can mechanical investigation add to  
521 biological?, *Med. and Biol. Eng. & Comp.*, 2010, 48(8):811-819.

522 [Checa2010] S. Checa D. P. Byrne J. Pendergast: Predictive modeling in mechanobiology : combining  
523 algorithms for cell activities in response to physical stimuli using lattice-modelling approach. *Computer*  
524 *methods in mechanics*, ASM 1, 2010, pp. 423-435

525 [Claes1997] Claes L, Augat P, Suger G, Wilke HJ. Influence of Size and Stability of the Osteotomy Gap  
526 on the Success of Fracture Healing. *Journal of Orthopaedic Research* 1997, 15:577-584

527 [Coïc2010]. M. Coïc, V. Placet, E. Jacquet, C. Meyer. Mechanical properties of collagen membranes  
528 used in guided bone regeneration: a comparative study of three models. *Rev Stomatol Chir Maxillofac.*  
529 2010 ;111(5-6):286-90. doi: 10.1016/j.stomax.2010.10.006.

530 [Dailey2021] Dailey HL, Schwarzenberg P, Webb lii EB, Boran SAM, Guerin S, Harty JA. Pilot study of  
531 micromotion nailing for mechanical stimulation of tibial fracture healing. *Bone Jt Open.*  
532 2021;2(10):825-833. doi:10.1302/2633-1462.210.BJO-2021-0121.R1

533 [Gardner1997] Gardner TN, Evans M, Hardy J, Kenwright J. Dynamic interfragmentary motion in  
534 fractures during routine patient activity. *Clin Orthop Relat Res* 1997(336):216–25.

535 [Hamandi2022] Hamandi, F.; Tsatalis, J.T.; Goswami, T. Retrospective Evaluation and Framework  
536 Development of Bone Anisotropic Material Behavior Compared with Elastic, Elastic-Plastic, and Hyper-  
537 Elastic Properties. *Bioengineering* 2022, 9, 9. <https://doi.org/10.3390/bioengineering9010009>

538 [Harris2008] M. Charles-Harris M. A. Koch M. Navarro D. Lacroix E. Engel J. A. Planell : A PLA/calcium  
539 phosphate degradable composite material for bone tissue engineering : an vitro study. *Mater Med,*  
540 2008,19:1503-1513.

541 [Hobatho1997] M.C. Hobatho, J.Y. Rho, R.B. Ashman. Anatomical Variation of Human Cancellous Bone  
542 Mechanical Properties In Vitro. *Studies in Health Technology and Informatics*, 1997, Vol. 40, 157-173

543 [Hwang2010] C.M. Hwang, S. Sant, M. Masaeli, et al. Fabrication of three-dimensional porous cell-  
544 laden hydrogel for tissue engineering. *Biofabrication.* 2010;2(3):035003.

545 [Isaksson2006] Isaksson H, Donkelaar CCv, Huiskes R, et al. 2006. Corroboration of mechanoregulatory  
546 algorithms for tissue differentiation during fracture healing: comparison with in vivo results. *J*  
547 *Orthopaed Res* 24: 898– 907.

548 [Lacroix2002] D Lacroix P.J. Prendergast. A mechano-regulation model for tissue differentiation during  
549 fracture healing: analysis of gap size and loading. *Journal of Biomechanics* 2002, 35, 1163-1171.

550 [Masquelet2012] A.-C. Masquelet, J. Sales de Gauzy, T. Bauer, A. Fabre, F. Fitoussi, D. Hannouche, J.-L.  
551 Jouve, C. Karger, D. Le Nen, H. Mathevon, P. Merloz, L. Obert, A. Poichotte, S. Rigal, et la Société  
552 française de chirurgie orthopédique et traumatologique. Reconstruction of post-traumatic diaphyseal  
553 bone defects : Preoperative planning, guideline, and future developments. *Revue de Chirurgie*  
554 *Orthopédique et Traumatologique*, 2012, 98, 1, Pages94-103.

555 [Milan2010] J. L. Milan, J. A. Planell, D. Lacroix. Simulation of bone tissue formation within a porous  
556 scaffold under dynamic compression. *Biomech Model Mechanobiol*, 2010, 9:583-596

557 [Munford2020] Munford, M. J., Ng, K. C. G., Jeffers, J. R. T., Mapping the Multi-Directional Mechanical  
558 Properties of Bone in the Proximal Tibia. *Adv. Funct. Mater.* 2020, 30, 2004323.  
559 <https://doi.org/10.1002/adfm.202004323>

560 [Navarro2006] M. Navarro, C. Aparicio, M. Charles-Harris, M.P. Ginebra, E. Engel, J. A. Planell :  
561 Development of a biodegradable composite scaffold for bone tissue engineering: Physicochemical,  
562 Topographical, Mechanical, Degradation, and Biological Properties. *Adv Polym Sci*, 2006, 200: 209-231

563 [Oliveira2010] S.M. Oliveira, R.A. Ringshia, R.Z. LeGeros, E. Clark, M.J. Yost, L. Terracio & C.C. Teixeira.  
564 An improved Collagen Scaffold for Skeletal Regeneration. *Journal of Biomedical Materials Research.*  
565 *Part A*, 2010, 94(2), 371–379. <http://doi.org/10.1002/jbm.a.32694>

566 [Palomares2010] K. T. S. Palomares, R.E. Gleason, Z.D. Mason, D.M. Cullinane, T.A. Einhorn, L.C.  
567 Gerstenfeld, & E.F. Morgan. Mechanical stimulation alters tissue differentiation and molecular  
568 expression during bone healing. *Journal of Orthopaedic Research : Official Publication of the*  
569 *Orthopaedic Research Society*, 2009, 27(9), 1123–1132. <http://doi.org/10.1002/jor.20863>

570 [Peng2006] L. Peng, J. Bai, X. Zeng, Y. Zhou. Comparison of isotropic and orthotropic material property  
571 assignments on femoral finite element models under two loading conditions. *Med Eng Phys*, 28 (2006),  
572 pp. 227-233

573 [Perier-Metz2020] Perier-Metz C, Duda GN and Checa S (2020) Mechano-Biological Computer Model  
574 of Scaffold-Supported Bone Regeneration: Effect of Bone Graft and Scaffold Structure on Large Bone  
575 Defect Tissue Patterning. *Front. Bioeng. Biotechnol.* 8:585799. doi: 10.3389/fbioe.2020.585799

576 [Perren1977] Perren SM, Cordey J. [Tissue differences in fracture healing (author's transl)].  
577 *Unfallheilkunde* 1977;80(5):161–4.

578 [Perren2015] Perren SM. Fracture healing: fracture healing understood as the result of a fascinating  
579 cascade of physical and biological interactions. Part II. Acta Chir Orthop Traumatol Cech.  
580 2015;82(1):13–21

581 [Pobloth2018]. Pobloth AM, Checa S, Razi H, et al. Mechanobiologically optimized 3D titanium-mesh  
582 scaffolds enhance bone regeneration in critical segmental defects in sheep. Science Translational  
583 Medicine. 2018 Jan;10(423). DOI: 10.1126/scitranslmed.aam8828. PMID: 29321260

584 [Prendergast1997] Prendergast PJ, Huiskes R, Soballe K (1997) Biophysical stimuli on cells during tissue  
585 differentiation at implant interfaces. J Biomech 30:539–548

586 [Richter2015] Richter, Henning; Plecko, Michael; Andermatt, Daniel; Frigg, Robert; Kronen, Peter W;  
587 Klein, Karina; Nuss, Katja M; Ferguson, Stephen J; Stöckle, Ulrich; von Rechenberg, Brigitte (2015).  
588 Dynamization at the near cortex in locking plate osteosynthesis by means of dynamic locking screws:  
589 an experimental study of transverse tibial osteotomies in sheep. Journal Bone Joint Surgery America,  
590 97(3):208-215.

591 [Rolland1995] E. Rolland, G. Saillant: La consolidation osseuse normale et pathologique. Ann  
592 Réadaptation Med Phys, 1995, 38, 245-251

593 [Sandino2010] C. Sandino S. Checa, P. J. Prendergast, D. Lacroix.: Simulation of angiogenesis and cell  
594 differentiation in a CaP scaffold subjected to compressive strains using a lattice modeling approach. C.  
595 Sandino S. Checa, P. J. Prendergast, D. Lacroix. Biomaterials Volume 31, Issue 8, March 2010, Pages  
596 2446–2452

597 [Sheikh2015] Z. Sheikh, S. Najeeb, Z. Khurshid, V. Verma, H. Rashid, M. Glogauer. Biodegradable  
598 Materials for Bone Repair and Tissue Engineering Applications. Materials 2015, 8, 5744-5794;  
599 doi:10.3390/ma8095273.

600 [Snyder1991] S. M. Snyder, E. Schneider. Estimation of mechanical properties of cortical bone by  
601 computed tomography. 1991. Journal of Orthopaedic Research, 3, 1991, Pages 422-431,  
602 <https://doi.org/10.1002/jor.11000903159>

603 [Taddei2006] Fulvia Taddei, Luca Cristofolini, Saulo Martelli, H.S. Gill, Marco Viceconti, Subject-specific  
604 finite element models of long bones: An in vitro evaluation of the overall accuracy, Journal of  
605 Biomechanics, Volume 39, Issue 13, 2006, Pages 2457-2467,  
606 <https://doi.org/10.1016/j.jbiomech.2005.07.018>.



607 [Taylor2002] Taylor, W.; Roland, E.; Ploeg, H.-L.; Hertig, D.; Klabunde, R.; Warner, M.; Tho, M.-C.H.B.;  
608 Rakotomanana, L.; Clift, S. Determination of orthotropic bone elastic constants using FEA and modal  
609 analysis. *J. Biomech.* 2002, 35, 767–773.

610 [Ün2016]. Kerem Ün & Ahmet Çalık (2016) Relevance of inhomogeneous–anisotropic models of human  
611 cortical bone: a tibia study using the finite element method, *Biotechnology & Biotechnological  
612 Equipment*, 30:3, 538-547, DOI:10.1080/13102818.2016.1154803

613 [Victoria2009] G. Victoria, B. Petrisor, B. Drew & D. Dick. Bone stimulation for fracture healing: What’s  
614 all the fuss? *Indian Journal of Orthopaedics*, 2009, 43(2), 117–120. [http://doi.org/10.4103/0019-  
615 5413.50844](http://doi.org/10.4103/0019-5413.50844)

616 [Wang2016] J. Wang, L. Wang, Z. Zhou, H. Lai, P. Xu, L. Liao, J. Wei. Biodegradable Polymer Membranes  
617 Applied in Guided Bone/Tissue Regeneration: A Review. *Polymers* 2016, 8, 115;  
618 doi:10.3390/polym8040115

619 [Wirtz2000] Wirtz, D.C.; Schiffers, N.; Pandorf, T.; Radermacher, K.; Weichert, D.; Forst, R. Critical  
620 evaluation of known bone material properties to realize anisotropic FE-simulation of the proximal  
621 femur. *J. Biomech.* 2000, 33, 1325–1330.

622 [Yang2010] Haisheng Yang, Xin Ma, Tongtong Guo, Some factors that affect the comparison between  
623 isotropic and orthotropic inhomogeneous finite element material models of femur, *Medical  
624 Engineering & Physics*, Volume 32, Issue 6, 2010, Pages 553-560, ISSN 1350-4533,  
625 <https://doi.org/10.1016/j.medengphy.2010.01.004>.

626 [Yue2020] Yue S, He H, Li B, Hou T. Hydrogel as a Biomaterial for Bone Tissue Engineering: A Review.  
627 *Nanomaterials (Basel)*. 2020 Jul 31;10(8):1511. doi: 10.3390/nano10081511. PMID: 32752105; PMCID:  
628 PMC7466535.

629 [Zhao2018] Zhao F, Mc Garrigle MJ, Vaughan TJ, McNamara LM. In silico study of bone tissue  
630 regeneration in an idealised porous hydrogel scaffold using a mechano-regulation algorithm. *Biomech  
631 Model Mechanobiol.* 2018 Feb;17(1):5-18. doi: 10.1007/s10237-017-0941-3

632

633