Trabecular bone remodeling in the ageing mouse: a micro-multiphysics agent-based *in silico* model using cell-type-specific mechanomic profiles

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Abstract

Bone remodeling is regulated by the interaction between different cells and tissues across many spatial and temporal scales. In silico models have been of help to further understand the signaling pathways that regulate the spatial cellular interplay. We have established a 3D multiscale micro-multiphysics agent-based (micro-MPA) *in silico* model of trabecular bone remodeling using longitudinal *in vivo* data from the sixth caudal vertebra (CV6) of PolgA$^{(D257A/D257A)}$ mice, a mouse model of premature aging. Our model includes a variety of cells as single agents and receptor-ligand kinetics, mechanotransduction, diffusion and decay of cytokines which regulate the cells' behavior. The micro-MPA model was applied for simulating trabecular bone remodeling in the CV6 of 5 mice over 4 weeks and we evaluated the static and dynamic morphometry of the trabecular bone microarchitecture. We identified a configuration of the model parameters to simulate a homeostatic trabecular bone remodeling. Additionally, our simulations showed different anabolic, anti-anabolic, catabolic and anticatabolic responses with an increase or decrease by one standard deviation in the levels of osteoprotegerin (OPG), receptor activator of nuclear factor kB ligand (RANKL), and sclerostin (Scl) produced by the osteocytes. From these results, we concluded that OPG inhibits osteoclastic bone resorption by reducing the osteoclast recruitment, RANKL promotes bone resorption by enhancing the osteoclast recruitment and Scl blocks bone formation by inhibiting osteoblast differentiation. The variations in trabecular bone volume fraction and thickness (BV/TV and Tb.Th) were relatively higher with variations of one standard deviation in the levels of RANKL compared to OPG and Scl. This micro-MPA model will help us to better understand how cells respond to the mechanical signal by changing their activity in response to their local mechanical environment.

Introduction

With aging, bone becomes more fragile with increasing fracture risk, leading to an increased morbidity and mortality and to an economic burden of € 57 billion in 2019 in the European Union plus Switzerland and the United Kingdom (Kanis et al. 2021). To better understand how aging affects

39   bone cellular behavior, *in vivo* and *in silico* studies have been used to analyze the tissue and cellular
40   properties of bone mechanobiology. It is known that bone remodeling is regulated by the interaction
41   between different cells and tissues across many spatial and temporal scales. Yet, bone remodeling has
42   not been quantitatively studied *in silico* across spatial and temporal scales and using 3D *in vivo* data
43   as reference. Hybrid modeling has been identified as a powerful modeling technique which combines
44   multiscale, multiphysics, and agent-based modeling to analyze in a unified way the organ, tissue, cell,
45   and gene scales over weeks, months or even years (Boaretti et al. 2022). Such an *in silico* model can
46   deal with the action of single cells while millions of cells are active and interact with each other in a
47   multiscale simulation, making this tool ideal to explore the hierarchical processes governing bone
48   mechanobiology. Furthermore, by enabling fine modelling of signaling pathways that regulate the
49   cell's activity, such models can help to estimate physiological cytokine production rates and explore
50   their effect on spatial cellular distribution, bone formation, and bone resorption, which is hardly
51   feasible *in vivo*.

52   Recent experimental studies have analyzed the effects of aging in mice and how loading can
53   counterbalance frailty (Scheuren et al. 2020a, b). Accelerated aging was provoked in
54   PolgA$^{(D257A/D257A)}$ mice due to systemic mitochondrial dysfunction caused by accumulation of
55   mitochondrial DNA point mutations (Trifunovic et al. 2004; Kujoth et al. 2005). The findings from
56   these works have mainly focused on cortical and trabecular bone morphometric parameters with
57   analyses of selected gene and protein expression on specific time points. The work of Dobson and
58   colleagues (Dobson et al. 2020) showed that in this PolgA$^{(D257A/D257A)}$ mouse model, osteoblast
59   density was reduced and the production of mineralized matrix by osteoblasts was significantly
60   impaired, leading to reduced bone formation rates. The mechanisms which lead to cellular alterations
61   in bone with aging remain to be further elucidated.

62   We have recently proposed an *in silico* multiscale micro-multiphysics agent-based (micro-MPA)
63   model of fracture healing in cortical bone adapted from the model of Tourolle (Tourolle 2019) and
64   adapted by Boaretti (Boaretti et al. 2018). This micro-MPA model is based on multiscale (from the
65   organ to the protein spatial scales and from weeks to minutes as temporal scales), multiphysics
66   (mechanical signal, reaction-diffusion-decay of cytokines), and agent-based modelling (single-cell
67   behavior and mechanotransduction). Each cell is modelled as a single agent, and signaling pathways
68   were modeled to regulate the cell behavior. In this model, osteoprotegerin (OPG), receptor activator
69   of nuclear factor kB ligand (RANKL), sclerostin (Scl) are cytokines that decay, diffuse in the
70   volume, and are produced by the cells. Transforming growth factor beta 1 (TGF-β1) is modelled as
71   cytokine stored in the bone volume and it can diffuse and decay after resorption. The signaling
72   pathways were modelled with receptor-ligand kinetics, with the receptors located on the cells'
73   surfaces. In addition, a free ligand can bind to another free ligand, e.g., OPG can bind to RANKL.
74   These pathways have been shown to be the main regulators of cell differentiation and proliferation
75   (Krishnan et al. 2006; Boyce and Xing 2008; Lin et al. 2009; Tang et al. 2009; Warren et al. 2015;
76   Elson et al. 2022). The osteocytes were considered the main mechanosensors of the mechanical
77   signal (Santos et al. 2009; Klein-Nulend et al. 2012, 2013) and they promoted bone formation or
78   resorption by releasing cytokines into the volume to regulate the signaling pathways affecting
79   eventually the osteoblasts and osteoclasts. These cells form and resorb bone on the surface,
80   respectively. Osteocytes, osteoblasts and osteoclasts were the basis for the regulation of bone
81   remodeling we can simulate using *in vivo* data. Indeed, a previous version of this model was used for
82   simulating denosumab treatment in human biopsies over 10 years, showing the potential of micro-
83   MPA models for designing optimal clinical trials (Tourolle et al. 2021).

84 In the current study, we used this micro-MPA model to simulate how bone adapts and remodels
85 through single-cell mechanotransduction in mice. We hypothesized that cells produce cytokines to
86 regulate the cellular actions as a response to the local mechanical signal they perceive, e.g., cells
87 release cytokines to promote anabolic or anticatabolic responses under high stress and cells release
88 catabolic or anti-anabolic cytokines under low stress. Furthermore, we hypothesized that osteocytes
89 are mainly responsible for regulating the other cells' activity through their single-cell cytokine
90 production.

91 The aim of this work is to show that the proposed *in silico* model can reproduce a homeostatic
92 condition similar to the longitudinal *in vivo* data, where a dynamic equilibrium between bone
93 formation and bone resorption maintains relatively constant bone volume fraction (Rodan 1998;
94 Nakahama 2010; Rauner et al. 2020). Moreover, we investigated the quantitative effect of
95 manipulating the cytokines levels involved in the signaling pathways on the bone morphometric
96 parameters. For this purpose, the proposed novel micro-MPA *in silico* model of bone remodeling was
97 applied to micro-computed tomography (micro-CT) *in vivo* data to test the effect of different
98 production values of RANKL, OPG, and Scl by osteocytes on the static and dynamic bone
99 morphometry data relatively to the homeostatic configuration.

100 **Materials and Methods**

101 *In vivo* **input data**

102 *In vivo* data of the control group of a study analyzing the effects of frailty and osteosarcopenia on the
103 bone microarchitecture of prematurely aged PolgA$^{(D257A/D257A)}$ mice (n=9) were used (Scheuren et al.
104 2020a). Briefly, at an age of 35 weeks, stainless steel pins were inserted at the sixth caudal vertebra
105 (CV6). At week 38, a sham cyclic loading regime was applied three times per week over 4 weeks.
106 The *in vivo* micro-CT images (vivaCT 40, Scanco Medical AG) were acquired and analyzed every
107 week at an isotropic voxel resolution of 10.5 um, see Figure 1A. The acquired images showed a
108 slight reduction in the normalized trabecular bone volume fraction over the course of the experiment
109 (2% at the end), see **Error! Reference source not found.Error! Reference source not found.**B.

110 *In silico* **micro-multiphysics agent-based model**

111 The micro-MPA model was originally developed for simulating fracture healing in the mouse femur
112 (Tourolle 2019). In the present work, we adapted this micro-MPA model for the simulation of
113 homeostatic bone remodeling in the mouse vertebra, including the bone response to physiological
114 loading. The overview of the adapted *in silico* model is shown in Figure 2.

115 In the current implementation, the cells are modeled individually using the agent-based paradigm,
116 where all cells of the same type share the same biological properties and follow the same prescribed
117 rules. The model implements the actions of each cell according to the local physiological, chemical,
118 and mechanical environment at the cellular spatial and temporal scale. In our implementation, the
119 changes in the bone microarchitecture are accumulated over the whole time of the experiment (4
120 weeks) which is much longer than the single temporal step of the cells (40 minutes). The model
121 simulated bone remodeling only within the trabecular region, while the cortical region was kept
122 constant during the simulation. This approach was used also in other works by Schulte and Levchuk
123 (Schulte et al. 2013; Levchuk et al. 2014; Levchuk 2015).

Modeling the cellular behavior

124

125 Osteocytes (Ot) are embedded in bone and produce RANKL, OPG, and Scl depending on the local
126 mechanical signal they perceive (Santos et al. 2009; Klein-Nulend et al. 2012, 2013). Osteoblasts
127 (Ob) produce osteoid, unmineralized matrix, in their neighborhood towards the surface of the bone,
128 based on the local effective strain they perceive. Moreover, Ob may become pre-Ot when the voxel
129 they reside in is at least 50% full of osteoid and there is an osteocyte in the normal direction towards
130 the bone (Franz-Odendaal et al. 2006). Lining cells are considered osteoblasts precursors. In addition,
131 Ob and lining cells also produce OPG and RANKL accordingly to the mechanical signal they
132 perceive. Mesenchymal stem cells (MSC) are present in the marrow space where they can move,
133 proliferate, undergo apoptosis, and can differentiate into an Ob or a lining cell if they are close to the
134 surface and close to an Oc. MSC, lining cells, and Ob have the Lipoprotein receptor-related protein
135 5/6 (LRP5/6) receptor which binds to Scl (Li et al. 2005; Bourhis et al. 2011). If the bound receptor is
136 higher than a user-defined threshold value, Ob differentiate into lining cell and MSC differentiate
137 into lining cell if they meet the condition mentioned above. MSC and Ob have also the TGF-β1
138 receptor on their surface, and if that receptor is highly bound then they can proliferate more
139 frequently. Pre-Ot differentiate into Ot if the voxel they reside in is at least 50% full of mineralized
140 matrix (Franz-Odendaal et al. 2006). The hematopoietic stem cells (HSC) are present in the marrow
141 space, they can move, proliferate, undergo apoptosis and differentiate into pre-Oc if their RANK
142 receptors are highly bound to RANKL (Nelson et al. 2012; Warren et al. 2015). Pre-Oc are motile
143 and can differentiate back into HSCs if the RANK receptor is not highly bound. Moreover, they can
144 differentiate into Oc if there are at least 3 osteoclastic cells (pre-Oc or Oc) in their neighborhood. Oc
145 resorb bone towards the bone surface, with the direction defined by the gradient of the mineral
146 concentration. We modeled the mineralization of the matrix in each voxel by changing its mineral
147 concentration to reach its osteoid concentration. Therefore, given a voxel, if there is more osteoid
148 than mineral, the mineral concentration will increase and if there is more mineral than osteoid, the
149 mineral concentration will decrease. In this way, when Ob release osteoid, there is a delay in bone
150 formation because osteoid leads to a change in the mineral concentration through the mineralization
151 process.

152 To simulate trabecular bone remodeling, we modeled TGF-β1, RANKL-RANK-OPG, and LRP5/6-
153 Scl signaling pathways at the receptor-ligand level and the Ob, lining cell, Oc, pre-Oc, Ot, pre-Ot,
154 MSC, HSC at the cellular level.

Application of the model to simulate bone remodeling in mouse vertebrae

156 The overview of the simulation algorithm can be seen in Figure 2B. Each voxel has a value of bone
157 mineral density from 0 to 1160 mg HA/cm^3 and this value is converted to greyscale values ranging
158 from 0 to 1 with linear scaling. We considered the grayscale bone density values from 0.5 to 1 as
159 bone tissue. The minimum value of 0.5 corresponds to a bone density value of 580 mg HA/cm^3
160 which is the same threshold value employed previously for the postprocessing of the original
161 corresponding *in vivo* data (Scheuren et al. 2020a).

162 The mechanical signal was obtained using the micro-finite element (micro-FE) analysis. For this
163 purpose, the bone tissue and marrow voxels of CV6 vertebrae were converted to Young's modulus
164 values as performed previously (Webster et al. 2008). The effective strain computed from this
165 analysis was used as the mechanical signal of the cells (Mullender et al. 1996; Schulte et al. 2013).
166 This variable was used for the single cell production: each cell produces an amount of cytokines
167 following a specific mechanotransduction curve, with a sigmoidal shape to represent the anabolic
168 (OPG) and catabolic (RANKL, Scl) response to the mechanical signal. For each cytokine-cell

169   production, the Hill curve was defined for both marrow and bone cells with a specific maximum
170   value, hill coefficient and $mech_{thres}$ which is the value of the mechanical signal corresponding to
171   half of the maximum value produced by a cell. These values were defined specimen-specific in order
172   to take into account the variability of the mechanical environment between different animals.

173   The definition of the Hill parameters was calibrated to have a higher production of cytokines in
174   regions where the local mechanical signal, Gaussian-dilated effective strain (ε), is relatively higher or
175   lower for anabolic, anticatabolic cytokines or catabolic, anti-anabolic cytokines, respectively. The
176    $mech_{thres}$ for bone cells is defined for every vertebra as follows:
177    $mech_{thres}^{bone} = 0.95 * median(\varepsilon|_{bone})$ where $\varepsilon|_{bone}$ is the local mechanical signal in the trabecular
178   region of the given vertebra. The mechanotransduction for the marrow cells that can release products
179   into the volume is defined using this mechanostat threshold:
180    $mech_{thres}^{marrow} = 0.45 * median(\varepsilon|_{marrow})$ where $\varepsilon|_{marrow}$ is the local mechanical signal in the
181   trabecular region of the marrow of the given vertebra. In particular, $\varepsilon|_{marrow}$ is the gaussian dilated
182   effective strain in the trabecular marrow voxels and is used as input of the mechanotransduction
183   function for the osteoclasts, determining how much bone is resorbed based on the signal.
184   Analogously, $\varepsilon|_{marrow}$ is used as input of the mechanotransduction function for the osteoblasts and
185   lining cells, determining how much cytokines and osteoid are added to the specific voxel.

186   The model was implemented according to the following multiscale temporal discretization. The
187   cellular behavior we described in 2.2.1 was simulated with a 40-minute time step (dt$_{cells-RDD}$).
188   Following the simulations obtained by Tourolle (Tourolle 2019), this value turned out to be
189   sufficiently similar to the values observed experimentally about motility and activity of the bone
190   cells. The proteins and other present chemical substances in the *in silico* simulation were simulated to
191   React, Diffuse, Decay (RDD, Figure 2C) with the same time step for the cells dt$_{cells-RDD}$, subdivided
192   into 10 equal temporal substeps of 4 minutes through Strang splitting (Strang 1968).

193   In comparison to the implementation introduced in the model of reference, a new parallelized
194   approach has been used where the chemical substances and the cells have been subdivided into
195   subdomains. The subdivision in subdomains was carried out to minimize the volume of data from
196   one subdomain to the other. Then, the model computes the spatio-temporal evolution of the cells and
197   signaling pathways and RDD with the timestep dt$_{cells-RDD}$ in parallel across the subdomains. The
198   micro-FE of the vertebra is computed for updating the mechanical signal perceived by the cells with
199   a predefined update interval of 8 hours (dt$_{micro-FE}$), which is higher than dt$_{cells-RDD}$ to simulate a delay
200   in the perception of the new mechanical signal.

Model generation

202   The trabecular region was automatically obtained for each sample as described previously (Lambers
203   et al. 2011) comprising a lattice of up to 200x200x300 voxels of the same resolution of *in vivo* data,
204   hence 12 million voxels. In a first step, the greatest connected component (GCC) of the vertebra was
205   defined. The bone phase of the trabecular region (bone mineral density greater than 580 mg HA/cm^3),
206   was then used for setting the initial mineral and osteoid concentrations (1). The marrow of the
207   trabecular region was seeded with MSCs and HSCs with a density of 1.25 x 10^7 cells/ml. Ob and Oc
208   were seeded randomly occupying a portion of the bone surface. The binding sites of the cells were
209   modified to make the multicellular system closer to a real state, where the cells' receptors are
210   partially or fully bound. Ot were seeded using an exponential distribution to have more osteocytes
211   embedded deeper in bone rather than close to the bone surface. The seeding of all cells in a cross-
212   sectional slice can be seen in Figure 2D. The cytokines were defined using experimental data
213   obtained from the literature, when available, see Supplementary Table S1. Missing concentrations

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214 were defined using calibrated values after running simulations and checking whether the cells would
215 reside and be active in corresponding biochemical regions, e.g. Oc resorb bone and lining cells are
216 present mainly in low strain regions whereas Ob release osteoid primarily in high strain regions. The
217 summary of the initial concentrations is defined in Supplementary Table S1.

218 The model started running without changes to the bone microarchitecture for 48 iterations
219 (corresponds to 2 days), see Figure 2B, to enable a more adequate spatial arrangement of the cells
220 and cytokines. This pre-processing step is needed because the micro-CT image used as input contains
221 only information regarding the bone microarchitecture. The final configuration after this initialization
222 is illustrated in Figure 2E, where for simplicity reasons only the surface cells, the RANK binding site
223 occupancy for osteoclasts and preosteoclasts and the spatial RANKL distribution in the trabecular
224 region are shown. The model then continued running with changes to bone microarchitecture for five
225 days to enter the active bone remodeling phase and to reduce the dependency of the initial data,
226 where the distributions of the cells, proteins and receptors were affected by uncertainty or absence of
227 such input data. The result of this phase was considered as the initial state for the homeostatic as well
228 as for the simulations where the maximum amount of OPG, RANKL and Scl produced by osteocytes
229 are changed.

230 **Design of simulations**

231 In this work, we simulated homeostatic remodeling and we tested the effects of different production
232 values of RANKL, OPG, and Scl by osteocytes on the static and dynamic bone morphometry data
233 relatively to the homeostatic remodeling. For each condition different from homeostatic, one
234 parameter was increased or decreased at the time and the parameters were kept constant. This
235 approach was used regardless of the simulated animal. Each condition was simulated using *in vivo*
236 data of 5 CV6 vertebrae. The output of the homeostatic condition was compared against the *in vivo*
237 data and against the simulated conditions of higher and lower production values of OPG, RANKL
238 and Scl separately.

239 First, homeostatic remodeling was simulated with a set of 41 parameters that were kept constant
240 regardless of the simulated animal (Supplementary Table S2). 37 parameters were optimized for a
241 balanced spatiotemporal evolution of the cytokines, cell differentiation, bone formation and bone
242 resorption, while other values were set accordingly to literature. They range from the frequency of
243 random movement of the cells, thresholds regulating the differentiation of the cells, binding site
244 numbers, osteoblast and osteoclast polarization factors, osteoblast and osteoclast cluster size,
245 proliferation and apoptosis rates of cells (especially osteoblasts, MSC, HSC), mechanostat
246 coefficients, the maximum single-cell production rate of cytokines, diffusion and decay coefficients
247 for the cytokines, mineralization rate. The motility parameters are presented as probability values of
248 movement of 1 voxel per $dt_{cells-RDD}$ in a range of 0 to 1. The Ob and Oc polarization coefficients are
249 values that represent the tendency of these cells to add osteoid or resorb bone, respectively, towards
250 the gradient of the mineral concentration from their position (Tourolle 2019). The competitive
251 reaction RANKL-RANK-OPG requires the definition of the forward and backward binding
252 coefficients for the RANKL-RANK and RANKL-OPG complexes, whereas the simple receptor-
253 ligand kinetics LRP5/6-Scl requires the definition of the forward and backward binding coefficients
254 for the complex LRP5/6-Scl.

255 After the simulations of homeostatic remodeling, we investigated whether OPG inhibits osteoclast
256 reportion. An increased OPG production was modelled as an anabolic response to higher effective
257 strains by means of reducing Oc recruitment. With higher or lower OPG levels, OPG binds to
258 RANKL leading to lower or higher availability of free RANKL that can bind to the RANK receptors

6

259 on Oc cells, thus leading to lower Oc recruitment. Then, we investigated whether RANKL promotes
260 bone resorption. RANKL was modelled as a cytokine produced by Ob, Ot and lining cells as a
261 catabolic response when these cells perceive lower values of effective strains to promote Oc
262 recruitment and eventually favor bone resorption. Lastly, we investigated whether Scl blocks bone
263 formation by inhibiting osteoblasts. Scl was modeled as cytokine produced by Ot as an anti-anabolic
264 response when these cells perceive lower values of effective strains. These three hypotheses were
265 tested separately by changing the maximum production of OPG, RANKL and Scl by a single Ot,
266 $(\beta_{Ot}^{OPG}, \beta_{Ot}^{RANKL}, \beta_{Ot}^{Scl})$ in the simulations using the values reported in Supplementary Table S3,
267 Supplementary Table S4 and Supplementary Table S5. Starting from the baseline value reported in
268 Supplementary Table S2, these higher and lower values were obtained by adding or subtracting the
269 rescaled standard deviation of the serum concentrations levels reported by Shahnazari and colleagues
270 (Shahnazari et al. 2012).

Bone morphometry and visualization

272 Static morphometric parameters analyzed were bone volume fraction (BV/TV), specific bone surface
273 (BS/BV), bone surface density (BS/TV), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp),
274 and trabecular number (Tb.N). We computed them for each day of the simulation. Dynamic
275 parameters were mineral apposition rate (MAR), mineral resorption rate (MRR), bone formation rate
276 (BFR), bone resorption rate (BRR), mineralizing surface (MS), and eroded surface (ES). They were
277 computed by overlaying the images with a time interval of 2 weeks as analyzed in recent publications
278 (Scheuren et al. 2020a, b), using the image processing language (XIPL, (Hildebrand et al. 1999)).
279 Our simulation involved only the trabecular region while the cortical region is unchanged over time,
280 contrary to the *in vivo* data which show cortical remodeling occurs as well. Static parameters between
281 different datasets were compared as percent changes by normalizing the values to the initial value of
282 the morphological parameter of interest while dynamic parameters were compared as absolute values.

283 The trabecular 3D bone microarchitecture was visualized using ParaView (Kitware, Version 5.10;
284 Clifton Park, NY). The formed, quiescent and resorbed (FQR) regions in the *in vivo* images were
285 obtained after registration of the acquired images and their overlapping. The FQR regions in the *in*
286 *silico* images were directly obtained by overlapping the images at different time points.

Software and platform used

288 A hybrid C++/Python code (Python Language Reference, Version 3.8) that expanded from the
289 original implementation of the model was used to perform the simulations (Tourolle 2019). The
290 implementation is made available through Python bindings with pybind11 (Jakob et al. 2017). Taking
291 advantage of the other packages used for image processing, analysis and parallelization, we used
292 Python as the front-end. In particular, we used the mpi4py package for managing the MPI parallel
293 distributed computing interface in Python (Dalcin et al. 2011). We employed distributed and shared
294 parallel computing paradigms (OpenMP and MPI) due to the high number of variables used in the
295 simulations. We used the MPI parallel version of an algebraic multigrid solver, AMGCL, to solve the
296 diffusion problem of the cytokines into the volume (Demidov 2019). The Swiss National
297 Supercomputing Center (CSCS, Lugano, Switzerland) computational platform (STATE SYSTEM)
298 was used for running the simulations. Each node has 36 cores which can be scheduled to work in a
299 customized way regarding memory and parallelization of tasks. The parallel solver for the spatio-
300 temporal step of the cells and cytokines required 8 nodes to have enough memory and sufficient
301 speedup. For these resources, we use 4 MPI tasks for each node and 9 OMP threads. Additionally, the
302 micro-FE vertebral models were solved using 2 nodes using ParOsol, a parallel solver designed for
303 micro-FE analysis based on micro-CT images (Flaig and Arbenz 2011). The number of nodes was

304   chosen in order to have a good trade-off between computational time and speed-up of the code. These
305   two solvers were combined to obtain a suitable environment for solving the interconnected
306   mechanical environment and the tissue, cellular and signaling pathways with a resolution of 10.5 um.

Statistical analysis

308   Statistical analysis was performed in R (R Core Team (2019), R Foundation for Statistical
309   Computing, Vienna, Austria). The R lmerTEST package (Kuznetsova et al. 2017) was used to
310   perform the linear mixed model. The linear mixed-effects models account for "intra-correlations"
311   between the *in silico* simulations and *in vivo* repeated measurements. The model is described in two
312   parts: fixed effects and random effects. The random effects part accounts for the intra-correlations of
313   repeated measures in in *in vivo* samples and *in silico* simulations or the high/medium/low production
314   level of OPG, RANKL and Scl. The fixed-effects part accounts for the impact of various covariates
315   over time on outcomes on an average level of the dynamic and static bone morphometric parameters.
316   Furthermore, the likelihood ratio test was performed to assess the goodness of fit of three nested
317   models based on the ratio of their likelihoods. All the nested model equations are in the
318   supplementary section (Supplementary Material 1.1). The significance level between the groups (*in
319   vivo* data/*in silico* simulation or high/low, high/medium, medium/low) was calculated using pairwise
320   comparisons with Tukey's post-hoc correction for multiple comparisons. The significance level for
321   the interaction between time and group was calculated using ANOVA with the linear-mixed model.
322   The mean and the standard error of the mean were plotted and p-values smaller than 0.05 were
323   considered significant (Lenth 2022).

Results

325   To study trabecular bone remodeling, we ran our micro-MPA model on five CV6 vertebrae from
326   mice obtained using micro-CT imaging. Thanks to the use of high-performance computing, the
327   computational capability and the efficiency of the computational code were increased, allowing the
328   simulation of thousands of cells and RDD of proteins in a complete trabecular volume. A simulation
329   of 4 weeks took usually 3 to 6 hours on a supercomputer. More time was required with a bigger
330   trabecular volume (TV) or when the finite-element analysis required more iterations for converging
331   to a numerical solution. A single cell and RDD step took usually up to 10 s, whereas solving a single
332   micro-FE analysis took less than a minute. First, we report the results of homeostatic remodeling
333   compared to the *in vivo* data. Then, to demonstrate that the osteocytes can regulate the other cells'
334   activity, we report the results obtained after the individual manipulation of the maximum production
335   levels of OPG, RANKL and Scl by the osteocytes.

Homeostatic remodeling

337   The *in silico* simulations were able to reproduce bone remodeling with realistic changes, compared to
338   the *in vivo* data, through single-cell mechanotransduction of the mechanical signal. Figure 3 shows a
339   representative *in vivo* sample and *in silico* model of homeostasis. In Figure 3A, the individual
340   normalized trabecular bone volume fraction (Norm. BV/TV) is shown for the complete original
341   dataset, illustrating high variability within the group and for each animal over time. On the other
342   hand, the *in silico* data of Norm. BV/TV were more stable after the initial remodeling phase. The
343   values of ε are shown in Figure 3B with regions of high and low mechanical signal perceived by the
344   osteocytes. *In vivo* data shows that bone formation and bone resorption occur throughout the bone
345   microarchitecture, whereas in the *in silico* results only bone formation occurs more widely in the
346   trabecular region, see Figure 3C. Indeed, bone resorption is more localized at the top of the trabecular
347   region which corresponds to the distal side of the CV6 where the mechanical force is simulated.

348 Moreover, bone formation and bone resorption can alternate with each other in the same local region
349 over time *in vivo*, whereas *in silico* bone resorption events can change their locations more frequently
350 compared to bone formation events. The static trabecular bone parameters are shown in Figure 3D.
351 BV/TV and Tb.Th changed over time in a similar way between the simulated data and the *in vivo*
352 data and these two groups had a similar average (n.s. between groups and for the interaction time-
353 group). Additionally, *in silico* Tb.Sp and Tb.N followed *in vivo* trends (n.s. for the interaction time-
354 group). While Tb.Sp and Tb.N remained relatively constant over time *in vivo*, *in silico* we observed a
355 significant decrease and an increase in Tb.Sp and Tb.N, respectively ($p < 0.05$ between groups and for
356 the interaction time-group). Additionally, we also observed an increase in BS/BV and BS/TV *in*
357 *silico* but *in vivo* these values remain closer to their initial value ($p < 0.05$ between groups and for the
358 interaction time-group). MAR obtained from the *in silico* simulations was significantly lower than
359 the *in vivo* values ($p < 0.05$ between groups) and MRR was much higher *in silico* compared to the *in*
360 *vivo* data ($p < 0.05$ between groups); the values of MAR and MRR developed differently between the
361 *in silico* and the *in vivo* groups ($p < 0.05$ for the interaction time-group). The simulation results
362 captured the BFR average value over time (n.s. between groups) but its temporal evolution was
363 significantly different compared to the *in vivo* data ($p < 0.05$ for the interaction time-group). BRR had
364 similar values *in vivo* and *in silico* (n.s. between groups) with a similar trend over time (n.s. for the
365 interaction time-group). On the other hand, MS and ES were lower in the *in silico* simulations
366 compared to the *in vivo* data ($p < 0.05$ between groups). MS presented a different temporal progression
367 ($p < 0.05$ for the interaction time-group) while ES did not show a significantly different temporal
368 evolution (n.s. for the interaction time-group).

High osteoprotegerin inhibits osteoclast resorption

370 The variation of OPG levels produced by the osteocytes led to a change in bone remodeling activity
371 from the cells, see Figure 4. The main effect of the spatial characterization of the remodeling regions
372 was a higher or lower catabolic activity in the distal end of the CV6 when OPG was reduced or
373 increased respectively, see Figure 4A. This cell activity was reflected in the Norm. BV/TV with
374 lower values when OPG was lower and higher values when OPG was higher ($p < 0.05$ between
375 groups, for all possible group comparisons and for the interaction time-group), see Figure 4b. Norm.
376 Tb.Th. showed no significant difference for the interaction time-group and in the pairwise
377 comparison between the values obtained with high and medium OPG levels (n.s.). Norm. BS/BV
378 showed an inverse relationship with the OPG levels($p < 0.05$ between groups, for all possible group
379 comparisons). In addition, Norm. BS/BV showed different curves over time ($p < 0.05$ for the
380 interaction time-group). Norm. BS/TV, Norm. Tb.Sp and Norm. Tb.N were not affected by the
381 changes in OPG levels (n.s. between groups and for the interaction time-group). BFR was not
382 significantly affected by the variations in OPG with a similar slightly decreasing trend over time
383 among the three levels. The BRR and ES showed a similar variability of Norm. BS/BV, with lower
384 values when OPG was higher and higher values when OPG was lower ($p < 0.05$ between groups, for
385 all possible group comparisons). The significance of these changes was observed for both BRR and
386 ES over time between groups data ($p < 0.05$ for the interaction time-group). MS showed significantly
387 different values between groups ($p < 0.05$), however only the comparisons of high OPG-medium OPG
388 and medium OPG-low OPG showed significant differences between the groups ($p < 0.05$). MAR and
389 MRR did not show differences among the groups when the OPG production level was changed in the
390 simulations. These results suggest that OPG produced by the osteocytes can inhibit the osteoclasts
391 recruitment and the consequent amount of resorption by the available osteoclasts. In addition, the
392 number of osteoclasts also influenced the number of active osteoblasts, as shown by the variability of
393 the MS. The net effect of these changes has an impact on the static parameters, primarily on Norm.
394 BV/TV where the homeostatic balance is lost with alterations of the OPG levels.

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High receptor activator of nuclear factor kB ligand promotes bone resorption

The variation of RANKL levels produced by the osteocytes led to a different bone remodeling activity from the osteoblasts and osteoclasts, see Figure 5. Similar to the variations observed for OPG, we observed primarily a lower or higher catabolic activity when RANKL in the distal end of the CV6 was reduced or increased respectively, see Figure 5A. This cell activity was reflected in the Norm. BV/TV, Norm. Tb.Th. with lower values when RANKL was higher and higher values when RANKL was lower ($p<0.05$ between groups, for all possible group comparisons and for the interaction time-group), see Figure 5B. Norm. BS/TV did respond differently to higher or lower RANKL values over time but the average values over time were not statistically significantly different (n.s. between groups and $p<0.05$ for the interaction time-group). Norm. BS/BV, Norm. Tb.N and BRR and ES were lower when RANKL was lower and they were higher when RANKL was higher ($p<0.05$ between groups, for all possible group comparisons and for the interaction time-group). Norm. Tb.Sp was not affected by the changes in RANKL over time and its average value was not affected (n.s. between groups and for the interaction time-group). The changes in RANKL had an impact on BFR, with higher or lower values when RANKL was higher or lower, respectively ($p<0.05$ between groups and for the interaction time-group). The only comparison which did not show a significant difference in BFR was between high and medium levels of RANKL. Osteoblastic activity was also affected by the changes in RANKL in a similar way of BFR ($p<0.05$ between groups, for all possible group comparisons and for the interaction time-group). MAR and MRR did not show differences among the groups with changing RANKL production levels. These results suggest that RANKL promoted the osteoclasts differentiation by the changes of ES which in turn led to a variability of the resorption. The changes in the Ob recruitment are reflected in the extent of the formed surface by the Ob. The change in the formed surface had a cumulative effect on different values of BFR. The net effect of these changes had an impact on the static parameters, with the parameters starting to differ from the baseline condition earlier compared to OPG, thus augmenting the diversion from the homeostatic condition.

High Scl blocks bone formation by inhibiting osteoblasts

The variation of Scl levels produced by the osteocytes led to a different bone remodeling activity from the osteoblasts and osteoclasts compared to what was observed for the variations of RANKL and OPG before, see Figure 6. In the proximal end of the CV6 we observed primarily higher or lower anabolic activity of the osteoblasts when Scl was reduced or increased respectively, see Figure 6A. Therefore, bone formation was affected, and similar effect was observed in the Norm. BV/TV with lower values when Scl was higher, see Figure 6B. Indeed, the variations of Scl were statistically significant for Norm. BV/TV when comparing high and low Scl values as well as high and medium Scl values ($p<0.05$). However, their curves were not significantly different over time (n.s. for the interaction time-group). The statistical findings for Norm. BV/TV also apply to Norm. Tb.Th which slightly decreased over time and stabilized afterwards with a high Scl level whereas it increased when Scl was medium or lower ($p<0.05$ for the group comparisons high-low and high-medium). Conversely, Norm. BS/BV and Norm. BS/TV increased over time. The statistical significance of the variations for Norm BS/BV was the same as of Norm. BV/TV, whereas Norm BS/TV showed differences only in the group comparison high-medium ($p<0.05$). Interestingly, no increase in the Norm. BV/TV was observed for lower values of Scl . Norm. Tb.Sp and Norm.Tb.N did not present significant differences between the variations of Scl (n.s. between groups) and their temporal evolution between different levels (n.s. for the interaction time-group) was not significantly different statistically. BFR showed a significant variability due to Scl ($p<0.05$ between groups) but only in the comparisons high-low and high-medium. Also, MS was affected by the variations of Scl in a more

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441 evident way (p<0.05 between groups, for all possible group comparisons). Scl did not change how
442 bone was deposited by osteoblasts and this outcome was visible from the absence of variability in
443 MAR with different Scl values (n.s. between groups and for the interaction time-group). BRR, MRR
444 and ES did not show differences when Scl levels changed, nor its effect was significantly visible over
445 time (n.s. between groups and for the interaction time-group). The changes in Scl levels had a net
446 effect on the Norm. BV/TV less visible compared to the changes in OPG and RANKL levels, with a
447 temporal separation from the homeostatic range of values similar to the OPG case.

448 **Discussion**

449 The proposed *in silico* micro-MPA model successfully simulated trabecular bone remodeling to
450 evaluate the static and dynamic morphometry of the trabecular bone microarchitecture. We
451 demonstrated that our model can simulate a homeostatic response similar to that observed with *in*
452 *vivo* data. Although some morphological aspects were not captured by the simulations, our model
453 showed adaptation towards a normalized trabecular bone fraction in all samples. Furthermore, the
454 variability observed *in vivo* was also partially reflected in our simulations. Capturing the static
455 parameters with *in silico* models has been proven to be challenging and the most frequent parameter
456 to be captured was BV/TV (Schulte et al. 2013; Levchuk et al. 2014; Levchuk 2015). This parameter
457 did not show significant differences on average between the *in silico* and the *in vivo* values, whereas
458 Tb.Sp and Tb.N were different in our simulations compared to the *in vivo* data. Our simulations
459 showed a similar evolution pattern of the static bone morphometric parameters across the samples in
460 the second half of the simulation, meaning that it was possible to simulate similar bone changes for a
461 group of samples with limited variability in the output. The single-cell activity and the signaling
462 pathways were additional information modelled and they could be further tuned to capture the
463 spatiotemporal evolution of the bone microarchitectures. This approach might be considered a step
464 further in modeling the action of single cells and the signaling pathways compared to models of bone
465 remodeling based on systemic ODEs where the spatial information of the cells is missing (Buenzli et
466 al. 2012; Pastrama et al. 2018; Martin et al. 2019). The parameters of the single cells' activity can be
467 directly linked to some parameters, e.g., MAR was correlated to the osteoblast synthesis rate of
468 osteoid and the mineralization rate and MRR is correlated to the osteoclast resorption rate of mineral
469 and osteoid (Supplementary Table S2). However, changing such parameters will also lead to changes
470 in BFR and BRR as a collective effect of the cells' activity. MS and ES could also be affected by
471 these changes to a reduced extent because those parameters can only extend or reduce the boundary
472 of the already present remodeled regions. The amount of remodeled surface is mainly linked to the
473 active spatial cellular distribution on the surface where the cells can remodel bone.

474 Tb.N, Tb.Th and Tb.Sp could be dependent on the apposition and resorption rates by osteoblasts and
475 osteoclasts as well as by the mineralizing and eroded surface. The mineral apposition and resorption
476 rates had to be higher to reduce the difference between *in silico* and *in vivo* bone formation and
477 resorption rates, meaning that eventually the trabecular parameters would be affected by these
478 changes by more accentuated changes in the local remodeling area. If MAR, MRR, MS and ES were
479 closer to the *in vivo* values, the static trabecular parameters could have better followed the
480 corresponding *in vivo* values. However, this aspect should be further inspected because, for example,
481 the *in silico* model of Levchuk and colleagues captured Tb.N, Tb.Th and Tb.Sp despite a significant
482 discrepancy in dynamic parameters (Levchuk et al. 2014), while the model of Schulte and colleagues
483 for ovariectomy and loading captured some dynamic parameters and BV/TV but not Tb.N, Tb.Th and
484 Tb.Sp (Schulte et al. 2013). The regulation of the dynamic parameters was not trivial because the
485 mineralizing and eroded surfaces were not easy to capture due to the mechanical environment, the
486 mechanotransduction and the subsequent cascade in the signaling process to regulate osteoblasts and

487 osteoclasts activity. The remodeled regions were rather large *in silico* compared to *in vivo* which
488 were more scattered throughout the trabecular volume. Thus, the trabecular remodeling by the single
489 cells was less localized *in silico*.

490 We demonstrated OPG inhibits excessive osteoclastic bone resorption as it was observed previously
491 (Kramer et al. 2010; Cawley et al. 2020). Cawley and colleagues found OPG to be secreted by
492 osteoblasts (Cawley et al. 2020) whereas Kramer and colleagues found osteocytes to be the cells
493 mainly producing OPG (Kramer et al. 2010). Our work highlighted osteocytes are the cells
494 responsible for the production of RANKL and the subsequent recruitment of osteoclasts and increase
495 of bone resorption, as it was shown in previous works (Nakashima et al. 2011; Xiong et al. 2015). In
496 our work, osteocytes were also identified as sources of sclerostin inhibiting the osteoblastic activity
497 and therefore reducing bone formation. This finding was experimentally obtained also by van
498 Bezooijen and colleagues (Van Bezooijen et al. 2004), where sclerostin protein was found to be
499 expressed by osteocytes and not by osteoclasts in cortical and trabecular bone. Further, they also
500 observed the inhibitory effect of sclerostin on osteoblasts, confirming its importance for regulating
501 bone formation (Winkler et al. 2003; Li et al. 2008; Colucci et al. 2011).

502 We observed that affecting the catabolic response would also imply a change in the anabolic activity.
503 This aspect was evident from the variations of RANKL and OPG which had a direct impact on the
504 osteoclasts' activity, but the osteoblasts' activity was subsequently affected by that. On the other
505 hand, the anti-anabolic response would also imply a subsequent change in the catabolic activity, as it
506 was seen from the variations of Scl which inhibited osteoblasts and a consequent slower resorption
507 activity. These findings confirm the existence of the coupling between bone formation and bone
508 resorption events as observed *in vivo*. Our *in silico* model represented bone formation mostly
509 occurring over time in the same region where the strains were high, until the osteoblasts back
510 differentiated into lining cells or became embedded into the osteoid and differentiate into
511 preosteocytes. On the other hand, bone resorption occurred starting from a region where the strains
512 were low because in this region RANKL was higher and osteoclasts were mainly recruited here.
513 TGF-β1 is thought to be responsible for coupling bone resorption and bone formation (Raggatt and
514 Partridge 2010; Kasagi and Chen 2013; Weivoda et al. 2016; Durdan et al. 2021) and osteoclasts
515 resorb bone in regions of low strains. MSCs proliferated closer to osteoclasts due to the presence of
516 TGF-β1 being released upon resorption in these regions of low strains. In addition, in such regions,
517 sclerostin was also produced by osteocytes to inhibit bone formation by the osteoblasts. Therefore,
518 the osteoblastic differentiation of such cells was inhibited and MSCs would directly differentiate into
519 lining cells instead of osteoblasts in these regions of low strains. Moreover, we included the
520 production of RANKL and OPG by osteoblasts and lining cells and their production levels
521 confluence in the total levels of OPG and RANKL An extensive calibration of the coupling would
522 dramatically scale the number of simulations required to calibrate all these aspects. Such calibration
523 should include the simulation of bone formation regions occurring over subsequent bone resorption
524 areas and vice versa as observed *in vivo*. It is still not clear how the cells are able to have this fast
525 (bi)weekly turnover of events in place when their lifespan in mice is relatively longer, e.g., at least
526 two weeks (Søe 2020). These complex dynamics remain to be further elucidated.

527 It is not yet fully understood when the osteoclasts stop resorbing bone (Filgueira 2010), but it is
528 assumed that this happens when the mineral phase of the bone underneath is degraded to a certain
529 extent (Kanehisa and Heersche 1988). Currently, this mechanism is not reproduced directly in the *in*
530 *silico* model. In addition, it was observed that collagen can decelerate the demineralization activity
531 when osteoclasts resorb bone in pit mode, protecting bone against excessive resorption (Delaisse et
532 al. 2021). In our *in silico* model, osteoclasts stopped being active, and therefore resorptive, when they

533   were not in a cluster or if they died by apoptosis or due to lack of bound RANK receptors. Further,
534   the resorption rate of mineral and collagen by osteoclasts was considered the same. This is the case
535   when osteoclasts resorb bone in trench mode (Delaisse et al. 2021). On the other hand, these rates are
536   usually different when osteoclasts resorb bone in pit mode, with solubilization of mineral being faster
537   than the evacuation of collagen fragments (Delaisse et al. 2021). The modelling of these events might
538   be an oversimplification of how osteoclasts stop being active and it does not represent the same
539   frequency of interruption of bone resorption by osteoclasts.

540   For initializing the cytokines in the model, we used values measured from experimental studies
541   which are closely in line with the modelled environment. However, it is likely that the values
542   appropriate for the mouse vertebra at a given age might differ from the experimental values due to
543   site, age, loading and phenotypic characterization of the data. This problem has been identified for *in*
544   *silico* models of bone mechanobiology (Checa and Prendergast 2009), where simple approaches were
545   adopted to overcome this lack of information (Perier-Metz et al. 2020, 2022; Borgiani et al. 2021).
546   This limitation might be partially overcome by taking advantage of the mechanical environment as it
547   was performed by Tourolle and colleagues (Tourolle et al. 2021). A more synergistic study where the
548   cytokines are experimentally obtained from the same site on a regular basis would help in the
549   calibration of this micro-MPA *in silico* model. In addition, the information on the cell densities is
550   scarce and they might change between different bones, age, sex, and physiological conditions.
551   Moreover, the cells might be distributed differently in bone and in the marrow. This aspect is
552   especially relevant for osteocytes which are not motile in bone, meaning that their initial distribution
553   is essentially preserved throughout the time of the simulation, except when they are no longer present
554   due to cell apoptosis or bone resorption. The initial cell densities and cytokine distributions will have
555   implications in the estimations of the proliferation and cell rates as well as the single-cell activity,
556   e.g., resorption and cytokine production rates. Consequently, the amount of unknown initial
557   conditions is very high, and it can take some time before the simulated cells and cytokines can reach
558   a spatio-temporal distribution which is more realistic and not affected by the assumed initial
559   condition. This limitation was addressed by the additional iterations of the model first without
560   changes and then with changes to the bone microarchitecture. However, the number of iterations
561   required for this purpose could be even higher.

562   The receptor-ligand kinetics in the context of micro-MPA *in silico* models is still very novel and
563   needs further exploration. Depending on the application, the formulations might differ to include
564   other aspects like trafficking and intracellular signaling (Cilfone et al. 2015). Moreover, there might
565   be molecular aspects that might be lost when using this kind of equation and coefficients might be
566   recalibrated depending on the environment. Indeed, some coefficients might be experimentally
567   obtained from analysis of the interactions between monomers (Nelson et al. 2012), but their usage
568   might not be straightforward due to the coexistence of other phenomena like spatial diffusion and
569   movement of the ligands from one receptor to the other in the proximity of the cell surface (Erbaş et
570   al. 2019). Furthermore, the cell response is usually achieved when most of its surface receptors are
571   still unoccupied (Lodish et al. 1999). It is still difficult to estimate *in vivo* the number of surface
572   receptors and the number of occupied receptors along with an accurate description of the receptor-
573   ligand kinetics, especially when competitive reactions are involved, e.g., the RANKL-RANK-OPG
574   axis. Hence, the receptor-ligand kinetics in bone remodeling remains challenging but crucial. *In silico*
575   models of bone remodeling use this information to regulate cell fate and differentiation by
576   parametrization of the coefficients used in the equations as well as the number of receptors per cell.
577   These factors are relevant at the cellular and gene scales that influence bone remodeling activities in
578   the whole volume. The numerical estimates are a starting point, but it is advisable to employ
579   experimental values detected from receptors on (bone remodeling) cells presenting such receptors.

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580 Different experimental approaches directly applied to the cells (Warren et al. 2015) or in a
581 multidimensional environment (Chesla et al. 1998) might help in estimating the coefficients to be
582 used for modelling such phenomena *in silico*.

Conclusion

584 This work showcases the use of single-cell mechanotransduction in a micro-MPA *in silico* model of
585 trabecular bone remodeling applied to *in vivo* data. We were able to reproduce homeostatic bone
586 remodeling and highlight how tuning the single-cell osteocyte production rates of OPG, RANKL and
587 Scl further induces anabolic, anti-anabolic, catabolic and anticatabolic responses from the baseline
588 model. The calibration with bone morphometric parameters was used in this work, however, it would
589 be helpful to employ a complete experimental dataset which can be directly assigned to the
590 parameters of the model, e.g., serum markers and cellular properties. By careful calibration of
591 biological parameters, we hope this model shed light on bone remodeling and associated diseased
592 states. Micro-MPA models over several scales will be needed in the future because only with them it
593 will be possible to unravel biological processes and their effects realistically. This will advance the
594 field of bone remodeling and our current understanding of its mechanisms.

Conflict of Interest

Author Contributions

599 DB, FM, CL, JK, YDB, FS and RM contributed to the design of the study. DAB, FM, CL and JK
600 contributed to the development of the model, assembly and assessment of data, AS and FS
601 contributed to the statistical analysis and interpretation of results, DB, FS, YDB and RM wrote the
602 main manuscript text. All authors contributed to revising the manuscript and approved the final
603 version to be submitted.

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899　**Figure captions**

900　**Figure 1**

901　Experimental design of the *in vivo* data used for the simulations of the *in silico* model. (**A**) Weekly
902　micro-computed tomography (micro-CT) images of the sixth caudal vertebra of PolgA[(D257A/D257A)]
903　mice. Half of the vertebra is shown with transparency of the cortical region over time for each
904　measurement. (**B**) The normalized trabecular bone volume fraction slightly decreased over the course
905　of the study in the group of mice (n=9).

Figure 2

The concept of the micro-MPA *in silico* model in the mouse vertebra. (**A**) The cells and cytokines modeled in the bone remodeling version of the model. (**B**) Overview of the simulation pipeline from the input data to the end of the simulation. (**C**) A schematic representation of the cell receptor-ligand kinetics modelled *in silico*. Here, the ligand can bind only to the targeted cell receptor with a forward rate k^f and it can dissociate from the receptor with a backward rate k^r. This is an example of the modelled receptor-ligand kinetics of Lipoprotein receptor-related protein 5/6 (LRP5/6)-sclerostin for an osteoblast, lining cell and mesenchymal stem cell. (**D**) A 2D slice from the trabecular region of an *in silico* simulation with osteocytes (black), osteoclasts (purple), preosteoclasts (blue), osteoblasts (orange), mesenchymal stem cells (green) and hematopoietic stem cells (brown). (**E**) A snapshot of the initial configuration of the *in silico* simulation at t=0. On the left, the osteoblasts (orange), osteoclasts (purple) and preosteoclasts (blue) are shown on the trabecular bone surface, in the middle the spatial distribution of the receptor activator of nuclear factor kB (RANK) binding site occupancy on the osteoclasts and preosteoclasts and on the right the spatial distribution of the RANK ligand (RANKL) configuration in the trabecular region.

Figure 3

The *in vivo* data and the comparison against the *in silico* modeling of homeostasis. (**A**) The normalized bone volume fraction (Norm. BV/TV) for the complete group of mice *in vivo* (left, n=10) and for the group of mice analyzed *in silico* (right, n=5). (**B**) Mechanical signal in the trabecular region for the representative mouse (number 5). (**C**) Bone formation, quiescent and resorption events images over time *in vivo* and *in silico*. (**D**) Static and dynamic bone morphometry values for the *in vivo* and *in silico* groups. In these plots, only the same selection of mice was plotted for both the *in vivo* and *in silico* data (n=5).

Figure 4

In silico results of variations of the maximum single cell production level of osteoprotegerin (OPG) by osteocytes. (**A**) Biweekly FQR regions of results obtained with higher and lower OPG production levels for the representative mouse (number 5. (**B**) Static and dynamic bone morphometry parameters of the results of the simulations of the same group of mice (n=5) under three different production levels of OPG, starting from the same initial condition of the medium level. High=higher production level of OPG, Baseline=medium production level of OPG as in the homeostatic configuration, low=lower production level of OPG.

Figure 5

In silico results of variations of the maximum single cell production level of receptor activator of nuclear factor kB ligand (RANKL) by osteocytes. (**A**) Biweekly FQR regions of results obtained with higher and lower RANKL production levels for the representative mouse (number 5). (**B**) Static and dynamic bone morphometry parameters of the results of the simulations of the same group of mice (n=5) under three different production levels of RANKL, starting from the same initial condition of the medium level. High=higher production level of RANKL, Baseline=medium production level of RANKL as in the homeostatic configuration, low=lower production level of RANK.

945 **Figure 6**

946 *In silico* results of variations of the maximum single cell production level of sclerostin (Scl) by
947 osteocytes. (**A**) Biweekly FQR regions of results obtained with higher and lower Scl production
948 levels for the representative mouse (number 5). (**B**) Static and dynamic bone morphometry
949 parameters of the results of the simulations of the same group of mice (n=5) under three different
950 production levels of Scl, starting from the same initial condition of the medium level. High=higher
951 production level of Scl, Baseline=medium production level of Scl as in the homeostatic
952 configuration, low=lower production level of Scl.

953 **Supplementary Material**

954 See additional file for Supplementary Material.

955 **Data Availability Statement**

956 The raw data supporting the conclusions of this article will be made available by the authors, without
957 undue reservation.

a) 6th caudal vertebra

b)

m1
micro-CT
Age=38 weeks

m2
micro-CT
Age=39 weeks

m3
micro-CT
Age=40 weeks

m4
micro-CT
Age=41 weeks

m5
micro-CT
Age=42 weeks

a)

b)

Start t = -2d

Definition of the parameters in the simulation

Addition of the cytokines to the lattice

Initial Micro-FE

Seeding of the cells

Timestep of cells and RDD without changes to bone

t=0?

False

True

Timestep of cells and RDD

$t=n \cdot dt_{\text{micro-FE}}$
n=1,2,3..?

False

True

Micro-FE and update of the mechanical signal

$t=t_{\text{end}}$?

False

True

End

c)

Ligand

k^f

Cell

Receptor

k^r

d)

e)

at t=0

% RANK occupancy

0.65
0.61
0.57
0.53
0.49
0.45

RANKL (pg/ml)

130
120
110
100
90
80