MULTIPLE VERIFICATION IN COMPLEX BIOLOGICAL SYSTEMS: THE BONE REMODELLING CASE STUDY

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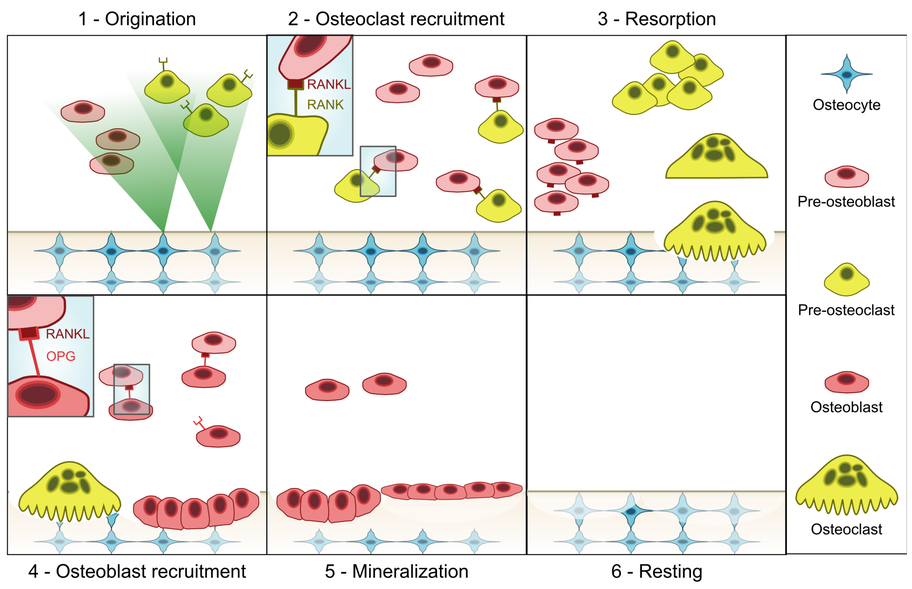
BONE REMODELLING AS A PARADIGM FOR ORGAN FUNCTIONAL MANTAINANCE

Bone remodelling (BR) is a process iterating throughout life, by which aged bone is continuously renewed in a balanced alternation of bone resorption (performed by cells called **osteoclasts**) and formation (performed by **osteoblasts**).
It is responsible for repairing micro-damages, for maintaining mineral homeostasis and for the structural adaptation of bone in response to mechanical stress. In other words, a regular remodelling activity ensures the mechanical quality of the bone.
Pathologies arise when the resorption and the formation phases are not in equilibrium: osteoporosis is an example of negative remodelling where resorption prevails on formation. In this situation even small negative changes in bone density become more and more critical as remodelling cycles follow one another.



Key phases during the bone remodelling process

Bone remodelling is **a paradigm for several other physiological systems**, since similarly to the epithelium renewal process, the haematopoiesis process and many others, it is characterized by a birth-death dynamics involving different populations of cells (osteoclasts and osteoblasts) which together contribute in maintaining the stability of the tissue level and of the organ level.
Furthermore, bone remodelling is a multiscale process where the molecular scale affects the cellular scale (e.g. RANKL induces osteoclasts' proliferation), and in turn the cellular scale affects the tissue scale (the number and the activity of bone cells determine tissue density and micro-structure).

EACH BIOLOGICAL PROPERTY, ITS MODEL

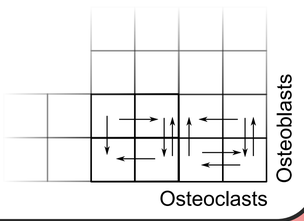
in this work we address the "property to semantics" problem: **given a biological property to be formally analyzed, which is the most suitable semantics for my model?** As a matter of fact, the type of questions a model can answer to depend both onthe model semantics (continuous, discrete, deterministic, stochastic, ...) and in turn on the analysis methods supported by that particular semantics.
We present a set of formal techniques and a methodology for a composite formal analysis at the tissue and organ levels, focusing on the verification of quantitative properties in the process of bone remodelling. Starting from a **differential equation model,** we derive a **stochastic model** and a **piecewise-multiaffine approximation** in order to perform model checking of stabilisation properties for the biological tissue, and to assess the differences between a regular remodelling activity and a defective activity typical of pathologies like osteoporosis.

MODELS AND ANALYSIS METHODS

Piecewise-multiaffine model

We approximate the non-linear ODE into a piecewise-multiaffine model, by an optimization algorithm [2] that ensures the best fitting, given the number of segments in the function.

Then, the continuous state space is partitioned into hyper-rectangles (determined by the segments) that give rise to a classical **discrete transition system**.



Model Checking

Parameter Synthesis

ODE model

Autocrine and paracrine regulation factors

Osteoclasts: $\dot{x}_1 = \alpha_1 x_1^{g_{11}} x_2^{g_{21}} - \beta_1 x_1$

Osteoblasts: $\dot{x}_2 = \alpha_2 x_1^{g_{12}} x_2^{g_{22}} - \beta_2 x_2$

Growth and death rates

Bone density: $\dot{z} = -k_1 x_1 + k_2 x_2$

Resorption and formation rates

Sensitivity Analysis

Simulation

Stochastic model

We employ **Continuous Time Markov Chains** (CTMC) and the probabilistic model checker **PRISM**. The model [1] is structured into modules that executes concurrently, and are equipped with a set of discrete finite-ranging state variables and by a set of guarded transitions.

Osteoclast module:

$$0 < x_1 < x_1^{max} \wedge x_2 > 0 \rightarrow \alpha_1 x_1^{g_{11}} x_2^{g_{21}} : x_1 = x_1 + 1$$
$$x_1 > 0 \rightarrow \beta_1 x_1 : x_1 = x_1 - 1$$

Osteoblast module:

$$0 < x_2 < x_2^{max} \wedge x_1 > 0 \rightarrow \alpha_2 x_1^{g_{12}} x_2^{g_{22}} : x_2 = x_2 + 1$$
$$x_2 > 0 \rightarrow \beta_2 x_2 : x_2 = x_2 - 1$$

Probabilistic Model Checking

BIOLOGICAL PROPERTIES AND RESULTS

Osteoclasts down-regulation — MC PMC

We verify the effectiveness of the negative regulation that osteoblasts apply on osteoclasts. It is a key feature in the bone remodelling system since it ensures that the resorption phase comes to an end, and consequently that bone is protected from excessive resorption. In other words, we verify that osteoclasts cannot increase when osteoblast concentration is above a given threshold.

Osteoclasts proliferation — MC PMC

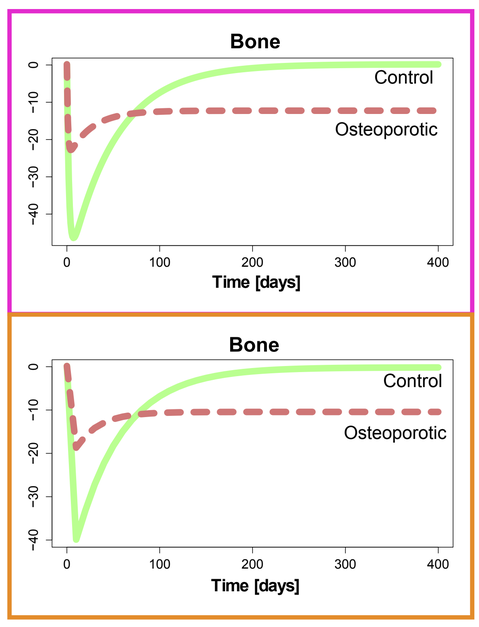
We verify that osteoclasts cannot decrease when the number of osteoblasts is below the same thresold used in the down-regulation property. This guarantees thato steoclasts can proliferate in presence of small perturbations of osteoblasts.

Boundedness of osteoclasts and osteoblasts — PS

We perform parameter synthesis on the piecewise-multiane model in order to find regions in the parameter space for which the concentrations of osteoblasts and osteoclasts are below fixed thresholds. The existence of an upper-bound ensures the bounded growth of bone cells and therefore, the absence of anomalous dynamics like the osteoclasts proliferation in bone metastases.

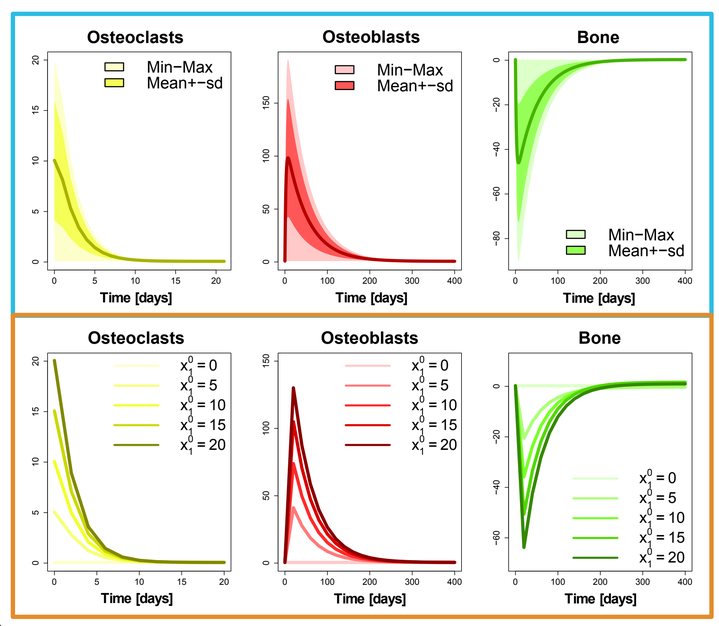
Osteoporosis — S PMC

We simulate defective remodelling, in order to reproduce bone pathologies like osteoporosis that are characterized by a lower bone density. This negative balance has been modelled by assuming an increased death rate for bone cells.



Robustness of stabilization w.r.t. initial conditions — SA PMC

We assess how changes in initial concentration of osteoclasts affect remodelling, in particular the stabilisation of bone cells and bone density, that is normally achieved with the original parameters.



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