PERSPECTIVES ON *IN SILICO* BONE MECHANOBIOLOGY: COMPUTATIONAL MODELLING OF MULTICELLULAR SYSTEMS

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Abstract

Bone mechanobiology is the study of the physical, biological and mechanical processes that continuously affect the multiscale multicellular system of the bone from the organ to the molecular scale. Current knowledge derives from experimental studies, which are often limited to gathering qualitative data in a cross-sectional manner, up to a restricted number of time points. Moreover, the simultaneous collection of information about 3D bone microarchitecture, cell activity as well as protein distribution and level is still a challenge. *In silico* models can expand qualitative information with hypothetical quantitative systems, which allow quantification, testing and comparison to existing quantifiable experimental data. An overview of multiscale, multiphysics, agent-based and hybrid techniques and their applications to bone mechanobiology is provided in the present review. The study analysed how mechanical signals, cells and proteins can be modelled *in silico* to represent bone remodelling and adaptation. Hybrid modelling of bone mechanobiology could combine the methods used in multiscale, multiphysics and agent-based models into a single model, leading to a unified and comprehensive understanding of bone mechanobiology. Numerical simulations of *in vivo* multicellular systems aided in hypothesis testing of such *in silico* models. Recently, *in silico* trials have been used to illustrate the mechanobiology of cells and signalling pathways in clinical biopsies and animal bones, including the effects of drugs on single cells and signalling pathways up to the organ level. This improved understanding may lead to the identification of novel therapies for degenerative diseases such as osteoporosis.

Keywords: Multiscale, multiphysics, agent-based, hybrid modelling, cells, reproducibility.

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List of Abbreviations

ABM	agent-based modelling
BMU	basic multicellular unit
CA	cellular automaton
FEM	finite element method
LRP	low density lipoprotein receptor-related protein
MSC	mesenchymal stem cell
ODE	ordinary differential equation
OPG	osteoprotegerin
PDE	partial differential equation
PTH	parathyroid hormone
RANK	receptor activator of nuclear factor-kB
RANKL	RANK ligand
RVE	representative volume element
SED	strain energy density
Sema3A	semaphorin 3A
SPECT	single-photon emission computed tomography
TGF-β	transforming growth factor-β
μCT	micro-computed tomography
μFE	micro finite element

Introduction

Bone is the material that gives the body its primary structure and stability (Clarke, 2008). The adaptation, renewal and maintenance of bone are vital for this purpose and they are tightly regulated by mechanoresponsive cells and intracellular signalling pathways (*e.g.* Wnt, oestrogen, Ca^{2+}). The mechanotransduction of an extracellular stimulus into intracellular biochemical responses can be divided into three cellular phases: mechanoreception of the stimulus, signal transduction to the nucleus, changes in gene/protein expression (Vogel, 2006; Vogel and Sheetz, 2006). Tightly regulated mechanotransduction is important for cell communication, *e.g.* in the context of bone remodelling driven by bone-forming

osteoblasts and bone-resorbing osteoclasts in the BMU. Unbalanced bone remodelling can lead to diseases such as osteoporosis, osteopetrosis and Paget's disease. Osteoporosis is the systemic loss of bone leading to increased fracture risk (Oden *et al.*, 2015); it is debilitating, and in 2010, the total direct cost of osteoporosis in the European Union was estimated to be 37 billion EUR (Hernlund *et al.* 2013). Furthermore, developing treatments is an expensive and time-consuming process. It could take as long as a decade between drug discovery and the entry of an approved treatment into the market (Morris *et al.*, 2011). The development of novel treatments for bone-related diseases depends upon the understanding of the fundamental bone mechanobiology and the computational models that are available to test hypotheses (Thorne *et al.*, 2007). Recent work has coupled systems biology with computer simulations to capture homeostasis and unbalanced bone remodelling (Hambli, 2010; Hambli *et al.*, 2011; Kameo *et al.*, 2020; Kameo and Adachi, 2014a; Schulte *et al.*, 2013) and to provide a platform for developing new treatments. The effects of drugs, dosage and frequency of treatment could potentially be studied and tracked over time and space down to the action of single cells with an *in silico* model. However, current understanding of bone mechanobiology might still be further improved through the development of new *in silico* models, including the modelling of the actions of single cells, proteins and signalling pathways. Therefore, research should focus on novel 3D approaches to examine bone mechanobiology, including more biological and physical details, to enable better direct validation with *in vivo* data over time, *e.g.* analysis of cytokine values depending on strains and age. For more information on this topic, please refer to the review by Levchuk and Müller (2013).

Bone remodelling occurs across varying length scales that are separated by several orders of magnitude from the organ to the gene level. A complete understanding of the mechanisms involving these scales has several challenges in terms of mathematical description (fidelity), computational implementation and resolution (accuracy) as well as obtaining consistent results using the same approach (computational reproducibility) (Martin *et al.*, 2019; Paoletti *et al.*, 2012). The *in silico* models should emulate the phenomena observed experimentally and integrate the missing information with a hypothesis. Non-invasive imaging techniques can be used to study bone remodelling at a μm resolution (Lambers *et al.*, 2015a; Schulte *et al.*, 2013; Willie *et al.*, 2013) and they focus on the observation of structural changes *in vivo* (Birkhold *et al.*, 2015; Christen and Müller, 2017). Nuclear imaging techniques, such as SPECT and positron emission tomography, allow the tracking of radioisotope labels. These labels can be attached to cells, allowing the location and density of cell populations to be tracked (Blackwood *et al.*, 2009; Mathavan *et al.*, 2019). However, using these tools, it is not possible to observe the 3D distribution and dynamics of proteins and individual cells. Endpoint histology can be used to investigate protein- and cellular-scale phenomena but these data are often limited to a few histological sections per sample and to qualitative or semiquantitative metrics. Moreover, the cross-sectional nature makes it impossible to detect changes in protein expression and cell phenotype/genotype over time (Currey *et al.*, 2015; Li *et al.*, 2014; Pavlos *et al.*, 2005). *In silico* models offer a different approach, in which biological hypotheses are tested virtually. The data produced are quantitative and are not limited to cross-sectional endpoints. In addition, such methods can use hypotheses and data from different studies with different animals, regions of interest and ages if this information is limited or unavailable for the analysis and can assess their applicability. Such methods can leverage existing experimental methods and data for parameterisation and validation but provide fine-grained insight into the underlying mechanism in a way that is currently impossible through experimentation. Indeed, with model tuning, it is possible to estimate the values that represent a particular aspect that is not yet quantified experimentally or available in the literature.

Computational mechanobiology is a continuously developing field in which *in silico* models are used to study how mechanical and biological phenomena affect each other (Giorgi *et al.*, 2016; Martin *et al.*, 2019). *In silico* models have the potential to connect the existing knowledge and methods of computational modelling and mechanobiology to overcome the obstacles by comprehensive and exhaustive data integration (Soheilypour and Mofrad, 2018) as opposed to individual disciplines. Out of all the *in silico* models, the agent-based, multiscale and multiphysics models are of particular interest. These modelling techniques represent cells as heterogeneous agents following a set of genotypically prescribed rules (Checa, 2018; Drasdo *et al.*, 2018). The spatial and temporal separation of scales in the same framework allows the coupling of local phenomena, such as bone remodelling, to organ-wide changes, such as oestrogen depletion, as well as the coupling of distinct physical phenomena involving entities of different natures, *e.g.* cells that sense mechanical deformation (*e.g.* fluid-induced deformations) and then produce proteins, such as RANKL, which diffuse and bind to other cells (George *et al.*, 2018; Nava *et al.*, 2013; Peyroteo *et al.*, 2020). To date, these techniques have been applied to different aspects of bone biology in an *ad hoc* way.

In the present review, the focus is on the use of each method to create new biological insights into bone mechanobiology and its possible improvement. Herein, the scientific application of interest is the modelling of bone mechanobiology. The existing models cover a variety of different scales and aspects of bone mechanobiology. To discuss them with a consistent approach, a model biological system was described. Then, for each method, the specific bone

properties as well as the techniques and applications for the modelling of bone and computational biology were identified. Multiscale, multiphysics, agent-based and hybrid modellings are all described in a similar manner. Reproducibility is discussed to identify the current state of the art in developing a possible multipurpose computational platform of *in silico* models of bone mechanobiology. Based on this analysis, future methods for modelling bone mechanobiology are proposed.

Multicellular reference system

A multicellular reference system was defined to enable better identification and mapping of the mechanobiological features of bone using the related *in silico* models. In this work, "multicellular system" is used to refer to what the numerical models represent, *e.g.* the different cell types and their interactions with other chemical components, hard and soft tissue (including the extracellular matrix) as well as the mechanical environment. Fig. 1 shows the system description, combining figures from other studies, to establish a framework for the subsequent considerations. This figure will be used as a reference throughout this section.

Bone is an organ (Fig. 1**a**) formed of different organic (*e.g.* collagen) and inorganic (*e.g.* hydroxyapatite) components. Bone continuously adapts to the external stimuli (Colloca *et al.*, 2014b; Lambers *et al.*, 2011; Lambers *et al.*, 2015b; Perry *et al.*, 2009; Wolff, 1892), such as experimentally applied forces through external fixators that allow the controlled application of mechanical loading (Wehrle *et al.*, 2020), due to the coordinated action of the cells in the BMU (Fig. 1**d**). Cells residing in bone show distinct characteristics and features, such as the ability to differentiate into multiple cell types (mesenchymal stem cells), motility (osteoblasts, osteoclasts) (Jiang *et al.*, 2002; Singh *et al.*, 2016) and mechanoresponsiveness (mesenchymal stem cells, osteocytes, osteoblasts, osteoclasts). Furthermore, some cells, such as mesenchymal stem cells in the bone marrow, osteoblasts and osteoclasts, are motile while others, such as osteocytes, are motionless. Osteocytes are believed to be the main mechanosensors in the bone that facilitate the mechanotransduction of extracellular stimuli into intracellular biochemical responses. The activation of intracellular signalling pathways such as Wnt-signalling (Downward, 2001; Frenk and Houseley, 2018) *via* cell membrane receptors (Häusler *et al.*, 2004; Pinzone *et al.*, 2009) (Fig. 1**h**) regulates the DNA-mRNA transcription of target genes in the nucleus (*e.g.* RANKL, OPG) (Boyce and Xing, 2008; Kuang *et al.*, 2018) and subsequent changes in ribosomal mRNA-protein translation (Fig. 1**l**). This well-regulated protein expression is required for cell communication within the BMUs during bone remodelling (Klein-Nulend *et al.*, 2013; Vaughan *et al.*, 2012). Proteins present in the extracellular matrix (Fig. 1**k**) allow spatiotemporal changes in the mechanobiological (micromechanical) tissue properties. These properties are modelled using techniques (Fig. 1**c**,**f**,**g**,**j**) that will be described in detail in the following sections. Overall, these mechanobiological properties and modelling techniques together create a multicellular reference system, which will be used as a guide in the following sections.

Based upon this multicellular reference system, a natural hierarchy is revealed. The spatial scales of this hierarchy can be classified as the "organ scale", which corresponds to the organ level (Badilatti *et al.*, 2016; Colloca *et al.*, 2014a); the "tissue scale", where the tissue characteristics are studied (Lerebours *et al.*, 2016; Linderman *et al.*, 2015); the "cell scale", which points to the cells *i.e.* the structures with a characteristic length of 1-10 μm (Albers *et al.*, 2013; Hashimoto *et al.*, 2015; Kaul *et al.*, 2015); and the "protein scale", where proteins and molecules characterised by a length scale of 1 nm are examined (Landis *et al.*, 1993; Rubin *et al.*, 2003). At the organ scale, the related length scales as a physical quantity may shift depending on the actual bone under investigation. For example, the human vertebral column is 600-700 mm long, whereas the length of a mouse spine is 75-90 mm (Wang *et al.*, 2015). However, cell and molecule scales are in a more consistently defined range within the context of bone biology. These scales should be appropriately represented in the *in silico* models of bone.

In silico models may include the same length scales but study different mechanobiological features (Colloca *et al.*, 2014b; Lerebours *et al.*, 2016; Martin *et al.*, 2019). For example, FEM has been used at the tissue scale (Lambers *et al.*, 2015a; Schulte *et al.*, 2013) as well as the nanoscale (Marino and Vairo, 2014; Nikolov and Raabe, 2008; Pradhan *et al.*, 2014; Vaughan *et al.*, 2012). Thus, using the same tools, it is possible to model different aspects involving different length scales. The following sections highlight how the length scales can be connected using different techniques, such as averaging, equations and agent-based models. It is important to note that the classifications of multiscale, multiphysics and ABMs often overlap, indicating that some models may belong to more than one category.

In silico computational mechanobiology

Multiscale modelling

Multiscale properties of bone

In multiscale modelling, one or more interlinked mechanisms that involve several characteristic scales (spatial, temporal or both) are studied. A biological system that presents a structure similar to the hierarchy mentioned in the previous section is suitable for multiscale modelling (Agur *et al.*, 2011; Budyn and Hoc, 2006; Carlier *et al.*, 2012). Bone is a classic example of such complex hierarchy, with distinct phenomena occurring at the organ (Fig. 1**a**,**b**), tissue (Fig. 1**d**,**e**), cell (Fig. 1**d**,**h**) as well as gene and protein (Fig. 1**h**,**i**,**k**,**l**) scale. These scales are connected

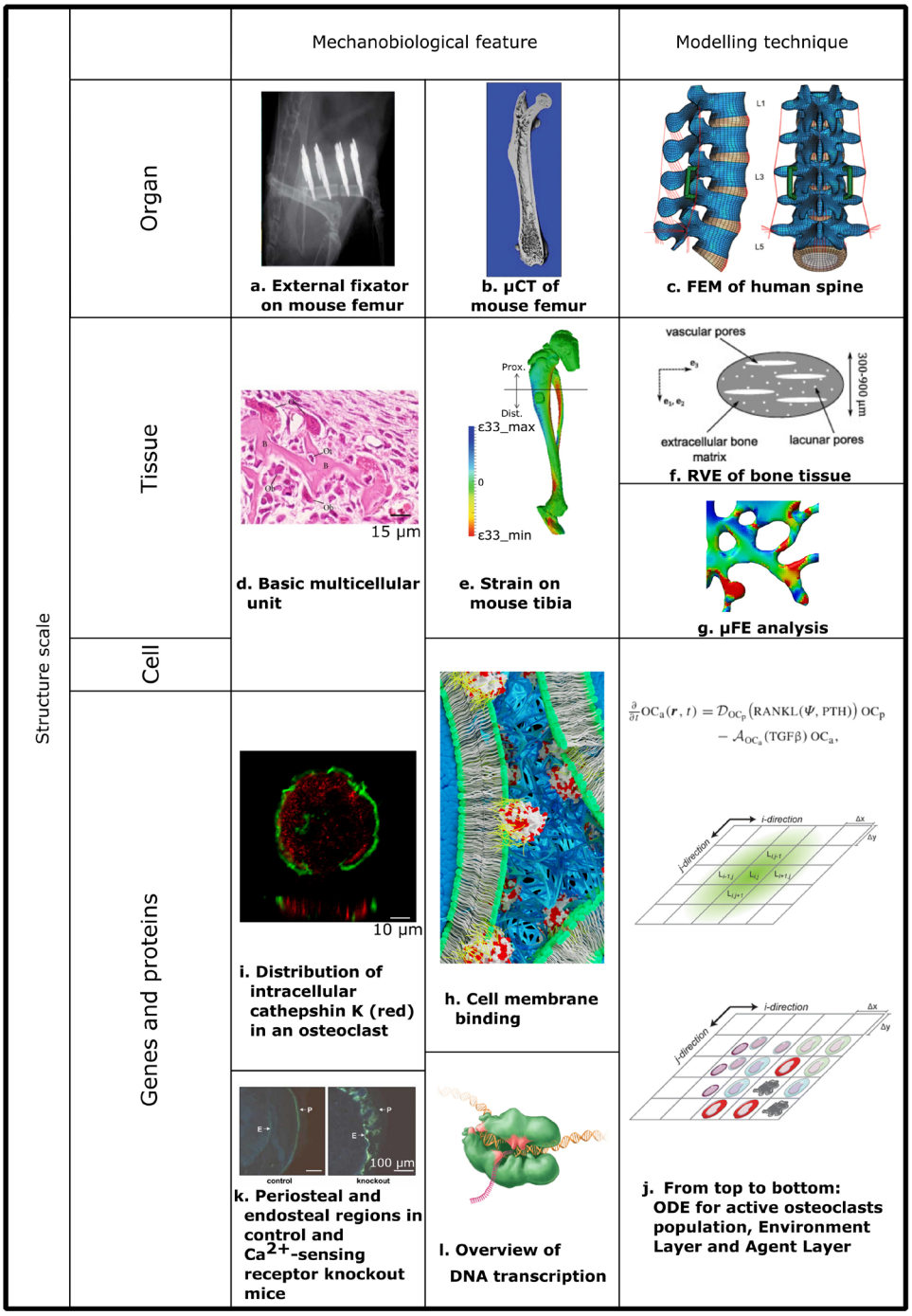

Fig. 1. The reference system of bone biology used in the analysis. The experimental mechanobiological features are shown with their structural scale(s) as well as the modelling technique used to study them. At the organ scale, bone can be experimentally studied with the application of an external fixator, such as (**a**) the mouse femur (Röntgen *et al.*, 2010), and (**b**) a µCT image can be acquired to measure bone density (Brommage *et al.*, 2014). (**c**) For this scale, the modelling technique used can be, for example, FEM (Rohlmann *et al.*, 2007). At the tissue level, (**d**) the cells in the BMUs remodel bone (Florencio-Silva *et al.*, 2015), (**e**) according to the strain (Torcasio *et al.*, 2012) they perceive. This mechanical stimulus can be computationally estimated (Torcasio *et al.*, 2012) and the mechanical properties of bone might be modelled through (**f**) RVE and (**g**) µFE analysis, as shown by Morin and Hellmich (2014) and Lambers *et al.* (2015a), respectively. (**h**) Receptor-ligand bindings occur at the interface of the cell (Deller *et al.*, 2019). From an experimental perspective, the (**i**) intracellular and (**k**) extracellular distributions of proteins and matrix components can be observed through staining (Morko *et al.*, 2019) and fluorochrome application (Dvorak-Ewell *et al.*, 2011). (**j**) Cells, genes and proteins can be numerically studied thanks to agent-based modelling and ODEs, as illustrated by Lerebours *et al.* (2016) and Cilfone *et al.* (2015), respectively. (**l**) Last, it is possible to analyse DNA transcription (Pollard *et al.*, 2017), which occurs inside a single cell and tunes protein expression. (**d**,**b**,**h**) Reproduced in compliance with the CC BY licence applied by Hindawi and Springer Nature; (**a**,**k**) reproduced with permission from John Wiley and Sons, (**c**,**e**,**j**) from Springer, (**f**,**g**,**i**,**l**) from Elsevier.

through mechanobiological changes that propagate through the hierarchy. For instance, cell activity is regulated by proteins, which reside on a lower scale (Frenk and Houseley, 2018; Häusler *et al.*, 2004; Pinzone *et al.*, 2009), and cells remodel the tissue, concomitantly changing the properties at the tissue (Fig. 1**d**) and organ scale. All these features reside not only on different spatial scales but also have their characteristic time scales range, from weeks to years for the treatment of osteoporosis and bone adaptation (Christen *et al.*, 2012; Gossiel *et al.*, 2016; Willie *et al.*, 2013) to minutes for cell activity (Shemesh *et al.*, 2017; Søe and Delaissé, 2017) and to seconds for protein activity (Luxenburg *et al.*, 2006; Sklar *et al.*, 1985). The multiscale nature of the model depends on the research question at hand. A simplified model might provide insights limited only to the chosen scale. For example, if a bone remodelling model defines bone formation and resorption rules based on the local strain distribution, then it might investigate the existence of a correlation between remodelling events and strains at the tissue scale only. Consequently, the model becomes highly complex as it includes more spatial and temporal levels in its hierarchy. Some multiscale techniques can reduce the problem size by reducing the complexity of the model. Overall, the study of bone from organs down to molecules implies the analysis of a multicellular system that is suitable for multiscale modelling due to the presence of spatiotemporal scales.

Multiscale modelling of bone

Broadly speaking, multiscale approaches can be classified based on the methods they use to analyse the length scales of interest, which are mainly concurrent or hierarchical. In concurrent methods, the scales of the problems are solved with compatibility conditions such as displacement compatibility and momentum balance in solid mechanics (Ghosh *et al.*, 2001; Silani *et al.*, 2014; Talebi *et al.*, 2014). This technique can be applied to study material failure, for instance. The hierarchical method is instead a technique that links one or more scales by passing information from the fine-scale to the coarse-scale and solving the coarse-scale together with the fine-scale. An example would be receptor-ligand kinetics where the cell state differentiation is regulated by the concentrations of ligands and receptors. Receptor-ligand kinetics occur at the nanoscale and an *in silico* model could use molecular concentrations to model these kinetics that regulate the cell state, which is microscale information. The concept of the RVE is often used to statistically represent a small volume (Ghosh *et al.*, 2001; Ilic *et al.*, 2010; Morin and Hellmich, 2014; Pivonka *et al.*, 2013; Scheiner *et al.*, 2013), including all its microscopical heterogeneities, within the context of a greater scale (Fig. 1**f**). For instance, it is possible to create a chain of RVE connecting systems residing in multiple length scales, as Estermann and Scheiner (2018) did by linking the cell scale to the tissue scale in their multiscale model of bone tissue. However, the RVE technique is most suitable for a model that does not involve motion of the living units. Averaging over a small volume of bone tissue may lead to loss of information about the localised properties of cells that are motile, such as osteoblasts and osteoclasts, or responsive to chemotaxis or other extracellular stimuli. This approach was used in the model developed by Pivonka *et al.* (2013). In this approach, a population model was used to study the effect of the geometrical properties of the regulation of new remodelling events on bone porosity and stiffness. This model was composed of equations defining the variation of cell concentrations that are dependent on cytokine concentrations, vascular porosity, bone surface and the mechanical signal. All these quantities were analysed over time without any local spatial characterisation, *e.g.* the cells were not described in terms of the local properties where they reside. Therefore, this model might be useful if spatial discretisation is not needed in the analysis. Furthermore, the cells themselves are complex systems and some multiscale models either do not include them (Estermann and Scheiner, 2018; Perrin *et al.*, 2019) or study only single cell characteristics across finer levels, such as cell shape or motility with a resolution of 50-100 nm (Borau *et al.*, 2014; McGarry and Prendergast, 2004). Finally, some models use a semi-concurrent method that is a combination of both concurrent and hierarchical methods (Andrade and Tu, 2009; Kouznetsova *et al.*, 2002; Marques *et al.*, 2020; Silani *et al.*, 2014; Talebi *et al.*, 2014). In this method, changes from lower scales are transferred to higher scales and *vice versa*. In addition, temporal scales can be modelled in many ways. For more details on temporal multiscale approaches please refer to the review by Chopard *et al.* (2014). The present review briefly mentioned that time step is the most commonly used time discretisation technique. More time steps can be used to model phenomena occurring at different scales. It is possible to use, for example, one time step of seconds to minutes for modelling protein and receptor-ligand kinetics, next time step of minutes to hours for modelling cell activity and finally a time step of hours to days for computing the distribution of bone mechanics. Hence, a multiscale model of bone remodelling, including the tissue and cell scales, should use a technique that preserves at least the multicellular description of the cells. If this model can also include activity of each cell, it would be a step towards single-cell analysis and tracking. In this way, the changes from the finest scale to the coarsest scale can be tracked with sufficient resolution and reasonable performance.

Simulations of bone across spatial and temporal scales are massive because they require solving equations with many degrees of freedom depending on scale and methodology. Hierarchical methods are more commonly used for multiscale modelling of bone than concurrent and semi-concurrent methods because they can be solved with less computational effort. Nonetheless, semi-concurrent methods might

be employed in the context of bone remodelling and adaptation because they are based on the same type of bottom-up and top-down connections (Badilatti *et al.*, 2016; Christen *et al.*, 2012; Holguin *et al.*, 2016; Li *et al.*, 2014; Schulte *et al.*, 2013): a mechanical signal, such as SED, is transferred to finer scales and algorithms prescribe whether the bone microarchitecture is resorbed or formed, leading to organ-scale adaptation to the load. Recently, *in silico* models have become more complex and comprehensive; therefore, comparatively more resources and improved methods are required to solve them. Furthermore, increased computational power and more efficient algorithms allow these models to efficiently solve larger problems in terms of resolution and complexity. Multiscale *in silico* models can study different (patho)physiological conditions in different contexts, focusing on characteristic mechanical properties (Budyn and Hoc, 2006; Colloca *et al.*, 2014a; Estermann and Scheiner, 2018) as well as the cause of pathologies such as osteoporosis and osteopetrosis (Lerebours *et al.*, 2016), the behaviour of cells (Vaughan *et al.*, 2015) and potential drugs for molecular targets such as RANKL and sclerostin. For example, denosumab is a drug that binds to RANKL and thereby inhibits its anabolic action. This treatment was simulated using a multiscale hybrid model proposed by Tourolle *et al.* (2021). In this model, bone mechanobiology and the signalling pathways involved in the treatment were simulated before and after the treatment over a period of 10 years in a representative region of human biopsies. The denosumab concentrations patterns and changes in bone mineral density predicted by the *in silico* model were in line with the clinical observations. Furthermore, Martínez-Reina and Pivonka (2019) investigated the action of denosumab in an extended version of the original model by Pivonka. Here, a one-compartment model of the absorption and elimination of denosumab was added to the previous version of the model and denosumab was added as a third competitive ligand to RANKL after OPG and RANK. This study highlighted the different outcomes arising from the region of application of the drug (lumbar spine *vs.* hip). The proposed model was further employed to study the effects of the dosage and the frequency of administration, *e.g.* prescribed drug holidays against uninterrupted treatment (Martínez-Reina *et al.*, 2021). Another drug is romozosumab, which binds to sclerostin, inhibiting its anti-catabolic effect. It was modelled similarly in another extended version of Pivonka's model by adding one-compartment model of absorption and elimination of romozosumab by Martin *et al.* (2020). In addition, the reaction-ligand kinetics of LRP5/6 and sclerostin were redefined to be competitive along with the action of the drug. These models have established foundations for the description of bone mechanics and remodelling across scales with continuum models. They emphasised the importance of including bone stimulus (modelled as a strain energy density or either strain or fluid flow in vascular pores) as an essential component to regulate cell activity in multiscale bone remodelling models. Borgiani *et al.* (2017) provided further insights on the biological aspects investigated in *in silico* models of fracture healing. Additionally, in the context of bone fracture, Sabet *et al.* (2016) shed light on modelling the tissue properties of bone, microcrack and crack propagations.

Multiphysics modelling

Multiphysics properties of bone

In multiphysics modelling, the focus is on a system where more than one process concurrently develop and involve different physical quantities that simultaneously obey different constitutive laws. The production of proteins by cells (Fig. 1**h**) in the same system is an example of a possible topic for multiphysics modelling (Fig. 1**j**) and experimental observations might suggest the possible relationships (Hu *et al.*, 2011; Kikuta *et al.*, 2013; Rumpler *et al.*, 2013). The production of proteins by cells can be modelled as a limited-volume source diffusing through space in 3D and decay over time. As another example, one might model tissues and cells together (Fig. 1**d**) using their independent constitutive laws or assumptions based on experimental findings (Dallas *et al.*, 2009; Tang *et al.*, 2006; Xiong *et al.*, 2011). The mechanical strain at the tissue scale (Fig. 1**e**) can be used as the mechanical signal locally perceived by the cells and it can be computed by taking into account the mechanical properties and the boundary conditions prescribed at the organ scale (Lambers *et al.*, 2013; Torcasio *et al.*, 2012). The mechanical strain locally sensed by the cells can be modelled to affect protein production by individual cells. In regions of high strain, cells tend to upregulate bone formation by releasing more proteins, such as OPG; while, in regions of low strain, bone resorption is increased through the release of RANKL. As a result, the coupling of mechanical properties and chemical reactions can be modelled following constitutive laws in the context of multiphysics modelling. In addition, reaction-ligand kinetics are essential in regulating the differentiation of osteoblastic and osteoclastic cells. This is another multiphysics aspect of the bone, as it couples chemical reactions and the cell genotype. Therefore, multiphysics modelling can be applied to study the biological and physical phenomena in bone, from strain distribution to signalling pathways and reaction-ligand kinetics.

Multiphysics modelling of bone

A multiphysics model is defined to include different phenomena in its modelling; therefore, if bone biology is modelled, even with simplified rules, it is considered to be a multiphysics model. In such case, even though the fidelity is not very high, the classification of the model is satisfied. However, in the present review, it is encouraged to enhance the biological fidelity of *in silico* models. One of the most

common formulations of multiphysics problems is PDEs. A mathematical term can be added to an equation to represent a distinct phenomenon such as advection, diffusion, decay or chemotaxis. These equations define the spatiotemporal evolution of the components through the representative variables and their derivatives. The terms that constitute the equations define different types of equations and their presence changes their solvability. The complexity of these equations increases as the equations become coupled or non-linear. In ODEs, derivatives with respect to a single independent variable are used in the formulation, whereas derivatives with respect to more than one independent variable are used in PDEs. ODEs employ one independent variable, which may represent space in one dimension, time or a combination of both. Therefore, ODEs represent a simplified case of PDEs and they can be formulated directly based upon assumptions in the model. The advantage of using ODEs is the reduced complexity. However, the information in a single ODE is not as detailed as in a single PDE. The intrinsic complexity of ODEs can still be high depending on whether the terms in the constituting the equations are linear and whether the system of equations is coupled. Examples of ODEs with such complex terms can be found in studies of cellular populations (Lerebours *et al.*, 2016; Martin *et al.*, 2019; Pastrama *et al.*, 2018; Scheiner *et al.*, 2012) that include apoptosis, differentiation and proliferation. A reaction-based model was employed in a simulator of cellular processes based on mass-action kinetics through a system of ODEs (Tangherloni *et al.*, 2017). ODEs can also be used for modelling mRNA translation and competitive or non-competitive reactions between proteins (Dimelow and Wilkinson, 2009; Skjøndal-Bara and Morrisb, 2007; Zinovyev *et al.*, 2010). ODEs, with time as the independent variable, are suitable for these studies because they provide a convenient way to study large systems of proteins interacting with each other with reduced spatial dimensionality and parameters. Finally, FEM is a numerical technique that can be used to study the mechanical properties of a musculoskeletal system composed of multiple bones and muscles (Fig. 1**c**) as well as to compute the strain (Fig. 1**e**) perceived by the cells that reside at the μm scale, especially osteocytes, which are believed to perceive mechanical cues. In the latter particular case, the technique is called μFE since it analyses the mechanical properties at the tissue scale (Marangalou *et al.*, 2012; Pistoia *et al.*, 2002; Van Rietbergen *et al.*, 2002; Tsubota *et al.*, 2009) with a resolution in the range of μm (Fig. 1**g**). The numerical resolution of the FEM requires a discretisation of the domain of interest. Given that the experimental data are discrete, the numerical discrete resolution of the desired fields must be at least the same as the experimental resolution to ease the comparison between these data.

Bone mechanobiology is a particular field in which multiphysics can be applied. Here, the interplay between bone multicellular units, tissue and proteins is regulated through complex processes (Fig. 1**h**-**l**) that can be modelled using a multiphysics approach. The model proposed and subsequently improved by the research groups of Pivonka and Scheiner included populations of bone remodelling cells that were able to produce RANKL, OPG, TGF-β and PTH. These cells could differentiate and change the bone microarchitecture through the usage of PDEs (Lerebours *et al.*, 2016; Martin *et al.*, 2019; Pastrama *et al.*, 2018; Scheiner *et al.*, 2012). In one of these versions, Pastrama *et al.* (2018) proposed a model employing continuum equations and including a poromicromechanical technique that assessed the influence of the pore pressure in the lacunae on the bone remodelling process. These models were able to simulate bone remodelling using data from human and mice samples. However, they lacked the multidimensional characterisation of bone remodelling because of its temporal nature that did not consider the spatial variability of bone microarchitecture and cellular populations. Another multiphysics model of bone remodelling was introduced by Kameo *et al.* (2020), who analysed the regulation of bone formation and bone resorption through the expression of RANKL, OPG, sclerostin and Sema3A secreted by osteocytes in the bone microenvironment. The regulation of the cytokines was modelled through PDEs, which were based on diffusion, production and degradation, and the reaction of the cytokines. Moreover, the activity of the cells was modelled using equations that were based on the mechanical signal and cytokines. For example, the mechanical signal sensed by the osteocytes was assumed to be dependent on the local density of the osteocytes. Furthermore, the production of sclerostin by osteocytes was assumed to be inversely proportional to the mechanical signal using a Hill function. Finally, the mechanical signal was assumed to directly increase the apoptosis rate of osteoclasts and reduce the same for osteoblasts. They studied scenarios of osteoporosis, osteopetrosis and drug treatment for such diseases, highlighting the capability of analysing mechanobiological processes in real 3D bone structures *in silico*. The PDE-based *in silico* model of fracture healing proposed by Geris and colleagues (Geris *et al.*, 2008; Geris *et al.*, 2010a; Geris *et al.*, 2010b) was implemented on simplified 2D domains-obtained *in vivo* images. It included mainly the cell activity related to bone formation and the parameters used for differentiation and proliferation of cells were modelled as dependent on either fluid flow or hydrostatic pressure. The model was able to emulate the results of overload-induced non-union formation (Geris *et al.*, 2010b). The multiphysics model of bone remodelling proposed by George *et al.* (2018) employed continuum equations and included external loads, cellular migration and differentiation as well as nutriment supply. The mechanobiological stimulus was determined based on different factors, starting with the concentration of nutrients, mechanical energy derived from the

application of mechanical loads, cell differentiation and proliferation as well as the addition or removal of bone. It was tested by predicting the kinetics of bone reconstruction on a simple 2D domain and the results showed that bone reconstruction depends not only on the mechanics but also on the biological phenomena and the distribution of bone density. Mullender and Huiskes (1995) proposed an *in silico* model of bone remodelling in which the mechanical signal perceived by the osteocytes was obtained from stress and strain. It was used to indirectly model the bone remodelling action of osteoblasts and osteoclasts because bone density varied where the mechanical signal differed from a reference signal value. A relationship between strain and relative fluid/solid velocity over time in bone was suggested by Prendergast *et al.* (1997): fibrous connective tissue, cartilage and bone were suggested to form based on the evolution of strain over time. Conversely, Claes and Heigele (1999) proposed a strain-hydrostatic pressure scheme for the outcome of fracture healing among endochondral ossification, connective tissue and intramembranous ossification. Another model of fracture healing was proposed by Carter *et al.* (1998). They aimed to demonstrate how mechanical forces can influence the basic induction process. They calculated stress and strain distribution in the callus and diaphyseal bone under compression and an initial period of distraction osteogenesis. Furthermore, the FEM was used in the version of μFE to compute the SED on bone samples (Cox *et al.*, 2011; Huiskes, 2000; Kameo and Adachi, 2014a; Ruimerman *et al.*, 2001) or on simplified trabecular structures (Adachi *et al.*, 2010; Kameo and Adachi, 2014b). The frequency distribution of SED was used, for instance, as data for the probabilities of formation, quiescence and resorption (Badilatti *et al.*, 2016; Schulte *et al.*, 2013; Webster *et al.*, 2008). These studies aimed to assess how bone remodelling is mechanically driven at the tissue scale. Advancing to the current state of the art, the focus should be on the mechanobiological properties starting from the mechanical regulation of cells to the protein expression of cells, with more quantitative and complete information in a 3D space, which should be as close as possible to real structures. Multiphysics modelling has the potential to analyse these properties on real bone samples using μCT data from animal as well as human experiments (Badilatti *et al.*, 2016; Christen *et al.*, 2012; Lafage-Proust *et al.*, 2015; Lambers *et al.*, 2011; Schulte *et al.*, 2011; Schulte *et al.*, 2013; Torcasio *et al.*, 2012). Overall, multiphysics models of bone mechanobiology have been used to study specific features of bone; however, comprehensive *in silico* models are required to include relationships between biological features and mechanical features, such as cell production and mechanical strain, respectively.

Agent-based modelling

Features of bone suitable for agent-based modelling

In agent-based modelling, individual entities, called agents, can represent single cells, agglomerations of cells or subcellular components (Borgiani *et al.*, 2015; Buenzli *et al.*, 2012b; Paoletti *et al.*, 2012; Seekhao *et al.*, 2016; Sun *et al.*, 2007). These models may have different genotypes where genes could be represented as internal parameters with specific properties and behaviour. As a result, agent-based modelling is a highly flexible technique and can be tuned depending on the application. The definition of the properties of agents and the laws to describe the behaviour of the cells might be based on experimental findings, limiting the number of hypotheses to introduce in the model. In this way, using simple rules, it is possible to model the behaviour of every cell with one-to-one mapping to the real cell (Sun *et al.*, 2007). This reasoning also applies perfectly to the presented model system (Fig. 1**d**), where different cell types coexist, evolve and interact with each other in the same microenvironment. Hence, agent-based modelling can numerically validate and analyse properties such as cell movements, cluster size and chemotaxis in a multicellular system.

Agent-based modelling of bone

Agent-based modelling can be seen as a technique because it studies individual agents of a population in a discrete way. ABMs use this technique to examine specific features of one or more cell types, *e.g.* an ABM to study the behaviour of osteoblasts or osteoclasts in a particular domain, without any other information from other scales or fields. It could be used to study the *ad hoc* properties of cells by reducing the additional information included in the model. These models can be used in combination with other techniques to create more complex models, such as hybrid models. ABMs can also analyse multicellular systems with different cell types focusing on the interactions and movements of the modelled agents (Borgiani *et al.*, 2015; Borgiani *et al.*, 2019; Checa *et al.*, 2011; Khayyeri *et al.*, 2009). An ABM may model several entities with little effort once the set of rules they follow are defined. CA is a special case of an ABM where the cells do not move but the modelled properties or fields can change spatially. A CA model analyses several static cells in the same domain, focusing on possible emerging patterns of clusters of cells (Van Scoy *et al.*, 2017). These cells may change their state among a limited set of possible states. Moreover, CA models are usually defined on a uniform grid, prescribing *a priori* the position of the cells. ABMs can also be defined over a lattice domain (Fig. 2**a**), in which case they are called lattice-based models (Callaghan *et al.*, 2006; Jasti and Higgs, 2006; Plank and Simpson, 2012; Simpson *et al.*, 2010). On the other hand, ABMs that can manipulate their entities on a domain without prescribed positions (Fig. 2**b**) are called "off-lattice" or "lattice-free" models (Drasdo *et al.*, 2007; Galle *et al.*, 2005). The definition of time in ABMs is not unique. If it is defined as a stochastic process, then a quantitative definition of the time step is required. For example, the time step might be associated with the cell cycle in the case of modelling biological cells (Fig. 1**h**-**l**). In the case

of an off-lattice ABM, the time step should be less than the cell growth time so that fine changes in cell deformation and growth are appropriately captured (Drasdo *et al.*, 2007; Galle *et al.*, 2005; Van Liedekerke *et al.*, 2015). This might be an important feature to model, as cells also show different morphologies in microenvironments with high or low strain. In addition, the algorithm should pay attention to the concurrency of events in neighbouring locations at the same time step. Moreover, the choice of the spatial domain in which these entities reside can differ depending on the application used. Off-lattice models can represent more complex deformations of cells and various cell sizes (Galle *et al.*, 2005; Van Liedekerke *et al.*, 2015; Van Liedekerke *et al.*, 2020), whereas lattice-based models are usually defined with a grid that constrains the position and size of the cells. An ABM might use a domain obtained from other experiments to insert and model cells in such an environment. For example, μCT scans might provide a 3D domain for a lattice-based ABM because those images have voxels that could represent cell positions. If the resolution is in the range of tens of μm, such a model may be directly used for modelling cells because this voxel size is close to a typical cell size. However, it is possible to increase the resolution of such images using a more refined lattice grid (Block *et al.*, 2007). Then, this ABM could study more refined cellular properties or behaviour. However, the position of the cell is less straightforward when the voxel size is less than the cell size.

Recently, *in silico* ABMs have started analysing bone remodelling (Buenzli *et al.*, 2012a; Paoletti *et al.*, 2012). Nonetheless, these models can still be improved in terms of accuracy and fidelity. They usually employ simplified domains in terms of dimensionality or representation of real bone structures. For example, some ABMs have focused on the mechanoregulation of fracture healing (Borgiani *et al.*, 2015; Borgiani *et al.*, 2019; Checa *et al.*, 2011) assuming that bone is a 3D cylinder. The model developed by Checa and Borgiani simulated the differentiation, proliferation, apoptosis, migration, matrix synthesis and degradation of osteoblasts, fibroblasts, chondrocytes and MSCs. A Taguchi design of the experiments was carried out to investigate the contribution of each cell-related parameter. Two possible set of values were measured, one for elderly and another for adult mice, leading to 16 experiments. This shows a possible way to explore how the parameters can be calibrated for an agent-based model. This model was able to predict the tissue patterning in the presence of rigid and semirigid fixation. Nonetheless, the later stage of bone remodelling was not captured despite the model being designed to capture that stage as well. Consequently, even with a simplified domain, it is difficult to capture bone mechanoregulation of the cells using ABMs. However, it is also possible to use *in vitro* data to analyse more specifically cell clusters of reduced size. For example, Van Scoy *et al.* (2017) validated a CA model of bone formation against *in vitro* data on osteoblastic cells, with a special focus on bone mineralisation. Another 3D ABM of osteoblastic behaviour was validated against *in vitro* data (Kaul *et al.*, 2015), with a particular focus on osteoblast polarity. The cell types included in the model were mesenchymal cells, preosteoblasts, osteoblasts and osteocytes. Matrix deposition and osteocyte embedding were analysed by changing the related parameters in the model, such as preosteoblast proliferation and matrix deposition rate. ABMs are a powerful tool with growing usage in bone mechanobiology, but a multicellular description, including several cell types and all cellular events from recruitment to differentiation, movement, production and regulation still needs to be developed to enhance the understanding of bone remodelling through ABMs.

Hybrid modelling

Hybrid properties of bone

A hybrid ABM is defined as a model that combines aspects of continuous and discrete model units (Cilfone *et al.*, 2015). Previous sections highlighted how multiscale, multiphysics and agent-based modelling reflect the properties of bone. Bone can be identified as a hybrid system (Frenk and Houseley, 2018; Häusler *et al.*, 2004; Pinzone *et al.*, 2009) of

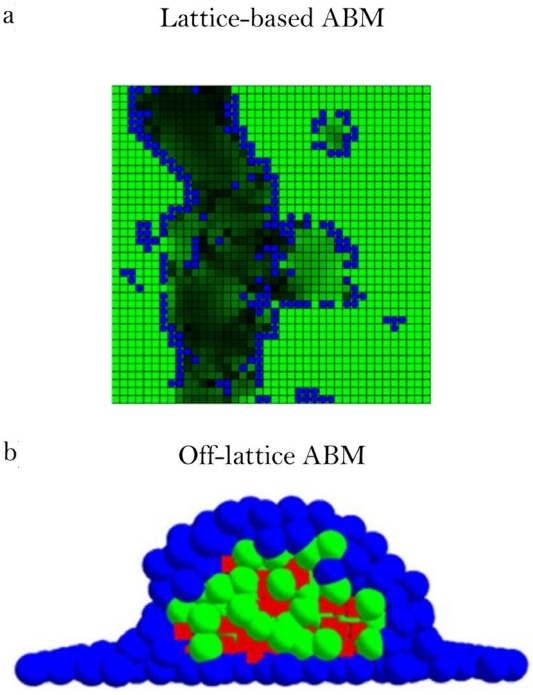

Fig. 2. A lattice-based ABM and an off-lattice ABM. The main difference is related to the positions of the agents. (**a**) The positions of the agents are constrained to the lattice (Stiegelmeyer and Giddings, 2013) (**b**) The agents do not occupy a predefined position in the space (Kaul *et al.*, 2015). (**a**,**b**) Reproduced in adherence with the CC BY licence applied by Springer Nature.

discrete and continuous components: cells are discrete (Sun *et al.*, 2007), while chemical concentrations and bone density are continuous (Bouxsein *et al.*, 2010; Dallas *et al.*, 2009; Tang *et al.*, 2006; Xiong *et al.*, 2011). Therefore, hybrid ABM is suitable for analysing such components concurrently in a multiscale manner to improve the understanding of bone mechanobiology.

Hybrid modelling of bone

In this review, the potential combination of multiscale, multiphysics and ABM modelling techniques into a hybrid ABM is highlighted due to a growing interest in such an approach (Chang *et al.*, 2015; Cilfone *et al.*, 2015; Kaul *et al.*, 2013; Wells *et al.*, 2015). Continuum models are employed to describe mechanobiological properties for each variable of interest, usually by employing differential equations. ABMs describe the behaviour and properties of discrete entities such as cells. A hybrid ABM might be called multiscale when the spatiotemporal scales between the continuum models and the ABM are different (Fig. 1**j**). The multiphysics component of a hybrid ABM model employs computational spatial and temporal discretisation, which might be greater than or equal to the corresponding discretisation used for analysing the agents.

There are very few examples of such techniques in the context of bone mechanobiology because of their innovative nature. Fracture healing was studied with a hybrid model that combined the paradigms of ABM and multiphysics simulations (Tourolle *et al.*, 2019). It included signalling pathways such as the RANKL-RANK-OPG axis and TGF-β signalling along with sclerostin to quantify their effect on osteoclastic and osteoblastic cell differentiation. The local mechanical signal was computed using μFE on a real bone microarchitecture obtained from μCT of murine femora and it was further mechanotransduced into the production of cytokines from cells modelled as individual agents. This model was developed for assessing pharmaceutical and tissue-engineered treatments. The mechanical stimulus sensed by the cell was defined as a linear combination of fluid flow and shear strain in a hybrid ABM of tissue differentiation and blood vessel growth (Checa and Prendergast, 2010). This model included stem cells, fibroblasts, chondrocytes and osteoblasts and it showed the influence of the initial distribution of the cells on angiogenesis. The initial distribution of the cells is likely to be important in other bone-related processes, such as fracture healing and bone remodelling. The mechanotransduction dynamics of osteoblasts and osteoclasts were analysed using a hybrid multiscale ABM, showing that osteoblast activity depends on the heterogeneity of mechanical stimulation of integrins (Shuaib *et al.*, 2019). This model included not only osteoblasts but also osteocytes through differentiation from osteoblasts to osteocytes. However, the intercellular activity between osteoblasts and osteocytes was not included because the scope of the work was particularly focused on the complex intracellular regulation of osteoblasts through multiple proteins. This highlights the implementations of a hybrid multiscale ABM for a multiscale cellular system including the information at the protein and gene scales.

Reproducibility

The reproducibility of models is a key feature to ensure that the model is appropriately validated. At the same time, such models can be further improved by duplicating the results, making it more accurate and acknowledged by the scientific community. Shared platforms that track the parameters chosen for simulations might be the first step for extending the reproducible models. These platforms should enable easier and better version control and cross-checking of the model, from the initial to the final implementation (Bradley *et al.*, 2011; Passini *et al.*, 2016). An open-source platform would be the best choice for this idea because it is accessible to all users and developers (Van Leeuwen *et al.*, 2009; Mirams *et al.*, 2011; Osborne *et al.*, 2017; Pitt-Francis *et al.*, 2009).

The (pseudo) code is more relevant than the software because it is often possible to adapt the code to the software. Most of the code used in these models is not publicly available because it is written using in-house technology such as C++, Python, MATLAB or another type of programming environment. With a flexible shared platform, it is possible to build *in silico* models that may progress towards a comprehensive multiscale approach for bone mechanobiology. Fracture healing and bone remodelling are very diverse processes that are based on different cell and biochemical mechanisms and they might include specific subprocesses, *e.g.* angiogenesis is present only in fracture healing. Nonetheless, the modelling of fracture healing and bone remodelling can benefit from code sharing and shared platforms. These aspects can ensure the reproducibility of simulations and can help in modelling subprocesses present in both fracture healing and bone remodelling. For example, the presence of osteoblasts and osteoclasts in both the remodelling phase of fracture healing and bone remodelling and their mathematical description can be encoded in a common platform. Moreover, the idea could even be incorporated into the larger context of biology or other mechanical, physical and biological studies.

Examples of reproducibility

Some examples of multipurpose platforms exist and have been used for projects in different fields that share the common modelling and implementation background (Hunter and Borg, 2003; Pitt-Francis *et al.*, 2009; Tomita *et al.*, 1999). Integrative models among several length scales have been developed and inserted into a web-based common platform, the IUPS Physiome Project (Hunter and Borg, 2003), where researchers can share and merge their code. This approach has emphasised its ability to integrate the benefits of each model and can also

be applied to existing models of bone remodelling. Chaste is an open-source software library aimed at multiscale, computationally demanding problems arising in the domain of biology (Pitt-Francis *et al.*, 2009). Its most relevant applications are in the fields of cancer, cardiac physiology and soft-tissue mechanics. For example, Osborne *et al.* (2017) showed an improved open-source C++ library for cell-based and multiscale modelling of multicellular systems based on Chaste. In this work, five classes of cell-based models were applied in four 2D case studies to analyse the influence of each method on the modelled cellular phenomenon. The authors illustrated appropriate mapping between models and related applications such as adhesion, proliferation, short-range and long-range signalling. These models were also implemented for 3D simulations but the results were not reported. CellML is a markup language for modelling equations of biological systems (Cuellar *et al.*, 2003) that are easily readable by humans and machines. It can also model the relationship and encapsulation of components along with the biochemical reactions. The general-purpose framework introduced by Zwart *et al.* (2009; 2013) is an example of a multiphysics object-oriented data model where the function calls are combined with physically based interfaces. Here, the authors illustrated the flexibility of their framework by applying it in the astrophysics domain. With this platform, simulations can be performed using different solvers and exchanged without completely refactoring the underlying codes. The differences in length scales and time steps required to simulate astrophysics problems lend to similar issues in simulating biology; protein interactions take place at the nanoscale in nanoseconds, while overall structural changes take place in weeks. Another example of software developed for reproducibility across several models is E-CELL (Takahashi *et al.*, 2003; Tomita *et al.*, 1999). It focuses on the implementation and simulations of biochemical and genetic processes, with the possibility of defining complex specific properties of cells such as protein-protein interactions and protein-DNA interactions. As an example, they presented a model of a cell with 127 genes for transcription, translation and other metabolic activities. E-CELL also has an interactive graphical interface. Overall, these platforms can provide a foundation for an improved understanding of bone mechanobiology.

Conclusion and future directions

Bone mechanobiology is a field that studies the interlinking of biological, physical and mechanical processes occurring in a complex hierarchical system, namely, the bone. While experimental tools can provide some insights into the (patho)physiology, the use of simulation models is vital in addressing existing quantitative gaps. The existing computational methods can be employed to obtain comprehensive data with better quantitative validation.

Hybrid models can be used as tools to study the different yet related biological responses to mechanical loading. Such models will be able to investigate mechanobiological properties, such as cell movement, apposition rate and bone growth. The modularity of the agent-based technique inside a hybrid model is ideal since it considers the natural heterogeneity among the cells. Moreover, the ease of the potential comparison with *in vivo* data was highlighted. The hybrid model by Tourolle *et al.* (2019) was used to perform bone remodelling simulations on a murine caudal vertebra (Boaretti *et al.* (2018) Studying how the link between mechanical stimulation and cellular activation effects bone microarchitecture; 25th Congress of the European Society of Biomechanics, Vienna, conference abstract; Boaretti *et al.* (2020) Improved initialisation of a multiscale in silico model of trabecular bone remodelling using *in vivo* murine data; American Society for Bone and Mineral Research's Annual Meeting, conference abstract). This is the first step towards the full integration of *in vivo* data into an *in silico* model of bone mechanobiology.

Experimental data should reflect the modelled properties and *vice versa*. This goal can be achieved if the experimental data is expressed quantitatively, *i.e.* in terms of numerical values, along with qualitative observations. In addition, *in silico* models need to use the biological knowledge available to run simulations and eventually validate their numerical data against the experimental data. Such synergy is fundamental to building upon the state of the art. Accordingly, a continuous collaboration between modellers and biologists is vital.

The techniques of agent-based, multiscale and multiphysics modelling each provide a framework in which biological phenomena can be directly translated into simulations. Then, knowledge can be expanded by testing the development of the systems they model against the sparse data available from experiments. Shared modelling platforms provide a basis for developing an *in silico* model from existing work, with the possibility of improvements and merging different models. A hybrid model that combines multiscale, multiphysics and agent-based techniques can describe bone mechanobiology across all length scales, *i.e.* from the organ to gene and protein scale. Such a model can validate the certainty of recent biological observations and potentially be used to discover new molecular targets for treatments.

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Editor's note: There were no questions from reviewers for this paper, therefore there is no Discussion with Reviewers section. The Scientific Editor responsible for this paper was Stephen Ferguson.