

Effects of mechanical stimuli on bone cells for regenerative medicine: A review of recent experimental and computational methods

Emilie Wiedemann-Fodé^{a,b,c,*} , Jessica Schiavi-Tritz^b , Halima Kerdjoudj^c,
Cédric Laurent^{a,*}

^a LEM3, CNRS URM 7239, University of Lorraine, F-54000 Nancy, France

^b LRGP, CNRS UMR 7274, University of Lorraine, F-54000 Nancy, France

^c BIOS, UR 4691, University of Reims Champagne Ardennes, Reims, France

ARTICLE INFO

Keywords:

Mechanobiology
Bone regeneration
Stem cells
In silico modelling
Ultrasounds

ABSTRACT

Mechanobiology integrates biological and mechanical cues to provide a comprehensive understanding of how physical forces influence tissue regeneration, specifically focusing on bone growth and repair. This review presents a comprehensive overview of the latest developments in bone mechanobiology and highlights the remaining scientific and methodological challenges. Following a brief presentation of the challenges facing cell therapy, and in particular the application of different types of mechanical stimuli, the article examines these various processes on cells and tissues such as compressors and bioreactors operating at low frequencies (in the Hz range). Special attention is given to cutting-edge ultrasound techniques, such as the LIPUS method with MHz frequencies and low intensities ranging from 0.5 to 100 mW/cm², and with an emphasis on the emerging application of acoustic levitation with frequencies ranging from 340 kHz to 2.12 MHz permitting non-invasive manipulation of cells and tissues in biological research, with initial beneficial results in cell therapy. Lastly, the review offers a detailed analysis of multi-scale and multi-physics *in silico* approaches that may contribute to interpret the obtained experimental results. Agent-based models capture the discrete behaviour of individual cells, while continuum models describe tissue mechanics through averaged properties, offering complementary approaches to study complex mechanobiological phenomena. Such approaches hold the potential to drive transformative advances in bone regeneration medicine, providing a roadmap for future research.

Introduction

Tissue engineering is a pivotal field within regenerative medicine [1], aiming to develop innovative solution to repair and regenerate damaged tissue. A key strategy involves the use of Mesenchymal Stromal Cells (MSCs), along with the application of specific mechanical stimuli to induce their differentiation into targeted phenotype. MSCs and Hematopoietic Stem Cells (HSCs) are primarily found in bone marrow [2], where they play a crucial role in tissue regeneration.

Among the tissues influenced by mechanical stimuli, bone is particularly notable for its remarkable ability to respond and adapt to physical forces. Beyond its mechanical role in enabling body motion and protecting vital organs, the skeleton also plays a key role in the metabolic regulation of skeletal cells [3]. While these various functions are essential, the present contribution primarily focuses on the physiological fate of bone, particularly regarding bone remodelling and stem cell

activity.

Indeed bone, a dynamic and composite material, is constantly being remodelled to repair micro-damage, reshape during growth, and maintain calcium and phosphate homeostasis. This balanced process maintains bone's strength and integrity throughout life and is driven by osteoblasts (formation), osteoclasts (resorption) and osteocytes (Fig. 1). In particular osteocytes serve as the primary mechanosensors and play a central role in sensing mechanical changes within the bone matrix. Such mechanical forces are detected through specialized structures like dendrites or cilia, which act as mechanoreceptors. In the field of mechanobiology [4], a mechanosensor refers to a cell capable of detecting mechanical forces such as pressure, compressive or shear stresses. These forces are converted into biochemical signals, regulating cellular functions such as differentiation and bone homeostasis. Given the complexity of mechanoregulation processes, dozens of comprehensive reviews have been published on the topic, and readers are encouraged to

* Corresponding authors.

E-mail addresses: emilie.wiedemann-fode@univ-lorraine.fr (E. Wiedemann-Fodé), cedric.laurent@univ-lorraine.fr (C. Laurent).

<https://doi.org/10.1016/j.medengphy.2025.104369>

Received 20 December 2024; Received in revised form 29 April 2025; Accepted 19 May 2025

Available online 22 May 2025

1350-4533/© 2025 The Authors. Published by Elsevier Ltd on behalf of IPPEM. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

consult these for further details [5–7].

The lacuno-canalicular system allows the dendrites of osteocytes to be connected within the bone structure, which plays a central role in sensing mechanical stimuli and coordinating bone remodelling through their secretion. The figure below (Fig. 2) provides a detailed view of the main structural components of bone, including osteons, lamellae and the lacuno-canalicular system.

However, in the presence of diseases and pathologies, the homeostasis becomes dysregulated, leading to increased skeletal fragility, as observed in disorders such as osteoporosis and osteoarthritis [9,10]. Regenerative medicine plays a crucial role in addressing these challenges, enabling the regeneration of healthy cells and the reconstruction of functional tissues. Various methods in bone engineering – such as scaffold design, stem cell therapy, growth factors, mechanical stimulation, gene therapy, vascularization techniques, and hybrid approaches – each offer unique strategies for promoting bone regeneration [11–14]. Among these, the key role of biomaterials has become increasingly important for bone repair, providing structural support while influencing cellular behaviour. However, fine-tuning its architecture and physico-chemical properties to mimic bone's complexity and support vascularization remains a challenge [15–18]. Combinations of these techniques are constantly tested to enhance our understanding of the impact of the mechanical environment [19–24]. The focus of the subsequent sections lies in understanding how stem cells respond to mechanical stimulation when applied to a given biomaterial: the reader interest by recent advances on biomaterials is referred to [25–27].

To better anticipate cellular behaviour, especially when the biomaterial is not designed and needs to be created, computational models are emerging to simulate the environmental conditions and apply specific mechanical conditions on the virtual scaffold. Such models aim at predicting cell behaviours via the data collected, opening a wide research area known as *in silico* modelling [28,29]. Some of the main strategies reported to integrate recent knowledge in mechanobiology within computational models to improve the understanding of biological response will be described subsequently. Moreover, while substantial data have been gathered concerning the link between a given cyclical load amplitude and the corresponding cell response, little is known about the effect of loading frequency and the techniques available to apply those stimuli.

The present contribution therefore aims to provide a recent and comprehensive review of:

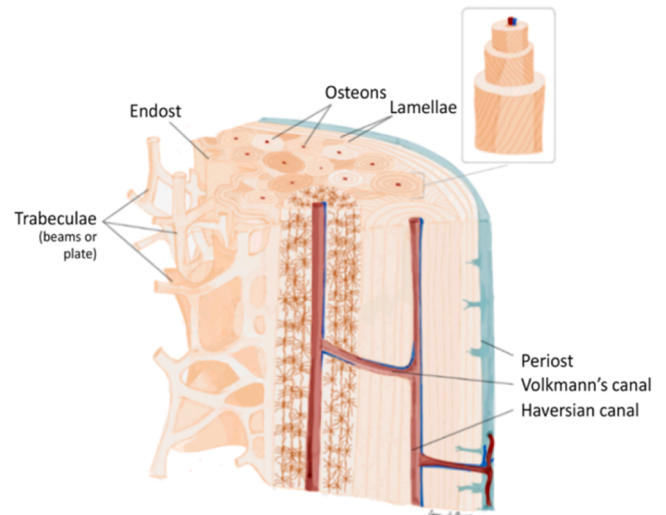


Fig. 2. Cortical bone and cancellous bone. Osteons are cylindrical structures with concentric lamellae in the cortical bone, while cancellous bone has trabeculae with less organized lamellae. Figure reproduced with permission of the author [8].

- The different techniques used to apply a dynamic cyclical loading to cells and tissues with a particular attention on bone tissue, with an extra focus on bioreactors and ultrasound techniques such as acoustic levitation to assess different frequencies, and the associated reported results concerning cellular responses;
- The use of *in silico* approaches and the associated modelling methods to understand and interpret the collected data, based on the mechanical loadings that are simulated.

This contribution aims to broaden the scope of existing literature reviews on the subject, which are typically limited to low-frequency stimuli and focus exclusively either on experimental or computational approaches, but rarely both.

Part I: experimental protocols and cellular responses

Previous studies highlight that bone cells, especially osteocytes, play a crucial role in the constant remodelling of bone tissue. Among the

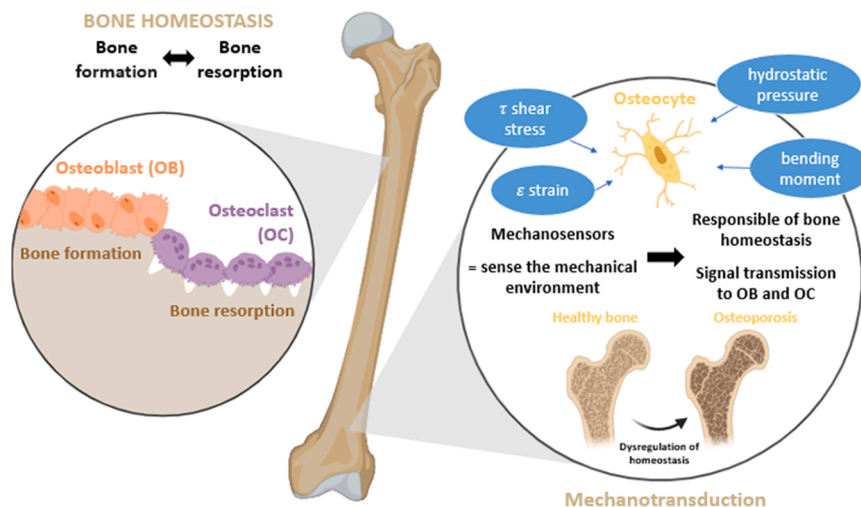


Fig. 1. Mechanotransduction in bone. Bone homeostasis is balanced between bone formation by osteoblasts and bone resorption by osteoclasts, is orchestrated by osteocytes that sense various mechanical stimuli, such as shear force (τ), strain (ϵ), hydrostatic pressure, and bending moment, and send biochemical signals in response to these mechanical changes.

different quoted methods such as stem cell therapy, this contribution focuses on the use of MSCs from the bone marrow to emphasize how mimicking their usual mechanical environment enhances proliferation, viability and differentiation. Such stem cell therapy could avoid allogeneic rejection from patients, by directly using autologous MSCs from their own bone marrow [30–32].

The importance of the cell environment

Reported studies in bone mechanobiology aimed to recreate in vitro biomimetic conditions and the mechanical environment of bone cells, to investigate the relations between cellular behaviour at the microscopic scale and its environment [33]. Tissue growth is dynamic since biological, physical and mechanical parameters constantly evolve over time. Therefore, it is important to understand which mechanical stimuli can be applied to mimic in vivo conditions to obtain the desired environment get positive results, while out-of-range stresses may lead to opposite cell behaviours. Mechanical stimuli can induce osteocyte apoptosis, which increases skeletal fragility [34] however this process can be reversed under specific conditions. Conversely, apoptosis also plays a beneficial role by supporting damage repair and skeletal replacement, although inhibition during necessary repairs may have adverse effects [35].

The in vitro culture of cells requires to reproduce their physiological conditions outside of their natural environment. Recent observations have shown that the microenvironment, or niche, is critical for MSCs well-being regulation, specifically from the bone marrow. In laboratory settings, several parameters are monitored and controlled such as cell proliferation and viability, and the nutrient supply, temperature, and environmental conditions (pH level, oxygen and carbon dioxide levels). Alteration of the culture microenvironment can rapidly degrade their properties and culture productivity, which refers to the effectiveness of cell growth. Different conditions must be respected to mimic niches, which can be classified into two main categories: (i) essential biochemical signals for survival and regeneration and (ii) the physical and mechanical properties of the niche.

The intensification of cell proliferation through external mechanical stimuli may allow to obtain large batches of MSCs in regenerative medicine, mitigating the challenges of painful harvesting and limited availability of autologous cells. The section briefly reviews existing setups known to biologists as “bioreactors”, and introduces acoustic levitation as an alternative and promising method due to its non-contact nature that allows for easily determined boundary conditions. To compare different methods, particular attention will be put on the associated cellular environments, stress states, targeted loading frequencies for cyclical loading and the characterization methods that have been used to assess cellular responses together with a brief report of results.

2D. cultures

Bone cells are mostly cultured in vitro in a 2D static culture environment on surfaces like glass or plastic substrate [36–38]. Various 2D models exist, such as the sandwich model, which some consider 3D due to the cells being seeded between two ECM or collagen layers [39,40]. However, this model still represents a 2D culture, as cells remain confined to a single plane, unlike true 3D models like the cell sheet, where cells interact in multiple dimensions. ECM can improve cell growth, motility and repair [41], while type I collagen provides physical scaffolding for bone and connective tissues [42]. On the basis of these 2D flat surfaces, micro-patterning, uses lithography to create surface patterns that guide cell behaviour, such as migration and differentiation [43]. Combined with mechanical stimulation setups, 2D culture has improved our understanding of osteocytes and bone mechanobiology [44].

Among the reported 2D culture surfaces, the use of spherical microcarriers rather than flat flasks is gaining in popularity for producing human Mesenchymal Stromal Cells (hMSCs). Benefits include

reduced contamination due to automatic monitoring, with a higher surface-to-volume ratio than microcarrier-free methods [45]. These carriers can be tailored to meet patient needs, providing varying quantities of cells for different conditions, such as skeletal disorders and osteogenesis imperfecta [46]. With a diameter of 100 to 250 μm , microcarriers can be developed with different physiochemical properties, for the production of several cell phenotypes and adaptable to bioreactor cultures. However, challenges as the homogenization of nutrients and oxygen is key as it can alter the proliferation of cells or lead to necrosis of tissues which highlight the need for further research. Reproducibility issues also remain when using primary cells despite using similar protocols [47–49].

3D. cultures

By removing MSCs from their natural 3D niche, 2D culture can alter growth kinetics and differentiation potential [50] highlighting the need for 3D culture systems that better mimic bone’s architecture [51–54]. Different models, such as spheroids, cell sheets, scaffolds or hydrogels simulate the 3D structure [55] allowing forces to be transmitted in an environment that mimics the native bone, unlike 2D cultures. Spheroids, for example, are aggregates of cells that form spheres to expand cell populations for therapies or produce tissue. However they present challenges such as size control and nutrient access [56–59].

Hydrogels with their water absorption capacity and similarity to ECM are of particular interest. They are generally made from natural polymers, cross-linked in networks [60], such as collagen [61], gelatine, alginate [62–65] or chitosan [66], and offer various mechanical properties, biodegradability and low toxicity, making them ideal for many applications [67]. Despite their advantages, hydrogels do not fully recreate bone’s physical properties (such as structural, mechanical, and biological) leading to the development of scaffolds to better mimic bone tissue. These scaffolds need features like adjustable porosity [68], mechanical strength and stiffness [69], cell-matrix-interaction, and distribution of ECM factors to support stem cell characteristics. Common scaffold materials range from natural substances like alginate and chitosan to synthetic polymers, which allow for controlled physical and mechanical properties, increasingly driven by computational methods to predict their overall properties and meta-material research [70]. For further information, the interested reader may refer to recent dedicated reviews [71–74].

What kind of mechanical stimuli exists

For mechanobiological studies, the responses of cells to mechanical stimulation are assessed and have been shown to enhance bone formation [75]. Parameters such as amplitude, frequency and the loading sequence (or duty cycle) are critical, especially for high-frequency stimulations like ultrasounds, which can raise harmonic frequencies or may be associated with temperature elevation.

A representation on a frequency scale (Fig. 4) illustrates the various ranges of amplitude of frequencies employed in mechanical stimuli,

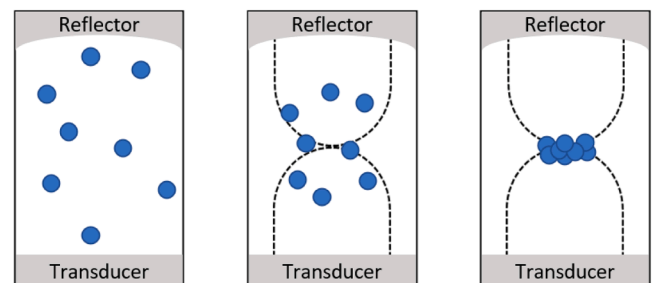


Fig. 3. Representation of the activation of the acoustic field leading to a displacement of the particles towards the pressure node.

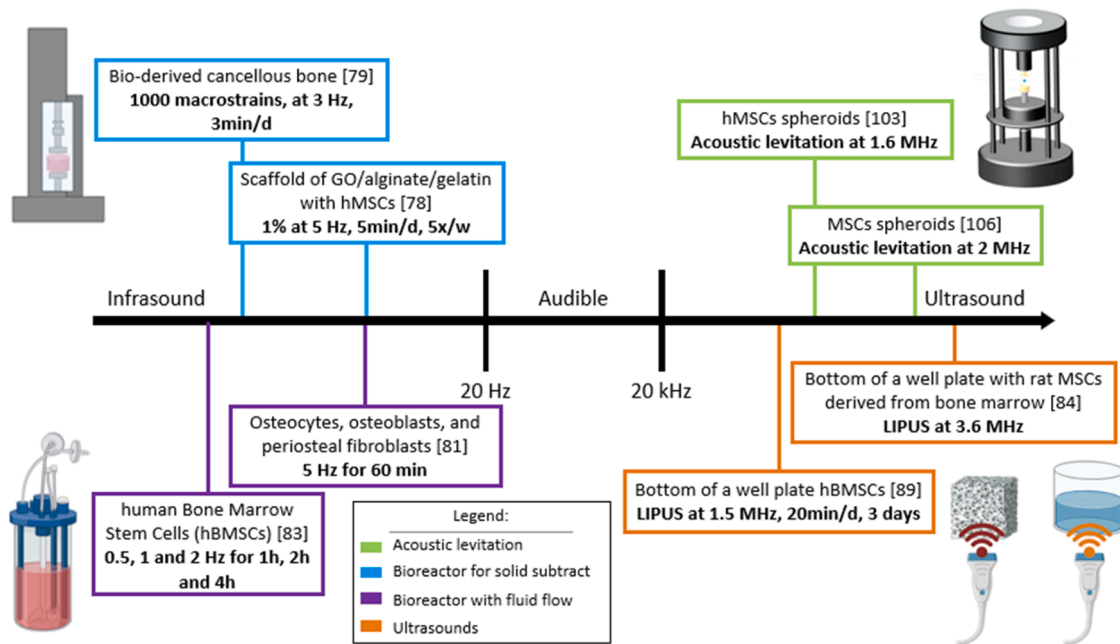


Fig. 4. Frequency range of mechanical stimuli at the macro/micro scale with four different groups: compressor (blue), bioreactor with pulsatile flow (purple), acoustic levitation (green), and ultrasounds (orange).

from low-frequency oscillations to high-frequency vibrations, highlighting the diverse mechanical environments to which cells and tissues are exposed.

Bioreactors are designed to control and monitor the physico-chemical environment of cells, and may also provide mechanical stimuli to homogenise the media and study cellular responses. Among these, “dynamic bioreactors” (or “cyclical bioreactors”) apply cyclical loads (compression, tension, flexion, etc....) to the substrate. Studies show that proper biochemical and physical signals in such bioreactors promote stem cell differentiation and ECM production [76,77]. Noteworthy, a recent automated system which applied mechanical compression to 3D cell culture at 5 Hz resulted in increased cell proliferation and ALP activity, indicating osteogenic differentiation [78]. Alternatively, another recent study highlighted that dynamic compression study at 3 Hz enhanced ECM production and improved cell distribution [79].

Alternatively, fluid-flow-based bioreactors aim to simulate the mechanical environment caused by fluid flow in the lacuno-canalicular system [80,81], which is known to constitute a key element in bone mechanobiology. Rotating walls or fluid perfusion systems have been proven to enhance cell culture efficiency by generating fluid-induced shear stress (FSS) [82]. Pulsatile fluid-flow bioreactors, replicating cyclic hydrostatic pressure (CHP), have demonstrated the ability to enhance osteogenic responses in human bone marrow stem cells (hBMSCs) by promoting Adenosine Triphosphate (ATP) release, collagen synthesis, and mineral deposition. Notably, even low pressure (10 kPa) at physiological frequencies (2 Hz) can induce osteogenic lineage commitment, highlighting the importance of pressure-driven fluid shear effects in bone mechanobiology [83].

Ultrasound-based mechanical stimulation uses wave propagation by placing a piezoelectric transducer in culture media, also generating FSS. This allows the study of high-frequency stimulation up to several MHz [84,85]. Ultrasound applications vary in intensity: low intensities (0.5 to 50 mW/cm²) are used for diagnostic, while high ones (0.2 to 100 W/cm²) are applied for therapeutic purposes, such as promoting bone healing or drug efficiency [86]. Among those techniques, Low-Intensity Pulsed Ultrasound (LIPUS) is a non-invasive, non-thermal method particularly effective for fracture repair, as it causes micromechanical stress at the fracture site and promotes bone formation, notably through

intramembranous and endochondral ossification [87]. Recent reviews highlight LIPUS applications for stem cells and bone, improving proliferation, migration, and osteogenesis [88–91]. Xie et al. studied LIPUS on hBMSCs using intensities at 30, 40, 50, 60 and 70 mW/cm² over 4 days, with parameters of 1.5 MHz frequency, 20 % duty cycle and 1 kHz pulse repetition frequency. Optimal intensities were at 50 or 60 mW/cm² for 5 or 10 min daily, resulting in increased ultimate load and stiffness compared to controls. Flow cytometry confirmed specific MSC markers, and cells cultured in osteogenic medium, a significant calcium production. The underlying causes of such positive effects require further investigation since the local mechanical environment related to such loading protocols is largely unknown.

Although studies show some effectiveness of ultrasound stimulation on cell culture, wound healing and anti-inflammatory effects, there are still limitations and controversies. Results remain inconsistent due to the diversity of parameters tested (intensity, frequency, exposure time). The cell type is also a parameter to be considered, as their reactions are particularly sensitive and can various to different results going from bone cells to neural cells [92]. While promising, the LIPUS method still lacks widespread clinical validation for many applications and therefore highlight the need for better standardization of protocols.

These common methods for mechanical stimulation applied via substrates often result in heterogeneous microscale stress fields that are difficult to control (highlighting the interest of associated *ad hoc* computational methods, mentioned in the next section). To address this, levitation is a non-contact technique and has gained popularity for controlled particle manipulation in a precise environment. Various levitation techniques exist including magnetic [93], optical [94], electric [95], aerodynamic [96] and acoustic levitation. Among these, acoustic levitation is increasingly being used in cell culture experiments due to its unique advantages including the ability to create 3D culture environments, reduced contamination risk and cost-effectiveness [97, 98]. It uses high-intensity sound waves to suspend objects by balancing gravity and acoustic radiation pressure. A piezoelectric transducer generates ultrasonic waves, which when reflected, create “standing waves”. Particles, including cells, are drawn to pressure nodes where movement ceases (Fig. 3), enabling controlled manipulation [99–102].

Acoustic levitation has numerous biological applications, including cell trapping [103], cell sorting [104], cell differentiation [105] and cell

proliferation [106]. In fact, it has been used to organize epithelial and fibroblast cells to recreate multi-layered tissues [104]. Initially, epithelial cells aggregated, but adjusting the conditions (1.95 to 2.12 MHz for 2 h) allowed them to form sheets on fibroblasts, promoting intercellular communication. Similarly, acoustic levitation has been shown to support the formation of 3D brain-like tissue by promoting the differentiation of human Embryonic Stem Cells (hESCs) at 380 kHz and 640 kHz into neurons [105], showing enhanced cell activity and stability after levitation. It also positively influences cell differentiation as demonstrated with the formation of spheroids at 2 MHz with adipose tissue-derived MSCs [106] which enhanced their differentiation into osteoblast and no adverse effects were reported on surface markers. In another study [103], similar to the previous study, hMSCs spheroids were rapidly formed at 1.6 MHz while maintaining the viability of cells. In addition, they showed that levitation promoted angiogenesis by upregulating paracrine factors essential for blood vessel formation which is essential for vascularized tissues like bone. Despite its potential for mechanical stimulation of cells, acoustic levitation remains under-explored in bone cell studies. Moreover, and uppermost, leveraging it for cyclic, non-contact mechanical stimulations across various frequencies could offer valuable insights into bone cell responses, a research gap worth addressing in future studies.

However, the examples quoted only focus on small volumes, and it is still difficult to control larger cell populations or tissue samples. Due to variations in acoustic pressure fields, it can lead to unpredictable cell drifting. Even if the results are conclusive, too much exposure could have the opposite effect, causing stress and even cell death, which has not been deeply investigated so far. Further testing is therefore required to fully understand the potential effects on membrane integrity, gene expression and viability. From a material point of view, even if the process itself is fairly simple, it requires precise tuning of frequency, intensity, and chamber conditions. Therefore, devices can be expensive and sensitive to temperature, humidity, and medium composition, and there is uppermost a need to scale-up the currently available devices in order to tend towards easier clinical application. The next section sets out some methodological considerations about the common methods reported for characterization of cellular response after the application of mechanical stimulation.

Effect of mechanical stimuli on cell's phenotype

Methods like viability tests and gene expression analysis are generally adopted to help assess cellular responses to mechanical stimuli (viability, proliferation, differentiation). Viability assays focus on specific markers, like mitochondrial function and apoptosis [107]. Gene expression analysis, critical in cell differentiation, helps define the lineage and differentiation phases in cells are [108]. Mechanical stimulation such as LIPUS at 1 MHz on MSC, have shown to induce ECM mineralisation and increase osteogenesis-related gene expression [91]. Similarly, mechanical loading at 5 Hz on MSC revealed osteogenic differentiation through analyses of bone markers, sclerostin and Young's modulus of the scaffold [78]. After LIPUS simulation at 1.5 Hz, the secretome was analysed to confirm the promotion of osteogenic differentiation [90] by assessing proteins such as ALP and sclerostin for osteoblasts and osteocytes, respectively [109].

These diverse methods help to establish links between mechanical stimuli to cellular responses (Table 1). In general, current research remains largely fundamental, focusing not quite yet on developing new therapeutic systems but rather on deepening our understanding of its mechanisms and biological impact. This link may make use of computational models to better understand stresses in tissues under a given macroscopic loading, which constitutes the focus of the next section.

Table 1

Summary of Experimental Conditions and Findings: This table presents key excerpts from various studies regarding the cell culture medium, mechanical stimulation methods, and observed results (same colour codes as Fig. 4).

Cell medium/ substrate	Mechanical stimuli parameters	Results	References
3D bioprinted hBMSCs cell-laden Graphene Oxide (GO) composite scaffold with osteogenic medium	Loading frequency of 5 Hz with a 1 % sinusoidal strain, for 5 min/per day and 5 days/for 3 groups: No Loading – Mechanical Loading day 1 – ML day 21	Increased organoid mineral density, organoid stiffness, osteoblast and osteocyte differentiation, collagen I maturation, lacuno-canalicular network formation and Yes-Associated Protein expression for ML01	[78]
Bio-derived cancellous bone scaffold, seeded with human fetal osteoblastic cell line	Cyclic compression at 1000 microstrain, at 3 Hz for 3 min/day for 24 days	Increased of osteoblast numbers by 36 % in 24 days, increased extracellular matrix deposition	[79]
Spheroids of hBMSCs cultured in standard growth medium or chondrogenic medium for 21 days, and then in osteogenic media for 21 days	Hydrostatic Pressure (HP) at 10 MPa, 1 Hz for 1 h/day for 3 weeks	Early bone formation (ALP activity) for growth medium + HP day 21 Late-stage mineralization (calcium) for chondrogenic medium + HP day 42 Collagen and bone matrix proteins for chondrogenic medium + HP day 21	[80]
Osteocytes, osteoblasts and periosteal fibroblasts isolated from fetal chicken then seeded on polylysine-coated glass slides with culture medium	Pulsatile fluid flow at 5 Hz with mean shear stress of 0.7 Pa for 1 h	Significant inhibition of osteoclast differentiation, and reduced bone resorption signalling via soluble factors produced by osteocytes	[81]
hBMSCs cultured on fibronectin coated glass slides	Cyclic Hydrostatic Pressure (CHP) of 10 kPa, 100 kPa and 300 kPa at 0.5 Hz, 1 Hz and 2 Hz for 1 h, 2 h and 4 h	At low pressure/frequency, hBMSCs differentiate towards an osteogenic phenotype; Collagen synthesis and mineral deposition at all magnitudes. Best pro-osteogenic response at the highest frequency of 2 Hz	[83]
rBMSCs in 24 well-plates, cultivated with osteogenic medium	Ultrasounds of 3.6 MHz at 30 mW/cm ² , 20 min/day with a 27.8 % duty cycle for 7 days	Increased osteogenic markers with osteopontin (OPN), Collagen-1, Runx2 (osteoblast-specific transcription factor) and E11 protein (early osteocyte marker) No apoptosis reported	[84]

(continued on next page)

Table 1 (continued)

Cell medium/ substrate	Mechanical stimuli parameters	Results	References
rBMSCs from the 3-month and 12-month old animals, seed in a 24-well plates with adipogenic or osteogenic medium	FLIPUS of 3.6 MHz at 11.7 or 44.5 mW/cm ² for 20 min/day for 14 days	At 11.7 mW/cm ² enhanced OCN gene expression and ECM mineralization for rBMSC of the 3-month animal, but decreased Runx2 expression by the cell of the 12-month old animal after 7 days of stimulation At 44.5 mW/cm ² , enhanced Runx2 expression for the 12-month cells after day 14 of stimulation	[85]
hBMSCs in 6 or 96 well-plates with a medium containing cell growth inhibitor	LIPUS at 1.5 MHz, with a 20 % duty cycle and at 30–40–50–60–80 mW/cm ² for 5, 10 and 20 min / day during 4 days	No differentiation but high expression of stem cell markers 50–60 mW/cm ² for 5–10 min/day promotes proliferation ≥50 mW/cm ² for 20 min/day inhibits growth	[89]
rBMSCs in a 6 well-plate, then replated in osteogenic or adipogenic medium	LIPUS at 1.5 MHz and 30 mW/cm ² for 20 min/d and 3 days, then split into wells with bone healing inhibitor (UAG) or without (UG)	UG enhanced MSC migration and fracture healing Ultrasound with or without inhibitor provided better bone healing outcomes than control group	[90]
rBMSCs suspension seeded on two titanium specimens (polished and Sandblasted, Large-grit, Acid-etched)	LIPUS at 1 MHz and 100 mW/cm ² for 10min	Increased cell proliferation, ALP activity, ECM mineralization as well as up-regulated osteogenic genes and proteins (OPN, BMP-2, Runx2)	[91]
hBMSCs for 2D culture and in the anti-gravity bioreactor	Anti-gravity bioreactor at a resonance frequency of 1.6 MHz for 12 h	Spheroids aggregation, with increased angiogenic paracrine factors, improved therapeutic efficacy and attenuation of fibrosis and inflammation	[103]
Epithelial cells and fibroblastic cells	Acoustofluidic bioreactor at 1.95 to 2.12 MHz for 24 h	Optimal levitation of 2 h for the formation of stable cell sheet with Ca ²⁺ Functional epithelial barrier without mixing layers	[104]
Embryonic stem cells derived from neuro-progenitors in a fibrin hydrogel, then placed in a neuronal	Bio-acoustic levitational assembly at 340 kHz and 680 kHz for 10 min until polymerization of the gel	Early neural precursor marker expression After a week in neural differentiation medium, differentiation still	[105]

Table 1 (continued)

Cell medium/ substrate	Mechanical stimuli parameters	Results	References
differentiation media hMSCs from adipose-tissue placed in adipocytic or osteoblastic medium after levitation	Acoustic levitation at 2 MHz for 24 h creating layers of cells at pressure nodes, with a perfused culture medium without altering the cells positions	ongoing but not complete Spheroid formation and aggregation occurred, with potential for osteoblastic differentiation	[106]

Part II: *in silico* modelling

For biomechanical characterization and mechanobiological insights, computational simulations have been crucial to understand the mechanical properties of bone or the local distribution of stresses and strains. Such computational models could link mechanical stimuli to biological responses, which facilitates the development and evaluation of mechanobiological models and provides insights into pathologies and their mechanical underpinnings including bone growth, ossification and degradation supported by experimental results [110]. They also may predict medical outcomes such as the evolution of pathologies and growth outcomes based on clinical data (bone shape, loading history) [111]. In surgical operations, computational models help to establish personalized protocols adapted to specific pathologies reducing the length of the surgery [112].

Advances in numerical methods and computing capacities have facilitated the emergence of complex models which now may account for bone multiscale composition, microstructure and material properties, non-mechanical phenomena (e.g., piezoelectric, magnetic, chemical) and fluid-structure interactions in the lacuno-canalicular network. Numerous reviews exist on computational bone modelling [112–115]. The following sections focus on the computational methods that elucidate the relations between external mechanical loading, cellular mechanical environments, and cellular responses following the mechanobiological phenomena.

Different experiments [116,117], can be replicated in computational models, generally focusing on FSS or solid substrate stresses. Some models recreate the stimulus of the fluid surrounding the osteocytes, while others predict stimuli related to bone deformation under external load. For stimulations induced by external mechanical loading, the Finite Element Method (FEM) solves elasticity equations, while Computational Fluid Mechanics (CFD) addresses fluid flow stimuli by solving flow equations. Difficulties arise when both the solid and fluid responses are modelled and coupled (fluid-structure interactions, or FSI) and when increasing degree of complexity of solid behaviour (anisotropy, damage, viscoelasticity, ...) of fluid behaviour (non-Newtonian) are taken into account. Approaches adapted to model acoustic pressure or wave propagation are less straightforward and lack consensus. These different points will be described in the following sub-sections.

The use of finite element method to predict solid-phase stimuli

FEM has become the predominant tool in mechanical engineering for solving continuum mechanics equations for solids. It divides complex systems into smaller elements connected at nodes, discretizing continuous equations and applying boundary conditions like pressure, forces, temperature, or displacements. Mechanical fields (stresses and strains) are calculated for each element according to its respective behaviour, providing input for mechanobiological models [118]. FEM has been used extensively over two decades to link external loads to the cellular microenvironment, offering insights into local stimuli sensed by

osteocytes, and aiding in the interpretation of biological data. Numerous reviews detail these studies [119–122]. Today's simulations can involve millions of elements with sophisticated constitutive laws, accurately reproducing experimental results. However, FEM primarily addresses solid mechanics, while CFD is more adapted to a spatial description of the continuum and therefore to fluid mechanics. In mechanobiology, both solid and fluid phases contribute to the stimuli cells respond to, necessitating the integration of FEM and CFD for modelling fluid-structure interactions.

Fluid-structure interactions, the use of CFD with FEM

FSI are critical for understanding how osteocytes respond to mechanical stimuli at both cellular and tissue levels. These interactions involve the coupling of fluid flow and structural deformation, typically modelled by integrating Computational Fluid Dynamics (CFD), based on the Finite Volume Method (FVM), with Finite Element Method (FEM), as illustrated below (Fig. 5). This approach has been employed to simulate osteocytes subjected to fluid flow in the lacuno-canalicular system (LCS) [123–126], predicting velocity, pressure and shear stress distribution. The geometry of osteocytes impacts fluid flow as well, with studies using simplified geometries yielding different stress/strain values than those obtained from imaging [127]. FSI models predict fluid flow velocity, pressure and shear stress on cells with varying geometries. These models demonstrate that fluid velocity and WSS are highly dependent on structural features such as canalicular spacing and surface roughness, which modulate the mechanical environment experienced by osteocytes [128].

At the cellular level, simulations of fluid-induced forces have been used to predict osteogenic responses under mechanical loading [28]. For example, Verbruggen et al. [129] performed 3D reconstructions of osteocytes with images from confocal microscopy to simulate fluid flow during intense physical activity. By assigning mechanical properties, such as Young's modulus and Poisson ratio, to both the ECM and the cell body, their model demonstrated that the highest velocities and shear stresses within the osteocyte canaliculi, influenced by surface roughness, highlighting the role of the canalicular structure in osteocyte

mechanotransduction and bone growth.

At the tissue scale, combining FEM and CFD within FSI frameworks has enabled the investigation of scaffolds under mechanical stimulation [130–134]. These models reveal the heterogeneity of mechanical microenvironments, identifying regions with varying strain and a dynamic balance between tension and compression. Such analyses may enable to optimize scaffold design or dynamic culture conditions for bone tissue engineering, as they allow for the control of parameters like flow velocity and strain rates. Mechanoregulation models, including the pioneering framework by Prendergast [135], link mechanical stimuli to tissue differentiation, offering a comprehensive view of how mechanical forces guide tissue differentiation. In dynamic culture systems, FSI models can be employed to study bone explants under osteogenic stimuli. These simulations demonstrate mechanical loading generates localized mechanical environments that significantly influence bone adaptation [136]. Compressive strains and shear stresses in the marrow were quantified, with higher surface strains in bone and increased shear stress near the surface in explants (difference higher than 1 % compared to <0.1 % in bone marrow). These insights are crucial for designing bioreactors and scaffolds to replicate these mechanical conditions, enhancing the efficacy of bone tissue engineering by targeting specific regions for optimized growth and resorption control.

Stimulation by acoustic pressure or wave propagation: proposed computational approaches

Experimental studies based on the propagation of acoustic waves (resulting in vibration and levitation), have increased in the last two decades. Accordingly, computational methods aiming at predicting the mechanical environment during vibration-induced stimulation are emerging. Particularly, LIPUS device has been used to computationally explore mechanotransduction at high frequencies, which differ from typical low-frequency mechanical stimuli like walking or running [137]. A 2D cortical model incorporating a 1 MHz ultrasound signal evaluated different flow models to describe WSS in the lacuno-canalicular network. These simulations demonstrated that frequencies exceeding physiological loading ranges (0–20 Hz) can activate osteocyte biological

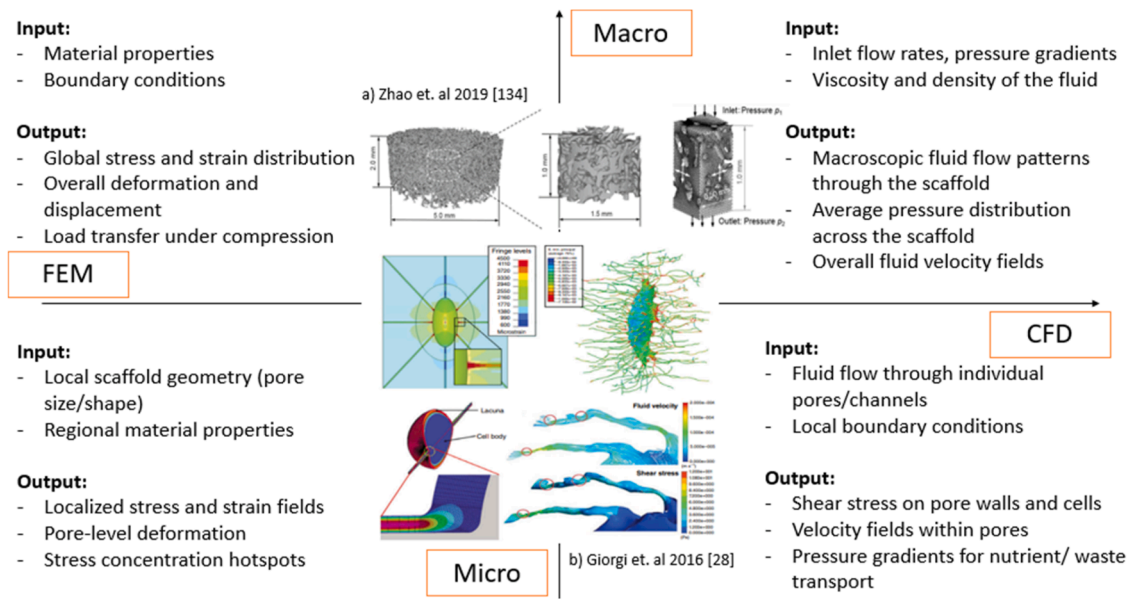


Fig. 5. Comparison between FEM and CFD methods on the macro/micro scale. a) Simulation of a scaffold with a more detailed close-up showing the porosity used in the study with CFD of a Representative Volume Element (RVE) of the scaffold's microstructure, illustrating fluid flow and transport characteristics within the porous architecture. Adapted from Zhao et al. [134] License Creative Commons Attribution 4.0 b) Progression from an idealized bone cell model (top-left) to a more complex and precise representation (top-right) and further advancements include CFD models (bottom-left) evolving toward FSI techniques, enabling the prediction of velocities and shear stresses (bottom-right). Adapted from Giorgi et al. [28] License Creative Commons Attribution 4.0.

responses. It also provides insight into how ultrasound might be spatially and temporally optimized in an in-dish ultrasonic bioreactor to influence cellular behaviour. Similarly, ultrasound propagation in a 3D fluid medium was modelled to quantify acoustic fields under varying water levels and with absorbing devices designed to prevent wave reflection. Similar studies explored the use of absorbent layers to modulate the acoustic field [138]. These two articles highlight the role of ultrasound in enhancing *in silico* models for studying cell and tissue responses to mechanical stimuli. The second article, examining cortical bone with varied porosities, extends this by showing how ultrasound interacts with complex tissue structures, addressing how mechanical forces influence bone at multiple porosity levels. Together these articles showcase the versatility and precision of ultrasound in computational models, supporting its potential as a non-contact, adaptable stimulus for simulating biological responses in various tissue engineering contexts. In summary, computational studies using ultrasounds or high frequencies are emerging to address gaps in understanding how bone cells respond to vibration-induced stimuli. These studies mainly quantify stimuli from macroscopic loading in 3D substrates to aid biological interpretation. To better understand cellular activation under stimulation, numerical methods are being developed focusing on single cells or cell groups to head towards predictive simulations of the cellular response to a given mechanical environment.

Computational models of the cellular response: agent-based methods vs continuum approaches

Cellular response to mechanical stimuli is modelled using two prominent modelling frameworks: either continuum approaches [139–141] or agent-based models (ABM) [142–144]. Continuum approaches use partial differential equations (PDEs) to model cell-density fields and tissue-level phenomena. In contrast, ABMs explicitly represent individual cells and their interactions, offering detailed insights into cellular behaviours at the microscale but at a higher computational cost [145]. Both frameworks have been instrumental in advancing the understanding of how mechanical stimuli influence cellular and tissue dynamic, with each offering unique strengths and limitations.

Continuum models are particularly well-suited for large-scale simulations, as they efficiently describe the mechanical and biochemical behaviour of tissues under various loading conditions. These models can incorporate tissue-specific properties, such as anisotropy and heterogeneity to simulate stress-strain distributions and fluid dynamics. In the context of tissue engineering, *in silico* models predict 2D and 3D cell growth depending on scaffold geometry, where features like pore size and curvature may impact the predicted growth. Studies utilizing level set (LS) methods have demonstrated that cell growth in scaffolds with hexagonal pores tends to be faster compared to those with triangular pores, emphasizing the critical role of pore geometry in designing effective tissue engineering scaffolds [146–149]. Beyond mechanical properties, continuum models often incorporate nutrient gradients to simulate the interplay between biochemical factors and cell growth. Nutrient availability is a key determinant of cell proliferation, as models have shown that higher cell density observed near scaffold core where nutrient levels are more abundant [150]. Carlier et al. extended this approach by integrating *in vivo-in silico* methods to study bone healing. Their model incorporated blood vessel formation, oxygen supply and growth factors, providing a comprehensive framework for understanding bone repair dynamics [151].

On the other hand, ABMs capture the heterogeneity and stochastic nature of cellular behaviours. By modelling individual cells as discrete agents with specific behavioural rules, ABMs can simulate a wide range of biological processes including proliferation, migration, apoptosis and differentiation. Open-source platforms like “PhysiCell” [152], simulate cell interactions in dynamic tissues offering submodels for processes such as necrosis, and cell cycle regulation. Similarly, tools like CompuCell3D [153] and Mopheus [154], are used to model multicellular

systems, with applications ranging from biomimetic bone tissue fabrication to endothelial cell self-organization [155].

Despite their advantages, both model types have significant limitations. Continuum models, while efficient at the tissue scale, cannot accurately represent the detailed cellular-level responses to mechanical stimuli and the complex interactions between cells, making them less suitable for studying mechanotransduction at the individual cell level. Additionally, they rely on simplified assumptions of uniform material properties, which may not capture the complexities of biological tissues. As for ABMs, while offering a high level of detail at the cellular level, they are computationally expensive especially for large-scale simulations and often lack integration of mechanical stimuli, limiting their ability to fully simulate the mechanical environments that influence cellular behaviour. Both approaches also face challenges in accurately modelling the perception of mechanical changes by cells and integrating these effects into the overall system dynamics. The following figure resumes the advantages and disadvantages of both methods (Fig. 6).

Despite these challenges, both continuum and agent-based models enhance the understanding of biological systems. Further research is needed to integrate mechanical stimuli more effectively and to address the remaining questions about mechanotransduction and the perception of mechanical changes in cells. Additionally, validating these models with experimental data remains a critical challenge, as it ensures their reliability and usability in clinical applications, while also addressing uncertainties and improving their predictive accuracy [156].

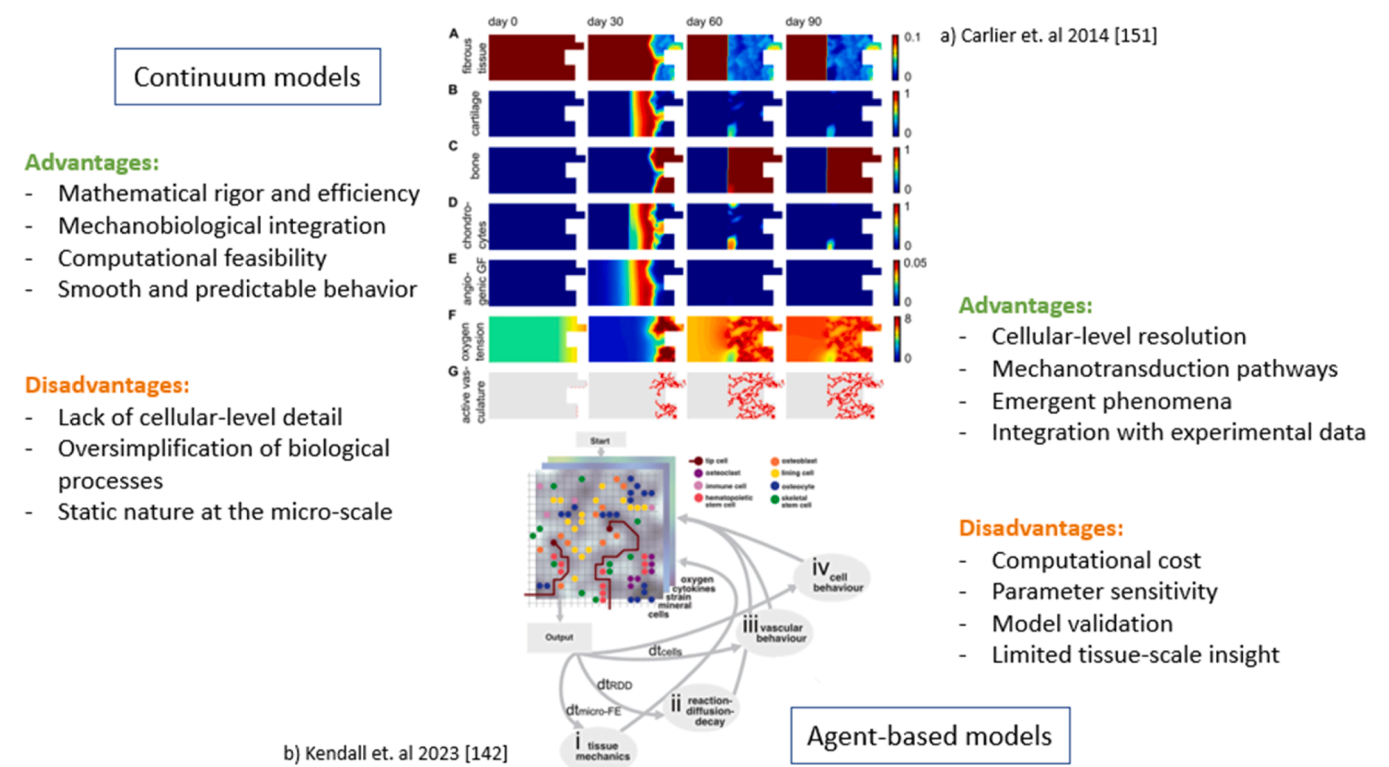
Conclusion and future directions

This article explores the advantages of the combined experimental and computational approaches to enhance our understanding of bone mechanobiology. By integrating experimental results with advanced *in silico* modelling, a comprehensive framework may be designed to explain the complex and still partially unknown links between the mechanical environments and the behaviour of bone cells.

On the experimental side, mechanical stimuli such as fluid shear stress, cyclic mechanical loading, and acoustic pressure have been applied on cells to mimic the conditions experienced in their native environments. Although these stimuli are crucial for understanding how mechanotransduction pathways are activated, particularly in MSCs and osteocytes, with positive effects on promoting osteogenic differentiation or enhancing tissue formation, the macro-micro links are still unclear and need to be clarified. This would include repeating the several experiments with a frequency sweep and establishing a comprehensive database of cellular responses to each case.

In addition, computational models have been developed to predict cellular responses under a given mechanical environment, providing a deeper understanding of how mechanical forces and fluid flow at both macroscopic and microscopic levels affect bone tissue. In addition, these models allow greater freedom with a wide range of parameters, such as scaffold geometry, pore size and material properties without material and experimental constraints, making analyses more flexible and in-depth. Nevertheless, the numerical database needs to be completed to better manage and include these biological behaviours and pathways as well as physico-chemical factors such as hydration, cross-linking, and diffusion capacity. Moreover, most studies focus on either solid mechanics or fluid dynamics, but investigating both simultaneously would provide a more comprehensive and realistic understanding of their combined effects on cellular behaviour. Thus, more future studies should be carried out with both fields to establish a complete mechanobiological understanding.

Ultrasound stimulation is generating a growing interest as it is advantageous in increasing cellular responses such as proliferation, differentiation and matrix production. Indeed, it is a non-invasive and localised method of application, with complementary numerical studies suggesting that appropriate adjustment of parameters such as duty cycle and frequency could optimize these cellular effects, leading to better



If your study involves human subjects you MUST have obtained ethical approval.

Please state whether Ethical Approval was given, by whom and the relevant Judgement's reference number

/

DOES YOUR STUDY INVOLVE ANIMAL SUBJECTS? Please cross out whichever is not applicable.

Yes

No

If your study involves animals you must declare that the work was carried out in accordance with your institution guidelines and, as appropriate, in accordance with the EU Directive 2010/63/EU. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063>

/

This information must also be inserted into your manuscript under the acknowledgements section prior to the References.

If you have no declaration to make please insert the following statements into your manuscript:

Conflict of interest

All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Acknowledges

This research was supported by a CNRS PhD fellowship, HyCareMat Project-ANR-23-CE51-0039 and the MECHABOOST ANR JCJC project ANR-23-CE51-0048.

References

- [1] Sampogna G, Guraya SY, Forgione A. Regenerative medicine: historical roots and potential strategies in modern medicine. *J. Microsc. Ultrastruct.* 2015;3:101–7. <https://doi.org/10.1016/j.jmau.2015.05.002>.
- [2] Bianco P. Stem cells and bone: a historical perspective. *Bone* 2015;70:2–9. <https://doi.org/10.1016/j.bone.2014.08.011>.
- [3] van Gestel N, Carmeliet G. Metabolic regulation of skeletal cell fate and function in physiology and disease. *Nat Metab* 2021;3:11–20. <https://doi.org/10.1038/s42255-020-00321-3>.
- [4] Jansen KA, Donato DM, Balcioglu HE, Schmidt T, Danen EHJ, Koenderink GH. A guide to mechanobiology: where biology and physics meet. *Biochim. Biophys. Acta (BBA) - Mol. Cell Res.* 2015;1853:3043–52. <https://doi.org/10.1016/j.bbmr.2015.05.007>.
- [5] Yavropoulou MP, Yovos JG. The molecular basis of bone mechanotransduction. *J Musculoskelet Neuronal Interact* 2016;16:221–36.
- [6] Franz-Odenaal TA, Hall BK, Witten PE. Buried alive: how osteoblasts become osteocytes. *Dev Dyn* 2006;235:176–90. <https://doi.org/10.1002/dvdy.20603>.
- [7] Liu Z, Wang Q, Zhang J, Qi S, Duan Y, Li C. The mechanotransduction signaling pathways in the regulation of osteogenesis. *Int J Mol Sci* 2023;24:14326. <https://doi.org/10.3390/ijms241814326>.
- [8] Sapin - de Brosse E., Mitton D. Biomécanique du tissu osseux et de ses interactions avec les tissus environnants. In: Laurent C, Verdier C, editors. *Mécanique des tissus vivants : imagerie, caractérisation et modélisation pour l'étude des tissus mous*. ISTE; 2023. p. 289–312.
- [9] Parle E, Tio S, Behre A, Carey JJ, Murphy CG, O'Brien TF, et al. Bone mineral is more heterogeneously distributed in the femoral heads of osteoporotic and diabetic patients: a pilot study. *JBM Plus* 2020;4:e10253. <https://doi.org/10.1002/jbm4.10253>.
- [10] He Z, Chu L, Liu X, Han X, Zhang K, Yan M, et al. Differences in subchondral trabecular bone microstructure and finite element analysis-based biomechanical properties between osteoporosis and osteoarthritis. *J Orthop Transl.* 2020;24:39–45. <https://doi.org/10.1016/j.jot.2020.05.006>.
- [11] Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. *J Orthop Surg Res* 2014;9:18. <https://doi.org/10.1186/1749-799X-9-18>.
- [12] Re F, Borsani E, Rezzani R, Sartore L, Russo D. Bone regeneration using mesenchymal stromal cells and biocompatible scaffolds: a concise review of the current clinical trials. *Gels* 2023;9:389. <https://doi.org/10.3390/gels9050389>.
- [13] Battafarano G, Rossi M, De Martino V, Marampon F, Borro L, Secinaro A, et al. Strategies for bone regeneration: from graft to tissue engineering. *Int J Mol Sci* 2021;22:1128. <https://doi.org/10.3390/ijms22031128>.
- [14] Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. *BMC Med* 2011;9:66. <https://doi.org/10.1186/1741-7015-9-66>.
- [15] Mamidi N, De Silva FF, Vacas AB, Gutiérrez Gómez JA, Montes Goo NY, Mendoza DR, et al. Multifaceted hydrogel scaffolds: bridging the gap between biomedical needs and environmental sustainability. *Adv Heal. Mater* 2024;13:2401195. <https://doi.org/10.1002/adhm.202401195>.
- [16] Mamidi N, Ijadi F, Norahan MH. Leveraging the recent advancements in GelMA scaffolds for bone tissue engineering: an assessment of challenges and opportunities. *Biomacromolecules* 2024;25:2075–113. <https://doi.org/10.1021/acs.biomac.3c00279>.
- [17] Huang J, Santos AC, Tan Q, Bai H, Hu X, Mamidi N, et al. Black phosphorous-based biomaterials for bone defect regeneration: a systematic review and meta-analysis. *J Nanobiotechnology* 2022;20:522. <https://doi.org/10.1186/s12951-022-01735-9>.
- [18] Mamidi N, Delgadillo RMV, Sustaita AO, Lozano K, Yallapu MM. Current nanocomposite advances for biomedical and environmental application diversity. *Med Res Rev* 2025;45:576–628. <https://doi.org/10.1002/med.22082>.
- [19] Shamsan GA, Odde DJ. Emerging technologies in mechanotransduction research. *Curr Opin Chem Biol* 2019;53:125–30. <https://doi.org/10.1016/j.cbpa.2019.08.002>.
- [20] Argentati C, Morena F, Tortorella I, Bazzucchi M, Porcellati S, Emiliani C, et al. Insight into mechanobiology: how stem cells feel mechanical forces and orchestrate biological functions. *Int J Mol Sci* 2019;20:5337. <https://doi.org/10.3390/ijms20215337>.
- [21] Mohammed D, Versaavel M, Bruyère C, Alaimo L, Luciano M, Vercruysse E, et al. Innovative tools for mechanobiology: unraveling outside-In and inside-out mechanotransduction. *Front Bioeng Biotechnol* 2019;9:7. <https://doi.org/10.3389/fbioe.2020.597661>.
- [22] Vining KH, Mooney DJ. Mechanical forces direct stem cell behaviour in development and regeneration. *Nat Rev Mol Cell Biol* 2017;18:728–42. <https://doi.org/10.1038/nrm.2017.108>.
- [23] Naqvi SM, McNamara LM. Stem cell mechanobiology and the role of biomaterials in governing mechanotransduction and matrix production for tissue regeneration. *Front Bioeng Biotechnol* 2020;8:597661. <https://doi.org/10.3389/fbioe.2020.597661>.
- [24] Tassinari R, Olivi E, Cavallini C, Taglioli V, Zannini C, Marcuzzi M, et al. Mechanobiology: a landscape for reinterpreting stem cell heterogeneity and regenerative potential in diseased tissues. *iScience* 2023;26:105875. <https://doi.org/10.1016/j.isci.2022.105875>.
- [25] Wei H, Cui J, Lin K, Xie J, Wang X. Recent advances in smart stimuli-responsive biomaterials for bone therapeutics and regeneration. *Bone Res* 2022;10:1–19. <https://doi.org/10.1038/s41413-021-00180-y>.
- [26] Winkler T, Sass FA, Duda GN, Schmidt-Bleek K. A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering. *Bone Jt. Res* 2018;7:232–43. <https://doi.org/10.1302/2046-3758.73.BJR-2017-0270.R1>.
- [27] Luo T, Tan B, Liao J, Shi K, Ning L. A review on external physical stimuli with biomaterials for bone repair. *Chem. Eng. J.* 2024;496:153749. <https://doi.org/10.1016/j.cej.2024.153749>.
- [28] Giorgi M, Verbruggen SW, Lacroix D. In silico bone mechanobiology: modeling a multifaceted biological system. *WIREs Syst. Biol. Med.* 2016;8:485–505. <https://doi.org/10.1002/wsbm.1356>.
- [29] Carlier A, Geris L, Lammens J, Van Oosterwyck H. Bringing computational models of bone regeneration to the clinic. *WIREs Syst. Biol. Med.* 2015;7:183–94. <https://doi.org/10.1002/wsbm.1299>.
- [30] Saeedi P, Halabian R, Fooladi AAL. A revealing review of mesenchymal stem cells therapy, clinical perspectives and modification strategies. *Stem Cell Investig.* 2019;6. <https://doi.org/10.21037/sci.2019.08.11>.
- [31] Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. *J. Inflamm* 2005;2:8. <https://doi.org/10.1186/1476-9255-2-8>.
- [32] Deo D, Marchioni M, Rao P. Mesenchymal stem/stromal cells in organ transplantation. *Pharmaceutics* 2022;14:791. <https://doi.org/10.3390/pharmaceutics14040791>.
- [33] Kartsogiannis V, Ng KW. Cell lines and primary cell cultures in the study of bone cell biology. *Mol. Cell. Endocrinol* 2004;228:79–102. <https://doi.org/10.1016/j.mce.2003.06.002>.
- [34] Tomkinson A, Reeve J, Shaw RW, Noble BS. The death of osteocytes via apoptosis accompanies estrogen withdrawal in Human bone*. *J. Clin. Endocrinol. Metab.* 1997;82:3128–35. <https://doi.org/10.1210/jcem.82.9.4200>.
- [35] Bonewald LF. The amazing osteocyte. *J. Bone Miner. Res.* 2011;26:229–38. <https://doi.org/10.1002/jbmr.320>.
- [36] Mertz EL, Makareeva E, Mirigian LS, Leikin S. Bone formation in 2D culture of primary cells. *JBM Plus* 2023;7:e10701. <https://doi.org/10.1002/jbm4.10701>.
- [37] Gallagher JA. Human osteoblast culture. *Methods Mol Med* 2003;80:3–18. <https://doi.org/10.1385/1-59259-366-6.3>.
- [38] Weyts F aA, Bosmans B, Niesing R, van Leeuwen JPTM, Weinans H. Mechanical control of human osteoblast apoptosis and proliferation in relation to differentiation. *Calcif Tissue Int* 2003;72:505–12. <https://doi.org/10.1007/s00223-002-2027-0>.

- [39] Sandwiches to better understand cells – Atlas of Science n.d. <https://atlasofscience.org/sandwiches-to-better-understand-cells/> (accessed May 15, 2023).
- [40] Ballester-Beltrán J, Lebourg M, Salmerón-Sánchez M. Sandwich-like microenvironments to harness cell/material interactions. *J Vis Exp* 2015:e53090. <https://doi.org/10.3791/53090>.
- [41] Yue B. Biology of the Extracellular matrix: an overview. *J Glaucoma* 2014:S20–3. <https://doi.org/10.1097/JG.0000000000000108>.
- [42] Makareeva E, Leikin S. Chapter 7 - collagen structure, folding and function. In: Shapiro JR, Byers PH, Glorieux FH, Sponseller PD, editors. *Osteogenesis* imperfecta. San Diego: Academic Press; 2014. p. 71–84. <https://doi.org/10.1016/B978-0-12-397165-4.00007-1>.
- [43] D'Arcangelo E, McGuigan AP. Micropatterning strategies to engineer controlled cell and tissue architecture in vitro. *Biotechniques* 2015;58:13–23. <https://doi.org/10.2144/000114245>.
- [44] Zhang C, Bakker AD, Klein-Nulend J, Bravenboer N. Studies on osteocytes in their 3D native matrix versus 2D in vitro models. *Curr Osteoporos Rep* 2019;17: 207–16. <https://doi.org/10.1007/s11914-019-00521-1>.
- [45] Badenes SM. Microcarrier-based platforms for in vitro expansion and differentiation of human pluripotent stem cells in bioreactor culture systems. *J. Biotechnol* 2016.
- [46] Tavassoli H. Large-scale production of stem cells utilizing microcarriers: a biomaterials engineering perspective from academic research to commercialized products 2018.
- [47] Tharmalingam T, Sunley K, Spearman M, Butler M. Enhanced production of Human recombinant proteins from CHO cells grown to high densities in macroporous microcarriers. *Mol Biotechnol* 2011;49:263–76. <https://doi.org/10.1007/s12033-011-9401-y>.
- [48] Couto PS. Expansion of human mesenchymal stem/stromal cells (hMSCs) in bioreactors using microcarriers: lessons learnt and what the future holds. *Biotechnol. Adv* 2020.
- [49] Koh B, Sulaiman N, Fauzi MB, Law JX, Ng MH, Idrus RBH, et al. Three dimensional microcarrier system in mesenchymal stem cell culture: a systematic review. *Cell Biosci* 2020;10:75. <https://doi.org/10.1186/s13578-020-00438-8>.
- [50] Bicer M, Cottrell GS, Widera D. Impact of 3D cell culture on bone regeneration potential of mesenchymal stromal cells. *Stem Cell Res Ther* 2021;12:31. <https://doi.org/10.1186/s13287-020-02094-8>.
- [51] Abbott A. Biology's new dimension. *Nature* 2003;424:870–2. <https://doi.org/10.1038/424870a>.
- [52] Ham J, Lever L, Fox M, Reagan MR. In vitro 3D cultures to reproduce the bone marrow niche. *JBMR Plus* 2019;3:e10228. <https://doi.org/10.1002/jbm.b.10228>.
- [53] Silva-Pedrosa R, Salgado AJ, Ferreira PE. Revolutionizing disease modeling: the emergence of organoids in cellular systems. *Cells* 2023;12:930. <https://doi.org/10.3390/cells12060930>.
- [54] Baruffaldi D, Palmara G, Pirri C, Frascella F. 3D Cell culture: recent development in materials with tunable stiffness. *ACS Appl Bio Mater* 2021;4:2233–50. <https://doi.org/10.1021/acsbm.0c01472>.
- [55] Yuste I, Luciano FC, González-Burgos E, Lalatsa A, Serrano DR. Mimicking bone microenvironment: 2D and 3D in vitro models of human osteoblasts. *Pharmacol. Res* 2021;169:105626. <https://doi.org/10.1016/j.phrs.2021.105626>.
- [56] Yamada Y, Okano T, Orita K, Makino T, Shima F, Nakamura H. 3D-cultured small size adipose-derived stem cell spheroids promote bone regeneration in the critical-sized bone defect rat model. *Biochem. Biophys. Res. Commun* 2022;603: 57–62. <https://doi.org/10.1016/j.bbrc.2022.03.027>.
- [57] Wang W, Itaka K, Ohba S, Nishiyama N, Chung U, Yamasaki Y, et al. 3D spheroid culture system on micropatterned substrates for improved differentiation efficiency of multipotent mesenchymal stem cells. *Biomaterials* 2009;30: 2705–15. <https://doi.org/10.1016/j.biomaterials.2009.01.030>.
- [58] Griffin KH, Fok SW, Kent Leach J. Strategies to capitalize on cell spheroid therapeutic potential for tissue repair and disease modeling. *Npj Regen Med* 2022;7:1–13. <https://doi.org/10.1038/s41536-022-00266-z>.
- [59] Prabhakaran V, Hawkswell R, Paxton J. Investigating the formation of 3d bone cell spheroids for scaffold-free bone tissue engineering. *Orthop. Proc.* 2023;105-B: 80. <https://doi.org/10.1302/1358-992X.2023.7.080>.
- [60] Xue X, Hu Y, Wang S, Chen X, Jiang Y, Su J. Fabrication of physical and chemical crosslinked hydrogels for bone tissue engineering. *Bioact. Mater.* 2022;12: 327–39. <https://doi.org/10.1016/j.bioactmat.2021.10.029>.
- [61] Antoine EE, Vlachos PP, Rylander MN. Review of Collagen I hydrogels for bioengineered tissue microenvironments: characterization of mechanics, structure, and transport. *Tissue Eng B Rev* 2014;20:683–96. <https://doi.org/10.1089/ten.teb.2014.0086>.
- [62] Yao R, Zhang R, Luan J, Lin F. Alginate and alginate/gelatin microspheres for human adipose-derived stem cell encapsulation and differentiation. *Biofabrication* 2012;4:025007. <https://doi.org/10.1088/1758-5082/4/2/025007>.
- [63] Lee KY, Mooney DJ. Alginate: properties and biomedical applications. *Prog Polym Sci* 2012;37:106–26. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>.
- [64] Liao S, Meng H, Zhao J, Lin W, Liu X, Tian Z, et al. Injectable adipose-derived stem cells-embedded alginate-gelatin microspheres prepared by electrospray for cartilage tissue regeneration. *J Orthop Transl.* 2022;33:174–85. <https://doi.org/10.1016/j.jot.2022.03.007>.
- [65] Distler T, Kretzschmar L, Schneider D, Girardo S, Goswami R, Friedrich O, et al. Mechanical properties of cell- and microgel bead-laden oxidized alginate-gelatin hydrogels. *Biomater Sci* 2021;9:3051–68. <https://doi.org/10.1039/D0BM02117B>.
- [66] Ahmadi F, Oveis Z, Samani SM, Amoozgar Z. Chitosan based hydrogels: characteristics and pharmaceutical applications. *Res Pharm Sci* 2015;10:1–16.
- [67] Nallusamy J, Das RK. Hydrogels and their role in bone tissue engineering: an overview. *J Pharm Bioallied Sci* 2021;13:S908–12. <https://doi.org/10.4103/jpbs.jpbs.237.21>.
- [68] Cheikh K, Laurent C, Ganghoffer JF. An advanced method to design graded cylindrical scaffolds with versatile effective cross-sectional mechanical properties. *J Mech Behav Biomed Mater* 2022;125:104887. <https://doi.org/10.1016/j.jmbbm.2021.104887>.
- [69] Cheikh K, Ganghoffer JF, Baldit A, Labbé E, Alix S, Kerdjoudj H, et al. A flexible design framework to design graded porous bone scaffolds with adjustable anisotropic properties. *J Mech Behav Biomed Mater* 2023;140:105727. <https://doi.org/10.1016/j.jmbbm.2023.105727>.
- [70] Chen G, Dong C, Yang L, Lv Y. 3D Scaffolds with different stiffness but the same microstructure for bone tissue engineering. *ACS Appl Mater Interfaces* 2015;7: 15790–802. <https://doi.org/10.1021/acsami.5b02662>.
- [71] Tang G, Liu Z, Liu Y, Yu J, Wang X, Tan Z, et al. Recent trends in the development of bone regenerative biomaterials. *Front Cell Dev Biol* 2021;9. <https://doi.org/10.3389/fcell.2021.665813>.
- [72] Stevens MM. Biomaterials for bone tissue engineering. *Mater. Today* 2008;11: 18–25. [https://doi.org/10.1016/S1369-7021\(08\)70086-5](https://doi.org/10.1016/S1369-7021(08)70086-5).
- [73] Abbasi N, Hamlet S, Love R, Nguyen N.-T. Porous scaffolds for bone regeneration 2020. <https://doi.org/10.1016/j.jsam.2020.01.007>.
- [74] Lee SS, Du X, Kim I, Ferguson SJ. Scaffolds for bone-tissue engineering. *Matter* 2022;5:2722–59. <https://doi.org/10.1016/j.matt.2022.06.003>.
- [75] Division of Biomechanics and Engineering Design, Leuven KU, Leuven Belgium, Torcasio A, Van Lenthe G, Van Oosterwyck H. The importance of loading frequency, rate and vibration for enhancing bone adaptation and implant osseointegration. *eCM* 2008;16:65–8. <https://doi.org/10.22203/eCM.v016a07>.
- [76] Chaudhuri J, Al-Rubeai M. Bioreactors for tissue engineering: principles, design and operation. Dordrecht: Springer Netherlands; 2005. <https://doi.org/10.1007/1-4020-3741-4>.
- [77] Partap S, Plunkett N.A., O'Brien F.J. 1×6 Bioreactors in tissue engineering n.d.
- [78] Zhang J, Griesbach J, Ganeyev M, Zehnder A-K, Zeng P, Schädli GN, et al. Long-term mechanical loading is required for the formation of 3D bioprinted functional osteocyte bone organoids. *Biofabrication* 2022;14:035018. <https://doi.org/10.1088/1758-5090/ac73b9>.
- [79] Zhang C, Zhang X, Dong X, Wu H, Li G. A loading device suitable for studying mechanical response of bone cells in hard scaffolds. *J. Biomed. Mater. Res. B: Appl. Biomater.* 2009;91B:481–8. <https://doi.org/10.1002/jbm.b.31462>.
- [80] Freeman FE, Schiavi J, Brennan MA, Owens P, Layrolle P, McNamara LM. Mimicking the biochemical and mechanical extracellular environment of the endochondral ossification process to enhance the In vitro mineralization potential of Human mesenchymal stem cells. *Tissue Eng. A* 2017;23:1466–78. <https://doi.org/10.1089/ten.tea.2017.0052>.
- [81] Tan SD, de Vries TJ, Kuipers-Jagtman AM, Semeins CM, Everts V, Klein-Nulend J. Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. *Bone* 2007;41:745–51. <https://doi.org/10.1016/j.bone.2007.07.019>.
- [82] Yeatts AB, Fisher JP. Bone tissue engineering bioreactors: dynamic culture and the influence of shear stress. *Bone* 2011;48:171–81. <https://doi.org/10.1016/j.bone.2010.09.138>.
- [83] Stavenschi E, Corrigan MA, Johnson GP, Riffault M, Hoey DA. Physiological cyclic hydrostatic pressure induces osteogenic lineage commitment of human bone marrow stem cells: a systematic study. *Stem Cell Res Ther* 2018;9:276. <https://doi.org/10.1186/s13287-018-1025-8>.
- [84] Puts R, Ruschke K, Ambrosi TH, Kadow-Romacker A, Knaus P, Jenderka K-V, et al. A focused low-intensity pulsed ultrasound (FLIPUS) system for cell stimulation: physical and biological proof of principle. *IEEE Trans Ultrason Ferroelectr Freq Control* 2016;63:91–100. <https://doi.org/10.1109/TUFFC.2015.2498042>.
- [85] Puts R, Albers J, Kadow-Romacker A, Geissler S, Raum K. Influence of donor age and stimulation intensity on osteogenic differentiation of rat mesenchymal stromal cells in response to focused low-intensity pulsed ultrasound. *Ultrasound Med Biol* 2016;42:2965–74. <https://doi.org/10.1016/j.ultrasmedbio.2016.08.012>.
- [86] Mundi R, Petis S, Kaloty R, Shetty V, Bhandari M. Low-intensity pulsed ultrasound: fracture healing. *Indian J Orthop* 2009;43:132–40. <https://doi.org/10.4103/0019-5413.50847>.
- [87] Claes L, Willie B. The enhancement of bone regeneration by ultrasound. *Prog. Biophys. Mol. Biol* 2007;93:384–98. <https://doi.org/10.1016/j.pbiomolbio.2006.07.021>.
- [88] Xia P, Shi Y, Wang X, Li X. Advances in the application of low-intensity pulsed ultrasound to mesenchymal stem cells. *Stem Cell Res Ther* 2022;13:214. <https://doi.org/10.1186/s13287-022-02887-z>.
- [89] Xie S, Jiang X, Wang R, Xie S, Hua Y, Zhou S, et al. Low-intensity pulsed ultrasound promotes the proliferation of human bone mesenchymal stem cells by activating PI3K/Akt signaling pathways. *J Cell Biochem* 2019;120:15823–33. <https://doi.org/10.1002/jcb.28853>.
- [90] Wei F-Y, Leung K-S, Li G, Qin J, Chow SK-H, Huang S, et al. Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing. *PLoS One* 2014;9:e106722. <https://doi.org/10.1371/journal.pone.0106722>.
- [91] An Y, Song Y, Wang Z, Wang J, Wu G, Zhu G, et al. Effect of low-intensity pulsed ultrasound on the biological behaviors of bone marrow mesenchymal stem cells on titanium with different surface topographies. *Am J Transl Res* 2018;10:67–76.
- [92] Amini A, Chien S, Bayat M. Impact of ultrasound therapy on stem cell differentiation - A systematic review. *Curr. Stem Cell Res. Ther.* n.d.;15:462–72. <https://doi.org/10.2174/1574888x15666200225124934>.

- [93] Lyu C, Tang D, Zhang C, Xie J, Zhang Q, Nie J, et al. Magnetic levitation for non-contact manipulation and measurement of cells. *Sens. Actuators B: Chem.* 2023; 385:133692. <https://doi.org/10.1016/j.snb.2023.133692>.
- [94] Kishimoto T, Masui K, Minoshima W, Hosokawa C. Recent advances in optical manipulation of cells and molecules for biological science. *J. Photochem. Photobiol. C: Photochem. Rev.* 2022;53:100554. <https://doi.org/10.1016/j.jphotochemrev.2022.100554>.
- [95] Lee S, Jo W, Chan Cho Y, Lee HH, Lee GW. Solution electrostatic levitator for measuring surface properties and bulk structures of an extremely supersaturated solution drop above metastable zone width limit. *Rev. Sci. Instrum.* 2017;88: 055101. <https://doi.org/10.1063/1.4982363>.
- [96] Hennet L, Cristiglio V, Kozaily J, Pozdnyakova I, Fischer HE, Bytchkov A, et al. Aerodynamic levitation and laser heating. *Eur Phys J Spec Top* 2011;196:151–65. <https://doi.org/10.1140/epjst/e2011-01425-0>.
- [97] Zang D, Yu Y, Chen Z, Li X, Wu H, Geng X. Acoustic levitation of liquid drops: dynamics, manipulation and phase transitions. *Adv Colloid Interface Sci* 2017; 243:77–85. <https://doi.org/10.1016/j.cis.2017.03.003>.
- [98] Vashi A, Yadav AS, Nguyen N-T, Sreejith KR. Parametric analysis of acoustically levitated droplet for potential microgravity application. *Appl. Acoust.* 2023;213: 109624. <https://doi.org/10.1016/j.apacoust.2023.109624>.
- [99] Andrade MAB, Pérez N, Adamowski JC. Particle manipulation by a non-resonant acoustic levitator. *Appl Phys Lett* 2015;106:014101. <https://doi.org/10.1063/1.4905130>.
- [100] Pazos Ospina JF, Contreras V, Estrada-Morales J, Baresch D, Ealo JL, Volke-Sepúlveda K. Particle-size effect in airborne standing-wave acoustic levitation: trapping particles at pressure antinodes. *Phys Rev Appl* 2022;18:034026. <https://doi.org/10.1103/PhysRevApplied.18.034026>.
- [101] Schlegel MC, Wenzel K-J, Sarfraz A, Panne U, Emmerling F. A wall-free climate unit for acoustic levitators. *Rev. Sci. Instrum.* 2012;83:055101. <https://doi.org/10.1063/1.4705968>.
- [102] Mondragon R, Hernandez L, Enrique Julia J, Carlos Jarque J, Chiva S, Zaitone B, et al. Study of the drying behavior of high load multiphase droplets in an acoustic levitator at high temperature conditions. *Chem Eng Sci* 2011;66:2734–44. <https://doi.org/10.1016/j.ces.2011.03.033>.
- [103] Park JH, Lee J-R, Park S, Kim Y-J, Yoon J-K, Park HS, et al. Subaqueous 3D stem cell spheroid levitation culture using anti-gravity bioreactor based on sound wave superposition. *Biomater Res* 2023;27:51. <https://doi.org/10.1186/s40824-023-00383-w>.
- [104] Tait A, Glynn-Jones P, Hill AR, Smart DE, Blume C, Hammarstrom B, et al. Engineering multi-layered tissue constructs using acoustic levitation. *Sci Rep* 2019;9:9789. <https://doi.org/10.1038/s41598-019-46201-z>.
- [105] Bouyer C, Chen P, Güven S, Demirtaş TT, Nieland TJF, Padilla F, et al. A bio-acoustic levitational (BAL) assembly method for engineering of multilayered, 3D brain-like constructs, using Human embryonic stem cell derived neuro-progenitors. *Adv Mater* 2016;28:161–7. <https://doi.org/10.1002/adma.201503916>.
- [106] Jeger-Madiot N, Arakelian L, Setterblad N, Bruneval P, Hoyos M, Larghero J, et al. Self-organization and culture of mesenchymal stem cell spheroids in acoustic levitation. *Sci Rep* 2021;11:8355. <https://doi.org/10.1038/s41598-021-87459-6>.
- [107] Olimpio RMC, de Oliveira M, Sibio MTD, Moretto FCF, Deprá IC, Mathias LS, et al. Cell viability assessed in a reproducible model of human osteoblasts derived from human adipose-derived stem cells. *PLoS One* 2018;13:e0194847. <https://doi.org/10.1371/journal.pone.0194847>.
- [108] Kulterer B, Friedl G, Jandrositz A, Sanchez-Cabo F, Prokesch A, Paar C, et al. Gene expression profiling of human mesenchymal stem cells derived from bone marrow during expansion and osteoblast differentiation. *BMC Genom.* 2007;8:70. <https://doi.org/10.1186/1471-2164-8-70>.
- [109] Akiva A, Melke J, Ansari S, Liv N, van der Meijden R, van Erp M, et al. An organoid for woven bone. *Adv Funct Mater* 2021;31:2010524. <https://doi.org/10.1002/adfm.202010524>.
- [110] Perier-Metz C, Duda GN, Checa S. Mechano-biological computer model of scaffold-supported bone regeneration: effect of bone graft and scaffold structure on large bone defect tissue patterning. *Front Bioeng Biotechnol* 2020;8. <https://doi.org/10.3389/fbioe.2020.585799>.
- [111] Alierta JA, Pérez MA, García-Aznar JM. An interface finite element model can be used to predict healing outcome of bone fractures. *J Mech Behav Biomed Mater* 2014;29:328–38. <https://doi.org/10.1016/j.jmbbm.2013.09.023>.
- [112] Comellas E, Shefelbine SJ. The role of computational models in mechanobiology of growing bone. *Front Bioeng Biotechnol* 2022;10.
- [113] García-Aznar JM, Nasello G, Hervás-Raluy S, Pérez MÁ, MJ Gómez-Benito. Multiscale modeling of bone tissue mechanobiology. *Bone* 2021;151:116032. <https://doi.org/10.1016/j.bone.2021.116032>.
- [114] dos Santos BC, Noritomi PY, da Silva JVL, Maia IA, Manzini BM. Biological multiscale computational modeling: a promising tool for 3D bioprinting and tissue engineering. *Bioprinting* 2022;28:e00234. <https://doi.org/10.1016/j.bprint.2022.e00234>.
- [115] Wang M, Yang N, Wang X. A review of computational models of bone fracture healing. *Med Biol Eng Comput* 2017;55:1895–914. <https://doi.org/10.1007/s11517-017-1701-3>.
- [116] A comparison of strain and fluid shear stress in stimulating bone cell responses—A computational and experimental study - McGarry - 2005 - The FASEB Journal - Wiley Online Library n.d. <https://faseb.onlinelibrary.wiley.com/doi/10.1096/fj.04-2210fje> (accessed October 27, 2023).
- [117] Computation | free full-text | computational modelling and simulation of scaffolds for Bone tissue engineering n.d. <https://www.mdpi.com/2079-3197/12/4/74> (accessed July 12, 2024).
- [118] Smit TH. Finite element models of osteocytes and their load-induced activation. *Curr Osteoporos Rep* 2022;20:127–40. <https://doi.org/10.1007/s11914-022-00728-9>.
- [119] Meslier QA, Shefelbine SJ. Using finite element modeling in bone mechanoadaptation. *Curr Osteoporos Rep* 2023;21:105–16. <https://doi.org/10.1007/s11914-023-00776-9>.
- [120] Zysset PK, Dall'Ara E, Varga P, Pahr DH. Finite element analysis for prediction of bone strength. *Bonekey Rep* 2013;2:386. <https://doi.org/10.1038/bonekey.2013.120>.
- [121] Parashar SK, Sharma JK. A review on application of finite element modelling in bone biomechanics. *Perspect. Sci.* 2016;8:696–8. <https://doi.org/10.1016/j.pisc.2016.06.062>.
- [122] Boccaccio A, Ballini A, Pappalettere C, Tullo D, Cantore S, Desiate A. Finite element method (FEM), mechanobiology and biomimetic scaffolds in bone tissue engineering. *Int J Biol Sci* 2011;7:112–32. <https://doi.org/10.7150/ijbs.7.112>.
- [123] Wu X, Wang X, Li C, Wang Z, Sun Y, Yan Y, et al. A fluid flow model in the lacunar-canalicular system under the pressure gradient and electrical field driven loads. *Appl Math Mech-Engl Ed* 2022;43:899–916. <https://doi.org/10.1007/s10483-022-2856-9>.
- [124] Zhou X, Novotny JE, Wang L. Anatomic variations of the lacunar–canalicular system influence solute transport in bone. *Bone* 2009;45:704–10. <https://doi.org/10.1016/j.bone.2009.06.026>.
- [125] Yang F, Yu W, Huo X, Li H, Qi Q, Yang X, et al. Effects of osteocyte shape on fluid flow and fluid shear stress of the loaded bone. *Biomed Res Int* 2022;2022: 3935803. <https://doi.org/10.1155/2022/3935803>.
- [126] Wittkowski C, Reilly G.C., Lacroix D, Perrault C.M. In vitro bone cell models: impact of fluid shear stress on bone formation. *Frontiers in bioengineering and biotechnology* 2016;4.
- [127] Anderson EJ, Knothe Tate ML. Idealization of pericellular fluid space geometry and dimension results in a profound underprediction of nano-microscale stresses imparted by fluid drag on osteocytes. *J Biomech* 2008;41:1736–46. <https://doi.org/10.1016/j.jbiomech.2008.02.035>.
- [128] Bacabac RG, Mizuno D, Schmidt CF, MacKintosh FC, Van Loon JJWA, Klein-Nulend J, et al. Round versus flat: bone cell morphology, elasticity, and mechanosensing. *J Biomech* 2008;41:1590–8. <https://doi.org/10.1016/j.jbiomech.2008.01.031>.
- [129] Verbruggen SW, Vaughan TJ, McNamara LM. Fluid flow in the osteocyte mechanical environment: a fluid–structure interaction approach. *Biomech Model Mechanobiol* 2014;13:85–97. <https://doi.org/10.1007/s10237-013-0487-y>.
- [130] Milan J-L, Planell JA, Lacroix D. Computational modelling of the mechanical environment of osteogenesis within a polylactic acid–calcium phosphate glass scaffold. *Biomaterials* 2009;30:4219–26. <https://doi.org/10.1016/j.biomaterials.2009.04.026>.
- [131] Zhao F., Vaughan T.J., McNamara L.M. Multiscale fluid-structure interaction modelling to determine the mechanical stimulation of bone cells in a tissue engineered scaffold 2015. <https://doi.org/10.1007/s10237-014-0599-z>.
- [132] Olivares AL, Marsal E, Planell JA, Lacroix D. Finite element study of scaffold architecture design and culture conditions for tissue engineering. *Biomaterials* 2009;30:6142–9. <https://doi.org/10.1016/j.biomaterials.2009.07.041>.
- [133] Jungreuthmayer C, Jaasma MJ, Al-Munajjed AA, Zanghellini J, Kelly DJ, O'Brien FJ. Deformation simulation of cells seeded on a collagen-GAG scaffold in a flow perfusion bioreactor using a sequential 3D CFD-elastostatics model. *Med Eng Phys* 2009;31:420–7. <https://doi.org/10.1016/j.medengphys.2008.11.003>.
- [134] Zhao F, Melke J, Ito K, van Rietbergen B, Hofmann S. A multiscale computational fluid dynamics approach to simulate the micro-fluidic environment within a tissue engineering scaffold with highly irregular pore geometry. *Biomech Model Mechanobiol* 2019;18:1965–77. <https://doi.org/10.1007/s10237-019-01188-4>.
- [135] Lacroix D, Prendergast PJ. A mechano-regulation model for tissue differentiation during fracture healing: analysis of gap size and loading. *J Biomech* 2002;35: 1163–71. [https://doi.org/10.1016/S0021-9290\(02\)00086-6](https://doi.org/10.1016/S0021-9290(02)00086-6).
- [136] Birmingham E, Niebur GL, McNamara LM, McHugh PE. An experimental and computational investigation of bone formation in mechanically loaded trabecular bone explants. *Ann Biomed Eng* 2016;44:1191–203. <https://doi.org/10.1007/s10439-015-1378-4>.
- [137] Baron C, Nguyen V-H, Naili S, Guivier-Curién C. Interaction of ultrasound waves with bone remodelling: a multiscale computational study. *Biomech Model Mechanobiol* 2020;19:1755–64. <https://doi.org/10.1007/s10237-020-01306-7>.
- [138] Rahimi A, Case N. Computational model to evaluate modulation of the acoustic field in an ultrasonic bioreactor by incorporation of a water layer bounded by an acoustic absorbent boundary layer. *Ultrasonics* 2020;103:106086. <https://doi.org/10.1016/j.ultras.2020.106086>.
- [139] Abbasi K, Janghorban M, Javanmardi F, Mobasseri S. Feasibility study of femur bone with continuum model. *J Med Eng Technol* 2023;47:355–66. <https://doi.org/10.1080/03091902.2024.2336512>.
- [140] Sanz-Herrera JA, Reina-Romo E. Continuum modeling and simulation in bone tissue engineering. *Appl. Sci.* 2019;9:3674. <https://doi.org/10.3390/app9183674>.
- [141] Zysset PK, Wolfram U. A rate-independent continuum model for bone tissue with interaction of compressive and tensile micro-damage. *J Mech Behav Biomed Mater* 2017;74:448–62. <https://doi.org/10.1016/j.jmbbm.2017.07.008>.
- [142] Kendall JJ, Ledoux C, Marques FC, Boaretti D, Schulte FA, Morgan EF, et al. An in silico micro-multiphysics agent-based approach for simulating bone regeneration in a mouse femur defect model. *Front Bioeng Biotechnol* 2023;11:1289127. <https://doi.org/10.3389/fbioe.2023.1289127>.
- [143] Faweya O, Desai PS, Higgs III CF. Towards an agent-based model to simulate osseointegration in powder-bed 3D printed implant-like structures. *J Mech Behav*

- Biomed Mater 2022;126:104915. <https://doi.org/10.1016/j.jmbbm.2021.104915>.
- [144] Boaretti D, Marques FC, Ledoux C, Singh A, Kendall JJ, Wehrle E, et al. Trabecular bone remodeling in the aging mouse: a micro-multiphysics agent-based in silico model using single-cell mechanomics. *Front Bioeng Biotechnol* 2023;11:1091294. <https://doi.org/10.3389/fbioe.2023.1091294>.
- [145] An G, Fitzpatrick BG, Christley S, Federico P, Kanarek A, Neilan RM, et al. Optimization and control of agent-based models in biology: a perspective. *Bull Math Biol* 2017;79:63–87. <https://doi.org/10.1007/s11538-016-0225-6>.
- [146] Bidan CM, Kommareddy KP, Rumpel M, Kollmannsberger P, Fratzl P, Dunlop JWC. Geometry as a factor for tissue growth: towards shape optimization of tissue engineering scaffolds. *Adv Heal. Mater* 2013;2:186–94. <https://doi.org/10.1002/adhm.201200159>.
- [147] Knychala J, Bouropoulos N, Catt CJ, Katsamenis OL, Please CP, Sengers BG. Pore geometry regulates early stage Human bone marrow cell tissue formation and organisation. *Ann Biomed Eng* 2013;41:917–30. <https://doi.org/10.1007/s10439-013-0748-z>.
- [148] Guyot Y, Papantoniou I, Chai YC, Bael SV, Schrooten J, Geris L. A computational model for cell/ECM growth on 3D surfaces using the level set method: a bone tissue engineering case study. *Biomech Model Mechanobiol* 2014;13:1361–71.
- [149] Guyot Y, Papantoniou I, Luyten FP, Geris L. Coupling curvature-dependent and shear stress-stimulated neotissue growth in dynamic bioreactor cultures: a 3D computational model of a complete scaffold. *Biomech Model Mechanobiol* 2016;15:169–80. <https://doi.org/10.1007/s10237-015-0753-2>.
- [150] Jeong D, Yun A, Kim J. Mathematical model and numerical simulation of the cell growth in scaffolds. *Biomech Model Mechanobiol* 2012;11:677–88. <https://doi.org/10.1007/s10237-011-0342-y>.
- [151] Carlier A, van Gastel N, Geris L, Carmeliet G, Oosterwyck HV. Size does matter: an integrative In vivo-In Silico approach for the treatment of critical Size bone defects. *PLoS Comput. Biol* 2014;10:e1003888. <https://doi.org/10.1371/journal.pcbi.1003888>.
- [152] Ghaffarizadeh A, Heiland R, Friedman SH, Mumenthaler SM, Macklin P. PhysiCell: an open source physics-based cell simulator for 3-D multicellular systems. *PLoS Comput. Biol* 2018;14:e1005991. <https://doi.org/10.1371/journal.pcbi.1005991>.
- [153] Multi-scale modeling of tissues using CompuCell3D - ScienceDirect n.d. <https://www.sciencedirect.com/science/article/abs/pii/B9780123884039000138?via%3Dihub> (accessed June 2, 2023).
- [154] Starrau J, de Back W, Bruschi L, Deutsch A. Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. *Bioinformatics* 2014;30:1331–2. <https://doi.org/10.1093/bioinformatics/btt772>.
- [155] Sasaki J-I, Hashimoto M, Yamaguchi S, Itoh Y, Yoshimoto I, Matsumoto T, et al. Fabrication of biomimetic bone tissue using mesenchymal stem cell-derived three-dimensional constructs incorporating endothelial cells. *PLoS One* 2015;10:e0129266. <https://doi.org/10.1371/journal.pone.0129266>.
- [156] Viceconti M, Pappalardo F, Rodriguez B, Horner M, Bischoff J, Musuamba Tshinanu F. *In silico* trials: verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. *Methods* 2021;185:120–7. <https://doi.org/10.1016/j.ymeth.2020.01.011>.
- [157] Lou S, Lv H, Li Z, Zhang L, Tang P. The effects of low-intensity pulsed ultrasound on fresh fracture. *Medicine (Baltimore)* 2017;96:e8181. <https://doi.org/10.1097/MD.00000000000008181>.
- [158] Romanò CL, Kirienko A, Sandrone C, Toro G, Toro A, Valente EP, et al. Low-intensity pulsed ultrasound in the treatment of nonunions and fresh fractures: a case series. *Trauma Care* 2022;2:174–84. <https://doi.org/10.3390/traumacare2020014>.
- [159] Rabiet L, Jeger-Madiot N, García DR, Tosca L, Tachdjian G, Kellouche S, et al. Improved functionality of hepatic spheroids cultured in acoustic levitation compared to existing 2D and 3D models. *Sci Rep* 2024;14:21528. <https://doi.org/10.1038/s41598-024-72059-x>.
- [160] Rabiet L, Arakelian L, Jeger-Madiot N, García DR, Larghero J, Aider J-L. Acoustic levitation as a tool for cell-driven self-organization of human cell spheroids during long-term 3D culture. *Biotechnol. Bioeng* 2024;121:1421–33. <https://doi.org/10.1002/bit.28651>.
- [161] Harrison A, Alt V. Low-intensity pulsed ultrasound (LIPUS) for stimulation of bone healing - A narrative review. *Injury* 2021;52(Suppl 2):S91–6. <https://doi.org/10.1016/j.injury.2021.05.002>.