



Review

Biomechanics in bone regeneration and mechanobiology in osteoblasts: Fundamental concepts and recent progress



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ABSTRACT

Bone is a hard, dynamically active tissue that continually rebuilds and maintains its mineral balance and structural integrity. Multiple mechanical properties of bone determine its ability to deform and fracture under load, as well as provide stable support and durability during motion. Meanwhile, the mechanical microenvironment within the bone can directly influence bone cells, whose perception of mechanical forces may enhance or inhibit various biological functions. Based on these mechanobiological processes, the mechanical design of biomaterials for bone repair and regeneration is becoming increasingly important. To achieve better clinical outcomes, bone repair materials should be selected based on the characteristics of the materials used in clinical application to achieve better outcomes. This review discusses the types of mechanical loading to which bone is subjected, how osteoblasts respond to such loading and the potential mechanisms involved, and the mechanical design of various biomaterials for bone repair. It then summarizes the progress made in bone-related biomechanical research and provides an outlook for future research efforts.

Introduction

Bone is a major supporting tissue that is dynamically remodeled throughout life [1]. Bone itself has numerous mechanical properties, including stiffness, viscoelasticity, strength, and fatigue, which underlie its support of motion and protection of vital organs. There is increasing evidence that mechanical stimulation plays a key role in bone remodeling, as physiological dynamic loading promotes bone formation and the absence of mechanical forces leads to bone loss [2]. Studying the mechanical properties of bone contributes to a deeper understanding of its behavior, which is essential for research on bone disease, fracture treatment, and bone engineering. Moreover, this knowledge aids the development of bone-substitute materials and improved treatments for bone repair and regeneration (see Scheme 1).

Bone remodeling is an ongoing dynamic process involving the destruction and regeneration of bone, playing a major role in maintaining bone structure and function, especially in the presence of bone defects. The bone matrix is composed of an organic component—primarily type I collagen—and an inorganic component in the form of hydroxyapatite

crystals, which undergo gradual mineralization [3,4]. Remodeling is ultimately mediated by cells within bone: osteocytes (maintaining bone homeostasis), osteoblasts (depositing bone matrix), osteoclasts (resorbing bone), and other interacting bone cells [5]. The mechanical microenvironment and cellular mechanobiology influence bone formation at multiple stages, beginning with mesenchymal stem cells (MSCs), which differentiate into skeletal progenitors, osteoblasts, and eventually osteocytes. This microenvironment affects cell behavior through biophysical, biochemical, and other mechanisms.

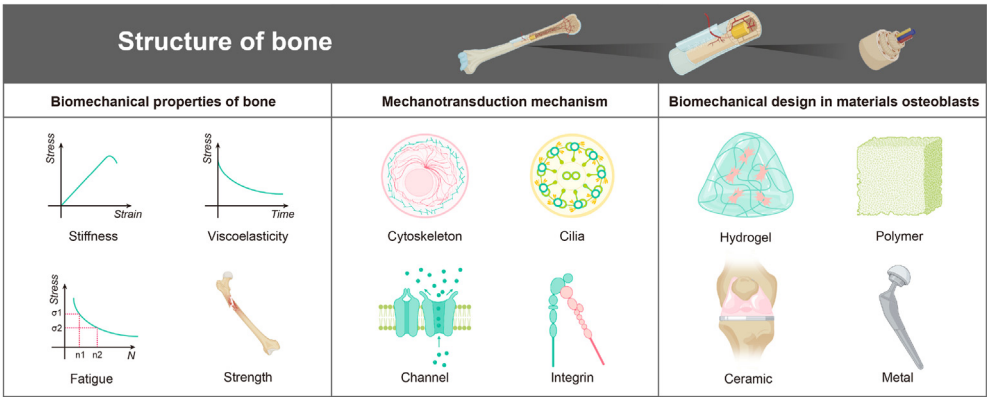
MSCs are pluripotent stem cells that can detect and respond to mechanical signals in their environment. Mechanical signals provided by the microenvironment to MSCs have a particularly pronounced effect on their physiological function, which can be converted into biochemical signals through mechanotransduction, affecting osteogenic differentiation, chondrogenic differentiation, and cell senescence. Mechanical stretching also influences the behavior of osteoblasts, promoting their differentiation via the extracellular signal-associated protein kinase (ERK) pathway and thus strongly impacting bone remodeling. Osteocytes exposed to mechanical stress produce signaling molecules that regulate the recruitment,

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Scheme 1. Biomechanics and mechanobiology of bone and biomechanical design of bone repair materials. The mechanical properties of bone include elastic modulus, viscoelasticity, strength, and fatigue properties. The mechanotransduction mechanism of bone cells includes cytoskeleton, channel, cilia, and integrin. The biomechanical materials include polymer, metal, ceramic, and hydrogel.

differentiation, and activity of osteoblasts and osteoclasts. Key signaling pathways activated by mechanical loading in osteocytes include the Wnt/ β -catenin and RANKL pathways, which are crucial regulators of bone metabolism. By adjusting bone formation and resorption, bone cells maintain optimal bone mass in response to mechanical stimuli. Coordinating the activities of osteoblasts, osteoclasts, and other bone cells preserves the dynamic balance of bone remodeling, with osteoblasts playing a central role [6,7]. Recent advances have clarified the cellular events triggered when bone is mechanically stimulated and how these lead to bone improvement. Cells reside in an extracellular matrix (ECM) composed of various proteins and polysaccharides; the tight connection between cells and ECM enables them to sense external changes. Mechanical forces generated through cell–matrix interactions are transmitted to membrane proteins, initiating intracellular signaling cascades that regulate cellular behavior [8]. Consequently, altering ECM mechanical properties elicits changes in cellular function and signal transduction. Not only do the ECM's own mechanical properties affect osteoblast behavior, but osteoblasts also respond to diverse mechanical stimuli—such as compressive forces and cyclic stretch—directly regulating the

production of ECM proteins through gene activation. In turn, this process influences osteoblast differentiation, growth, and apoptosis [9]. Currently, the main bone-repair materials include metals, bioceramics, polymers, tissue engineering, and related composite materials. Each category has advantages and limitations; therefore, clinical applications require optimization and adjustment to achieve superior outcomes. Biomechanical investigations of bone can guide the design of material properties that better facilitate bone repair.

Biomechanical properties of bone

The mechanical properties of bone include the elastic modulus (stiffness in the elastic region), viscoelasticity (time-dependent deformation during loading and unloading), strength (the maximum stress bone can withstand without fracturing), and fatigue properties (the ability to resist failure under repeated loading). These multi-scale properties determine the ability of bone to deform and fracture under load, providing stable support and durability during movement [10–13] (Fig. 1). They also directly influence the biological function of bone cells

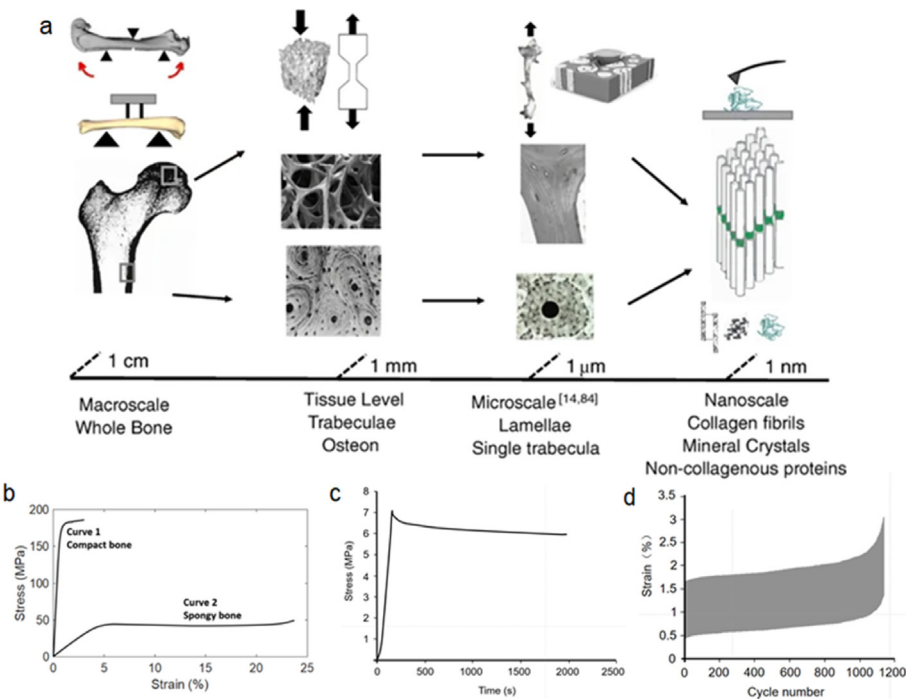


Fig. 1. Biomechanical properties of bone. (a) Mechanical properties of bone at different scales and corresponding mechanical test methods. Mechanical characterization of bone: state of the art in experimental approaches—what types of experiments do people do and how does one interpret the results? Current Osteoporosis Reports [10], Copyright 2018, with permission from an open-access source. (b) The typical stress–strain curves of compact and spongy bones. Mechanical properties of compact bone defined by the stress–strain curve measured using uniaxial tensile test: a concise review and practical guide. Materials (Basel) [11], Copyright 2021, with permission from an open-access source. (c) The stress relaxation curve of bone. New microscale constitutive model of human trabecular bone based on depth sensing indentation technique. Journal of the Mechanical Behavior of Biomedical Materials [12], Copyright 2018, with permission from an open-access source. (d) The cyclic strain curve of bone. An investigation of composition, morphology, mechanical properties, and microdamage accumulation of human type 2 diabetic bone. Bone [13], Copyright 2024, with permission from an open-access source.

by regulating how cells perceive and respond to mechanical loading, as well as influencing cell activity during bone remodeling. The stress and strain generated by exercise and weight-bearing can alter cell signaling and gene expression through intra- and intercellular interactions, ensuring stable bone function in a dynamic environment and promoting overall bone health.

Elastic modulus of bone

Bone is a heterogeneous, anisotropic material with mechanical properties that vary depending on the loading direction. The elastic modulus (ratio of stress to strain) is commonly used to describe bone stiffness or rigidity. At low stress levels, there is a linear relationship between stress and strain, known as the elastic region. Once this region is exceeded, the bone undergoes permanent deformation, referred to as the elastic limit or yield point. Stresses beyond the elastic limit can cause bone fractures. At the macro level, long bones are composed of cortical bone (dense bone) and trabecular bone (cancellous or spongy bone). Cortical bone has a dense structure with a porosity between 5 % and 30 %, while trabecular bone has a spongy, lightweight, cellular structure with a porosity of approximately 70 % [14]. Microscopically, bone can be classified as either primary (woven) bone, found in newly formed immature bone at fracture callus sites, or lamellar bone, a more mature form typically found in adults. The elastic modulus of osteonal bone in femur samples of children is lower than that of interstitial lamellae, with values of 14.1 ± 0.8 GPa and 15.5 ± 1.5 GPa, respectively. In the transverse direction, the modulus is 11.8 ± 1.7 GPa. In fibula specimens of children, osteonal bone has a modulus of 13.5 ± 1.6 GPa, while interstitial lamellae exhibit a modulus of 15.8 ± 1.5 GPa. Adult specimens have higher moduli, with osteons at 18.1 ± 2.6 GPa, interstitial lamellae at 21.3 ± 2.3 GPa, and the transverse direction at 13.8 ± 1.7 GPa. The elastic modulus of interstitial bone is significantly higher than that of osteonal bone in both adults and children, and the longitudinal modulus is consistently higher than the transverse modulus in both types of bone [15].

The relationship between tissue density and elastic modulus has been derived from micro-computed tomography (CT) bone scan data. The elastic modulus of bone is directly proportional to its calcium content, with tissue density significantly influencing the elastic modulus [16]. Bone density and elastic modulus are also related at different scales. At larger scales, they are positively correlated; however, bone density only partially explains the variance in elastic modulus. At the tissue scale, this relationship is more complex, with variations observed across studies [17,18]. The elastic modulus of cortical bone decreases with increased porosity across three types of bones: untreated, demineralized, and deproteinized. The untreated bone exhibits a higher modulus in the longitudinal direction compared to the transverse direction. Similarly, demineralized and deproteinized bone show higher longitudinal moduli than transverse moduli, but their values are lower than those of untreated bone. The elastic modulus of bone is linked to its structure and composition, with the greater longitudinal modulus attributed to the structural arrangement of bone trabeculae [19].

Viscoelasticity of bone

Viscoelasticity refers to the ability of a material to delay deformation and recover when subjected to stress or strain. Bone exhibits viscoelastic properties, including creep, relaxation, and dynamic response [20]. A constitutive model incorporating viscoelastic and viscoplastic components was developed to capture the mechanical behavior of cortical bone at different strain rates. Analysis of experimental data identified two viscoelastic mechanisms and one viscoplastic mechanism [21]. The relationship between viscoelastic damping in cortical bone and its microstructural and compositional characteristics was studied using 52 bone samples from 26 older pelvic bones. Resonant ultrasound spectroscopy was employed to measure viscoelastic damping, while

synchrotron radiation high-resolution computed tomography was used to analyze the vascular pore network structure and tissue mineral density. Simultaneously, Fourier transform infrared microspectroscopy quantified the mineral/organic phase ratio, mineral maturity, crystallinity, and collagen maturity, with biochemical methods used to evaluate collagen cross-linking. Results showed that viscoelastic damping increased with higher vascular porosity and decreased with reduced ECM mineralization, with minimal influence from collagen [22]. Studies on the viscoelasticity of human cortical bone and demineralized cortical bone have found that bone elasticity primarily originates from the mineral phase, while viscosity is mainly derived from the organic phase. Water content significantly influences bone viscoelasticity, and collagen molecular movement is a critical determinant of the viscoelastic response [23]. Research on human trabecular bone has investigated the relationship between tissue composition and viscoelasticity using experimental methods (nanoindentation, Raman spectroscopy, and micro-CT) and finite element analysis. Tissue-level viscoelasticity was found to be influenced by collagen content, collagen cross-linking, and crystallinity, while macroscopic (apparent) viscoelasticity was primarily affected by bone microstructure characteristics [24]. Wu et al. examined the viscoelastic properties of human cortical bone and explored the effects of sex and elastic moduli on these properties. The viscoelastic time constant was negatively correlated with elastic moduli, with females exhibiting a significantly higher intercept than males. However, no gender-related differences in collagen cross-linking were observed. This suggests that while gender influences the viscoelasticity of bone tissue, the specific mechanisms remain unclear [25]. The interactions between the ECM, bone, and implanted composite materials allow viscoelastic properties to co-regulate cell behavior and tissue regeneration [26]. For example, in fracture treatment, matching the viscoelasticity of the implant material to that of the bone and ECM can promote fracture healing and tissue regeneration. Furthermore, modulating the viscoelasticity of implant materials can optimize their interaction with surrounding tissues, thereby improving implant performance and tolerability [27,28].

Strength of bone

Bone strength refers to the ability to withstand fractures and is determined by various biomechanical properties. These include material properties—such as crystal structure, degree of mineralization, collagen properties, and osteoblast viability—and structural properties—such as cortical bone diameter, thickness, and porosity, as well as trabecular bone connectivity, anisotropy, thickness, stress concentration, and microfractures. These factors influence bone strength in different ways. Bone strength requires a balance between stiffness and flexibility to optimize fracture resistance. Biomechanical tests, such as compression and bending tests, are commonly used to assess bone strength. Understanding the fundamental biomechanical properties of bone helps determine fracture mechanisms and the potential for reduced fracture resistance [29]. Bone remodeling also affects bone structure and material properties by regulating bone resorption and formation rates, which directly or indirectly influence bone strength [30].

The mechanical properties of bone microstructures are anisotropic. Fibers in bone are arranged in parallel bundles, forming a layered structure. This arrangement provides higher strength under longitudinal (fiber-aligned) stress and lower strength under lateral (perpendicular) stress. For instance, the axial tensile strength is 0.35 ± 0.05 GPa under a strain of 1.8 ± 0.2 %, while the lateral strength is 0.13 ± 0.02 GPa under a strain of 1.3 ± 0.3 %. Bone also exhibits greater strength under compression than tension, with compressive strength reaching 0.75 ± 0.06 GPa along the major axis and 0.59 ± 0.04 GPa transversely [31,32]. Age and sex significantly influence bone stiffness and strength. Patton et al. reported the following examples: a 27-year-old female exhibited femur stiffness of 1391 N/mm and strength of 6103 N, whereas for a 90-year-old female, these values were 1429 N/mm and 2407 N. For a 33-year-old male, femur stiffness was 1317 N/mm and strength 6393 N,

while for a 77-year-old male, these values were 1429 N/mm and 4253 N. Bone stiffness and strength are generally higher in men and younger individuals compared to women and older individuals [33]. Physical activity enhances bone strength by improving bone microstructure and geometry, while prolonged sitting time negatively correlates with bone strength [34]. Metzner examined the compressive strength of cancellous bone using femoral borehole samples. The strength of “normal” bones (14.1 ± 7.3 MPa) was significantly higher than that of bones with “osteopenia” (6.8 ± 4.5 MPa) or “osteoporosis” (6.1 ± 3.1 MPa), demonstrating that increased severity of osteoporosis reduces bone compressive strength [35].

Bone strength is a key indicator for assessing fracture risk, but material properties like toughness and fatigue strength also significantly impact fracture susceptibility. Therefore, evaluating fracture risk requires considering not only bone density but also other material properties [36]. Tai et al. explored nanoscale sources of bone strength, finding that friction between mineral particles and cohesion within the organic matrix contribute to compressive yield strength. These findings emphasize the importance of bone structure in predicting fractures and developing clinical strategies to address biomechanical degradation [37].

However, while increased stiffness and hardness enhance bone strength, they reduce toughness and resistance to crack propagation. Water content acts as a plasticizer, improving ductility and contributing to toughness [38,39]. The stress intensity factor is often used to describe bone fracture toughness. Merlo investigated cortical bone using the four-point notch bending method, finding that stress intensity factors for vehicle-treated specimens were 4.98 ± 0.56 MPa, while ribose-treated specimens exhibited significantly lower values (2.20 ± 0.47 MPa). These results link increased advanced glycosylation end products with reduced cortical bone fracture toughness [40]. Gauthier compared fracture toughness at various anatomical sites (femoral diaphysis, femoral neck, tibial diaphysis, and radial diaphysis) under quasi-static and fall-like strain rates. Quasi-static fracture toughness values were 2.75 ± 0.48 , 2.46 ± 0.52 , 3.15 ± 0.60 , and 3.11 ± 0.56 MPa, respectively, with tibial and radial diaphysis showing significantly higher toughness than femoral sites. At fall-like strain rates, fracture toughness increased to 3.23 ± 0.56 , 3.15 ± 0.63 , 3.19 ± 0.51 , and 3.23 ± 0.70 MPa, respectively, with femoral sites showing a more significant increase than tibial and radial sites. No significant differences in toughness were observed across sites at fall-like strain rates [41].

Fatigue property of bone

Fatigue properties refer to the behavior and performance of bone under repeated loading. Repeated stress leads to minor bone damage and cracking, resulting in fatigue damage, which is a major cause of bone fractures [42]. Phase Space Warping (PSW) has been employed to monitor and predict bone fatigue properties and differentiate fatigue damage patterns. PSW-calculated eigenvectors reveal damage modes and their timescales, with high-frequency/fast timescales corresponding to brittle damage and low-frequency/slow timescales corresponding to ductile damage [43]. In one study, investigators performed *in vivo* fatigue loading on rat ulnas, inducing varying levels of fatigue damage through different loading cycles. Bone remodeling activation was assessed by measuring stiffness loss, microdamage content, and osteoblast integrity. Results showed that ulnas with both microdamage types exhibited greater stiffness loss compared to those with diffuse damage only [44].

Two osteoblast populations are associated with fatigue damage: apoptotic osteoblasts near fatigue cracks and surviving osteoblasts resistant to apoptosis. Fatigue loading induces osteoblast apoptosis and signaling activation, triggering bone resorption processes [45]. To evaluate fatigue damage in long bones, Bai used a two-dimensional finite difference time-domain method to simulate ultrasound wave propagation in bones with varying elastic moduli. Guided waves propagating in a solid plate, known as Lamb waves, are vibrations under non-tractive boundary conditions. Lamb waves are classified into antisymmetric

(A0, A1, A2 ...) and symmetric (S0, S1, S2 ...) types based on their vibration characteristics. In bovine bone plate experiments, the phase velocities of A1 and S1 modes decreased as fatigue damage increased. After 20,000 cycles of fatigue loading, both modes showed average velocities below those of intact bone. These findings suggest that ultrasound-guided waves could be a potential tool for assessing fatigue damage in long bones [46].

The effects of bone fatigue on microstructure and clinical parameters were assessed using uniaxial compression fatigue tests on isolated porcine vertebral samples, combined with dual-energy X-ray absorptiometry and micro-CT imaging. Sustained fatigue injuries were strongly correlated with bone microstructure, evidenced by reduced stiffness [47]. Vascular channels also influence bone fatigue life. For secondary bone, a linear relationship between vascular channel size and fatigue life is present, whereas no such relationship exists for reticular bone. Additionally, vessel size and volume under load were strongly correlated [48]. Studying bone fatigue provides insights into bone's adaptability and repair mechanisms under different stresses. This knowledge is critical for developing effective prevention and treatment methods, especially for athletes, the elderly, and individuals with osteoporosis, who are at higher risk of fatigue-related fractures.

The effects of external stress on bone vary at different stages. The healing process of a fracture can be divided into the following stages, each with its own specific characteristics and adaptations to external stress. During the hematoma mechanization phase (early stage) and primary scab formation phase (intermediate stage), external stress may cause displacement of the fracture end or aggravate the injury; therefore, strenuous exercise and weight bearing should be avoided. In the late stage, as the strength of the fracture end gradually returns, moderate external stimulation such as functional exercise can be gradually increased to promote fracture healing and functional recovery. In addition, the rate of fracture healing and adaptability to external stress may vary according to different fracture types, sites, and individual differences. Therefore, external stress stimulation must be appropriately controlled according to the fracture healing process and patient's specific situation to promote fracture healing and functional recovery.

Effects of different mechanical stimuli on osteoblasts

Types of bone cells

Bone is a tissue composed of different types of bone cells and ECM, and its active metabolism is regulated by various factors. The 4 cellular elements of bone are: osteoblasts, bone lining cells, osteocytes, and osteoclasts [49,50]. Further bone cell differentiation results from the presence of a variety of stem cells. Osteoblasts, osteocytes, and bone-lining cells are derived from MSCs known as osteoprogenitor cells, while osteoclasts are derived from hematopoietic stem cells [51]. Different types of bone cells are distributed in different parts of the skeleton with different structures and specific functions, and each plays an important role in bone growth, repair, remodeling, and regulation of calcium and phosphorus homeostasis. Skeletal metabolism and modeling processes are mainly dependent on the combined action of osteoclasts, which are responsible for the dissolution of bone salts and the breakdown of organic components of bone, and osteoblasts, which are responsible for the synthesis and secretion of organic components of the bone matrix and the formation of osteoids (Fig. 2) [52,53]. However, other types of bone cells (especially osteoblasts and bone lining cells) also influence the functions of osteoclasts and osteoblasts. These bone cells work together to ensure the health and stability of the skeleton, adapting to the body's growth, and changing its requirements. Chondrocytes and osteoblasts derived from bone marrow MSCs are involved in bone formation and repair and, to some extent, regulate the adaptability of the skeleton to different physiological demands. In contrast, osteoclasts derived from hematopoietic stem cells maintain mineral homeostasis by promoting bone resorption (mainly of cancellous bone) [49]. Osteoblasts

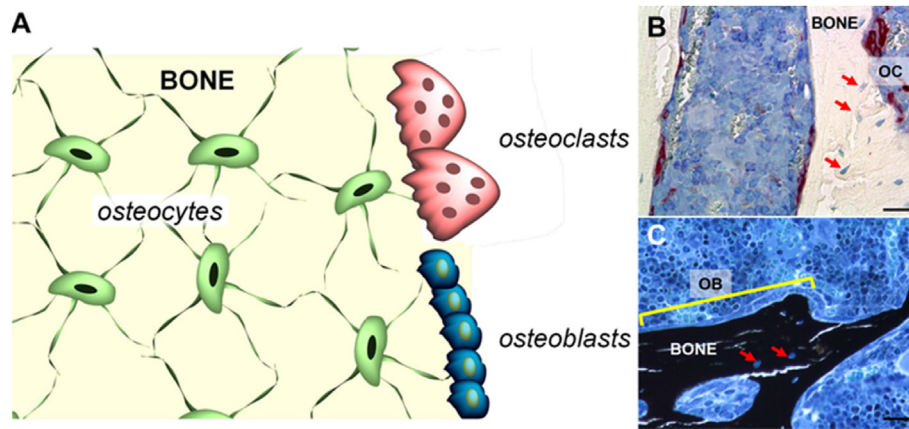


Fig. 2. The combined action of osteoclasts and osteoblasts on bone formation. Molecular signaling in bone cells: regulation of cell differentiation and survival. Adv Protein Chem Struct Biol [53], Copyright 2019, with permission from an open-access source.

(approximately 46 % of all bone cells) are monolayers of cells located on the bone surface that are responsible for the synthesis, secretion, and mineralization of the bone matrix. Osteoblasts undergo four stages of bone formation: proliferation, maturation, mineralization, and apoptosis. Factors such as the degree of differentiation and maturation of stem cells and the secretion of a series of regulatory cytokines can regulate the development and maintenance of bone size, shape, and integrity, ultimately regulating bone formation [54]. Therefore, the following discussion focuses on the effects of mechanical microenvironment on osteoblasts.

Effect of stiffness and viscoelasticity of ECM on osteoblasts

ECM stiffness has a significant effect on cell behavior and is a key stimulus for osteoblasts during bone tissue formation. Bone matrix synthesis mainly involves the differentiation, proliferation, and secretory activities of osteoblasts. Osteoblasts are the main functional cells involved in bone formation and differentiate from mesenchymal progenitor cells within the stroma of the inner and outer periosteum and bone marrow. The process of bone matrix synthesis is divided into two steps: (1) secretion of collagen and non-collagenous proteins; and (2) mineralization and formation of hydroxyapatite [49,55]. It is possible to culture neonatal rodent calvarial osteoblasts, which form mature bone nodules *in vitro* in 2D [56,57]. This process was improved when primary osteoblasts were cultured in dense collagen (10 %) scaffolds [57]. However, culturing primary osteoblasts in three-dimensional (3D) dense collagen resulted in a significant increase in mineralization and formation of large bone nodules compared with culturing in 2D [58]. Wu et al. first constructed cell 3D culture environments of varying hardness using GelMA hydrogels with different degrees of amino substitution. The hard matrix significantly promoted the expression of Piezo1 channels in osteoblasts, leading to increased cytosolic calcium influx [59]. Distinct differences in cell shape and vinculin expression were observed in osteoblasts grown on different polydimethylsiloxane (PDMS) substrates. The expression of alkaline phosphatase, Runx2, and osteocalcin was identified as a representative of OB function and showed a decreasing trend with substrate softening, which is associated with the Rho/ROCK signaling pathway [60]. The reduced stiffness of 3D gelatin hydrogels promotes osteogenic differentiation of MC3T3 cells and increases the deposition of minerals in primary osteogenic cells [60,61]. Gao prepared polyacrylamide (PAAm) substrates with varying stiffness and coated the surface of the substrate with type I collagen to simulate the microenvironment of bone regeneration at different stages. In addition, we found that focal adhesion formation was dependent on stiffness. When the stiffness of the substrate was increased from 15 to 150 kPa, the focal adhesions formed by pre-osteoblasts were longer [62].

Viscoelasticity is an important mechanical parameter with a time dimension that is mainly reflected in the stress-strain responses, such as stress relaxation, creep, and rate correlation. Compared with MG63 cells cultured on tissue culture polystyrene, osteoblast-like MG63 cells showed more than 3-fold higher alkaline phosphatase upregulation. Calcium phosphate nodules accumulated on the secreted ECM of MG63 cells grown on 3D “HAp/Col sponges”, a porous HAp/Col having sponge-like viscoelasticity [63]. Berner et al. reported the use of a viscoelastic sodium alginate-collagen interpenetrating network hydrogel for 3D cultures of mouse osteoblast-like IDG-SW3 cells. On day 14, a significant increase in some osteogenic markers (alkaline phosphatase [ALP], Dmp1, and hydroxyapatite) and intercellular gap junctions was observed in the slow relaxation hydrogel. Fast-relaxation hydrogels promote cell spreading at an early stage, whereas slow-relaxation hydrogels promote osteogenic differentiation [60].

Effect of external mechanical stimuli on osteoblasts

Mechanical signaling dominates bone formation, a process in which osteoblasts synthesize, secrete, and promote the mineralization of bone-like materials. Different mechanical effects regulate the biological properties of osteoblasts, such as proliferation, differentiation, and apoptosis, through hormones, cytoskeletal proteins, and microRNAs. Various mechanical effects regulate the biological properties of osteoblasts, such as proliferation, differentiation, and apoptosis, through hormones, cytoskeletal proteins, and microRNAs. Mechanical stimulation significantly downregulated *Sost* gene expression and sclerostin protein levels, while upregulating *Opg* gene expression, and increasing the OPG/RANKL ratio. Mechanical stimulation significantly downregulated *Sost* gene expression and sclerostin protein levels. Upregulated *Opg* gene expression and increased the OPG/RANKL ratio. The effect of compressive stress on osteoblasts depends on the intensity of the load and the time for which it is applied. Appropriate compressive stress promotes osteoblast proliferation and differentiation, which is beneficial for bone formation and repair. Excessive compressive stress can lead to osteoblast apoptosis and the destruction of bone tissue. The application of different types of tension to osteoblasts results in different biological effects. Studies have shown that strains in the range of 0–12 % have a stimulating effect on osteoblast proliferation. The mechanical environment within the mineralized ECM where bone cells reside is a dynamic combination of various biophysical stimuli [6]. Osteoblast-like cells were activated and ALP activity was increased after applying a mechanical strain of 2500 μe (0.25 %) at 0.5 Hz to MC3T3-E1 cells [64].

Studies have shown that compressive strain can stimulate osteogenic differentiation and mineralization of cells. Rath applied compressive strains of 10 % and 20 % to electrostatically spun polycaprolactone

scaffolds implanted with cranial osteoblasts and found that the 10 % compressive strain promoted the osteogenic differentiation of cells [65]. Osteoblasts mature on a compact osteoblast-derived ECM, whereas osteoclasts mature on a loose osteoclast-derived ECM. Furthermore, ECM crosslinking is a potential force for bone reconstruction [66].

Signaling pathways regulating osteoblast mechanobiology

Mechanical stimuli play a vital role in the mechanobiological signaling pathways in bone, where osteoblasts sense and respond to physical forces, thereby regulating osteogenesis and bone resorption. However, the exact underlying mechanisms remain unclear. One of the most critical issues in regulating bone metabolism—particularly in conditions like weightlessness during space flights or bone loss due to prolonged bed rest—is how bone cells sense, transmit, and resolve mechanical signals. Li's team screened several differentially expressed long-chain non-coding RNAs, including *Neat1*, through deep sequencing analysis of osteoblast RNAs in a microgravity rotation simulation. They found that *Neat1* exhibited the most significant changes. By simulating various mechanical stimuli—such as weightlessness, overweight conditions, fluid shear, and soft or hard matrix gels—they observed a positive correlation between *Neat1* expression and osteoblast function. The response of osteoblasts to mechanical stimuli was closely related to the number and morphology of paraspeckles, phase-separated structures formed by *Neat1*. Under rotational simulated weightlessness, the number and area of paraspeckles in osteoblast nuclei decreased. In contrast, under overweight conditions, the number and area of paraspeckles increased, with a cohesive state observed. Additionally, both fluid shear and hard matrix gels promoted the formation of paranuclear plaques.

Osteoblasts are the primary mechanosensory cells in bone tissue, sensing mechanical signals through the lacunar-canalicular network and regulating bone remodeling at the surface by releasing osteoanabolic molecules into the bone matrix. Key mechanosensors in osteoblasts include the cytoskeleton, dendritic processes, integrin-based focal adhesions, connexin-based intercellular junctions, primary cilia, ion channels, and the ECM (Fig. 3) [67,68]. The origin of the mechanical properties of the cell and its response to extracellular mechanical stimuli depends mainly on the cytoskeleton, in particular the mechanical

properties of the cytoskeleton [69]. The cytoskeleton comprises actin filaments, microtubules, and intermediate filaments. Microtubules are the main source of intracellular scaffolding and the direct transport of substances within the cell. Intermediate filaments provide the cell with tension and shear resistance, whereas microfilaments allow the cell to move and contract. Microfilaments, microtubules, and intermediate filaments are composed of protein monomers linked by non-covalent bonds to form multimeric proteins. Most multimeric fibrous proteins in the cell can be depolymerized and repolymerized and are therefore involved in the regulation of cellular functions. Although the three types of cytoskeletal filaments function differently in terms of the mechanical response of osteoblasts, they contribute to the mechanical properties of the cells.

In osteoblasts, primary cilia are essential sensors of mechanical stimuli, coordinating load-induced bone adaptation [70]. Acting as hubs for cellular signaling integration, primary cilia are involved in multiple signaling pathways that regulate processes such as cell proliferation, differentiation, migration, and metabolism. They sense mechanical stimuli, such as pressure and fluid shear, and translate these signals into intracellular pathways that drive adaptive responses. For instance, fluid shear induces membrane depolarization in osteoblasts, activating voltage-gated ion channels and Ca^{2+} channels, ultimately increasing intracellular calcium concentration and promoting calcification [71]. Circulatory stress also enhances matrix mineralization in adult MSCs through extracellular signal-regulated kinase pathways [72]. Compared to motor cilia, the axonemal structure of primary cilia lacks components such as kinesin arms and connecting chains, resulting in lower bending stiffness [73]. When exposed to fluid shear, primary cilia upregulate the expression of factors involved in bone matrix synthesis, including cyclooxygenase-2, osteopontin, and prostaglandin E2 [74]. Microgravity inhibits osteogenic differentiation by reducing primary cilia formation, suggesting that primary cilia could serve as therapeutic targets to prevent microgravity-induced bone loss [75].

Integrins are transmembrane heterodimers composed of two non-covalently bound transmembrane subunits: the α subunit is responsible for extracellular ligand specificity, while the β subunit contributes to internal signaling pathways [76]. As transmembrane junctions, integrins act as bidirectional liaisons between the ECM and the intracellular actin

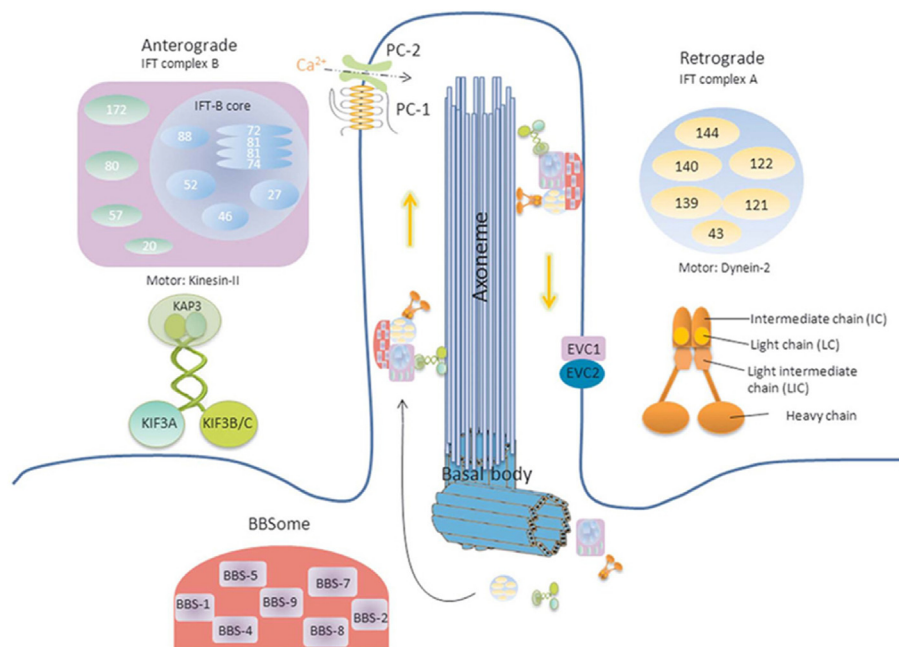


Fig. 3. The primary cilia in Osteoblasts. Function and regulation of primary cilia and intraflagellar transport proteins in the skeleton. Ann N Y Acad Sci. Copyright 2015 [68], with permission from an open-access source.

cytoskeleton, linking the ECM to the intracellular cytoskeletal network. In skeletal tissues, integrins are commonly expressed in a wide range of osteoblasts, with integrin $\beta 1$ predominantly found on osteoblast cell bodies and integrin $\beta 3$ predominantly found in cell processes [77]. When the extracellular structural domain of an integral protein binds to an extracellular ligand, it induces a conformational change at the end of the intracellular structural domain of the integral protein, which in turn alters the interactions of the end of the cytoplasmic structural domain of the integral protein with neighboring proteins, as in the case of the adhesion plaque kinase (FAK) effect, and as a result FAK induces a number of proteins and a cascade of signal amplification, causing a cascade reaction for signal amplification [78,79]. In contrast, increased amounts of integrins promote phosphorylation of focal adhesion kinase (FAK) phosphorylation thereby regulating cell differentiation [80]. Both integrin $\beta 1$ and $\beta 3$ are critical for mechanistically stimulated transduction of osteoblasts. When mouse osteoblast-like cells were stably transfected with vectors expressing a dominant-negative vector containing only the transmembrane structural domain of integrin $\beta 1$ and the $\beta 1$ subunit of the cytoplasm ($\beta 1DN$), the adhesion plaque (integrins are the major component of the adhesion plaque) complex was reduced [81]. Integrins $\beta 1$ and $\beta 3$ are associated with the formation of osteocyte adhesion plaque complexes and mechanostimulation transduction. Experimental studies targeting integrins in osteoblasts *in vivo* are scarce, using mostly mice with specific integrin $\beta 1$ knockout. Reduced bone formation after osteoblast-specific integrin $\beta 1$ knockout suggests that integrin $\beta 1$ plays an important role in mechanical stimulus-induced bone formation [82].

Piezo possesses a trimeric three-bladed propeller-like ion channel with a central pore for ion entry and exit, and three peripheral paddles for sensing mechanical stimuli [83]. Piezo1 is mainly distributed in non-sensory tissues and can sense a variety of stresses, including compressive, tensile, and shear stresses [84]. Piezo1 and Piezo2 are mainly expressed in osteoblasts, while Piezo1 is also expressed in hypertrophic chondrocytes, suggesting that they may be involved in the transduction of mechanical stimuli during skeletal development [85]. Piezo1 can act through several different pathways, including the Akt-Sost and YAP/TAZ-Wnt pathways, to regulate the biological behavior of osteoblasts [86]. In response to mechanical stimulation, Piezo1 in osteoblasts regulates the expression of different proteins (including several collagens) in the bone matrix via the YAP signaling pathway. Piezo1 regulates the expression of Wnt1 and other osteogenic genes in osteoblasts via YAP1 and TAZ [87].

The piezoelectric effect significantly influences cellular activation when mechanical forces are applied to bone collagen. Mechanosensitive ion channels, including piezoelectric channels, are crucial for transmitting mechanical signals. The activation of voltage-gated calcium channels triggers a cascade of signaling pathways, promoting extracellular calcium influx and activating calmodulin. This, in turn, stimulates the Ras-ERK signaling pathway, which activates Runx2 and induces growth factors essential for osteogenesis. The piezoelectric effect enhances bone cell sensing, accelerates repair, and maintains the electrophysiological microenvironment critical for shaping, remodeling, and functional performance. Electroactive materials used in bone repair scaffolds can mimic the electrical properties of bone tissue, providing necessary physiological signals for regeneration [88]. Biocompatible piezoelectric materials, which convert mechanical deformation energy into surface charges, represent a promising class of smart materials. Their electrical signals promote tissue regeneration through specialized ECM pathways, offering potential applications in advanced bone repair [89, 90].

Biomechanical design in biomaterials for bone repair

Currently, the main bone repair materials include metal materials, bioceramics, polymer materials, tissue engineering, and related composite materials (Fig. 4). Different materials have certain advantages and limitations; therefore, it is necessary to optimize the selection according

to the characteristics of various materials in clinical applications to achieve better clinical results [91].

External stress (mechanical stimuli), the ECM, and implanted materials influence bone regeneration, and there is a stacking effect of these different factors. External stress influences the bone remodeling process by stimulating the periosteum and regulating the activity of osteoblasts and osteoclasts. The extracellular matrix, through its complex structure and function, provides a favorable microenvironment for the formation of new bone tissue, and implant materials serve as an important tool to support and guide the growth of new tissue. The interactions and synergies among these three elements promote the smooth progression of bone regeneration. The mechanical adaptation of implanted materials is also critical and must match the mechanical environment of the bone. If the mechanical properties of the implanted material are significantly different from those of the surrounding bone tissue, stress concentration, material loosening, or fracture can occur.

Metal materials

Metals are the main choice for load-bearing implants because of their high tensile and compressive strengths, and good impact resistance, ductility, stability, and reliability [92]. Typically, these materials include stainless steel, cobalt-chromium (Co-Cr) alloys, titanium (Ti), and titanium alloys [93,94]. Stainless steel is mainly used to make parts such as splints, bone hooks, and bone screws for the fixation of fracture sites, and is also used to make artificial bones and joints. Although the Co-Cr alloy has excellent fatigue and wear resistance, it is currently a better orthopedic alloy material. Ti and its alloys have the best corrosion resistance, but their wear resistance is poor, and artificial joint wear debris accumulates in the body, causing human tissue mutations [95]. Several Ti alloys, such as Ti-6AL-4V and Ti-6AL-7Nb, have sufficient strength (0.8–1.3 GPa) and corrosion resistance, which make titanium and its alloys favorable for load-bearing applications [96]. However, the high cost, poor wear properties, diffusion of oxygen to Ti during manufacturing and heat treatment, and Ti embrittlement due to dissolved oxygen need to be addressed [97]. The difference in elastic modulus between metal materials and bone causes changes in the mechanical stress field when the implant is subjected to physical stress instead of bone, resulting in adaptive remodeling, local bone density reduction, implant loosening, and even failure [98]. In metal implants, titanium alloys have a lower modulus of 50–118 GPa, but are still 2–5 times higher than that of sclerocortical bone (10–30 GPa) and much higher than that of cancellous bone (1–15 GPa). The elastic modulus of the metal implant should be similar to that of the natural bone to minimize stress shielding. In addition, adverse effects such as fatigue fracture and corrosion caused by metallic materials implanted into the human body need to be reduced [96]. Porous metal implants made with additives to achieve a low modulus of elasticity allow new bone tissue to grow inwards and blood vessels to form. The final density, pore size, material type, and preparation parameters significantly affect the mechanical properties of the porous scaffolds [83]. For example, the strength of spongy bionic titanium scaffolds decreases from 120 MPa to 35 MPa when the porosity increases from 55 % to 75 % [98].

Polymer materials

Polymers generally refer to macromolecules composed of repeated monomers by covalent bonds, which can control the shape, structure, and chemical composition of the implant, and can be used to manufacture biological scaffolds as artificial biological materials [99]. Depending on the source, polymers can be classified as natural or synthetic. Biodegradable polymers can undergo structural damage in the physiological environment, and the degradation products can be absorbed by the body or excreted through normal metabolism, which is crucial for bone defect repair [100]. Natural biodegradable polymers such as cellulose, hyaluronic acid, collagen, gelatin, and sodium alginate have been widely

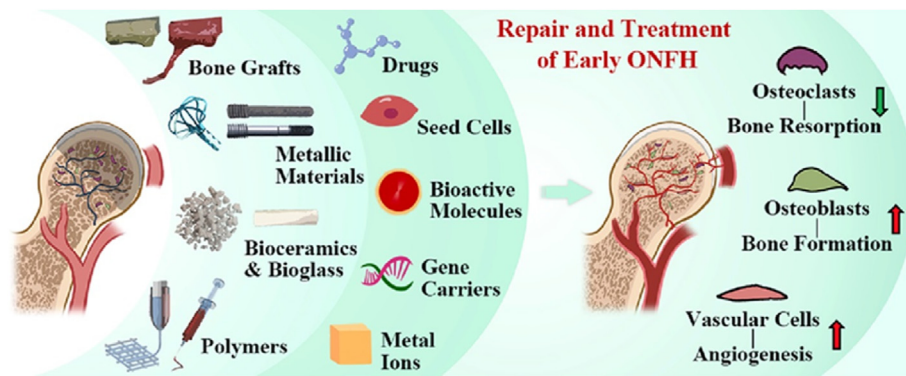


Fig. 4. The main biomaterials for the bone repair. Application of biomaterials in treating early osteonecrosis of the femoral head: Research progress and future perspectives. *Acta Biomaterialia*. Copyright 2023 [91], with permission from an open-access source.

investigated for use as bone-defect repair materials because of their good physicochemical and excellent biological properties. However, they also have some shortcomings, such as unstable sources, difficulty in controlling and predicting the degradation rate *in vivo*, poor mechanical properties, and possible immunogenicity [101,102].

Unlike natural polymers, synthetic polymers have more possibilities for chemical modification and can be adapted for specific applications. The advantages of synthetic polymers ensure their ability to be customized and designed for different needs at the time of application, including their mechanical and biological properties. Methyl methacrylate (PMMA) is a commonly used biomaterial as bone cement, which has the advantages of convenient preparation and injection, and strong biomechanical properties; however, there are some inherent and unavoidable defects in its clinical application, such as easy leakage during injection, resulting in spinal cord and nerve damage, poor integration with bone tissue, easy loosening and falling off of the prosthesis, excessive mechanical strength, which can easily cause fractures of adjacent vertebrae, and poor biological activity, which cannot induce the growth of new bone tissue in the human body. Therefore, by modifying PMMA bone cement and adding other materials, the mechanical properties of PMMA are improved and its biological activity is enhanced; for example, researchers reduce the elastic modulus of PMMA bone cement by adding hyaluronic acid to PMMA and using sodium hyaluronate aqueous solutions with different volume fractions to combine PMMA with PMMA to produce porous bone materials, which can reduce the hardness of the modified PMMA, and the texture becomes softer, close to human cancellous bone, which is more suitable for clinical practice than conventional PMMA. Researchers have used mineralized collagen particles to modify PMMA to test the surgical operability, mechanical properties, and biological activity of composite bone materials, which not only changed the hardness of PMMA, but also endowed PMMA with the ability to guide bone tissue regeneration and reduce the incidence of prosthesis loosening.

Bioceramic materials

Ceramics are biomaterials with crystalline or partially crystalline structures that are rigid and brittle. Their elements and structure confer temperature resistance, desirable mechanical properties, and excellent chemical resistance. In general, the chemical elements used to make ceramics come from a small subset of the periodic table—mainly aluminum oxide, zirconium oxide, carbon, silicon, and calcium [102, 103]. Because ceramics are both biologically functional and biocompatible, apatite can form on their surface after implantation, allowing better adhesion between internal tissues and the implant.

Hydroxyapatite (nHAp) is a calcium phosphate bioceramics containing CaO and P₂O₅, an important inorganic component for bone regeneration. After being implanted into the human body, nHAp's surface can

bond with human tissues to achieve strong affinity, making it widely used in biomedical applications and bone defect repair. Among various forms, nanoscale HAp exhibits higher surface activity and enhanced resorbability and biological activity; it is one of the most common nanomaterials in bone tissue engineering, and its preparation methods and applications have been extensively studied [104,105]. However, conventional nHAp repair materials differ from natural bone in orientation, size, and crystallinity, leading to issues like low strength, poor toughness, and difficulties in molding. Additionally, nHAp nanoparticles tend to agglomerate due to their large surface energy, resulting in poor material stability and rapid degradation of mechanical strength after implantation, which limits clinical use [106]. Although multifunctional nHAp loaded with various growth factors or bone-promoting drugs can partly address these shortcomings, traditional physical and chemical modification methods are cumbersome and may interfere with nHAp's biological activity. In recent years, biomimetic synthesis methods leveraging the molecular recognition and self-assembly capabilities of biological macromolecules or living microorganisms have simplified production while yielding more stable nanomaterials.

Bioglass materials

Bioglass is a biomaterial known for its excellent degradability and stability. Bioactive glasses primarily composed of CaO, SiO₂ and P₂O₅ offer high bioactivity, osseointegration, and mechanical properties, making them widely used in bone repair. Recent findings highlight bioactive glass's unique osteoinduction capability to upregulate host osteogenic genes and guide bone growth. It also possesses biodegradability, biocompatibility, and bone conduction properties. However, many commercial bioactive glass products still do not meet the standards required for ideal bone tissue repair materials [107]. Studies have shown that adding boron can enhance the performance of bioactive glass. Using a high-temperature melting process, base glass with different mass fractions of B₂O₃ was fired, and its mechanical properties were tested using uniaxial compression and three-point bending. Increasing the B₂O₃ mass fraction raised the compressive and flexural strengths of the porous bioactive glass scaffold, gradually improving its mechanical performance.

When boron is incorporated into bioactive glass, boron ions act as network formers that bind to and occupy interstitial sites within the glass matrix, producing B–O–B and Si–O–B bonds, thus increasing structural density. Boron doping also improves crystallization in the glass matrix, converting part of the calcium silicate phase into a sodium borosilicate phase with higher strength and a dense microstructure, which boosts the glass's mechanical properties [108]. In addition, unlike silicate glass and phosphate glass, the network modifier Na₂O in boron-containing bioactive glass does not initially convert bridge-oxygen bonds into non-bridge-oxygen bonds, but instead makes the boron in the network

structure exist in the form of a boron-oxygen triangle (BO_3^{3-}) group, and with the gradual incorporation of boron, a BO_4 group emerges, which has stable properties and effectively enhances the mechanical properties of the glass structure [109]. In addition, annealing can affect the mechanical properties of bioactive glass scaffolds. In this experiment, the glass powder attached to the sponge skeleton needs to be reheated between the softening temperature and the initial temperature of crystallization, and the mechanical properties of the glass are changed after the annealing process. However, unlike the decrease in the mechanical properties of the crystalline material after annealing, the mechanical properties of the bioactive glass are improved because, for the crystalline material, the crystal density will increase owing to the increase in bond density (structural density) after annealing, which reduces the mechanical properties of the material. In contrast, annealing improved the mechanical properties of the glass by increasing its structural density without affecting its density.

Hydrogel materials

Tissue engineering using hydrogels has emerged as an attractive strategy for bone tissue reconstruction [110]. Hydrogels can be derived from both natural and synthetic materials. Natural hydrogels primarily include collagen, chitosan (CS), and hyaluronic acid, while synthetic hydrogels encompass polyvinyl alcohol (PVA), polyacrylamide, and polyethylene glycol [111,112]. Natural materials vary in molecular weight, influencing the mechanical properties of the gels. Higher molecular weights form stiffer network structures that provide better mechanical support. Cross-linking and other material interactions play critical roles in determining implantable materials' mechanical properties and biocompatibility. Cross-linking strengthens and toughens the material, while beneficial interactions enhance its stability and reduce complications [113]. By adjusting the degree of cross-linking and interaction, implantable materials with excellent mechanical properties and biocompatibility can be achieved. Synthetic materials depend on the highly oriented structure of polymers, which influences the hydrogel's mechanical behavior. Natural materials generally have superior biological activity but weaker mechanical properties, whereas synthetic materials often exhibit stronger mechanical performance but lower biological activity. Combining these two material types can improve both mechanical properties and biocompatibility, ensuring that hydrogels meet different application requirements [114].

Hydrogels used in bone tissue engineering require excellent mechanical properties, such as strength and elasticity, to ensure that the tissue has appropriate support properties after repair. Currently, it is difficult for some hydrogels to meet the requirements of bone-defect repair, especially at bone-defect sites subjected to high loads or complex loading environments. To overcome these shortcomings, researchers are continuously researching and developing new materials to improve the mechanical properties, increase the bond strength with the bone substrate, optimize the degradation properties, and enhance bio-responsiveness to meet the needs of bone defect repair. Xu et al. prepared a new porous polyvinyl alcohol/sodium alginate/hydroxyapatite (PVA/SA/HA) composite hydrogel with a tunable structure and mechanical properties using the double cross-linking method [115]. By adjusting the mixing ratio of each component, the elastic modulus and compressive strength of the hydrogel were improved compared to those of conventional hydroxyapatite scaffolds. Its excellent compressive strength makes it a promising hydrogel material for bone tissue engineering applications. Another approach involved dopamine-modified nano-HA, introduced into a Schiff base reaction between sodium alginate oxide and gelatin under mild conditions. The resulting injectable hydrogel reached about 0.5 MPa in compressive strength at 80 % deformation, effectively improving rabbit femoral condyle defect repair [116]. In another study, an injectable chitosan/silk fibroin/bioactive glass nanoparticle hydrogel (prepared via a thermal triggering method) significantly promoted osteogenic and angiogenic differentiation of

MC3T3-E1 cells, improving bone defect repair *in vivo*. The bioactive glass enhanced the hydrogel's mechanical properties, encouraging BMSC osteogenic differentiation [117]. Bi et al. constructed a dual-network hydrogel by physically cross-linking medical-grade PVA and chitosan in a potassium hydroxide/urea dissolution system, yielding a material with high compressive strength that effectively repaired bone defects in rabbits [118]. Zhang and colleagues prepared a double-network hydrogel containing magnesium ions via optical cross-linking of methacryloyl gelatin, thiolated chitosan, and modified polyhedral oligomeric sesquioxane nanoparticles. Active magnesium ions were introduced through coordination bonds, significantly improving compressive and tensile properties compared to a single-network hydrogel. This enhanced mechanical performance promoted BMSC proliferation, osteogenic differentiation, and skull defect repair in rats [119].

Conclusion

This study systematically describes the mechanical properties of bone and their roles in the regulation of osteoblast function and related mechanisms to provide guidance for the mechanical design of bone repair materials. Not only can the mechanical properties of the bone itself affect the overall function, but the mechanical properties of its ECM can also directly affect the mechanical microenvironment around cells, thereby regulating the biological functions of osteoblasts. However, there are still many unresolved roles and mechanisms in the synergistic regulation of osteoblast function by ECM mechanical properties and external loading. Further studies on the mechanisms by which the mechanical properties of ECM regulate cells will provide new ideas and avenues for therapeutic approaches to tissue regeneration and repair. The study of bone mechanics can be used to guide the design and optimize the selection of restorative materials in terms of their properties to achieve better clinical outcomes.

CRedit authorship contribution statement

Wenbin Cai: Writing – original draft, Data curation, Conceptualization. **Yaya Huo:** Writing – original draft, Data curation. **Yu Liu:** Writing – original draft, Data curation. **Yan Su:** Validation, Data curation. **Haobo Guo:** Validation, Data curation. **Lijie Wang:** Conceptualization. **Bin Li:** Writing – review & editing, Funding acquisition, Conceptualization. **Ting Liang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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