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**Review**

# Multi-omics insights into bone tissue injury and healing: bridging orthopedic trauma and regenerative medicine

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## Abstract

To preserve functionality, bone is an active tissue that can constantly reconstruct itself through modeling and remodeling. It plays critical roles in the body, including maintaining mineral homeostasis, serving as the adult human body's core site of hematopoiesis, and supporting the structures of the body's soft tissues. It possesses the natural regeneration capacity, but large and complex lesions often require surgical intervention. Multiple omics integrate proteomics, metabolomics, genomics, and transcriptomics to provide a comprehensive understanding of biological processes like bone tissue injury and healing in bone tissue regeneration and engineering. Recently, bone tissue engineering and regenerative medicines have offered promising tools for bone regeneration using a multi-omics approach. Thus, this article will highlight the role of multiple omics in understanding bone tissue injury and healing. It will discuss the role of bone tissue engineering in developing bone substitutes that can replace translational medicine. Lastly, new developments in bone tissue engineering and regenerative medicine, along with multi-omics approaches, offer promising tools for bone regeneration.

**Keywords:** Multi-omics; Bone tissue healing; Orthopedic trauma; Regenerative medicine

### Highlights

- Three-dimensional cultures of osteogenic and chondrogenic cells provide a tissue engineering approach that mimics bone and cartilage *in vitro*, offering new insights into bone and cartilage regeneration.
- Polymer-based drug delivery systems show promise for bone tissue regeneration, with ongoing research exploring their progress, prospects, and potential applications.
- Strontium functionalization of biomaterials enhances osteogenic differentiation, making them a promising option for bone tissue engineering purposes.
- Multi-omics approaches, including materiomics and mechanomics, offer new perspectives for understanding bone biology and engineering bone tissue, with implications for regenerative medicine and disease treatment.
- The integration of clinical phenomes with molecular multi-omics, known as clinical trans-omics, presents a comprehensive approach for understanding and treating complex bone diseases, offering new avenues for personalized medicine.

## Background

The organs of the skeletal system, primarily bones, provide form, protection, and mechanical support for the body while facilitating movement. Additionally, recent studies indicate that bones contribute to the body's mineral homeostasis and are involved in the endocrine regulation of energy metabolism. Bone tissue is an active organ with substantial regenerative capacity. It undertakes various essential biological functions, such as maintaining hematopoietic cell homeostasis, shielding internal organs, and serving as a reservoir for mineral replenishment [1,2]. Despite bones' impressive regenerative abilities, particularly in chronic conditions or after severe injuries, a better comprehension of the fundamental molecular mechanisms is necessary. This highlights the importance of advanced biological tools, such as multi-omics, which aid in unraveling the cellular and molecular processes that transpire during bone injury and repair. Multi-omics is a rapidly developing field that combines multiple "omics" technologies to analyze extensive biological data concurrently. It aligns with the principles of systems biology. This approach employs various bioinformatics tools to investigate genomes, proteomics, metabolomics, and transcriptomics in conjunction with other omics disciplines (such as microbiomics) to obtain a thorough understanding of intricate biological systems (Figure 1) [3,4]. Integrating multi-omics data allows researchers to create a comprehensive and potent understanding of human health and disease. Each omics layer offers distinct insight, and their interconnections yield a richer comprehension of the intricate molecular processes underlying diverse conditions [5].

The advent of high-throughput technologies, including liquid chromatography–mass spectrometry (LC-MS), RNA sequencing (RNA-seq), whole genome sequencing, and

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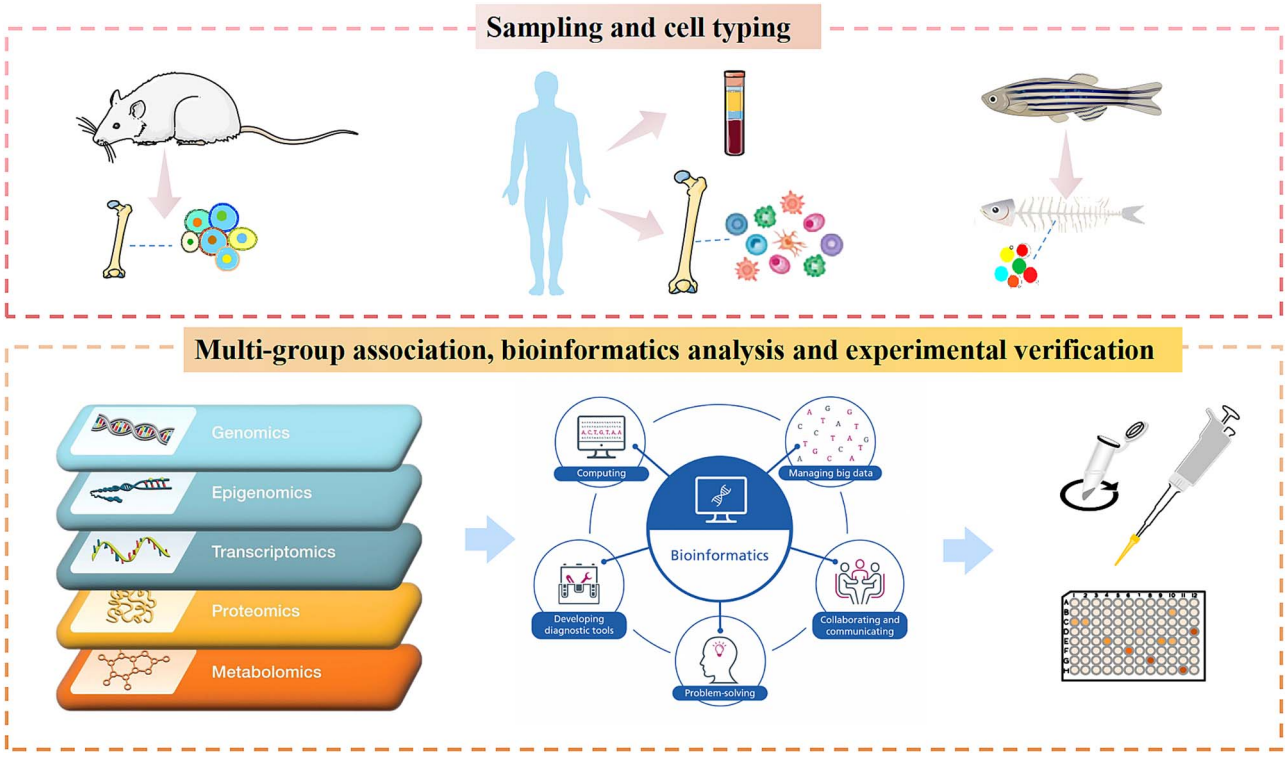


Figure 1. A range of specially designed bioinformatics tools exist to delve into the complex details of bone diseases by studying various areas of biology. These tools play an important role in research in other areas of omics such as genomics, proteomics, metabolomics, and transcriptomics. By utilizing these advanced technologies, researchers can gain a comprehensive understanding of the genetic makeup, protein interactions, metabolic pathways, and gene expression patterns associated with bone diseases. This holistic approach enables in-depth analysis and better understanding of the complex biological processes that lead to the onset and development of bone-related diseases (by Figdraw 2.0)

reduced-representation bisulfite sequencing, has significantly advanced our capability to perform in-depth analyses of diverse molecular characteristics across multiple omics levels. These advanced techniques allow researchers to gather and interpret vast amounts of biological data that were previously inaccessible, thus paving the way for more in-depth studies of complex biological systems. In parallel to these technological advancements, numerous statistical integrative methods have been developed to effectively combine the molecular biomarkers identified through independent analyses of each omics layer. This integration facilitates a more cohesive understanding of the data and leads to the holistic discovery of critical biological insights. Ultimately, by combining these efforts, we can better comprehend the complex molecular networks and pathways that lead to the development and progression of human diseases, which in turn helps us understand the mechanisms behind them.

Throughout the postnatal stage, bone, known as metabolically active tissue, goes through constant cycles of regeneration and resorption to substitute and repair the skeleton [6]. The "remodeling" process is regulated by the coordinated actions of bone-resorbing osteoclasts, bone-forming osteoblasts, and mechanosensitive osteocytes. In skeletal disorders, ranging from metabolic conditions causing bone loss like osteoporosis to rare high bone mass diseases such as sclerosteosis, there is an imbalance in the number and activity of bone cells [7]. Recent breakthroughs in isolating bone cells and high-resolution omics technologies have enabled researchers to systematically and objectively examine bone biology. Most studies in bone omics have utilized a singular omics platform to assess qualitative and quantitative variances in genes, epigenetics, RNA transcripts, proteins, and metabolites. While each omics platform can provide insights into a bone cell's lifespan or disease condition, they fall short in capturing comprehensive and spatiotemporal changes within cells and tissues [8]. Multi-omics strategies have been incorporated to investigate subsets of molecular networks related to bone biology and explore bone injury and recovery [6].

Recent years have seen tremendous advancements in the fields of nanotechnology and the use of nanomaterials in regenerative medicine [9]. The majority of these studies have examined particular topics; for example, Boccaccini *et al*. examined the use of glass-based nanocomposites in bone regeneration [10], Gu *et al*. examined the use of nanotechnology in targeted drug delivery for bone regeneration [11], another review paper assessed the advancements in nanotechnology for osteoporosis treatment [12], and Wang *et al*. examined the interaction of nanomaterials with cells and growth factors for bone repair in their review study [13]. For bone regeneration to be effective, the properties of nanostructured biomaterials should be thoroughly investigated. The mechanical characteristics, biocompatibility, and osteoinductivity of biomaterials are recognized to be significant factors influencing bone regeneration [14,15]. Changing the mechanical environment can also modify the response of bone growth. Furthermore, biomaterials' ability to promote bone healing is significantly influenced by their biocompatibility, and the most crucial characteristic that promotes the production of new bone is their osteoinductivity. Orthopedic trauma surgeons continue to face significant hurdles when dealing with

bone abnormalities that have poor outcomes, such as delayed or unexpected bony repair or high infection rates. Although autogenous bone graft and allograft are considered the gold standard for bone repair in clinical settings among currently approved therapies, their effectiveness is nevertheless constrained by a number of issues, including a shortage of donor sources [16]. The last several decades have seen the fast growth of biomaterials and nanomedicine, which opens up new avenues for improving bone regeneration techniques and increasing their efficacy.

## Review

### Current challenges in bone tissue injury healing

The bone tissue's capacity to withstand pressure reduces in certain extended and stressful compression circumstances. Fracture happens whenever these pressures are greater than the bone tissue's tolerance. Stable fractures and other minor bone injuries can heal on their own without the help of orthopedic surgeons [8]. Although bone fracture healing and soft tissue healing share many similarities, bone fracture healing is distinct because it can be finished without developing scar tissue. A series of processes are involved in bone fracture healing, such as the production of hemoglobas, inflammation, the formation of soft cartilaginous calluses, neovascularization, the mineralization of soft calluses, the formation of hard calluses, and the osteoclastic remodeling of the hard callus to distinguish it from the lamellar bone [17].

Current fracture fixation techniques have advanced to a level of technological proficiency that ensures the methodological validity of the operation and, on a worldwide scale, a high grade of medical care. However, significant clinical issues still need to be addressed [18]. The main challenges for successful bone regeneration are still bone loss, deformities, lack of vascularization, soft-tissue injury, inadequate mechanical stability, infections, and tumors [19]. Although there is still much to learn about the incredibly complex fracture healing process, new research has shown correlations between several variables that influence the healing process and repair outcome [1,20].

When there are significant bone defects, bone fracture repair is insufficient and can become more problematic due to diabetes, age, neoplastic lesions, infection, and reduced blood flow. Under these conditions, autografts, in particular, are the gold standard for replacing tissue with new tissue [17]. The autografts do have certain drawbacks, though. Illness, pain, and donor site appearance are some of the drawbacks. Another drawback of the time-consuming process is that it requires a second operation to harvest the graft, which raises costs. More significantly, it is impossible to obtain an appropriate autograft to fill in such extensive holes in the bone caused by such injuries [17].

Large fractures of the bone may be repaired with allografts; however, this form of graft has several drawbacks. One of the biggest drawbacks of this graft may be the spread of diseases like hepatitis and the human immune deficiency virus. The graft's durability may also be questioned, meaning it might not integrate with the healing response. Instead, it might be taken up by the host immunological defense system, a process referred to as rejection. Additional restrictions on these kinds of transplants include ethical considerations [21]. Another bone graft, a xenograft, is taken from the animal's body to rebuild the damaged area. It is theoretically true that xenografts have greater drawbacks than auto- and allografts. It is possible that they have a faster resorption rate and that this exacerbates the inflammatory response, which could be detrimental to bone recovery [22]. The well-known drawbacks of employing autografts and allografts in clinical settings continue to motivate research into developing bone graft alternatives based on tissue engineering and biomaterials concepts. The public health burden of bone abnormalities that can be carried on by cancer, trauma, and bone illnesses is enormous. Large bone deficiencies remain a major clinical concern even when autologous, allograft, or xenografts are used in clinical settings [23]. Additionally, allografts may not integrate well or may even be rejected [24]. The great prevalence of major defects of segmental bone arising from trauma, inflammation, or tumors creates a great demand for tissue-created bone [25]. The ability of the human body to sufficiently regenerate automatically most, if not all, of its main tissues and structures is diminished when the original tissue is seriously impaired by medical conditions, including tissue dysfunction or crippling impairments [26].

### Current treatments in bone healing

Despite these drawbacks, xenografts have gained interest over auto- and allografts due to their accessibility and affordability. Consequently, they are employed in producing various biologic-based biomaterials through tissue engineering technologies [27]. Numerous biomaterials are available for bone repair and cell seeding [28]. Emerging strategies for enhancing biomaterials involve the development of bioinspired composite matrices, biogenic hydroxyapatites, and biomimetic organoids [18].

It has been recognized that bone tissue engineering (BTE) has the potential to address the limitations of autografts and allografts. Thanks to the groundbreaking integration of engineering principles with concepts from bone biology, scaffolds populated with stem cells can effectively repair significant bone defects [29,30]. The primary objective of bone tissue engineering involves replacing scaffold materials with new bone tissue once these scaffolds are implanted in an area with a deficiency. This process is facilitated through scaffolds, seed cells, and cytokines. A scaffold, often described as a temporary and artificial extracellular matrix, plays a crucial role in influencing cell division and proliferation, potentially aiding in the development of new bone. The best scaffolds for tissue engineering in bone are those capable of fostering angiogenesis, promoting cell adhesion, and satisfying the demands of clinical applications regarding factors such as porosity, mechanical strength, surface activity, biocompatibility, and surface area ratio [31,32].

Regenerative medicine refers to re-growing lost or damaged tissues or organ components. Consequently, both tissue engineering and regenerative medicine present alternative therapeutic strategies that might encourage bone regeneration in response to increasing instances of metabolic disorders, severe trauma, progressive diseases, and various other conditions. Additionally, these methods could help develop novel biological therapies for various diseases that are now incurable [33]. Effective study into bone regeneration depends on developing a new bone transplantation system that incorporates functional scaffold materials and cell sources since tissue engineering integrates concepts from engineering, materials science, biology, and medicine [34].

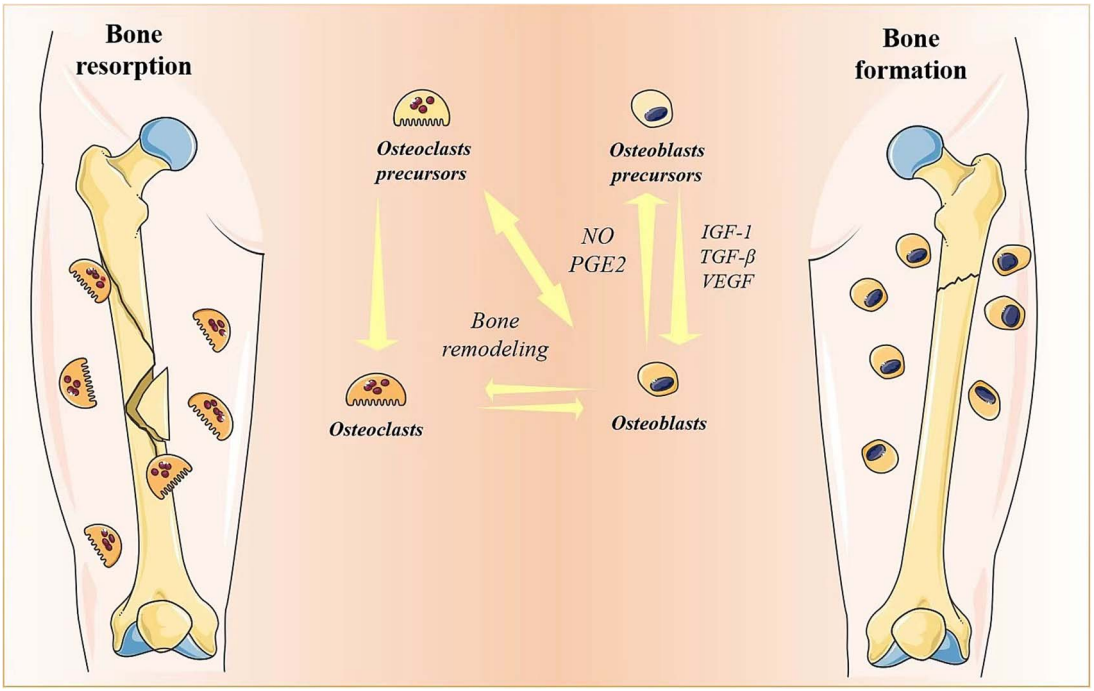


Figure 2. The biological process in bone remodeling. The structural characteristics of bone can be described by two main types of bone tissue: dense bone and cancellous bone. The continuous renewal process of bone tissue, called bone remodeling, is essential for maintaining bone density and regulating mineral balance. During the bone remodeling cycle, osteoclasts, the cells derived from blood stem cells, are responsible for breaking down old or damaged bone tissue. At the same time, osteoblasts, which are derived from mesenchymal stem cells, are directed to these areas to replenish bone tissue that has been cleared by osteoclasts (by Figdraw 2.0). *PGE2* prostaglandin E2, *IGF* insulin-like growth factor, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor

## Remodeling of bone

Modeling is the process of forming and depositing bone on surfaces without the requirement for prior reabsorption [35]. The long bones' longitudinal development in the metaphysis and diaphysis illustrates the modeling process. Studying this process and remodeling it helps to comprehend the effect of hormones, growth factors, and other agents [36]. Signaling molecules directly interfere with physiological processes that involve modeling and remodeling bones [36]. Each regulates them either favorably or unfavorably, depending on their function in the autocrine, endocrine, and paracrine systems [37]. Osteoclasts produce and resorb trabecular bone in a controlled process known as remodeling, which creates space for osteoblast activity [38]. This control can be influenced by both biochemical and mechanical elements, including growth factors and hormones [39]. As a result, bone tissue can grow or resorb depending on a biological reaction. The cells that produce bone are called osteoblasts, and during resorption, older osteocytes and bone matrix are broken down by osteoclasts [40]. Bone disease results from an imbalance brought on by excessive resorption between bone production and resorption (Figure 2).

Osteoclasts play an important role in the bone remodeling process. Osteoprotegerin (OPG) attaches to RANK to initiate a series of events that include gene expression and signaling that ultimately produce osteoclasts. Osteoblasts secrete OPG, a cytokine that functions as a competitive endogenic ligand for additional molecules, including prostaglandin E2 (PGE2), estrogen, and parathyroid hormone. It also inhibits the production of osteoclasts, which in turn prevents bone resorption [41,42].

Bone remodeling is a highly dynamic and self-regulating process that facilitates the restoration of aged or damaged bone in adults. This process is crucial for mitigating the impacts of aging and regular physical stress. Over 3 to 6 months, it promotes bone regeneration. It includes the ability to adjust to a variety of changes, including changes in load distribution, variations in nutrition and metabolism, and the replacement of damaged or necrotic tissues [42].

## Mult-omics techniques and their insights into bone tissue injury and healing

In recent years, technologies within the omics field have been recognized as powerful tools for monitoring disease progression and exploring the molecular mechanisms that drive biological processes such as bone healing [43]. Additionally, these technologies facilitate the identification of specific proteins linked to particular diseases, which could serve as promising targets for therapeutic interventions. Proteomics, transcriptomics, and epigenomics are the chief omics platforms used to examine bone regeneration in healthy and compromised conditions (Figure 3) [16]. DNA, RNA, proteins, and metabolites are among the molecules examined by omics technologies, including genomics, proteomics, transcriptomics, and metabolomics [6]. Increasingly, omics research is being utilized not only for drug development but also in evaluating toxicity and efficacy while also enhancing our comprehension of the molecular mechanisms implicated in healthy and pathological bone repair [7].

The transformation of human dermal fibroblasts into cells resembling osteoblasts was examined by Pihlström *et al*. using multi-omics methodologies. Their multi-omics

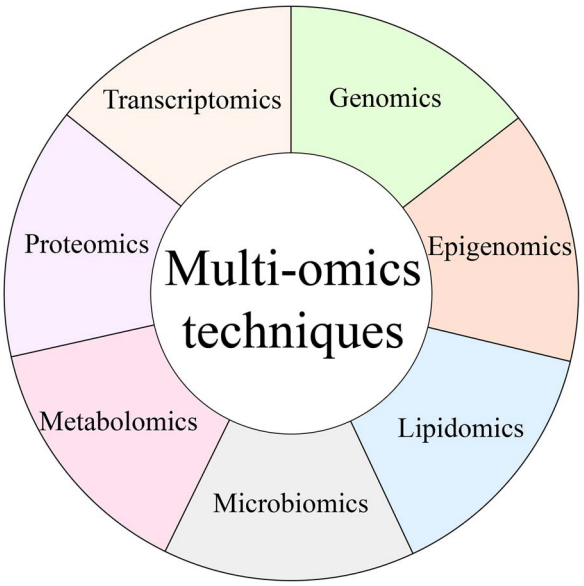


Figure 3. Proteomics, transcriptomics, and epigenomics are the chief omics platforms used to examine bone regeneration in healthy and compromised conditions (by Figdraw 2.0)

analysis, including transcriptomics, proteomics, and phosphoproteomics, revealed significant gene and protein expression changes linked to osteogenesis. This research offers an innovative *in vitro* model that aids in the study of bone biology and skeletal tissue engineering, contributing valuable insights into potential therapeutic approaches for bone regeneration [44].

Similarly, a genome-wide association study (GWAS) found genetic variants linked to bone mineral density (BMD) and the risk of osteoporosis. This research, approved under the Protection of Animals Act (Reference number: V 54-19 c 20-15 (1) GI 20/28 Nr. 108/2011), involved 2094 women and 6463 participants from three European cohorts. The investigators pinpointed two single nucleotide polymorphisms (SNPs) with significant correlations: rs4355801, located near the TNFRSF11B gene, and rs3736228, within the LRP5 gene. Both SNPs correlated with reduced BMD and a heightened risk of osteoporosis and fractures. Notably, the SNP rs3736228 in the LRP5 gene prominently impacted BMD in the femoral neck and lumbar spine and contributed to an increased susceptibility to osteoporotic fractures. According to the study, these genetic variants may impact bone density and fracture risk through critical bone health pathways. Understanding this genetic information offers potential targets for personalized therapies and innovative approaches to osteoporosis treatment [45].

Research on the role of DNA methylation in bone healing discovered that the control of the SOST gene, which produces sclerostin, is critical. Osteocytes are the primary source of sclerostin, a strong inhibitor of bone formation. Researchers found two CpG-rich areas in the SOST gene: one in the proximal promoter (region 1) and another surrounding exon 1 (region 2). Through quantitative methylation-specific Polymerase Chain Reaction (PCR) and pyrosequencing, they discovered that region 1 was hypomethylated in osteocytes, allowing for high SOST expression but hypermethylated in osteoblasts, resulting in reduced SOST expression. This differential methylation pattern suggests that DNA methylation is critical in the osteoblast-to-osteocyte transition, regulating SOST production. Treatment with the demethylating drug 5-Aza-2″-deoxycytidine (AzadC) boosted SOST expression in osteoblastic cell lines by lowering DNA methylation, demonstrating the causal link. These findings imply that epigenetic alterations can be used to improve bone repair by changing the expression of important regulatory genes such as SOST, indicating a possible treatment method for bone-related illnesses [46].

## Genomics and osteoporosis risk factors

The phrase "genomic technology" encompasses a wide array of techniques and tools for analyzing and manipulating the genetic material of an organism, particularly its DNA. These technologies have advanced significantly in recent decades, allowing researchers to examine the composition, operation, and control of genomes [47]. The genome evolution, storage, and functionality exploration fall under the molecular biology discipline termed genomics. These genomic technologies are essential for comprehending the molecular processes involved in the healing of bones and for creating targeted treatments for disorders related to bone health. Genomic strategies aid in the identification of new drug targets and therapeutic options aimed at improving bone healing [48]. By pinpointing genes or signaling pathways that are vital for bone regeneration, scientists can devise precise therapies, which may include small molecule drugs, biologics, or gene therapies, to enhance the speed and effectiveness of bone healing [49]. The GWAS examines the relationship between genetic variations and specific traits by analyzing genetic data from large groups of individuals and employing statistical methods. Research on GWAS related to osteoporosis primarily examines how genetic factors influence bone mineral density [50]. In their study, Richards *et al*. analyzed fracture data from a forward-looking cohort of 5974 participants. Their findings revealed that SNPs located at the loci TNFRSF11A, TNFRSF11B, SOST, SPP1, ITGA1, LRP5, LRP5, ESR1, and TNFSF11 were associated with BMD across various loci, with SNPs in the LRP5, SOST, SPP1, and TNFRSF11A regions strongly correlated with fracture risk [51].

## Epigenomics in bone development

The investigation into reversible modifications to DNA or proteins associated with DNA, including histone acetylation and DNA methylation across the entire genome, is called epigenomics [52]. Programs for gene expression, which are tightly regulated, oversee changes in cellular identity. Research has shown that engineered biomaterials can influence gene expression and modify cell identity [53]. Gene expression regulation occurs through the binding of transcription factors and the assembly of RNA polymerase complexes at DNA regulatory elements, reliant on the chromatin configuration (i.e. whether the chromatin is open or closed) [54]. The field of epigenetics focuses on reversible posttranslational modification (PTMs), such as methylation and acetylation of DNA and histones. These epigenetic modifications affect downstream gene expression profiles, either directly or indirectly [55].

Epigenomic studies have identified dynamic changes in DNA methylation patterns during bone development, injury, and healing. Alterations in DNA methylation profiles at specific gene promoters or enhancers can influence gene expression linked to osteoblast differentiation, extracellular matrix

formation, and inflammation, affecting the outcome of bone healing [56,57].

### Transcriptomics

The term "transcriptomics" describes the analysis of every RNA transcript produced by a cell in a certain situation. More often than not, it refers to recording and measuring every polyadenylated mature RNA transcribed from active genes [58]. As RNA molecules are ultimately translated into proteins, the RNA expression levels of every gene in the genome give an indirect readout of the protein stages of these genes. The transcriptome's steady-state expression at any given time can be seen via RNA-seq. To be more precise, RNA-seq reports on mutations, gene fusions, alternatively spliced transcripts, and variations in gene expression [59]. RNA-seq, as opposed to traditional RNA microarrays, may be performed without *a priori* genomic information and does not require preselected transcript probes. As a result, it offers an impartial, worldwide snapshot of the amounts of RNA expression both before and after biomaterial treatment [60].

Transcriptomic studies involve profiling gene expression patterns in bone tissue during different stages of injury and healing. By comparing gene expression profiles between normal and injured bone, researchers can identify key genes and pathways that are differentially regulated during the healing process [61]. This helps uncover the molecular mechanisms underlying bone regeneration and remodeling. By profiling gene expression in diverse cell types elaborate in the healing process, such as osteoblasts, osteoclasts, chondrocytes, and immune cells, the coordinated interactions between these cell types and their roles in tissue regeneration can be elucidated by researchers [62].

### Proteomics

Proteomics is a prominent subfield of multi-omics that focuses on the complete study of proteins, including their relationships, expression patterns, and posttranslational modifications (PTMs) within biological systems [63]. While transcriptomics methods offer valuable insights into global transcriptional changes during interactions between cells and biomaterials, evaluating protein global expression and modifications is equally critical. Proteins are important for the cell's structure, signaling, and function. [64]. It is critical to understand that proteomics involves more than just linking particular proteins to observable biological reactions [47]. In addition to imparting physiologic stimuli through fleeting interactions with other proteins, proteins can also be pleiotropic [65]. As a result, three distinct investigation areas have emerged: post-translational protein modifications, proteome-wide protein interactions, and large-scale protein identification using protein expression mapping [66].

Changes in the extracellular matrix's (ECM) structure and organization during bone healing are explained by proteomic studies. Proteins involved in ECM remodeling, such as collagen isoforms, proteoglycans, and glycoproteins, are identified and quantified using proteomic techniques [67]. This information provides insights into how the ECM environment evolves throughout the healing process and influences cell behavior and tissue regeneration. Proteomic analyses uncover potential drug targets for enhancing bone healing and regenerative therapies [68]. Proteomic approaches facilitate the discovery of novel therapeutic agents, including small molecules, biologics, and gene therapies, enhancing bone healing [69].

Key proteins that play a role in the healing of bone tissue encompass fibroblast growth factors (FGF), which are responsible for controlling the growth of blood vessels in calluses. Another important element is vascular endothelial growth factor (VEGF), which is influenced by hypoxia-inducible factor-1 alpha (HIF-1$\alpha$) and is necessary for blood vessel reconstruction throughout endochondral bone formation. Additionally, angiopoietins (Ang-1 and Ang-2) are crucial for regulating the stability of blood vessels and the process of angiogenesis, with Angiopoietin 1 exhibiting increased activity, particularly during the process of fracture healing. Additionally, platelet-derived growth factor (PDGF), produced by activated platelets via thrombin and subendothelial collagen, promotes the migration of mesenchymal cells, the development of new blood vessels, the attraction of acute inflammatory cells, and fracture repair [70].

### Metabolomics

Metabolomics is the field that investigates small molecules (with a molecular weight <2000 Da) involved in numerous biological functions and metabolic pathways [71]. In addition to providing important information about the metabolic changes associated with diseases and treatment outcomes, these compounds can serve as direct indicators of cellular activity [72]. Consequently, monitoring these biomolecules offers essential insights into individual cells' properties, functions, and developmental stages. Metabolites are small molecules generated through various processes, including energy production, molecular transport, and cellular signaling. Assessing and analyzing the concentrations of these metabolites is the primary objective of metabolomics [73].

In contrast to transcriptomics, genomics, and proteomics, metabolomics considers metabolites as end products that link downstream phenotypes to upstream biological processes. Metabolomics is widely utilized to detect disease biomarkers, disease progression, and potential treatment targets because it can accurately and comprehensively explain the dynamic reactions of biological organisms to environmental and genetic stimuli [74].

Metabolomic studies identify metabolites associated with the inflammatory response following bone injury. Metabolites derived from immune cell activation, such as prostaglandins, leukotrienes, and reactive oxygen species, may contribute to tissue damage and inflammation or promote resolution and tissue repair. Metabolomic profiling of inflammatory metabolites offers insights into the pathophysiology of inflammatory bone disorders and informs the development of anti-inflammatory therapies [70].

### Microbiomics

The microbiome is made up of several components, including the host immune system, a source of genetic variation, and an important component influencing medication metabolism [75]. Simultaneously, the microbiome is increasingly recognized for its ability to regulate the physiological connections and operations of nearly every bodily organ and to assist the host in coordinating vital survival activities [76].

The microbiome, particularly the gut microbiome, has been implicated in modulating systemic immune responses and inflammation, which can indirectly influence bone health and healing. Many disorders affecting the bones, such as osteoporosis and inflammatory bone diseases, have been connected to dysbiosis or changes in the microbiome's

composition and function [77]. Modulating the microbiome through fecal microbiota transplantation, probiotics, or prebiotics has emerged as a potential therapeutic strategy for enhancing bone healing [78].

### Lipidomics

Comprehensive lipidomic analysis in clinical trials is now enabled by recent advancements in LC-MS technology, which analyzes large sample groups for various lipids and lipid intermediaries. Targeted or untargeted LC-MS may be the most suitable method, depending on the particular lipids [79]. Due to the limited dynamic range of high-resolution mass spectrometry, the untargeted technique concentrates on the more abundant lipids, such as triglycerides, whereas detecting scarcer compounds, such as lipid mediators, is impossible. On the other hand, it can potentially analyze the whole lipidome in a single run [80].

Lipids have a major influence on the ECM's structure and functionality throughout the bone-healing process. Studies in lipidomics reveal associations between specific lipids and ECM constituents, including glycosphingolipids, glycerophospholipids, and cholesterol, all of which affect the matrix's organization, mineralization, and mechanical characteristics [37,81]. Lipid-mediated interactions between cells and the ECM are essential for cell adhesion, migration, and matrix remodeling in tissue repair [82]. Table 1 presents a variety of bone-related diseases and their associated key cell types [83–91].

### Integration of genomic and proteomic in bone injury healing

Genomic and proteomic methods serve as powerful instruments for monitoring shifts in the expression of genes and proteins during the healing of bone injuries and fractures (Figure 4) [92]. Gaining insight into the signaling processes involved in bone healing enables us to influence the healing process to mitigate instances of inadequate or unsuccessful recovery [93]. Factors such as FGF, VEGF, and angiopoietins 1 and 2 play pivotal roles in the ingrowth of blood vessels within developing calluses. The process of bone healing involves the production and action of angiopoietin 1, while VEGF is synthesized, released, and activated later, primarily during the endochondral bone formation process [94]. Current research highlights the significant role of HIF-1$\alpha$ in bone healing and VEGF activity throughout revascularization. Hypoxic environments regulate the movement of mesenchymal stem cell progenitors through the action of HIF-1 [95]. The production of PDGF and TGF-$\beta$ by platelets stimulated by thrombin and subendothelial collagen promotes angiogenesis, the migration of mesenchymal cells, fracture healing, the movement of acute inflammatory cells, and platelet aggregation [94]. It may be beneficial to use genomic and proteomic methods to find key markers linked to transcriptional and translational alterations in cell differentiation, proliferation, and skeletal development [92].

## Clinical studies of multi-omics in bone healing

The biological processes that occur during human bone regeneration have only been partially described in clinical studies due to clear ethical considerations. This restriction results from the requirement to gather tissue samples at different stages of the healing process in order to use omics techniques to describe the bone regeneration process [96]. Several preclinical and clinical studies have used reverse transcription PCR with specific primers to look at the expression levels of specific genes in bone tissue samples. Nonetheless, these investigations provide restricted insights, possibly overlooking the broader context of biological mechanisms and signaling pathways. Recent advancements in microarray technology and whole genome sequencing facilitate the simultaneous assessment of expression levels of thousands of genes, enabling a more thorough analysis of dysregulated genes. This includes the proteins generated and the entire array of RNA transcripts produced by the genome in certain cells or under specific conditions. Despite the difficulties associated with protein extraction, progress in techniques and methodologies has improved the characterization of bone samples regarding protein expression [97].

A study examined the ability of rat bone marrow stem cells (BMSCs) to differentiate in conditions lacking estrogen. In the osteogenic group, there was a substantial increase in alkaline phosphatase activity and calcium crystal staining. In contrast, the lipogenic group exhibited higher red lipid droplets and elevated lipogenic markers. The proteins expressed differently in the OP group were primarily enriched in functions associated with the ECM structural constituent and categories related to biological process and molecular function. The findings indicated that estrogen deficiency–induced osteoporosis is largely due to defects in the BMSC matrix, which impair their ability to differentiate. Additionally, BMSCs from the OP group showed a significant decrease in BMP2 gene expression, implying that proteins from the BMP family may also play a role in osteoporosis. The research proposes that osteoporosis might not solely result from diminished osteogenic activity and increased osteoclastic capability. This creates new possibilities for investigating the pathogenesis of osteoporosis and possible treatments using multi-omics approaches [98].

## *In vitro* or *in vivo* modeling of multi-omics for bone healing

Various regeneration models, such as the fracture model, have been introduced to investigate the bone regeneration process. These include extraction sockets, critical and non-critical size defects, union and non-union fractures, distraction osteogenesis, and *de novo* bone formation models with and without various kinds of barrier membranes and bone grafts [16]. The majority of the current understanding of bone regeneration comes from studies on fracture repair, most of which used small rodents [99]. To mimic clinical scenarios, various bone-healing models with unique experimental conditions have been created [100]. Regardless of ethical concerns, every preclinical research on the immune system [97] and bone regeneration must include animal model validation [101]. This article explores the most commonly utilized small and large animal models for examining the immune system's role in bone fracture repair. The modelers' point of view is used to highlight key findings with the aim of developing *in silico* counterparts to these studies. Modelers must consider physiological differences among skeletal regions when collecting data retrospectively from animal studies to estimate input parameters. Nevertheless, future studies must judiciously select bone regeneration models to ensure sufficient suitable samples are available for comprehensive analysis. Additionally, it is important to recognize that differences in bone metabolism and composition may be necessary

Table 1. Omics insights into bone-related diseases and affected cell types

| Disease | Animal | Location | Phenotype driving gene/materials in mice models | Important affected cell types | Reference |
|---|---|---|---|---|---|
| Bone injury and regeneration | Mice | Rib | Smo | Cxcl12-Expressing SSPCs | Serowoky *et al.*, 2022 [83] |
| | Mice | Frontal bones | P75(Ngfr) | ITGB1-Expressing mesenchymal, IL1a-, IL10-, and TNF-expressing mesenchymal, and immune cells | Xu *et al.*, 2022 [84] |
| | Mice | Long bone | – | Osteoblast lineage cells, chondrocytes, fibroblasts, Fabp5+ Mmp9+ septoclasts | Sivaraj *et al.*, 2022 [85] |
| Congenital skeletal dysplasia | Mice | Hind limb | – | HC | Wang *et al.*, 2022 [86] |
| | Mice | Calvarium | TrkA | Mesenchymal progenitor cells | Tower *et al.*, 2021 [87] |
| Adolescent idiopathic scoliosis | Human | Spinal cancellous bone tissues | – | MSC-IGFBP5, CPC-PCNA, and OC-BIRC3 | Yang *et al.*, 2021 [88] |
| Heterotopic ossification | Mice | Muscle | rhBMP2–Matrigel mixtures pre-immune antibody (referred to as BMP2/IgG) or neutralizing activin A antibody (BMP2/nActA.Ab) | Sox9-expressing skeletal progenitors and Acan- and Col2a1-expressing clusters | Mundy *et al.*, 2021 [89] |
| | Mice | Achilles tendon | – | $Prg4^{+}$ TSPC | Tachibana *et al.*, 2022 [90] |
| Osteoporosis | Human | Femoral head | – | Osteoclasts and immune cells | Wang *et al.*, 2022 [91] |

*SSPC* skeletal stem and progenitor cell, *MSC* mesenchymal stem cell, *IGFBP5* insulin-like growth factor binding protein 5, *CPC* chondroprogenitor cell, *PCNA* proliferating cell nuclear antigen, *OC* osteoclast, *BIRC3* baculoviral IAP repeat containing 3, *BMP* bone morphogenetic protein, *TSPC* tendon stem/progenitor cell

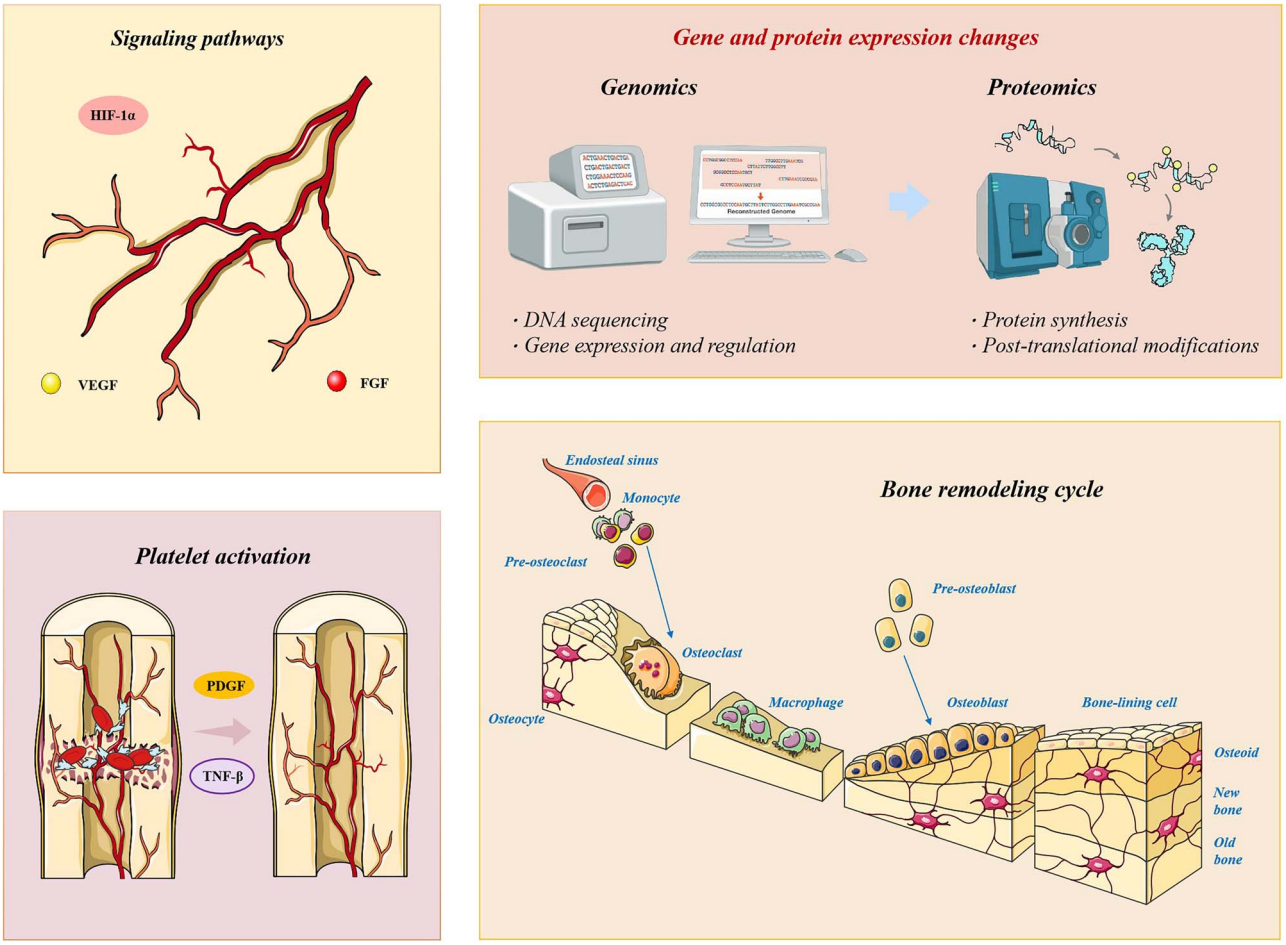


Figure 4. Genomic and proteomic methods serve as powerful instruments for monitoring shifts in the expression of genes and proteins during the healing of bone injuries and fractures (by Figdraw 2.0). *VEGF* vascular endothelial growth factor, *HIF-1α* hypoxia-inducible factor-1 alpha, *FGF* fibroblast growth factors, *PDGF* platelet-derived growth factor, *TGF* transforming growth factor

since animal models may not accurately reflect the biology of human diseases [16].

### Small animal models

Murine models are widely used to investigate human physiological processes and diseases. Although there are differences in immune responses and fracture healing mechanisms, these models can yield valuable insights applicable to clinical settings. For example, researchers used mouse models to validate the clinical finding that a better rate of fracture healing is linked to higher levels of $CD8^+$ T cells in the peripheral circulation [102]. The investigators discovered that manipulating the levels of $CD8^+$ T lymphocytes—either by depleting or enhancing them—in the mouse model affected the fracture healing process. Therefore, when considering the immune system's function in bone regeneration, the number of $CD8^+$ T cells in peripheral blood may be a measure of a patient's immune response [102].

### Large animal models

Animal models of a larger scale offer the most accurate representation of human biology and thus play a crucial role in the preclinical phase of translational research [103]. Although nonhuman primates serve as the human immune system's most representative model, pigs and sheep are commonly utilized to replicate bone healing due to their similar bone structure, mineral makeup, regenerative abilities, and biomechanical characteristics compared to humans [104]. Large animal models elicit a broader range of biological responses compared to small animal models. Larger animal models are used to investigate the full biological response and its implications on the entire organism, known as systemic impacts. In contrast, small animal models only offer mechanistic insights, such as comprehending the repercussions of removing a particular cell type. Pro-inflammatory cytokine levels fluctuated at the fracture site and in the peripheral circulation, according to an *in vivo* investigation done on pigs. Consequently, validating *in silico* models that use blood cytokine levels as input necessitates confirmation through large animal models [105]. Another noteworthy benefit of using large animal models is their ability to apply clinically relevant mechanical loads to the fracture site. T Cells at the fracture site, prolonged inflammatory signaling in the periosteum, and reduced angiogenesis were all correlated with mechanical loads that hinder bone healing. Therefore, substantial animal models must be utilized to investigate the correlations among the immune system, bone healing, and mechanical stressors [106].

## Bone tissue engineering in orthopedic trauma and regenerative medicine

Bone tissue engineering's aim is to create new, useful bone tissues. Success in this field relies on our comprehension of bone

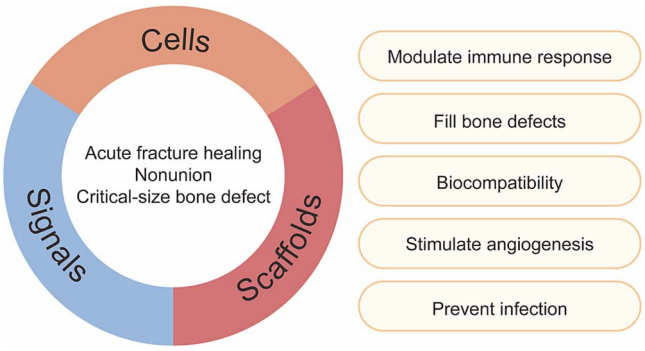


Figure 5. The functional properties and possible uses for the science of potential applications of bone tissue engineering (by Figdraw 2.0)

structure, its mechanics, and the processes of tissue development. In other words, grasping the biology and evolution of bone is essential for the successful regeneration or repair of bone [107]. For instance, various components involved in BTE, such as biomaterials, cells, signaling molecules, and the promotion of vascularization, are utilized to heal bone injuries (Figure 5). "Smart" or osteoinductive biomaterials have the potential to stimulate ectopic bone growth by interacting with the *in vivo* environment to initiate bone formation. Although the biological mechanisms behind this process remain somewhat obscure, it is broadly recognized that these materials hold significant promise for bone tissue regeneration. Different categories of biomaterials, including hydroxyapatite (HA) and various calcium phosphate structures present in both natural and synthetic ceramics, along with their combinations, such as HA/poly(lactic-co-glycolic acid) (PLGA), have demonstrated osteoinductive properties [108].

Bone tissue engineering research aims to develop materials that outperform autografts and allografts in function. The primary goal is to prepare materials that can be inserted into areas of bone loss and subsequently rebuilt by the patient's cells. These materials are frequently used to create scaffolds, which act as frameworks for cell attachment and mineralized matrix deposition. The aim of these scaffolds is to temporarily substitute the ECM in the developing tissue. To confirm the practicality of the selected approach, various structural and functional properties of the materials must be tailored based on the defect's location and the patient's overall health. Key features of healthy bone tissue essential for its functionality, such as a porous structure that permits cellular and vascular infiltration, along with the hierarchical multiscale organization of the bone matrix, play a crucial role in directing the design of materials utilized in bone tissue engineering [109].

In particular, when the natural healing response to an injury is inadequate, the creation of osteoconductive materials is the aim of bone tissue engineering (promoting the growth of bone on a surface), osteoinductive (drawing osteoprogenitor cells to a specific area), and osteogenic (encouraging osteoprogenitor cells to mature into osteoblasts) [110].

The diamond model introduced by Giannoudis *et al*. elucidates the essential elements that go into the development of bone tissue throughout the fracture healing process. They list four primary methods for improving acute fracture repair: providing osteogenic cells, using scaffolds that are either osteoconductive or osteoinductive, giving growth factors, and making sure the mechanical environment is appropriate [111]. The vascularity of the tissue in this situation is critical to healing and is intimately related to the other factors at play. Recent advancements have focused on optimizing implant design for improved biocompatibility, fostering tissue regeneration via immunomodulation, encouraging both angiogenesis and osteogenesis, and administering bioactive substances to prevent and treat infection [112]. While most fractures undergo spontaneous healing, these methods may accelerate the recovery process and could be particularly beneficial for patients with compromised baseline healing responses, including those with unhealthy lifestyles, concurrent medical conditions, or nutritional deficits [113].

## Application of BTE in fracture healing

In addition to having an unlimited supply, BTE provides a personalized therapy option with reduced risks of infection, immunogenicity, and disease transmission. While the intricate mechanisms that govern and influence the healing capacity of bone are not yet completely understood, existing strategies aim to enhance bone repair by establishing a suitable microenvironment that complements and boosts native regenerative activities without interference [114]. Typically, matrices are made of hydrogels, whereas scaffolds for bone tissue engineering are made of a strong support structure with interconnected pores. Both scaffold types need to possess appropriate biophysical and chemical characteristics, including surface properties, biodegradability, mechanical strength, and stiffness, to ensure effective tissue development and the capacity to endure and adapt to mechanical stressors [115]. Novel multimodal and *in silico* imaging and tracking techniques are being developed to determine the best scaffold design criteria for bone applications [116–118].

Implants for the treatment of acute fractures are frequently made up of metal plates, rods, and screws that are created from substances such as titanium, stainless steel, or other alloy-based materials [119]. In most cases, patients are able to immediately resume their regular daily activities after using these devices, which offer a significant amount of mechanical stability [120]. Ideally, the implants should not pose any harm, inflammation, allergies, or cancer risks. Not only must implants be able to give mechanical support, but they must also be able to facilitate and promote bone healing [23]. To achieve this, materials that enhance the adhesion and growth of osteoblasts and rough surfaces that expand the area for host–implant interaction are commonly employed in implant design. Several kinds of bone grafts, whether autologous or allogenic, are also routinely used to assist in the healing of bone in acute conditions [109].

At injury sites or during bone remodeling, biochemical signals such as growth factors, hormones, and cytokines are released, which cause progenitor and inflammatory cells to migrate and osteoblasts and osteoclasts to become activated. In order to promote remodeling or healing, this procedure helps to facilitate the formation of new bone tissue [38,121,122] (Table 2).

Incorporating cells and bioactive substances into bone tissue engineering has garnered significant attention in the past decade to improve the *in vivo* osseointegration of scaffolds lacking cells. This approach utilizes the natural regenerative capabilities of cells while promoting various physiological processes. The combined biomimetic scaffolds are referred to as "smart scaffolds." Recent studies show synthetic peptides are more effective than growth factors [123]. At the same time, the secretome of mesenchymal stem cells (MSCs) or

Table 2. Growth factors are being investigated for bone tissue engineering

| Growth factor | Tissues studied | Effect on growth |
|---|---|---|
| BMP (-2, -7) | Bone, cartilage | Osteoblast differentiation and migration, with faster bone healing observed |
| FGF (-1, -2, -18) | Bone, muscle, blood vessel | Migration, proliferation, and survival of endothelial cells while enhancing the osteogenic differentiation of MSCs |
| IGF-1 | Bone, cartilage, muscle | Proliferation and differentiation of osteoprogenitor cells |
| PDGF (-AA, -BB) | Bone, cartilage, blood vessels, muscle | The proliferation, migration, and growth of endothelial cells support osteoblast replication *in vitro* and promote the synthesis of type 1 collagen |
| PTH | Bone | Intermittent dosing stimulates osteoblasts, resulting in enhanced bone formation<br>Continuous administration leads to bone resorption |
| TGF-$\beta$3 | Bone, cartilage | The proliferation and differentiation of bone-forming cells enhances hyaline cartilage formation *in vivo* and exhibits antiproliferative effects on epithelial cells |
| VEGF | Bone, blood vessel | Enhanced vasculogenesis and angiogenesis occur, though vascular functionality depends on the concentration. Bone formation may increase or decrease based on the concentration when combined with BMP-2 delivery |

*BMP* bone morphogenetic protein, *FGF* fibroblast growth factor, *IGF* insulin-like growth factor, *PDGF* platelet-derived growth factor, *PTH* parathyroid hormone, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor

Table 3. Potentially helpful synthetic peptides as substitute agents to promote osteogenesis and cell adhesion

| Synthetic peptide sequence | Equivalent molecule |
|---|---|
| REDRV, LDV | Fibronectin |
| DGEA, GFOGER, $^{766}$GTPGPQGIAGQRGVV$^{780}$ | Collagen type I |
| GLRSKSKKFRRPDIQYPDATDEDITSHM | Osteopontin |
| FHRRIKA | Bone sialoprotein |
| KIPKASSVPTELSAISTLYL | BMP-2 |
| $^{105}$YKRSRYT$^{111}$, $^{119}$KRTGQYKLGSKTGPGQK$^{135}$ | FGF-2 |
| YIGSR, IKVAV | Laminin |
| RGD | Integrin-binding proteins |

*BMP* bone morphogenetic protein, *FGF* fibroblast growth factor

stromal cells can facilitate osteoinduction, modulate inflammation, and influence immunity during the regeneration process through the action of extracellular vesicles or ECM components, as illustrated in Table 3 [124].

### Angiogenesis and osteogenesis

Angiogenesis and osteogenesis are crucial components in BTE research and play a vital role in the healing process following acute fractures. While these processes were once considered distinct, recent investigations have revealed a significant interaction between the signaling pathways of angiogenesis and osteogenesis [125]. Numerous angiogenic growth factors, including insulin-like growth factor (IGF), PDGF, fibroblast growth factor 2 (FGF-2), VEGF, and angiopoietins, promote the differentiation of osteoblasts and stem cells and improve blood flow to the afflicted tissues, thereby facilitating both angiogenic and osteogenic processes. The primary osteogenic cells used in bone tissue engineering are MSCs [126]. These cells are part of a broad category of multipotent stem cells, capable of differentiating into adipogenic, chondrogenic, and osteoblastic lineages. Additionally, during the healing of an acute fracture, they instinctively migrate to the injury site, aiding in bone repair through mechanisms such as angiogenesis, immunomodulation, and paracrine signaling [127].

### Modulate immunology

Numerous biological systems are involved in the intricate and varied fracture healing process. This process is typically categorized into three main biological phases, extensively studied in various research: the inflammatory, repair, and remodeling [123]. There is a close connection between the vasculature and the inflammatory response associated with fractures. Initially, blood flow at the fracture site decreases due to damage to local soft tissues and the rupture of periosteal and/or endosteal blood vessels; however, it ultimately increases as arterial circulation improves [128]. Even though an initial temporary decrease in blood flow might still cause healing of the bones, angiogenesis, and revascularization are necessary for the process to be effective. Fracture hematomas are particularly abundant in leukocytes, angiogenic growth factors, and various pro-inflammatory and anti-inflammatory cytokines. The processes of angiogenesis, along with the interaction between inflammatory cells and bone cells, contribute to the generation and activation of osteoprogenitor cells. Although immunomodulation remains a relatively emerging field in bone tissue engineering, it holds significant potential for enhancing the speed of fracture healing [129].

### Biocompatibility

Key areas of focus in the field of BTE and biomaterials, specifically about the healing of acute fractures, include the development of bone scaffolds and tissue-engineered transplants as well as enhancements in the biocompatibility of existing prosthetic and fixation devices [130]. To promote bone regeneration and improve biocompatibility, implants may be modified to become more hydrophilic or treated with chemical agents such as hydroxyl and amine groups, bisphosphonates, or growth factors like VEGF or BMP-2. Bone tissue engineers have devoted significant efforts to the creation of polymer scaffolds, which hold promise for complementing or even replacing metallic implants by closely imitating the mechanical characteristics of bone [131].

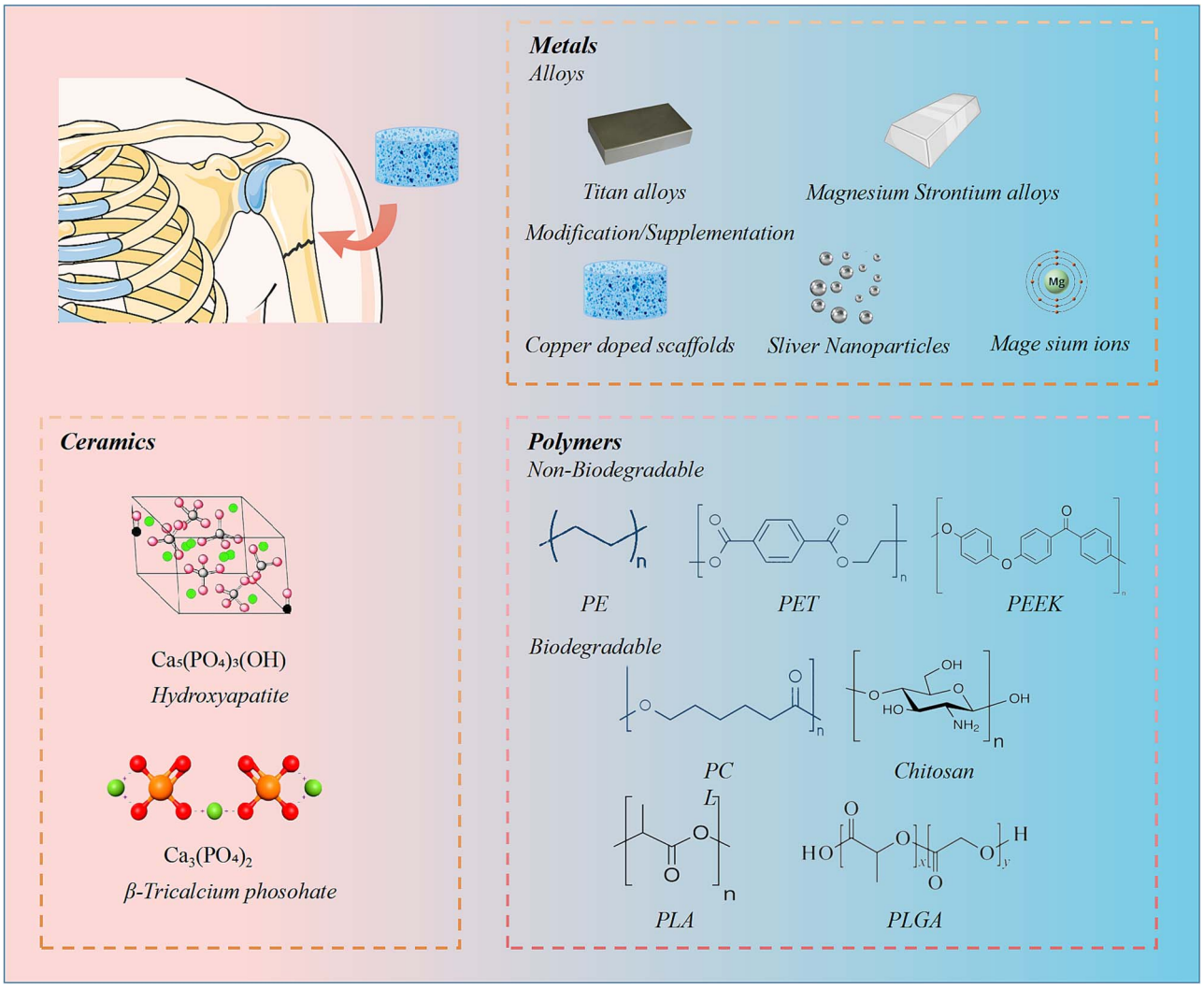


Figure 6. Material classes utilized in the development of innovative bone substitutes. Reproduced with permission from Figdraw 2.0

### Prevent infection

Although peri-implant infections are mainly examined about joint replacements within orthopedics, their importance in orthopedic trauma is substantial, as they can hinder the healing of fractures and lead to complications such as osteomyelitis, chronic wound infections, reoperations, and other severe issues [132]. Additionally, tissue engineering approaches have been employed to treat peri-implant infections. Engineers have created surfaces that possess either inherent antibacterial properties or anti-adhesive characteristics to prevent bacterial attachment to implants. Furthermore, researchers have investigated various synthetic and natural bactericidal coatings that can slowly release antibiotics or respond to specific triggers [133].

## Regenerative medicines

This section reviews new research on significant bone abnormalities, therapeutic resources, and potential screening approaches. The literature on materials can be categorized into three main categories based on the materials utilized to create novel bone graft replacements. These three groups are metals, ceramics, and polymers [134,135]. Figure 6 displays the materials employed in experiments to make innovative bone transplants.

### Biomedical material application in bone regeneration

Materials employed in many research fields that have favorable mechanical qualities but were not made expressly to interact with blood or surrounding tissues are frequently the source of biomaterials [136]. Because life expectancy has grown and society has a duty to give a higher quality of life, the use of ceramics and glasses for bone repair and rebuilding has expanded. They can be classified as either bioinert or bioactive, and the bioactive ceramics can be either resorbable or non-resorbable, depending on the kind of ceramics used and how they interact with the host tissue [137]. Numerous preclinical investigations into materials and AM methods for BTE are under underway [138]. Table 4 discusses several combinations of materials and production methods for clinical use. Since the performance of the materials or AM machines alone is irrelevant for a therapeutic application, the materials are placed in connection to the manufacturing procedures. Instead, more comprehensive information on the possibility for clinical translation is provided by the performance of the coupling material/machine [138–152].

Table 4. Translational potential for different types of materials associated to a given fabrication technique

| Type of material | Material | Scaffold modifications | Animal | Site of implantation | Ref |
|---|---|---|---|---|---|
| Synthetic biodegradable polymers | PCL | rhPDGF-BB | Human | Periodontal | [139] |
| | | | Pig | Condylar ramus unit | [140] |
| | | | Rabbit | Lateral epicondyle of the femur | [141] |
| | PLA | – | Rat | *Subcutaneous* | [142] |
| | PLGA | – | Rat | Iliac crest | [143] |
| | | – | Rat | Tibia | [144] |
| | | Osteoblast-like cells derived from bone or period | Sheep | Calvaria | [145] |
| Ceramics | $\alpha$-TCP | – | Human | Maxillofacial, facial | [146,147] |
| | | | Dog | Skull | [148] |
| | $\beta$-TCP | Bioglass or mesoporous bioglass coating [124] | Rabit | Mandible, alveolar, skull | [149] |
| | DCP | – | Goat | Lumbar | [150] |
| Composites | PCL/PLGA | Filling with collagen containing rhBMP-2 | Rabbit | Calvaria, radius | [151] |
| Metals | Ti and Ti6Al4V | – | Human | Maxillofacial, alveolar, orbital wall | [152] |

*Metals*. Metals are essential for osteosynthesis in mechanically stressed bones because of their superior mechanical stability and established biocompatibility [153,154]. Nevertheless, most metals that have historically been utilized as alternatives to bone grafts are not biodegradable, produce harmful metal ions *in vivo*, and have an elastic modulus higher than that of bone tissue that occurs naturally [154]. Restoring a patient's tissue with a physiologically flexible material is the goal of tissue engineering. A magnesium (Mg), strontium (Sr) alloy (Mg and 1.5 wt.% Sr) that was altered by micro-arc oxidation was used by Wang *et al*. [155] and Sun *et al*. [156] to create bone grafts because of its biodegradability and good machine-driven qualities. According to Sun *et al*., strontium was used due to its ability to promote bone growth and stop osteoporosis [156].

*Ceramics*. Ceramics, which are rigid and non-metallic inorganic materials, are extensively utilized as biomaterials for bone replacement. They are divided into two categories: bioactive ceramics and bioinert ceramics. Bioinert ceramics, including zirconia and alumina, are capable of forming a thin fibrous layer at the interface with bone, which may lead to an immune reaction [133]. Consequently, these bioinert ceramics are not considered suitable materials to address bone deficiencies. On the other hand, bioactive ceramics—often referred to as osteoconductive—possess the ability to create bonds with bone. Some of the primary bioactive ceramics employed as bone substitutes are bioglass (BG), $\beta$-tricalcium phosphate ($\beta$-TCP), hydroxyapatite (HA), whitlockite (WH), biphasic calcium phosphate (BCP), and octacalcium phosphate (OCP) [157]. The following Table 5 [23,127,158–160] provides examples of various ceramics.

*Polymers*. Since ceramics have very low mechanical stability and most metals are biologically inert, polymers have gained increased attention in recent years. Additionally, polymers offer diverse chemical functionalization options that are beneficial in BTE [161]. This enables the material properties to be tailored to the application, for instance, by changing its mechanical strength, biodegradability, antibacterial activity, or osteogenic qualities. Co-polymerization, the development of polymer mixtures or unique shapes, the chemical alteration of monomers, or the addition of inorganic nanocomposites into polymers are examples of possible changes accessible for polymers [162]. Since ceramics have very low mechanical stability and most metals have the critical drawback of being biologically inert, polymers have recently attracted more interest. Additionally, polymers provide a variety of chemical functionalization choices that are helpful in the field of BTE [163]. This enables the material's behavior to be tailored to the application, for instance, by changing its mechanical strength, biodegradability, antibacterial activity, or osteogenic qualities [164].

## Integration of multi-omics and materials science

Materiomics encompasses various scientific fields. Defined as the comprehensive exploration of material systems, the term "materiomics" fuses "material" with the suffix "omics." This interdisciplinary approach is vital for studying biological material systems, merging natural functions and mechanisms (which include biological or "living" interactions) with the classical perspectives found in materials science, such as physical characteristics, chemical constituents, hierarchical architectures, and mechanical properties [165]. Developmental bone biologists are currently equipped with two significant methodologies for examining individual cells' molecular and cellular characteristics: single-cell omics and multi-omics. These methods can reveal the diversity of skeletal cells and forecast developmental trajectories, complemented by *in vivo* lineage-tracing techniques that facilitate spatiotemporal validation of computational models. Understanding the intricacies of skeletal lineage cells has advanced knowledge regarding the essential mechanisms of bone growth, potentially unveiling new perspectives on bone-related diseases. Rapid advancements in platforms and methodologies for single-cell omics analysis are becoming more prominent. Such techniques facilitate a deeper comprehension of both the condition and dynamics of individual cells [166]. For example, omics technologies have been utilized in various preclinical investigations on bone regeneration, including studies on

Table 5. Different characteristics of various ceramics used in BTE

| Ceramics | Symbol | Formula | Advantage | Disadvantage |
|---|---|---|---|---|
| Hydroxyapatite | HA | $Ca_{10}(PO_4)_6(OH)_2$ | Osteoconduction, osteoinduction, biocompatibility | Lower biodegradability, poor mechanical properties |
| $\beta$-Tricalcium phosphate | B-TCP | $Ca_3(PO_4)_2$ | Osteoconduction, osteoinduction, biocompatibility | Higher biodegradability, poor mechanical properties |
| Whitlockite | WH | $Ca_9Mg\ (HPO_4)\ (PO_4)_6$ | Osteoconduction, osteoinduction, biocompatibility, and mechanical properties contain magnesium ions | Synthesize difficultly |
| Bioglass | BG | $SiO_2Na_2OCaOP_2O_5$ | Osteoconduction, osteoinduction, biocompatibility | Lower biodegradability, poor mechanical properties |
| Biphasic calcium phosphate | BCP | Mixture of HA and TCP | Osteoconduction, osteoinduction, biocompatibility | Poor mechanical properties |
| Octacalcium phosphate | OCP | $(Ca_8H_2(PO_4)_6\ 0.5H_2O)$ | Osteoconduction, osteoinduction, biocompatibility | Higher biodegradability and innate brittleness make it hard to sustain its shape |

*HA* hydroxyapatite, *β*-TCP *β*-tricalcium phosphate, *WH* Whitlockite, *BG* bioglass, *BCP* biphasic calcium phosphate, *OCP* octacalcium phosphate

fracture healing, alveolar bone recovery, and critical and non-critical size defect models. According to the findings of these studies, coagulation cascade activation, cytokine release, and inflammatory pathway engagement are all necessary for bone formation. During the initial phases of healing, markers and pathways associated with bone formation are identified, as osteoblast precursors discharge the bone matrix. Reductions in cellular and metabolic activity and an increase in the expression of proteins involved in extracellular matrix remodeling and bone mineralization are characteristics of the later stages of the bone formation process [16].

A multifaceted process that includes several cell types and their corresponding microenvironments is bone remodeling induced by mechanical forces. The precise mechanisms of cells reacting to mechanical stimuli are not fully understood. Integrative strategies have been recently devised to investigate bone mechanobiology at various scales, focusing on the interplay between molecular responses at the cellular level and mechanical stress at the organ level. Progress in imaging and mice loading models has made it easier to observe dynamic bone (re) modeling in living things at the tissue and cellular levels. Nevertheless, methodologies are needed to correlate the tissue-level processes involved in bone remodeling and the molecular responses of various cell types with their local mechanical microenvironments. To investigate the transcriptome profiles of individual cells in connection to their *in vivo* local 3D mechanical environment, a suggested mechanomics approach combines single-cell "omics" technology with tissue-scale models of bone mechanobiology [167].

Single-cell technologies are crucial in identifying biologically significant cell variability, including intrinsic stochastic differences and those influenced by external factors such as the local microenvironment. Nevertheless, the application of single-cell methods in bone biology is still emerging, primarily due to the challenges associated with extracting cells from bone tissue [168]. Imaging techniques have been used in previous studies to examine single cells exposed to fluid flow *in vitro*, such as individual osteoblasts and osteocytes generated from embryonic chick calvaria. Single osteocyte-like MLY-O4 cells' complex 3D deformation properties under fluid flow circumstances have been visualized using a quasi-3D microscopy technique [169]. Analyzing single cells is essential for understanding specific gene regulatory mechanisms that govern cell functionality *in vivo*. However, in order to sort cells, the entire tissue must be broken down either mechanically or enzymatically, which could alter gene expression and result in a loss of spatial context. To fully understand the molecular pathways underlying these actions, spatial context must be maintained [170].

## Limitations and challenges

High-throughput technologies have revolutionized bone research and significantly advanced the field. Various forms of omics data uncover differences in genes, proteins, and metabolites linked to physiological or pathological processes, presenting opportunities for potential disease markers or therapeutic targets. Current evidence indicates that omics could be instrumental in characterizing the early molecular processes involved in bone regeneration and addressing dysregulated mechanisms in pathological conditions. Nonetheless, relying solely on single-omics data can lead to a lack of completeness and may not accurately represent true causal relationships. While single-level omics techniques are capable of identifying mutations specific to diseases and epigenetic modifications, they fall short in demonstrating causal links between molecular indicators and disease expressions. Researchers are now incorporating multi-omics approaches to better understand complex physiological and pathological systems [100]. One significant challenge in processing multi-omics data is the management of large datasets and preventing false positives arising from insufficient power and design [171]. The implementation of multi-omics necessitates a comprehensive pipeline to combine data from various platforms, which presents difficulties due to inconsistencies in data collection, preparation, and measurement conditions [172]. While recent developments regarding bone formation and its possible regulators have been confirmed, these advancements have yet to lead to reliable regeneration in challenging situations, such as vertical augmentation in the maxillofacial area or non-union fractures. Current treatment protocols still lack customization to meet the individual needs of patients. Conducting human studies presents difficulties in control, and the availability of samples can be restricted due to various confounding factors, including dietary and lifestyle choices. When appropriately aligned with the medical condition, animal models offer benefits like reproducibility, accessibility to critical tissues, precise phenotyping, and controlled environmental conditions [171]. Future investigations need to meticulously choose models of bone regeneration to ensure

sufficient samples for comprehensive analysis. Recognizing that changes in bone metabolism and composition must be taken into account and that animal models may not fully represent human disorders.

## Discussion and prospects

Regenerative medicine and BTE have emerged as promising new avenues for bone regeneration. The term "multiple omics" denotes the amalgamation of genomes, transcriptomics, proteomics, and metabolomics. This collectively augments our comprehension of biological mechanisms associated with bone tissue injury and healing within bone engineering and regeneration. This review examines the interface studies of multi-omics within orthopedic trauma and regenerative medicine, outlining various techniques for bone repair and the physiological aspects of bones. The findings presented provide valuable insights for developing innovative biomaterial-based products. However, it is essential to assess the implications of each research study. A variety of methods are available for mending fractured bones, including bone graft replacements, the use of implantable materials and scaffolds, optimization of three-dimensional structures, and customization of surface properties. Bone grafting (BTE) is the most reliable approach for treating critical-sized bone defects. The scaffold is essential to BTE because it offers mechanical support and a substrate for cell attachment, development, and differentiation. The selection of biomaterial and the methods utilized for scaffold fabrication are the two most pivotal factors in achieving these aims. The combination of cells, scaffolds/biomaterials, and bioactive substances in regenerative medicine approaches has attracted considerable interest, particularly regarding bone repair and regeneration. Therefore, this article aims to provide a general review of bone tissue while describing the roles played by bone cells and the different bioactive compounds involved. The development of biomimetic substitutes for bone, which could potentially contribute to translational medicine, may be enhanced through BTE. Attention toward the utilization of cells, biomaterials, and bioactive substances in regenerative medicine, especially pertaining to bone repair and regeneration, is on the rise.

In the "postgenomic" era of bone research, cooperation among multidisciplinary teams, including Big Data projects, plays a vital role in acquiring and sharing biomedical digital information, thereby fostering biological discoveries. The goal of future omics studies is to enhance outcomes in bone regeneration by tailoring treatments to the specific needs of individual patients. The systematic organization of genetic, biochemical, and functional data related to the musculoskeletal system into searchable databases is essential to allow thorough studies in bone regeneration research [173,174]. There are several notable deficiencies in the foundational scientific understanding of tendon biology, including aspects related to disease, healing, and regeneration. Addressing these gaps could significantly advance tissue engineering, orthobiologic methods, and various treatment strategies—all of which could benefit from analysis using multitopic platforms. For example, a deeper comprehension of tendon conditions across many anatomical regions is necessary due to the intricate hierarchical organization of tendons and the wide variety of cells implicated in tendon degeneration. Furthermore, it is imperative to describe tendons precisely, identify the conditions necessary for tendon differentiation and regeneration, and develop basic standards to standardize treatment evaluations (e.g. markers, concentration profiles, cellularization, matrix-to-cell ratios). These knowledge deficiencies, compounded by the complexity of tendon biology, hinder the development of fully effective strategies for tendon repair. Therefore, we recommend the implementation of "omics" approaches that could effectively address these knowledge gaps [175].

As previously stated, the various structural types of bone tissue correspond to its complexity. The process of bone formation and remodeling requires the function of three distinct adult cell types (osteoblasts, osteocytes, and osteoclasts), the utilization of MSCs, a variety of growth hormones, and other as-yet-unidentified substances. Consequently, this aspect represents one of the most challenging elements in creating bioartificial bone tissue. Despite significant advancements in understanding bone biology to date, further efforts are essential to gain a clearer insight into what is necessary to produce commercially viable tissue-engineered bone.

The initial phase involves understanding the interactions among growth factors, their impact, the specific intracellular pathways they trigger, and the mechanisms through which they can be activated or deactivated. Additionally, exploring the mechanisms that facilitate the migration of cells to areas requiring bone healing would be intriguing. MSCs require further cell and molecular biology research. As mentioned earlier, the potential of MSCs is significant, especially when contrasted with embryonic stem (ES) cells. At the moment, MSCs are the most promising cell type for BTE. Nevertheless, these cells' origins and differentiation processes are still poorly understood, highlighting the need for new techniques to enhance purification and growth. To overcome some challenges associated with MSCs derived from bone marrow, examining alternative cell sources that can provide a substantial yield of osteoprogenitor cells is essential. While the authors do not intend to suggest that ES cells lack utility, utilizing them in clinical trials poses considerable challenges in the medium term. Regardless, as previously mentioned, their potential is still significant, and it is likely that they will be used in conjunction with MSCs as another option for bone tissue engineering applications.

The second domain in need of enhancement pertains to materials science. In order to provoke particular molecular-level biological responses, a new wave of biodegradable biomaterials is now under development [169]. These third-generation biomaterials result from molecularly altering resorbable polymer systems to facilitate certain interactions with cell integrins, promoting cell proliferation and differentiation and creating and organizing the ECM [176]. Another class of materials that can be applied to tissue engineering is self-assembled materials [177].

The concluding aspect pertains to scaffold processing techniques. Recent regenerative potential (RP) methods have shown potential in addressing certain drawbacks of earlier techniques, positioning them as promising candidates for upcoming applications in tissue engineering. Nonetheless, innovative processing methods that facilitate the creation of scaffolds with enhanced mechanical properties while maintaining porosity and interconnectivity warrant further exploration and development. Researchers are also of the opinion that RP must progress to accommodate less symmetrical scaffolds, necessitating the introduction of updated software and advanced RP machinery, including next-generation 3D dispensing plotters.

Insights from developmental biology will significantly influence future strategies in tissue engineering. For example, upcoming methods might involve using suitable ECM components or adhesive ligands that specifically engage stem cells in the preliminary tissue remodeling and regeneration phases. Additionally, to foster angiogenesis, BTE will focus on creating scaffolds that integrate growth factors while maintaining the essential porosity for vascular infiltration. Moreover, engineering scaffolds with micro- and nano-scale surface topology is vital for influencing cellular adhesion, spreading, and proliferation. On a larger scale, achieving success in bone tissue engineering necessitates the creation of a scaffold that draws inspiration from the natural mechanisms of developmental biology and enhances tissue remodeling rather than merely supporting the final form and function of the tissue [107]. Multi-omics is combined with clinical phenomes in a new concept called "trans-omics." While multi-omics delves into networks of genes, proteins, and metabolites, trans-omics seeks to provide a holistic view of molecular networks based on patient phenomes, thus facilitating personalized treatment strategies [178]. Trans-omics is a rapidly developing field in integrative medicine that has the potential for improving patient profiling, locating targets and biomarkers unique to a disease, and uncovering drug response mechanisms.

## Conclusions

This article reviews the progress and prospects of polymer-based drug delivery systems in the field of bone tissue regeneration. Studies have shown that polymer composites show great potential in BTE and drug delivery applications. By functionalizing biomaterials such as strontium, their application in BTE can be further enhanced. Multi-omics analysis provides insights in developmental bone biology, while single-cell mechanics studies provide a new dimension to understanding the mechanobiology of osteocytes. In addition, the application of multi-omics methods in disease research and the challenges of bioinformatics in processing big data provide new perspectives for the study of bone tissue regeneration. In summary, the combination of polymer-based material innovation and multi-omics technology has opened up a new path for the development of BTE and regenerative medicine.

## Author contributions

Liyu Yang (Investigation [equal]), Zhijie Xu (Formal analysis [equal], Methodology [equal]), Jie Liu (Data curation [equal], Software [equal]), Xiyue Chang (Data curation [equal], Formal analysis [equal]), Zhaozhou Ren (Project administration [equal], Supervision [equal]), and Wan'an Xiao (Funding acquisition [equal], Project administration [equal], Supervision [equal]).

## Conflict of interest

None declared.

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## Consent for publication

All authors read and approved the final manuscript.

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