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Review

Single-cell technologies drive mechanistic insights and therapeutic translation in skeletal system research

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SUMMARY

A clear mechanistic understanding of the skeletal system's physiological and pathological processes is essential for therapeutic research. Traditional bulk analysis methods cannot resolve cellular heterogeneity and dynamic intercellular signaling in bone microenvironments, limiting insights into skeletal biology. This review focuses on the hierarchical application of single-cell technologies: transcriptomics for cellular identity, spatial omics for tissue context, and multi-omics integration for holistic exploration. These technologies resolve undetectable cellular heterogeneity, identify key cell subpopulations and intercellular communication pathways, and illuminate mechanistic insights. We synthesize their applications, address implementation challenges, and highlight their potential to guide future skeletal research and treatment.

INTRODUCTION

The skeletal system serves as the structural and functional foundation of vertebrates, underpinning physiological posture, motor coordination, support, protection, and hematopoietic-immune functions—the latter of which intersects closely with osteoimmunology, a field focused on the cross-regulation between bone and immune systems.[1] Dysfunction or failure of this system leads to severe physical impairment, reduced adaptability, and compromised quality of life, affecting critical activities such as locomotion, feeding, and social behavior.[2] Traditional research methods have advanced our understanding of skeletal roles in physiological and pathological processes, yet their technical limitations—restricted to single-variable analysis—hinder comprehensive, high-resolution characterization of cellular heterogeneity and dynamic intercellular networks.

Single-cell technologies, including transcriptomics, spatial omics, and multi-omics integration, have revolutionized biomedical research by enabling unprecedented resolution of cellular and molecular landscapes. Unlike conventional approaches, these methods provide non-averaged data, minimizing the neglect of cellular heterogeneity.[3] Single-cell sequencing has reshaped our understanding of tumor microenvironment dynamics in cancer research,[4,5] and this ability to resolve complex microenvironmental interactions and cellular heterogeneity translates seamlessly to skeletal system studies—where these technologies offer transformative insights into bone marrow microenvironment integrity, cell population evolution, and intercellular communication. These core capabilities (deciphering microenvironmental complexity, resolving cellular heterogeneity, and capturing dynamic intercellular crosstalk) are critical for advancing therapeutic translation in skeletal diseases.

Applying single-cell technologies to skeletal research addresses long-standing gaps in mechanistic understanding, from bone genesis and development to injury repair and degeneration. For example, these technologies have uncovered novel cell subpopulations[6] and redefined regulatory networks,[7] directly informing the design of biomimetic scaffolds[8] and nano-therapeutics.[9] As single-cell technologies become increasingly prevalent, systematic summarization of their applications in skeletal physiology, pathology, and translational medicine is essential to foster interdisciplinary innovation.

Guided by these considerations, this review synthesizes how single-cell technologies drive mechanistic insights and therapeutic translation in the skeletal system. We systematically evaluate the applications of these technologies in key skeletal processes, including bone genesis, injury repair, and degeneration, while integrating analyses of their technical characteristics with progress in clinical practice and bioengineering translation. Beyond highlighting breakthroughs in resolving cellular heterogeneity and

Table 1. Functional descriptions of common single-cell analysis tools

	Analytical workflow	Core function	Reference
Cellular heterogeneity and lineage resolution	pseudotime analysis (RNA velocity, Slingshot, Monocle, Monocle2, Monocle3)	reconstruct cell differentiation trajectories and clarify differentiation stages and driver genes	He et al.[6]; Guo et al.[8]
	CytoTRACE and scVelo	track cell differentiation potential and dynamic changes, complementing trajectory analysis	Tu et al.[34]
	species comparative analysis	compare conservation and specificity of cell lineage characteristics across different species	He et al.[6]
Gene regulation and epigenetic analysis	single-cell regulatory network inference (SCENIC)	decode gene regulatory networks and identify key transcription factors	He et al.[6]
Spatial and intercellular interaction analysis	CellChat and CellPhoneDB	analyze intercellular communication networks via ligand-receptor pairs	Swahn et al.[35]; Wang et al.[36]
Protein and multidimensional analysis	weighted gene co-expression network analysis (WGCNA)	screen hub genes associated with specific phenotypes	Ji et al.[37]
	Mendelian randomization (MR) analysis	explore causal relationships between genes and diseases	Ji et al.[37]

regulatory networks, we also address the unique challenges of applying single-cell technologies to bone research (such as issues related to bone's dense mineralized matrix) and outline a roadmap for future bone-specific technical innovation. By doing so, we aim to advance skeletal disease research, accelerate the translation of single-cell-derived insights into clinical solutions, and provide a comprehensive framework that aligns with the findings and scope of the referenced document.

COMMON METHODOLOGIES AND RESEARCH PARADIGMS OF SINGLE-CELL TECHNOLOGIES

Core omics technologies and spatial tools

Single-cell technologies applied in skeletal research encompass a suite of functional omics tools and specialized analytical platforms, each tailored to address distinct research objectives with targeted resolution.

Key technologies include single-cell genomics (scDNA-seq), which identifies genomic variations such as single-nucleotide variants (SNVs), copy number alterations (CNAs), and structural variations (SVs) to unravel genetic drivers of skeletal pathologies like osteosarcoma.[10,11] Single-cell transcriptomics (scRNA-seq), the most widely utilized tool, captures dynamic gene expression patterns to resolve cellular heterogeneity—such as identifying subpopulations of skeletal stem cells (SSCs)—and detect rare cell types critical to skeletal biology.[12] Single-cell epigenomics integrates single-cell DNA methylation sequencing (scBS-seq) and single-cell chromatin accessibility sequencing (scATAC-seq) to reveal epigenetic regulation of cell fate decisions, including the differentiation of mesenchymal stem cells into osteoblasts[13–15]; ChIP-seq complements these approaches by identifying transcription factor binding at open chromatin sites, enabling comprehensive annotation of regulatory elements. Single-cell proteomics leverages cytometry by time-of-flight (CyTOF) and multiplex immunofluorescence to overcome spectral overlap limitations, providing orthogonal protein-level validation of transcriptomic data.[16–19] Single-cell metabolomics profiles small molecules to capture real-time functional states of cells, such as metabolic shifts in osteocytes during bone remodeling, complementing upstream genomic and transcriptomic analyses.[20,21]

Spatial omics technologies, including spatial transcriptomics (ST), multiplexed error-robust fluorescent *in situ* hybridization (MERFISH), and co-detection by indexing (CODEX), address the loss of spatial information inherent to dissociation-based single-cell methods. ST links gene expression to tissue architecture, which is essential for understanding bone development and tumor microenvironments,[22–29] while MERFISH (RNA-focused) and CODEX (protein-focused) achieve single-cell spatial resolution to decode intercellular communication in complex skeletal tissues.[30–32] Supporting these technologies are mature analytical tools such as CellChat for intercellular communication analysis, Monocle3 for reconstructing differentiation trajectories, and SCENIC for decoding gene regulatory networks,[33–37] which are continuously refined through interdisciplinary collaboration (Table 1).

Multi-omics technology integration

Multi-omics integration transcends the limitations of individual technologies by unifying genomic, transcriptomic, epigenetic, proteomic, metabolic, and spatial data, enabling a holistic characterization of skeletal biology.[38] Its core value lies in generating synergistic insights that would be unattainable through isolated analyses.

For example, the integration of transcriptomics and epigenomics has uncovered key regulatory networks driving human skeletal development: scRNA-seq identifies cell type-specific gene expression patterns, such as markers of the chondrocyte lineage, while scATAC-seq reveals dynamic chromatin accessibility, together highlighting ZEB2-mediated regulation of chondrocyte differentiation by linking transcriptional output to

Table 2. Summary of strengths and limitations of single-cell technologies[12–15,18,19,21,22,29,48]

Technology	Key strengths	Key limitations	Applications in skeletal tissues
scRNA-seq	high resolution of cellular heterogeneity; captures gene expression dynamics; identifies rare cell populations (e.g., stem cells)	loss of spatial information; low mRNA capture efficiency (~10–15%); no direct protein-level data	characterization of skeletal cell subpopulations and their differentiation; uncovering tumor heterogeneity in bone cancers; studying chondrocyte development
Spatial transcriptomics	preserves spatial organization of cells; links gene expression to tissue architecture; identifies cell-cell interactions *in situ*	lower resolution compared to scRNA-seq (55–100 μm spots); limited gene coverage in some platforms	mapping spatial distribution of osteoblasts/osteoclasts in bone; analyzing tumor-stroma interactions in skeletal metastases; studying cartilage matrix organization
Single-cell proteomics (MS-based)	direct measurement of protein expression; captures post-translational modifications; complements transcriptomic data	low throughput (hundreds of cells/day); technical challenges with trace protein amounts (~100–200 pg/cell); limited coverage (~3,000 proteins/cell)	profiling osteogenic markers (e.g., RUNX2, COL1A1); studying protein signaling in bone regeneration; identifying biomarkers in skeletal disorders
Single-cell epigenomics	reveals chromatin accessibility and methylation patterns; uncovers regulatory mechanisms of cell fate determination	data sparsity; high amplification bias; limited coverage of histone modifications	analyzing epigenetic regulation of mesenchymal stem cell differentiation into osteoblasts; studying DNA methylation in age-related bone loss
Single-cell metabolomics	captures small molecule dynamics (milliseconds to seconds); reflects immediate functional state of cells; complements upstream omics (genome, transcriptome)	high sparsity (only ~10% metabolites identifiable); technical challenges with small cell volumes (5–25 μm diameter) difficulty in quantifying low-abundance metabolites	profiling metabolic shifts in osteocytes during bone remodeling; studying metabolite changes in chondrocytes during osteoarthritis; identifying metabolic biomarkers of skeletal tumors

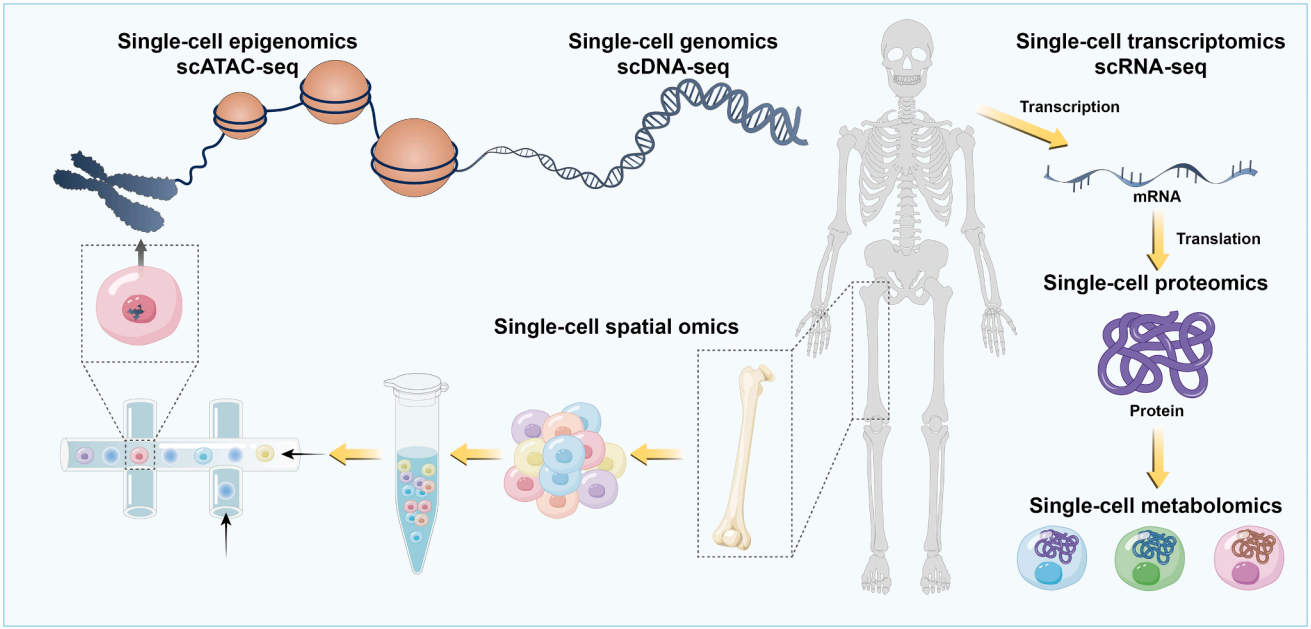
epigenetic control.[39] ST combined with proteomics, via cyclic immunofluorescence (CycIF), enhances intercellular communication analysis in formalin-fixed paraffin-embedded (FFPE) skeletal tissue samples, clarifying tumor-stroma interactions in osteosarcoma by correlating spatial gene expression with protein signaling gradients.[40,41] Lineage tracing integrated with multi-omics data reconstructs cellular lineage trees, mapping progenitor cell states during bone regeneration; mathematical modeling of these trees deciphers dynamic fate decisions, such as the commitment of mesenchymal stem cells to osteogenic or adipogenic lineages in age-related bone loss.[42–47]

While individual technologies have inherent limitations—including scRNA-seq's lack of spatial information and spatial omics' lower resolution[48]—multi-omics integration mitigates these constraints to address complex biological questions, from the origins of skeletal development to the pathological mechanisms of diseases like osteoporosis (Table 2). This paradigm shift enables precise definition of "cell types" based on integrated molecular signatures, providing a robust foundation for translating mechanistic insights into therapeutic strategies (Scheme 1).

SKELETAL SYSTEM SCENARIOS FROM A SINGLE-CELL PERSPECTIVE

Bone genesis and development

Research on bone genesis and development focuses on the overall developmental processes, ossification patterns, and directional growth of long bones, cranial bones, and appendicular bones in vertebrates. Specifically, the development of long bones is driven by endochondral ossification via growth plates, which traditional studies divide into three zones—resting, proliferative, and hypertrophic—composed of multi-layered chondrocytes. These zones promote longitudinal bone growth through the proliferation and hypertrophy of chondroprogenitors to chondrocytes, regulating long bone elongation. However, previous studies struggled to provide comprehensive analyses of the functional characteristics, transcriptional regulation, and interzonal interactions within these regions. Single-cell technologies now enable in-depth investigations of growth plate zones at single-cell resolution, deepening our understanding of long bone formation and revealing the key roles and regulatory



Scheme 1. Common methodological approaches and research paradigms of single-cell technologies

mechanisms of specific cell subpopulations and molecules in bone development.[49] Additionally, single-cell technologies have clarified the cellular heterogeneity and lineage hierarchy in the earliest appendicular skeleton formation within limb buds, including the anterior-posterior (AP) axis, proximal-distal (PD) axis, and core limb bud mesenchyme. Through systematic analysis of cellular heterogeneity and lineage hierarchy across multiple bone compartments, researchers have identified distinct skeletal stem/progenitor cells (SSPC)—along with their differentiation processes and molecular signatures—that coordinate endochondral and intramembranous ossification in human embryonic long bones and cranial bones. Specifically, scRNA-seq analysis of human embryonic limb buds (5 WPC) and long bones (8 WPC) has facilitated the identification of such cells[6] (Figure 1A). This resolves whether SSPC with similar molecular characteristics exist in embryonic long bones and cranial bones. Overall, single-cell technologies enhance our holistic understanding of developmental processes, patterns, and directions through high-resolution characterization and regional discrimination.

On another front, single-cell technologies have significantly advanced our understanding of the diversity and roles of developmental and reparative stem/progenitor cell subpopulations, as well as the complexity of their dynamic relationships in kinetics, hierarchy, and interactions. For example, Hanbo Li et al. used RNA sequencing (RNA-seq) to observe the aggregation of connective tissue subpopulations toward pluripotent skeletal progenitor subpopulations during salamander limb regeneration.[50] Another study achieved comprehensive cell subpopulation-level understanding of the murine musculoskeletal system's cellular composition and lineage hierarchy via scRNA-seq.[51] Furthermore, a recent study successfully mapped the whole-cell atlas of human embryonic skeletogenesis, offering solutions for issues such as bone system consistency, regional heterogeneity, molecular marker specificity, and holism. It also discusses how single-cell multiomics (transcriptomics combined with epigenomics) has revealed gene regulatory networks underlying human skeletal development, with ZEB2 in the chondrocyte lineage serving as an example[39,52] (Figure 1B). Among various cell subpopulations, the origin, responsiveness, and driving forces of osteogenesis-related stem cell subpopulations are often research priorities. Previous studies using prospective isolation and genetic lineage tracing techniques discovered SSCs with pluripotency and self-renewal capacity in the growth plates of early postnatal mice, periosteum of long bones and cranial bones in slightly older postnatal mice, and growth plates of 17-week human fetal long bones. This not only confirms SSC existence in higher vertebrates but also, via single-cell technologies, reveals their relationship with bone marrow stromal cells maintaining adult bones at earlier developmental stages. Additionally, the consistency between adult bone repair mechanisms and embryonic development helps explain the critical roles of embryonic SSCs in early bone formation, development, and individual repair processes.[6] Although understanding of these processes requires further depth, the lineage flexibility and plasticity of osteoblast origins challenge the traditional concept that strictly single, self-renewing, pluripotent cells drive bone tissue formation and regeneration. These findings will further advance modern understanding of stem cell functions and niches, emphasizing stem cell diversity and complexity in bone tissue formation and regeneration.

The dynamics, directions, and trajectories of stem cell development have been precisely reconstructed and predicted through single-cell pseudotemporal analysis, trajectory inference, and multi-omics integration. Notably, bone marrow mesenchymal stem cells (BM-MSCs), as core cell types in basic

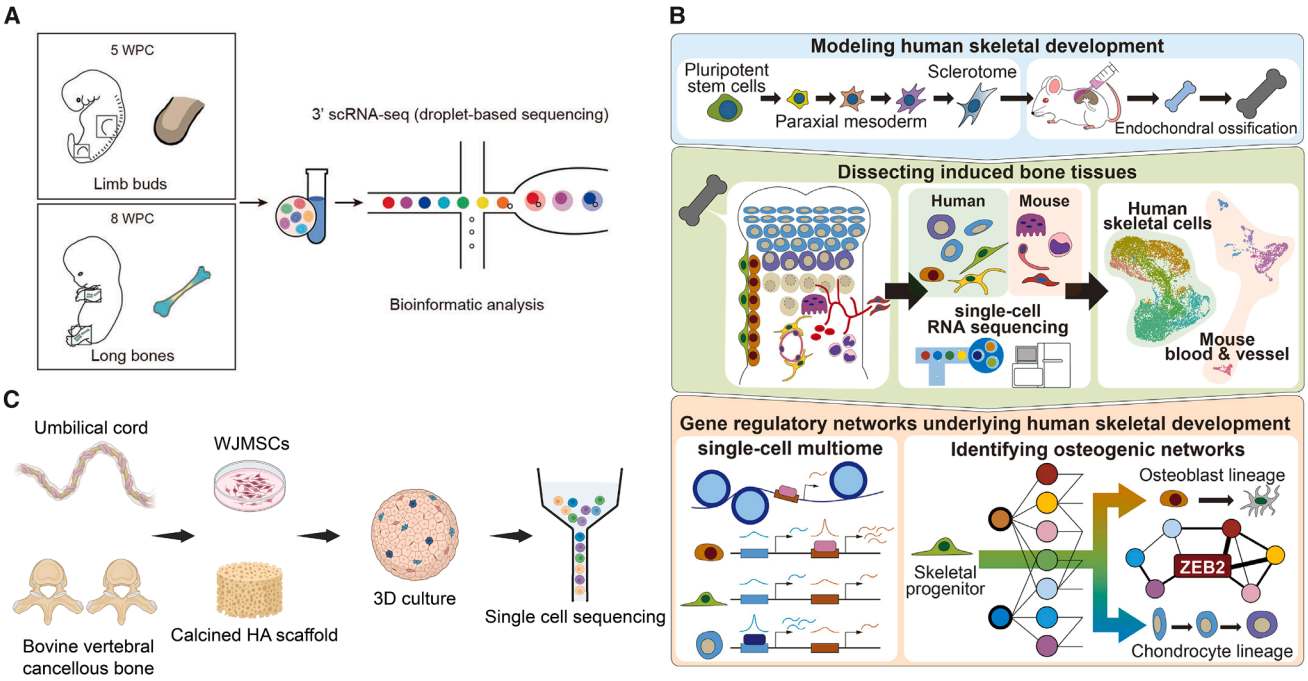


Figure 1. Applications of single-cell technologies in bone genesis and development

(A) Sampling procedures and experimental protocols for droplet-based scRNA-seq of human embryonic cells isolated from limb buds and long bones in Jian He et al.'s study.

(B) Shoichiro Tani et al. developed a method to simulate endochondral ossification at the embryonic stage using human pluripotent stem cells via single-cell multiomics, investigating dynamics of cell type-specific gene regulatory networks during development.

(C) Schematic of 3D co-culture of hydroxyapatite and cell isolation for scRNA-seq on the 10X genomics platform by Peng Guo et al.

and translational bone research, attract extensive attention due to their potential for multi-lineage differentiation (osteogenesis, chondrogenesis, and adipogenesis). Single-cell technologies have challenged existing research paradigms with high resolution—for example, D. Trompet et al. performed more detailed classification of BM-MSCs based on surface markers and differentiation trajectories, encoding them into distinct subpopulations corresponding to osteogenic, chondrogenic, and adipogenic differentiation pathways, as well as terminal differentiation states, which is highly instructive and innovative.[53] Meanwhile, Ken To's team mapped the differentiation trajectories of limb and cranial bone progenitors in different embryonic skeletal regions, elaborated a detailed dynamic regulatory map of skeletal and cartilage maturation, and constructed molecular networks governing intramembranous and endochondral ossification.[38] Additionally, single-cell technologies offer robust validation for target cell subpopulations, molecular marker functional ontology, and microenvironmental spatiotemporal characteristics during bone formation and resorption—for instance, scRNA-seq has validated the roles of the RANKL/OPG pathway in osteoclast activation and characterized the spatial localization of bone progenitor cells.[54] In summary, single-cell technologies facilitate increasingly systematic dissection of developmental processes and deepen our understanding of congenital and developmental abnormalities.[55]

In the field of bone regeneration and repair materials, bioactive components play a crucial role in promoting the osteogenic microenvironment. Previous studies typically relied on detecting phenotypic changes in cells or tissues after material treatment, along with associated transcriptional and protein molecular signaling pathways, to infer and validate the specific mechanisms of biomaterials in bone regeneration and repair. However, these studies often lacked high-resolution insights into the specific contributions and functional processes of biomaterials, limiting the depth and breadth of biomaterial development and application. Single-cell technologies offer new research tools for this field. For example, Peng Guo et al. performed in-depth comparative studies on 3D hydroxyapatite scaffolds and osteogenic media. Utilizing scRNA-seq, they finely mapped the *in vitro* transcriptional profiles of mesenchymal stromal cells after corresponding treatments at single-cell resolution, which contributes to optimizing osteogenic biomaterials—specifically, the hydroxyapatite scaffolds optimized based on such single-cell transcriptional profiling of mesenchymal stromal cells[8] (Figure 1C). This work not only enhances our understanding of how osteogenic culture conditions and hydroxyapatite scaffolds regulate cell activity but also provides new perspectives and database support for optimizing osteogenic biomaterials. With ongoing advancements and applications of single-cell technologies, they are expected to play an increasingly important role in promoting bone regeneration and repair with biomaterials, offering deeper molecular insights for precise biomaterial design and functional optimization (Table S1). These single-cell studies collectively decode the cellular heterogeneity, lineage hierarchy,

and regulatory networks underlying skeletal development, while providing precise molecular guidance for optimizing osteogenic biomaterials—laying a foundation for understanding congenital bone abnormalities and advancing regenerative strategies.

Heterotopic ossification

Heterotopic ossification (HO) is a complex clinical pathological process characterized by abnormal bone formation in soft tissues (e.g., muscles, periarticular regions, ligaments, and tendons), often occurring as a complication of trauma, surgery, explosions, spinal cord injuries, and other stress-induced injuries. New bone formation involves two modes—endochondral and intramembranous ossification—mediated by transcription factors such as IHH (Indian Hedgehog), RUNX2 (Runt-related transcription factor 2), and Osterix, which promote mesenchymal stem cell differentiation into osteoblasts. Single-cell RNA sequencing has played a pivotal role in this field, enabling in-depth understanding of cellular subpopulation composition, origin, heterogeneity, and specific mechanisms at lesion sites. By analyzing high-resolution patterns of characteristic gene expression during osteogenic, chondrogenic, fibroblastic, and macrophage differentiation/maturation, along with intercellular communication and ligand-receptor pairing via scRNA-seq, potential targets for early diagnosis and treatment of HO have been identified. This approach has enabled the resolution of cellular heterogeneity (e.g., osteogenic and chondrogenic clusters) in HO tissues and the characterization of ligand-receptor interactions driving HO progression[56] (Figure 2A). For example, in studies of tendon HO, traditional methods struggled with qualitative/quantitative analysis of different tendon-resident cell subpopulations. Junxin Lin et al. used single-cell technologies to reveal the osteogenic fate of tendon progenitor cells during HO in mouse tendons. They found that inhibiting angiogenesis counteracts the upregulation of pro-angiogenic genes in tendon cell clusters, thereby suppressing HO. This was demonstrated in a rat model, presenting a novel therapeutic strategy for tendon HO[57] (Figure 2C). Another study employed a gene reporter mouse model of tendon-related traumatic HO and performed single-cell transcriptome sequencing. It identified that abnormal osteochondral differentiation of synovial/tendon sheath progenitor cells promotes post-traumatic HO, with Tppp3$^+$ synovial/tendon sheath progenitor cells being key drivers of such abnormal differentiation—these cells contribute to heterotopic bone formation after trauma[58] (Figure 2B). Additionally, Michael Sorkin et al. used single-cell technologies to analyze the relationship between monocyte/macrophage populations and abnormal chondroprogenitor differentiation in traumatic HO. Their research revealed that monocyte/macrophage populations interact with chondroprogenitors via paracrine signaling to regulate HO occurrence, and targeting this intercellular crosstalk can reduce abnormal bone formation.[59] Further research combining scRNA-seq, ST, and lineage tracing has revealed the dynamic process and mechanisms by which quiescent MSCs transition to a cycling state under inflammatory exposure and then differentiate into cartilage/bone lineages, emphasizing the critical contribution of MSC dynamic fate in inflammatory microenvironments to HO progression (Table S2). Integrating single-cell and spatial omics has clarified the cellular origins (e.g., Tppp3$^+$ progenitors) and intercellular crosstalk (e.g., macrophage-MSC paracrine signaling) driving HO, enabling the identification of targeted therapies such as anti-angiogenic agents and macrophage reprogramming strategies.

Bone injury, healing, and repair

Fracture healing is a complex dynamic process involving continuous changes in pre-healing, healing, and post-healing stages, which traditional studies have struggled to observe systematically through single or limited reporter gene models. To address this, Yiming Liam Liu et al. systematically studied osteogenic activities in the periosteum after fracture using single-cell technologies. By integrating multiple reporter genes, they developed a technical platform for synchronous tracing of stem/progenitor cell fates, enabling precise differentiation of BM-MSCs, cambium periosteal cells, and fibrous periosteal cells across fracture healing stages.[60] Additionally, Ran Liu et al. used single-cell sequencing to elucidate and validate the involvement of mechanical factors in fracture healing.[61] The bone marrow microenvironment, comprising immune cells, endothelial cells, hematopoietic stem cells, bone marrow stromal cells, osteoblasts, and osteoclasts, plays a critical role in fracture healing. Previous research focused on stromal cell functions in bone injury, healing, and repair, while immune cell contributions were often overlooked due to microenvironmental complexity, leading to limited systematic studies on immune cell trajectories and their communication with stromal cells. A key discovery in this field: Hao Zhang et al. used scRNA-seq to analyze fresh fracture tissues and revealed that B cells play a critical regulatory role in fracture healing—specifically, they inhibit excessive bone regeneration by producing multiple osteoblast inhibitors, with B cell-osteoblast crosstalk mediated via IL-6 and TNF-α (tumor necrosis factor-α) secretion being involved[7] (Figure 3A). Specifically, during the hematoma stage, B cells are depleted by inflammatory responses to promote mesenchymal stem cell recruitment, osteogenic differentiation, and angiogenesis. During osteophyte formation, osteoblasts promote B cell maturation, which in turn inhibits osteoblast differentiation and abnormal osteophyte proliferation. B cells also promote osteoclast differentiation, driving the body into the osteophyte healing stage. During osteophyte healing, B cell-secreted cytokines reduce osteoblast and vascular endothelial cell numbers, which gradually recover thereafter. Beyond hard bone tissue, cartilage degradation and removal are equally important in fracture injury and healing. The mediator of this process was once controversial, but single-cell technologies provided a solution: Kishor K. Sivaraj et al. identified that fracture-induced cartilage degradation is mediated by rare septoclasts derived from mesenchymal stem cells (distinct from osteoclasts), and this process is potentially influenced by aging[62] (Figure 3B). Beyond the composition of cell subpopulations and molecular interaction relationships, spatial relationships are equally crucial for understanding the mechanisms of fracture healing. The work of Weiqiang Lin et al. and Xue Xiao et al. respectively, utilized spatial omics technologies to explain the site specificity of bone tissue repair from the perspectives of spatial gradient regional differences in bone remodeling (such as immune cells and inflammatory signaling pathways like NF-κB (nuclear factor κB)/TNF-α)[24] and

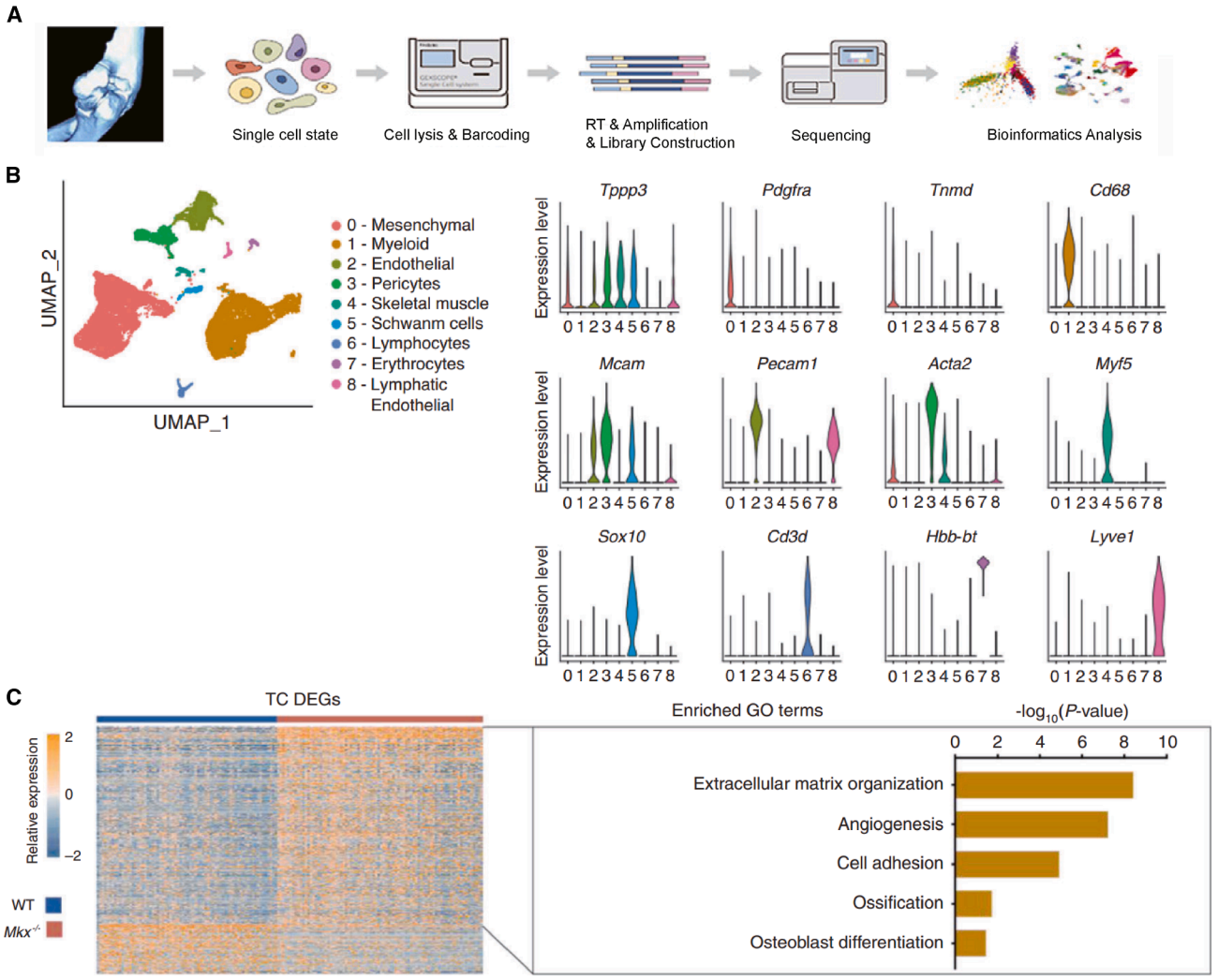


Figure 2. Applications of single-cell technologies in heterotopic ossification

(A) Roadmap of single-cell transcriptomic analysis and uniform manifold approximation and projection (UMAP) analysis of heterotopic ossification tissues in the elbow joint by Chi Zhang et al.

(B) Modeling process of post-traumatic heterotopic ossification and studies on synovial/tendon sheath progenitor cells by Ji-Hye Yea et al.

(C) Single-cell analysis of inhibiting angiogenesis to attenuate heterotopic ossification in the Achilles tendon of traumatized rats by Junxin Lin et al.

distribution differences of key cell subpopulations (SSPC subtypes).[25] Neashan Mathavan et al. through spatial omics technology, identified mechanical response genes such as Wnt7b and Cd44 as potential therapeutic targets, providing a multi-scale integrated basis of "mechanical environment—gene expression—tissue morphology" for optimizing fracture fixation methods and mechanical stimulation parameters.[26] Jonathan J. Rios et al. further clarified the spatial regulatory characteristics of gene expression and signaling pathways in various transcriptomic clusters during normal fracture healing (e.g., the TGF-β (transforming growth factor β) pathway is active near the fracture plane, the BMP pathway is enriched in ossification regions, and the WNT (wingless-type MMTV integration site family) pathway is expressed in the cartilage-bone junction, collectively regulating endochondral ossification).[27] All these studies have provided new ideas and theoretical foundations for clinical treatment of fractures and promotion of fracture healing.

Clinically, internal fixation is commonly used for severe fractures. During bone integration with implants, the impact of the foreign body response-related osteoimmune microenvironment is critical for bone regeneration efficiency and implant performance. Thus, high-resolution technologies are essential to analyze cellular heterogeneity and kinetic changes in implant-mediated osteoimmune microenvironments. Zhuqing Wan et al. employed scRNA-seq and ST to characterize the osteoimmune microenvironment surrounding classic collagen/nano-hydroxyapatite (Col+nHA) bone repair materials, including key cell populations (MSCs, macrophages, osteoblasts, and osteoclasts) and their interactions. This research expanded understanding of functional MSC subpopulations in

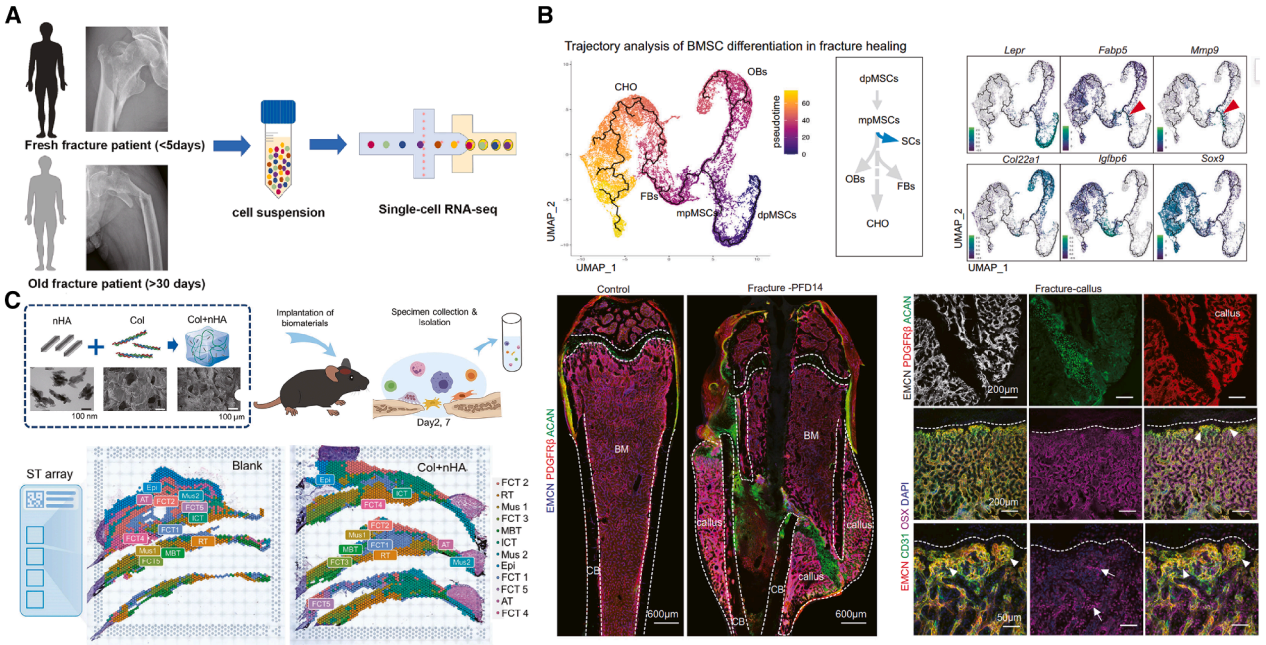


Figure 3. Applications of single-cell technologies in bone injury, healing, and repair

(A) Experimental strategies and analyses for single-cell profiling of human fracture tissue cells by Hao Zhang et al.

(B) Study by Kishor K. Sivaraj et al. on cartilage resorption by mesenchymal stromal cell-derived septoclasts during fracture healing, analyzed using single-cell technologies.

(C) Expression profiling and spatial transcriptomic analysis of bioactive Col+nHA hydrogel composite for repairing mouse cranial bone defects by Zhuqing Wan et al. using scRNA-seq.

biomaterial-mediated early bone regeneration, identifying Mgp^{+} MSCs as critical players in osteoimmune crosstalk—specifically, Mgp-high expressing MSCs that orchestrate the osteoimmune microenvironment in Col+nHA-mediated bone regeneration[63] (Figure 3C). Another team compared stainless steel and titanium implants using single-cell technologies to analyze bone marrow microenvironment heterogeneity, dynamic changes, and implant-modulated immune responses, particularly the role of neutrophils in bone integration.[64] These findings advance osteoimmunology and inspire designs for future "osteoimmune intelligent" materials. Furthermore, active repair materials designed based on key signaling pathways—such as the transplantable bandage containing 3D Wnt-induced osteogenic tissue models developed by Yoshihisa Okuchi et al.—have revealed via single-cell technologies that Wnt signaling mediates hematopoietic stem cell (HSC) asymmetric cell division to regulate osteogenesis.[65] This not only enhances understanding of human osteogenesis but also provides new insights into osteogenic regulation beyond traditional Wnt pathways. These advances lay important biological foundations for bone implant design and application, opening new avenues for in-depth osteoimmune microenvironment research (Table S3). Single-cell technologies have uncovered key cellular regulators (e.g., B cells, septoclasts) and spatial-temporal signaling dynamics in fracture healing, while elucidating the osteoimmune microenvironment of bone repair materials—providing a multi-scale basis for optimizing fracture fixation, implant design, and regenerative therapies.

Bone tumors

Osteosarcoma, the most common type of primary bone malignancy, is associated with poor prognosis due to insufficient understanding of its pathogenesis. Single-cell technologies have enabled high-resolution identification of specific cell subpopulations, building on traditional transcriptomic studies of mixed cell populations. This provides new avenues for in-depth research into the transcriptomic characteristics, regulatory factors, and dynamic features of osteosarcoma malignant cells and their tumor microenvironment, particularly stromal and immune cells. Yan Zhou et al. analyzed copy number variations and cell differentiation trajectories, revealing that malignant osteoblasts transdifferentiate from malignant chondrocytes in osteosarcoma, with metastasis driven by the ETS2/IBSP axis (Figure 4A). They also explored the potential to enhance immunotherapy by blocking inhibitory checkpoint markers (e.g., PD-1) highly expressed on NKT (natural killer T cells) cells *in vitro*, identifying these markers as promising immunotherapy targets.[66,67] For osteosarcoma patients with lymph node metastasis, scRNA-seq has deepened our understanding of lymphatic metastasis mechanisms and the lymphatic microenvironment. This includes transcriptional signatures and subtypes of osteoblasts during osteosarcoma progression, their potential differentiation pathways during lymph node metastasis, and specific molecular signaling axes. Additionally, studies have revealed interactions between osteoblasts and various components of the lymphatic microenvironment (e.g., myeloid cells, cancer-associated fibroblasts [CAFs], and NK/T cells). These interactions, which involve specific

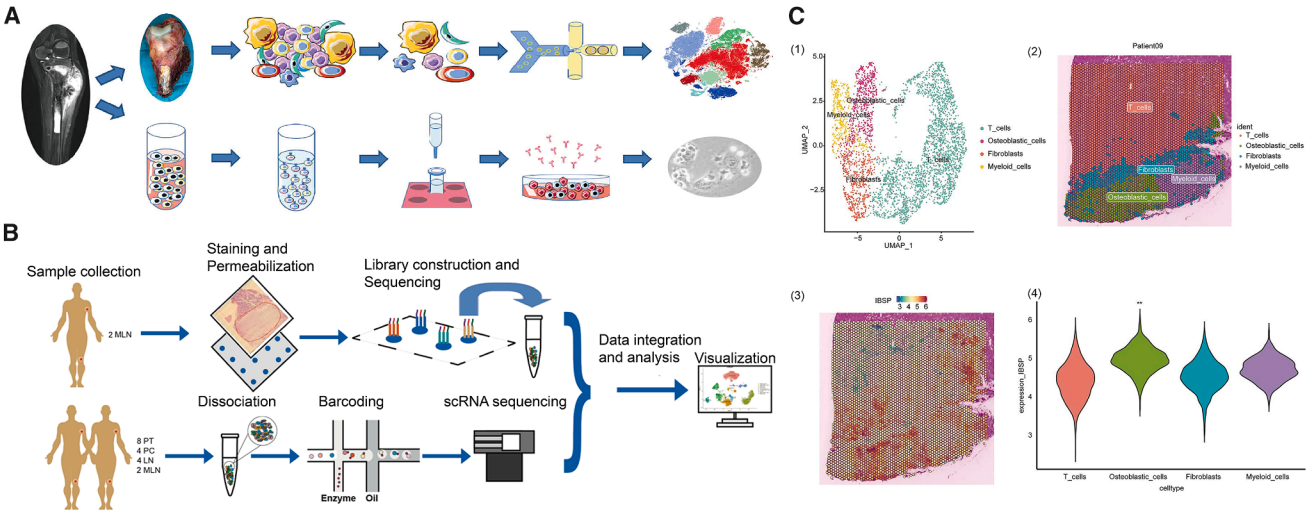


Figure 4. Applications of single-cell technologies in bone tumors

(A) Roadmap of scRNA-seq studies by Yan Zhou et al., collecting single-cell suspensions from osteosarcoma lesions of 11 patients on a 10X Genomics platform. (B and C) Roadmap and representative spatial visualization of single-cell and spatial transcriptomics revealing lymph node metastasis mechanisms and microenvironmental remodeling in osteosarcoma by Yun Liu et al.

signaling axes such as ETS2/IBSP in the crosstalk between osteoblasts and myeloid cells or CAFs, reshape the lymphatic microenvironment and uncover transcriptional signatures of osteoblasts during lymph node metastasis. Such findings provide new insights for blocking lymphatic metastasis of osteosarcoma[68] (Figures 4B and 4C). These findings not only enhance our understanding of osteosarcoma pathogenesis but also provide important molecular targets for developing new therapeutic strategies.

Beyond primary tumors, bone metastasis of cancer is a common and fatal complication. Traditional low-resolution analytical methods have limited understanding of metastasis processes and the characteristics of metastatic lesion microenvironments, hindering in-depth insights into disease mechanisms and contributing to refractoriness to immunotherapy. Single-cell technologies have provided a new high-resolution perspective by comparing single cells from bone metastatic tumors, affected and unaffected bone marrow, and bone marrow from cancer-free controls, enhancing understanding of disease mechanisms and therapeutic strategies. For example, Youmna Kfoury et al. performed high-resolution single-cell analysis of prostate cancer bone metastasis, revealing exhaustion of specific T cell subsets and the specific role of macrophages in this process. These findings offer potential for developing targeted therapies to alleviate local immune suppression.[69] Another study used single-cell analysis to identify spontaneous cell fusion in the bone marrow during cancer cell bone metastasis, leading to the formation of special myeloid-tumor hybrid cells. These cells not only promote bone metastasis but also exhibit resistance to docetaxel and ferroptosis while maintaining a highly immunosuppressive microenvironment, though they show sensitivity to radiotherapy.[68] These discoveries deepen our understanding of the molecular mechanisms of tumor bone metastasis microenvironments and provide potential targets for new therapeutic strategies.

In the field of tumor therapy, targeted and immunotherapies have become key tools for inhibiting tumor growth and recurrence, especially for refractory tumors. However, current drug therapies are often accompanied by potential adverse effects, largely due to previous single-findings-oriented research and insufficient understanding of the tumor microenvironment. Giant cell tumor of bone (GCTB), composed mainly of active osteoclast-like giant cells expressing receptor activator of nuclear factor κ-B (RANK) and tumor mononuclear stromal cells expressing RANK ligand (RANKL), serves as an example. Denosumab (DMAB), a human monoclonal antibody against RANKL, is widely used in clinical targeted therapy for GCTB. Although multiple clinical trials have shown improved tumor response with DMAB, inhibition of RANKL may inactivate T cells, promoting immune escape—a major challenge in both treatment and understanding. Single-cell RNA sequencing has filled the gap in studying immune cell dynamics during DMAB treatment for GCTB by comprehensively profiling the tumor microenvironment. Studies have revealed that after DMAB treatment, the proportion of M2-type tumor-associated macrophages (TAMs) in GCTB increases. These M2-TAMs promote $CD8^+$ T cells to express immune checkpoint molecules such as LAG3 and PDCD1 via pathways like IL-10/IL-10R and PVR/TIGIT, while reducing the expression of cytotoxic genes such as GZMB, leading to the gradual differentiation of $CD8^+$ T cells into an exhausted state. Meanwhile, POSTN in tumor cells is highly expressed under the regulation of AP-1 family transcription factors; by binding to ITGAV/ITGB5 receptors on the surface of TAMs, it further promotes M2 polarization, forming an immunosuppressive microenvironment that collectively contributes to T cell exhaustion. This finding shows that DMAB treatment in GCTB correlates with $CD8^+$ T cell exhaustion (high LAG3/PDCD1), providing a basis for combining RANKL inhibition with immune checkpoint blockers.[70] These findings enhance our

understanding of GCTB pathogenesis and provide important molecular insights for optimizing treatment regimens and improving therapeutic outcomes.

In addition to traditional approaches (surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy), bioactive materials offer a new bioengineering perspective for tumor treatment. Single-cell technologies provide meticulous guidance for material design and composition, demonstrating significant potential in treating drug-resistant and refractory bone tumors. For instance, Li Wang et al. used single-cell transcriptome sequencing to meticulously map cell type-specific gene expression in chemotherapy-resistant osteosarcoma, identifying stem-like progenitors and characteristic molecules. This enabled targeted nanoparticle-enhanced combination therapy—specifically, nanoparticles targeting VEGFR2/JMJD3 in chemotherapy-resistant osteosarcoma—to enhance apoptosis in such cells.[71] This strategy not only improves treatment efficacy but also provides a new molecular solution for chemotherapy-resistant osteosarcoma. In the field of tumor bone metastasis, another research team constructed an interaction model between tumor cells and osteoclasts through single-cell spatiotemporal coupling analysis.[9] Based on this model, they designed a tetracycline-modified nano-lipid complex that achieves *in situ* decoupling and killing, effectively preventing bone metastasis. This innovative therapeutic approach offers a new strategy for early intervention in bone metastasis, demonstrating the broad application prospects of single-cell technologies in tumor therapy (Table S4). By resolving intratumoral heterogeneity and tumor-stroma interactions, single-cell analyses have revealed pathogenic mechanisms (e.g., ETS2/IBSP-driven metastasis, T cell exhaustion) and guided the development of targeted nano-therapeutics and combination immunotherapies for osteosarcoma, GCTB, and bone metastases.

Arthritis

Osteoarthritis (OA) is a complex, dynamically progressive disease characterized by multi-structural lesions in articular cartilage, menisci, synovium, and subchondral bone. Typical pathological changes include cartilage destruction, subchondral bone sclerosis remodeling, synovitis, and meniscal degeneration, ultimately leading to joint deformity. Limited by technical approaches and research perspectives, traditional studies cannot provide high-resolution maps of regional cell subpopulation composition (including cartilage, synovium, menisci, and subchondral bone) like single-cell sequencing technologies, thus failing to comprehensively reveal cell types and states in heterogeneous lesional tissues that reflect OA physiological disorders. For example, Su'an Tang et al. used single-cell RNA sequencing analysis of joint components to reveal the developmental homology between infrapatellar fat pads and synovial tissues, as well as key regulators of arthritis progression and potential targeted therapies.[72] Another team focused on cartilage, using scRNA-seq to conduct in-depth regulatory network and clustering analyses of osteoarthritis chondrocytes. They identified 11 chondrocyte subtypes, including novel effector chondrocytes, innate chondrocytes, abnormally differentiated chondrocytes, dedifferentiated subsets, and pro-inflammatory InfC chondrocytes ($CD74^{+}CXCL8^{+}$), as well as depleted $METRNL^{+}$ regenerative chondroprogenitors[73] (Figure 5A). The study elaborated on the transcriptional regulatory differences and specific functional characteristics of these subtypes during OA progression and cartilage repair, highlighting their distinct regulatory networks. In contrast, Fiorella Carla Grandi et al. used single-cell proteomics to subdivide bulk chondrocytes into distinct subpopulations, including those that dampen inflammation and those that amplify it. This subdivision enabled the design of precise pharmacological treatment strategies targeting anti-inflammatory and pro-inflammatory processes, respectively[17] (Figure 5B). Another research team used single-cell technologies to analyze gene set variations across different subpopulations, with a focus on the transcriptional profiles of hypoxia-inducible factor 1α (HIF-1α) in chondrocyte subpopulations. They validated that HIF-1α upregulation in oxygen-degraded hypertrophic chondrocyte subpopulations of the subchondral bone activates OA signaling pathways, thereby promoting OA progression. This work shows that such HIF-1α upregulation in these hypertrophic chondrocytes activates OA pathways, while maintaining hypoxia alleviates OA progression.[74] These findings provide new insights into the molecular mechanisms of osteoarthritis, not only enabling detailed analysis of multicellular interactions but also offering potential targets for personalized clinical drug therapy and demonstrating the potential for tissue engineering translation.[75]

Rheumatoid arthritis (RA) is a typical articular inflammatory disease characterized by inflammation and destruction of joint bone and cartilage. Triggered by autoimmune responses, RA leads to abnormal synovial tissue activity, promoting osteoclastogenesis and bone resorption while impairing osteoblast bone formation capacity, resulting in bone loss. Previous theoretical research and treatment strategies for RA were mainly based on the traditional view of interactions among the immune system, synovial fibroblasts, and bone. Specifically, activated T cells, B cells, and macrophages produce proinflammatory cytokines that stimulate synovial fibroblasts (tissue-resident mesenchymal cells in joints) to differentiate into proinflammatory and tissue-destructive subpopulations. These tissue-destructive synovial fibroblasts express receptor activator of nuclear factor κB ligand (RANKL), inducing osteoclasts to promote bone destruction, and express matrix metalloproteinases (MMPs) that accelerate cartilage degradation. However, existing RA treatments are not effective for all patients and may be associated with undefined potential adverse effects. Therefore, constructing an objective cognitive map based on high-throughput technologies to reveal intercellular communication—especially the new, comprehensive interaction among the immune system, synovial fibroblasts, and bone—is crucial for changing the existing treatment landscape. Through single-cell technology analysis and research integration, a complete map of the immune cell-fibroblast-bone triad in RA has been revealed, in which immune balance regulation between immunomodulatory Treg cells and autoinflammatory Th17 cells not only inhibits inflammation but also suppresses osteoclast bone resorption and promotes osteoblast bone formation. This is of great significance for further clarifying the mechanisms of immune cell-fibroblast-bone interactions and their effects on joint destruction and pathogenic synovial fibroblasts.[76] For RA, a dynamically progressive

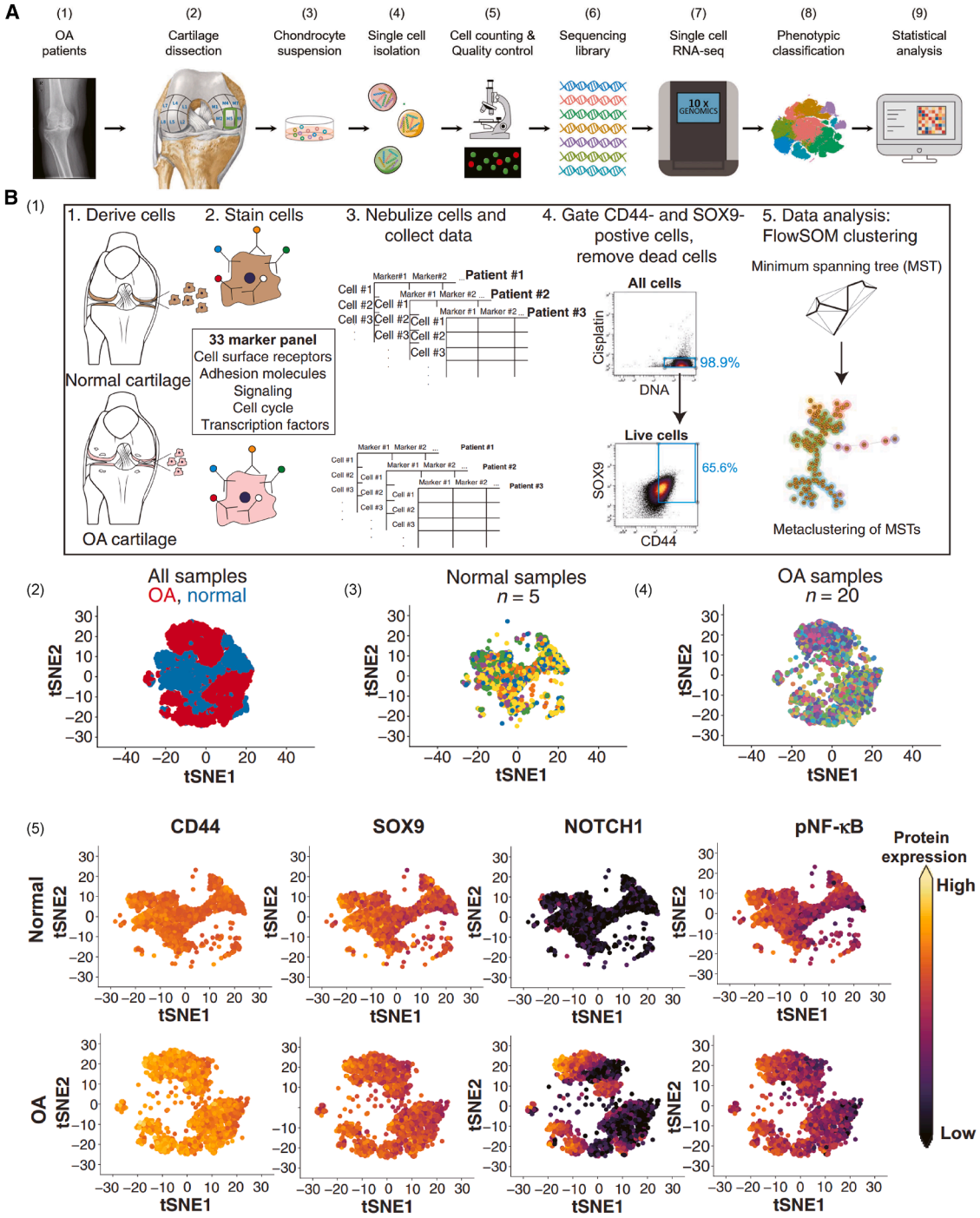


Figure 5. Applications of single-cell technologies in joint diseases

(A) Flowchart of experimental strategies for scRNA-seq sampling from femoral bones of control and OA groups by Zewen Sun et al.

(B) Process of high-dimensional analysis of normal and OA chondrocytes using bulk cytometry by Fiorella Carla Grandi et al.

disease, systematically revealing the characteristics of each developmental stage helps understand disease mechanisms and discover potential treatment strategies. Stefano Alivernini et al. conducted detailed analyses of single-cell data from patients with early/active RA, treatment-refractory/active RA, and sustained remission RA, summarizing the characteristics of synovial tissue macrophage subpopulations in each clinical state and the possibility of treating corresponding key specific subpopulations.[77] During treatment, single-cell technologies can also provide effective feedback on treatment responses and

mechanisms. For example, Qiuyan Guo et al. employed a multi-omics approach integrating single-cell RNA sequencing, proteomics, and transcriptomics to elucidate the mechanism of dihydroartemisinin in treating RA. Their research demonstrated that dihydroartemisinin regulates the MMP-mediated microenvironment—findings derived from scRNA-seq analyses—thereby reducing synovial inflammation and fibroblast activation in RA, providing a scientific basis for its clinical application.[78] Therefore, these research advances provide new perspectives and strategies for precision therapy of RA.

Another type of arthritis worthy of attention is inflammatory arthritis induced by immune checkpoint inhibitors (such as PD-1 inhibitors), which shares some clinical similarities with RA.[79] Although clinical evidence has confirmed heterogeneity between these two diseases, the application of single-cell technologies is particularly critical for in-depth analysis of their differential mechanisms. Ziyue Zhou et al. conducted comparative analyses at single-cell resolution on RA and PD-1 inhibitor (PD-1I)-related inflammatory arthritis (PD-1IA). In the synovial fluid of active PD-1IA, IL1Bhi macrophages are significantly enriched (almost absent in RA patients); these cells highly express chemokines, interferon-induced genes, and M1-type markers, with enrichment of multiple inflammatory pathways. Meanwhile, the proportion of $CD8^+$ exhausted T cells (Texs) is significantly increased, which can be divided into subsets such as terminally exhausted and progenitor exhausted cells. In contrast, synovial fluid from RA is enriched with monocyte-like macrophages and $SPP1^+$ macrophages, with more prominent involvement of B cells. In terms of molecular pathways, the TNF-NFκB pathway in PD-1IA is closely related to disease activity; RA involves a broader range of inflammatory pathways, including IL-6-JAK-STAT3, etc. Serologically, most PD-1IA patients are negative for anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor, while RA often presents with seropositivity. In addition, single-cell technologies have provided important cognitive supplements for other autoimmune diseases, such as psoriatic arthritis, juvenile idiopathic arthritis, and ankylosing spondylitis, correcting previous misunderstandings and biases. This is of great significance for basic scientific research, translational application of clinical treatment strategies, and formulation of individualized treatment plans for patients.

To optimize the treatment of joint diseases, including osteoarthritis and RA, significant research progress has been made in the field of biomaterials under the guidance of single-cell technologies. For example, through analysis of single-cell data in the GEO database and combined with a series of experimental validations, Haifei Cao et al. identified the cartilage-protective role of the key protein SOD3. They developed a promising new therapy for OA by purifying SOD3-rich extracellular vesicles and combining them with hydrogel microsphere delivery vectors.[80] Similarly, when developing combination treatment regimens for the core pathological processes of RA, Siyue Tao et al. obtained single-cell sequencing data of synovial $CD45^+$ $CD11b^+$ $Ly6G^-$ mononuclear macrophages from the published dataset GSE134420. They found that excessive activation of the complement system leads to impaired macrophage barrier function, thereby causing disruption of the macrophage niche. Based on these findings, the team designed a dual-targeted therapeutic nanoplatform that combines traditional anti-osteoclastic zoledronic acid therapy with novel therapies. Guided by scRNA-seq analysis of synovial $CD45^+CD11b^+Ly6G^-$ macrophages, this platform targets both complement overactivation and osteoclasts, aiming to restore macrophage niche function and reverse the core pathological processes of RA. This approach provides a new strategy for RA treatment.[81] These research advances demonstrate the powerful potential of single-cell technologies in deciphering the complex pathological mechanisms of joint diseases and provide a scientific basis for the development of novel biomaterials and treatment strategies, holding promise for improving patient treatment outcomes and quality of life (Table S5). Single-cell profiling has dissected the cellular and molecular heterogeneity of OA and RA, identifying disease-specific cell subtypes (e.g., InfC chondrocytes and IL1Bhi macrophages) and regulatory pathways—facilitating the design of personalized therapies, biomaterials, and precision pharmacology for joint diseases.

Bone-related systemic diseases

Osteoporosis

Beyond local bone and joint diseases, systemic disorders—especially those involving immune system disruption, such as osteoporosis—require comprehensive investigation of immune-skeletal interaction networks. Previous consensus on osteoporosis pathogenesis focused on the immune cell-proinflammatory cytokine-RANKL-osteoclast/osteoblast/bone marrow stromal cell axis, often overlooking the precision and high-resolution specificity of single-cell heterogeneity and intercellular communication. Thus, studies based on whole bone cell populations rather than single bone cell microenvironments are now feasible via scRNA-seq. Unlike single-cell methods like flow cytometry, magnetic-activated cell sorting (MACS), immunohistochemistry (IHC), and *in situ* hybridization (ISH), which focus on specific molecular information, scRNA-seq provides broader transcriptomic profiling with higher resolution and accuracy, identifies cell-specific regulatory modules, and reveals key underlying drivers. For example, Yuxin Wang et al. performed scRNA-seq on different subtypes of monocytes, T cells, and B cells in the bone microenvironment of osteoporotic femoral heads, revealing the immune landscape of osteoclast progenitors and osteoblasts. They reconstructed the communication cell network of human femoral head tissue, inferred how different femoral head cell types collaborate, and identified new bone metabolism-related gene profiles such as zinc finger protein 36 (ZFP36L1) and defensin 3 (DEFA3), offering new perspectives for comprehensively understanding osteoporosis molecular mechanisms and developing valuable therapeutic strategies.[33] In the molecular mechanisms of osteoporosis, single-cell technologies have also enabled more efficient and accurate novel predictions in previously explored research areas such as the miRNA axis. Specifically, they have identified the palbociclib/miR-141-3p/STAT4 axis in the context of oxidative stress, where miR-141-3p inhibits STAT4 to reduce osteoclastogenesis[37,82,83] (Figure 6A). In emerging fields such as long non-coding RNAs, single-cell technologies have yielded unexpected insights. For example,

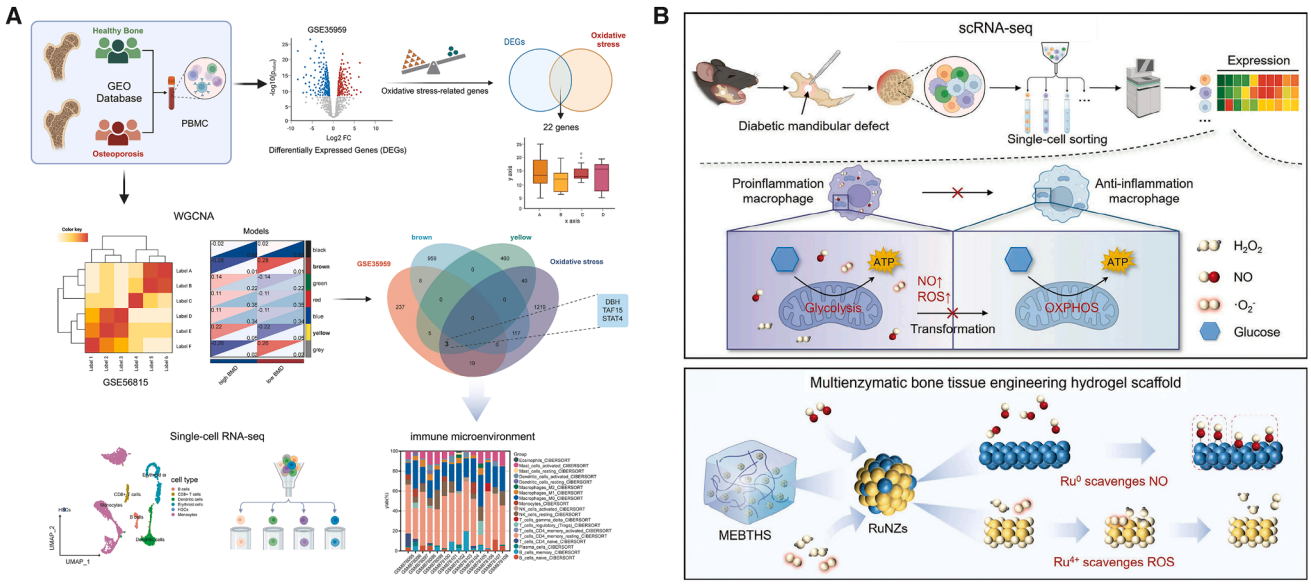


Figure 6. Applications of single-cell technologies in bone-related systemic diseases

(A) Jiajia Ji revealed a novel molecular mechanism axis of oxidative stress in osteoporosis using single-cell RNA-seq data and datasets from the Gene Expression Omnibus (GEO) database.

(B) Peihua Lin et al. guided the design of multi-enzyme hydrogel materials for self-regenerative repair of diabetic mandibular defects using single-cell RNA sequencing.

the highly conserved nuclear long non-coding RNA MALAT1, which is widely expressed across multiple tissues, has been confirmed via single-cell RNA sequencing to be downregulated in osteoporosis. This research also identified ZFP36L1^{+} osteoclast progenitors and DEFA3^{+} monocytes in osteoporosis, highlighting the protective roles of lncRNA MALAT1 in this context.[82] Additionally, during drug screening, single-cell technologies offer biological function references for target candidates,[84] providing insights into traditional treatment modalities and potential innovative therapies. For example, bisphosphonates, traditional osteoporosis medications, may cause atypical femoral fractures. Nianye Zheng et al. used single-cell sequencing to reveal that long-term bisphosphonate treatment leads to the abnormal transition of progenitor cells into fibroblast clusters. They also discovered that Mg-based biodegradable orthopedic implants can eliminate these fibroblast clusters and promote the healing of atypical femoral fractures.[85] These findings not only deepen our understanding of osteoporosis and its treatment but also provide a scientific basis for developing new therapeutic strategies and applying bioactive materials. Single-cell studies have unraveled the immune-skeletal crosstalk and molecular axes (e.g., palbociclib/miR-141-3p/STAT4) underlying osteoporosis, while clarifying the side effects of traditional therapies and guiding the development of Mg-based implants and targeted molecular interventions.

Diabetic osteopathy

As a typical representative of systemic bone diseases, diabetic osteopathy highlights the importance of osteoimmunology research. Although previous studies have focused on the "osteoimmune system" function formed by the coexistence and interactions of bone cells (including osteoclasts, osteoblasts, and osteocytes) and immune cells in the bone marrow microenvironment, and reported the effects of diabetic status on bone remodeling and underlying mechanisms, high-throughput systematic research on the impact of diabetes on osteoimmunology and bone metabolism in the bone matrix microenvironment remains insufficient. Single-cell RNA sequencing technology can effectively characterize the global profile of bone marrow cells and the unique osteoimmune microenvironment under both type 1 and type 2 diabetic conditions. In type 1 diabetes (T1D), it characterizes bone marrow cell profiles in T1D mice, identifying oxidative stress-related differentially expressed genes and impaired osteoclast differentiation in diabetic osteopathy.[86] For type 2 diabetes, similar single-cell analyses[87] further elaborate on details such as the heterogeneity of specific cell clusters (including neutrophils, B lymphocytes, monocytes, etc.) and trends in osteoclast inhibition, collectively enriching the understanding of diabetic osteopathy across different diabetes types. Similarly, single-cell technologies guide the design of regenerative repair materials for diabetic conditions. Addressing the impaired repolarization of pro-inflammatory macrophages to anti-inflammatory phenotypes in diabetic patients, single-cell analyses revealed the critical role of nitric oxide (NO) and reactive oxygen species (ROS) in hindering macrophage repolarization during diabetic physiological bone remodeling. Based on these findings, Peihua Lin et al. designed a multi-enzyme bone tissue engineering hydrogel scaffold (MEBTHS) that simultaneously scavenges NO and ROS to coordinate macrophage reprogramming. This scaffold revitalizes bone marrow-derived mesenchymal stem cells and endothelial cells in diabetic mandibular defects, enabling the generation of new bone with quality comparable to normal bone. Validated

by single-cell RNA sequencing, this work highlights that multi-enzyme hydrogels scavenge NO/ROS, restoring macrophage repolarization and the function of MSCs/endothelial cells in diabetic bone defects. It also identifies $CD36^+$ monocyte/macrophage subsets with impaired osteoclast differentiation in diabetic osteopathy, which guided the development of the multi-enzyme hydrogels[88] (Figure 6B). This achievement not only marks a major advance in bone tissue engineering but also provides new strategies and hope for treating diabetic osteopathy. Single-cell analyses have characterized the impaired osteoimmune microenvironment in diabetic bone, identifying $CD36^+$ macrophages as key mediators and guiding the design of multi-enzyme hydrogels that restore macrophage repolarization and bone regeneration in diabetic defects.

Immune-mediated bone marrow failure

In the research field of immune-mediated bone marrow failure, single-cell sequencing technologies have overcome the limitations of traditional methods—including cell culture, functional assays, immunophenotyping, and molecular biology approaches using blood/bone marrow samples and animal models—which were often constrained by limitations in cell quantity and type. The application of single-cell genomics and bioinformatics have enabled extensive multi-dimensional analysis of extremely limited cell numbers, revealing the scarcity of bone marrow cells, heterogeneity of hematopoietic and immune system components, and the complexity and variability of cell-intrinsic behaviors and intercellular interactions. The dense, complex data generated by these technologies provide high-resolution, molecular-level insights into the pathophysiology of diseases like immune-mediated bone marrow failure, their genetic developmental history, and the cell subpopulations targeted by therapeutic agents. Single-cell sequencing allows researchers to meticulously resolve dynamic changes in immune cell subpopulations and their specific roles in pathological states, illuminating the pathogenesis of immune-mediated bone marrow failure and aiding the development and evaluation of new therapeutic strategies.[89] By precisely identifying and targeting key cell subpopulations with high resolution, these technologies lay the foundation for personalized medicine and precision therapy (Table S6). Single-cell genomics has overcome limitations of traditional methods to resolve the heterogeneity of hematopoietic and immune cells in bone marrow failure, illuminating pathogenic mechanisms and enabling precise targeting of key cell subpopulations for personalized therapy.

Degeneration and aging

Bone aging

Aging in vertebrates is inevitable, associated with degeneration of regenerative stem cells and their niche environments. Unlike certain species, these stem cells cannot be continuously replenished by the stem cell system with age. Bone aging plays a pivotal role in overall senescence. Understanding how stem cell changes and interactions occur at the local bone niche level and their impacts on multi-organ physiological aging will significantly enhance our comprehension of the aging process (Table 3). Single-cell technologies have demonstrated remarkable capability in addressing such complex, multi-dimensional questions.

At the stem cell master regulation level, single-cell technologies have shed light on how aging-related degeneration of specific signaling pathway networks or key bone factors leads to dysfunction in stem/progenitor cells, thereby triggering a comprehensive decline in bone tissue structure and regenerative capacity. For instance, comparative analyses of bone cells from young versus middle-aged mice using these technologies have revealed an aging-related loss of Notch signaling in SSCs. This reduction in Notch signaling impairs osteogenic differentiation (the process by which stem cells develop into bone-forming cells) while promoting adipogenic differentiation (the formation of fat cells), contributing to the age-associated deterioration of bone health and regenerative potential.[93,94] These changes originate from cell-intrinsic alterations at the SSC level and clonal defects in functionally heterogeneous subpopulations, ultimately distorting bone and hematopoietic lineage outputs, reducing the integrity of bone anabolism and catabolism, and limiting healing capacity.[90] Thus, new insights provide a comprehensive framework for developing therapies to reverse age-related bone decline: locally targeting both autonomous and non-autonomous activities of senescent stem cells can restore aging-impaired bone regeneration, which is significant not only for bone but also for tissues like articular cartilage and menisci.[35] In the context of identifying and eliminating senescent cells, single-cell technologies provide precise definitions of aging markers. Specifically, they have identified $BCL\text{-}2^+$ senescent cells in aged bones that exhibit DNA damage (marked by $\gamma H2AX^+$) and express senescence-associated secretory phenotype factors such as IL-6 and TNF-α. These precise characterizations of senescent cell profiles guide the development of targeted senolytic strategies, enhancing the accuracy and efficacy of interventions aimed at clearing senescent cells to mitigate age-related bone deterioration[95] (Figure 7A). Similarly, they guide the design and efficacy evaluation of biomaterials for aging-related bone diseases. For example, Ye Sun et al. leveraged single-cell technologies to develop synovial mesenchymal cell organoid hydrogels tailored for addressing cartilage aging. Specifically, they created miR-24 mimic-loaded synovial mesenchymal cell organoid hydrogels (MSOH), which were shown to reduce chondrocyte senescence and enhance cartilage repair in a mouse model—validated through single-cell RNA sequencing. This work demonstrates the potential of single-cell approaches in both designing biomaterials for degenerative tissues and evaluating anti-aging effects, highlighting their utility in advancing regenerative strategies for age-related cartilage deterioration[96] (Figure 7C). These advances not only deepen our understanding of bone aging mechanisms but also provide a basis for developing novel anti-aging strategies. Single-cell technologies have revealed age-related changes in SSCs (e.g., Notch signaling decline and senescent SSC accumulation) and identified senolytic targets, while guiding the development of anti-aging biomaterials—providing strategies to reverse age-related bone loss and regenerative dysfunction.

Intervertebral disc degeneration

Intervertebral disc degeneration (IVDD) is a major cause of low back pain, with its pathological process primarily involving degenerative changes in the nucleus pulposus and annulus fibrosus. However, the specific mechanisms underlying the

Table 3. The relationship between clonal stem cell defects and clinical aging phenotypes in skeletal tissues

Skeletal tissue type	Clinical aging phenotypes	Clonal stem cell defects	Mechanistic links	Reference
Bone	osteoporosis (low BMD, fractures); delayed fracture healing	senescent SSPC clones with SASP (p16^{INK4a}, IL-6, TNF-α); epigenetic silencing of osteogenic genes (RUNX2, BMP2); mitochondrial dysfunction (reduced ATP, increased ROS)	SASP factors promote osteoclastogenesis (RANKL upregulation) and inhibit osteoblasts; notch signaling bias toward adipogenesis; impaired mechanosensitivity (reduced PC1-TAZ axis activity)	Wang et al.[33]; Tang et al.[72] Cao et al.[80] Ambrosi et al.[90] Liu et al.[61]
Cartilage	osteoarthritis (ECM degradation, joint pain)	clonal expansion of pathogenic chondrocytes (MMP13^{high} IL-1β^{high}); depletion of METRNL^{+} regenerative chondroprogenitors; epigenetic hypomethylation of NF-κB targets	pathogenic clones disrupt ECM via catabolic enzymes; loss of METRNL^{+} cells impairs matrix repair; inflammatory crosstalk with synovial macrophages (APOE-LRP1 axis)	Tang et al.[72] Sun et al.[73] Grandi et al.[17] Swahn et al.[35]
Tendon/ligament	tendinopathy (fibrosis, rupture risk)	clonal dominance of COL1A1^{high}fibroblasts; reduced Tppp3^{+}progenitor cells; impaired TGF-β/Smad signaling	disorganized collagen deposition (fibrosis); loss of tenogenic differentiation capacity; reduced ECM elasticity (ELN downregulation)	Lin et al.[57] Yea et al.[58]
Intervertebral disc	IVDD (nucleus pulposus degeneration, back pain)	clonal expansion of Fibro-NPCs (FBLN1^{+}, SRGN^{high}); depletion of CD24^{+}regenerative progenitors	fibro-NPCs drive ECM fibrosis and inflammation; reduced proteoglycan synthesis (ACAN downregulation); pyroptosis (GSDMD, NLRP3 activation)	Tu et al.[34]; Wang et al.[36]; Chen et al.[91] Sun et al.[92]

molecular complexity and cellular heterogeneity of these structures remain poorly understood. In clinical diagnosis and treatment—especially with the advancement of precision medicine—staging, grading, and personalized therapy for diseases have become increasingly important. For IVDD, there is an urgent need for an objective, systematic, comprehensive, and convenient tool to dynamically monitor gradient changes in degenerated tissues with high resolution and map detailed profiles. Single-cell technologies have demonstrated unique advantages in this field, effectively undertaking this task. Through single-cell analysis of nucleus pulposus cells across different grades of intervertebral disc degeneration, researchers have not only accurately mapped the dynamic cellular composition of degenerated intervertebral discs but also identified multiple previously unknown cell types. For instance, studies have characterized the transcriptomic changes in nucleus pulposus cells across Pfirrmann grades, identifying novel cell types such as Fibro-NPCs, which drive extracellular matrix fibrosis in IVDD. Fan Chen et al.'s work further elaborates on these findings, highlighting Serglycin^{+} late-stage nucleus pulposus cells as potential biomarkers for IVDD. Additionally, their research points to pyroptosis (mediated by GSDMD) as a key therapeutic target in addressing intervertebral disc degeneration, underscoring the value of single-cell analyses in uncovering both cellular heterogeneity and actionable mechanisms in this condition[34,36,91] (Figure 7B). These studies have revealed the phenotypic characteristics and complexity of nucleus pulposus and annulus fibrosus cells, their regulatory networks with immune cell subsets, specific biomarkers, and related processes, providing new insights and potential therapeutic clues for IVDD treatment. Building on this, Kaiqiang Sun et al. developed an antioxidant catalytic manganese-containing nanomedicine with the ability to inhibit nucleus pulposus cell pyroptosis for IVDD under the guidance of single-cell technologies.[92] This achievement not only provides an effective bioengineering strategy for IVDD treatment but also demonstrates the significant value of single-cell technologies in decoding disease mechanisms and developing bioengineering therapeutic strategies. These research advances have deepened our understanding of IVDD and hold promise for improving patient outcomes and quality of life (Table S7). Single-cell analyses have mapped the dynamic cellular composition of degenerated discs, identifying fibro-NPCs and pyroptosis as key pathological drivers, and guiding the development of catalytic nanomedicines and regenerative strategies for IVDD.

DISCUSSION

This review highlights how single-cell technologies have revolutionized skeletal research by enabling high-resolution mechanistic

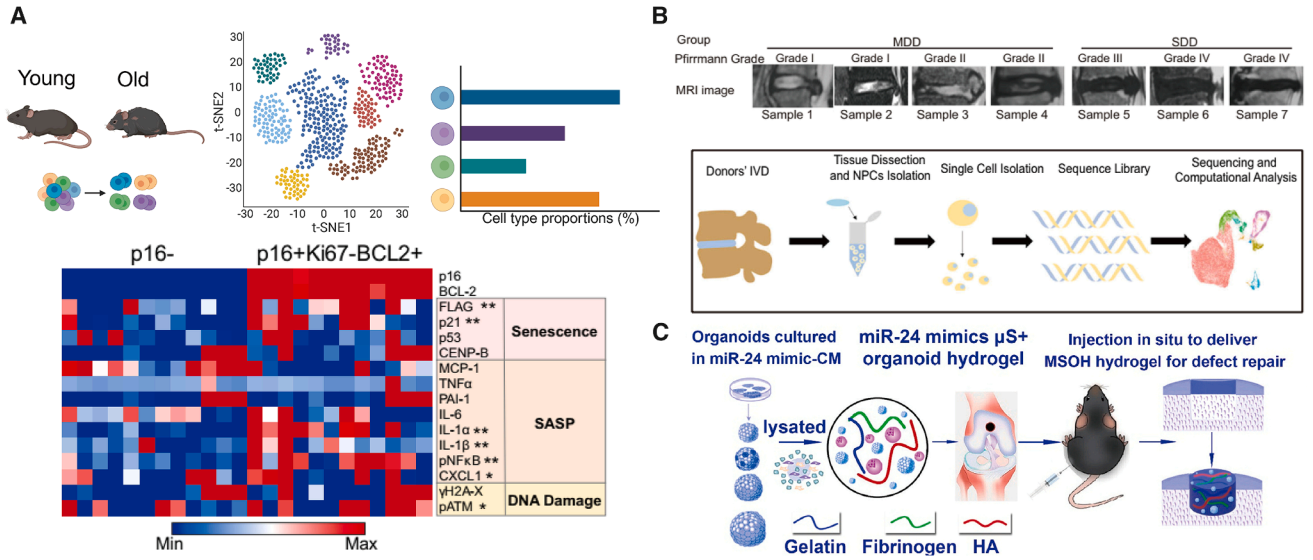


Figure 7. Applications of single-cell technologies in bone degeneration and aging

(A) Single-cell visualization and clustering of senescent characteristic cell pools from young and aged mice by Madison L. Doolittle et al., revealing that BCL-2 expression is involved in determining cellular senescent characteristics.

(B) Fan Chen et al. similarly performed single-cell analysis of nucleus pulposus in degenerative intervertebral discs at different degrees.

(C) Guided by single-cell technologies, Ye Sun et al. designed a composite hydrogel targeting senescence to repair cartilage defects and prevent joint degeneration by improving chondrocyte homeostasis.

insights and driving therapeutic translation. The integration of spatial relationships and multi-omics approaches has not only enhanced our understanding of physiological and pathological processes but also provided a framework for developing innovative therapies and biomaterials. Single-cell technologies have dismantled traditional research limitations by resolving cellular heterogeneity in bone microenvironments. For example, scRNA-seq has identified novel cell subpopulations such as mesenchymal stromal cell-derived septoclasts in fracture healing[62] and synovial progenitor cells in HO,[58] redefining disease mechanisms. ST has further revealed the spatial heterogeneity of tumor-immune interactions in osteosarcoma,[66] guiding the design of spatially targeted nano-therapeutics.[9] These technologies have also facilitated the development of biomimetic materials, such as hydroxyapatite scaffolds optimized via single-cell transcriptional profiling[8] and multi-enzyme hydrogels for diabetic bone repair,[88] demonstrating their direct translational value.

Bone tissue presents unique technical challenges in single-cell and spatial omics analyses: its dense extracellular matrix complicates single-cell isolation, often resulting in low cell yields and compromised RNA integrity.[97] Notably, while spatial omics technologies offer excellent explanatory power for gene expression in bone—tissue closely linked to morphological features[27] and mechanical properties[26]—spatial omics analysis typically requires decalcification, a process that can distort genetic signals. Emerging solutions, such as undecalcified fresh-frozen sectioning for spatial metabolomics, show promise in preserving tissue architecture while avoiding the artifacts introduced by decalcification. Additionally, a key limitation of current ST platforms is their relatively low resolution, with capture spots often containing multiple cells. This drawback highlights the need for technological advancements to better balance spatial precision with single-cell-level gene expression profiling,[98] ensuring more accurate and detailed insights into the spatial organization of bone cellular heterogeneity.

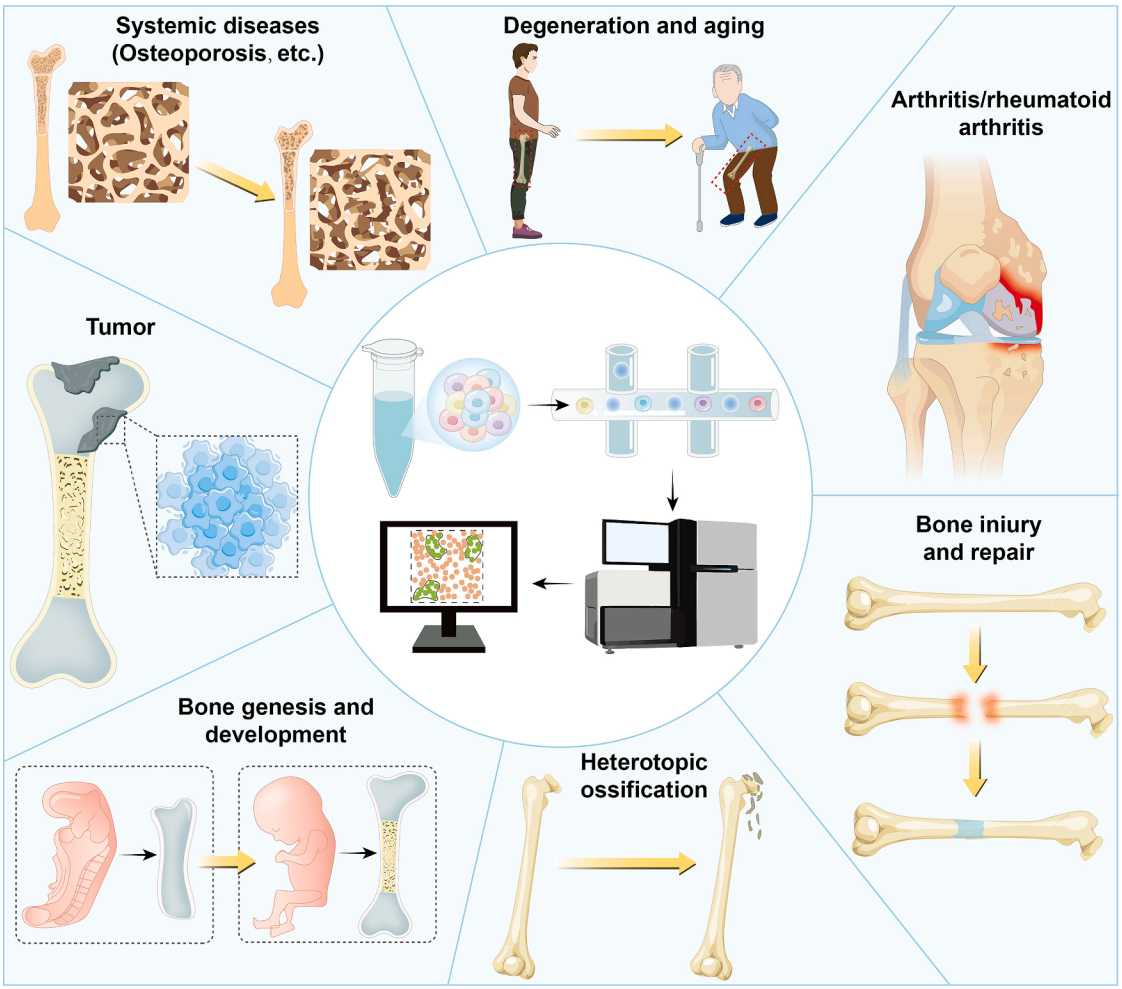
In addition, it can be noted that current research is still mostly dominated by scRNA-seq, while the applications of single-cell DNA sequencing, epigenomic scATAC-seq, proteomic CyTOF, and spatial omics are not common. Besides sorting out the advantages and disadvantages of various omics in application at the theoretical level (Table 2), we have also analyzed the reasons behind the current research pattern dominated by scRNA-seq as follows. First, in terms of technical characteristics, scRNA-seq can directly capture the gene expression status of cells, reflect cell functions and phenotypes, and has a more direct correlation with many biological issues (such as cellular heterogeneity, differentiation trajectories, etc.), making it easy to interpret its biological significance. In contrast, single-cell DNA sequencing mainly focuses on genomic variations, which is less targeted for exploring functional issues such as gene expression regulation; scATAC-seq focuses on chromatin accessibility, and its data interpretation requires combining information such as gene expression, resulting in high analysis complexity; although CyTOF can detect cell surface proteins, the number of markers that can be detected simultaneously is limited, and it is highly dependent on specific antibodies for proteins; spatial omics faces technical challenges such as resolution and data analysis difficulty, which have higher requirements for experimental design and analysis methods. Second, from the perspective of research needs, the core of many basic and clinical studies is to understand the functional changes of cells under specific physiological or pathological states, and gene expression is a

Table 4. Under single-cell technologies: Connecting mechanistic findings from different skeletal disease contexts to clinical applications

Skeletal disease context	Mechanistic findings (from scRNA-seq/spatial transcriptomics)	Therapeutic translation (biomarkers/drug targets/ strategies)
Osteoporosis	osteocyte ferroptosis via Nrf2/Dnmt3a/RANKL axis in PMOP.[72] T1D: BM-neutrophil_1 enrichment and B lymphocyte depletion drive bone loss.[86] senescent SSCs overexpress CSF1/RANKL, losing osteogenic potential.[90]	biomarker: neutrophil/B lymphocyte ratio for T1D osteopenia. drugs: Nrf2 agonists (e.g., bardoxolone methyl) to mitigate ferroptosis; anti-IL-17 to suppress neutrophil inflammation. strategies: dual targeting of SSCs (senolytics + PDGFRβ agonists) for age-related bone loss.
Osteoarthritis	pathogenic senescent cells in cartilage/meniscus overexpress ZEB1, disrupting matrix.[35] synovial APOE from Inflamm.Mφ and fibroblasts promotes chondrocyte ECM degradation[72] loss of METRNL^{+} regenerative chondrocytes; PRG4^{+} cells drive abnormal repair via Hippo-TAZ[73]	biomarker: ZEB1 expression in cartilage for OA severity. drugs: ZEB1 inhibitors/senolytics (navitoclax); anti-APOE antibodies to block synovial-chondrocyte crosstalk. strategies: HIF-1α stabilizers (DMOG) + Hippo inhibitors (verteporfin) to restore chondrocyte homeostasis.
Fracture healing	FL-PCs differentiate into neo-SSPCs but form poor-quality bone.[60] mechanical loading upregulates Wnt7b/Cd44 in high-strain regions; low-strain areas enrich TNF-α[26]; B cells secrete exosomes inhibiting osteoblasts via RANKL.[7]	biomarker: B cell exosome levels for healing delay. drugs: TGF-β agonists to enhance neo-SSPC formation; Wnt7b mimetics for high-strain regions. strategies: personalized fixation + anti-CD20 (rituximab) to reduce B cell-mediated delays.
Heterotopic ossification (HO)	Tppp3^{+} progenitors secrete BMPs/VEGF, driving chondrogenesis/osteogenesis.[58] macrophage-derived TGF-β1 promotes MSC differentiation; CD47 activation suppresses HO.[59] Mkx$^{-/-}$ mice: TPC skewing + angiogenesis via VEGF.[57]	biomarker: Tppp3 expression in synovium/tendon sheath for HO risk. drugs: BMP inhibitors (noggin); CD47 agonists (TT30); anti-angiogenics (BIBF1120). strategies: targeting Tppp3^{+} progenitors + macrophage reprogramming to block aberrant ossification.
Intervertebral disc degeneration	fibro-NPCs overexpress SRGN and collagen genes, driving fibrosis.[85] fibro-NPC pyroptosis (GSDMD, CASPASE3) correlates with IVDD severity.[92] CD90^{+}Fibro-NPCs retain stem potential but lose chondrogenic capacity.[87]	biomarker: SRGN levels in NP tissue for IVDD staging. drugs: SRGN siRNA; NLRP3 inhibitors (MCC950); catalytic nanodots (MCDs) to suppress pyroptosis. strategies: CD90^{+}Fibro-NPC transplantation with TGF-β3 priming for NP regeneration.

direct reflection of function, so scRNA-seq can better meet this demand. Research on DNA-level variations, epigenetic regulation, or protein expression is often further in-depth based on gene expression research, belonging to more subdivided research fields with relatively narrow application scenarios. Third, cost and technical maturity are also important factors. scRNA-seq technology has developed earlier, with relatively mature experimental procedures and data analysis methods, and its cost has gradually decreased, making it more easily accepted and applied by research teams. In contrast, other technologies such as spatial omics have higher equipment and reagent costs, complex technical processes, and their data analysis methods are still being continuously improved, which limits their widespread application. However, with the continuous development of technology, the applications of these technologies are gradually increasing. Their combination with scRNA-seq can provide a more comprehensive perspective for biological research.

Therapeutic translation in skeletal diseases is advancing rapidly, driven by mechanistic insights gleaned from single-cell technologies. By targeting cell-specific pathways—such as ferroptosis in osteocytes and APOE-related mechanisms in the synovium—and leveraging spatial dynamics (e.g., mechanical strain gradients and progenitor niches), a range of novel therapies is emerging, spanning small molecules to cell-based interventions (Table 4). Notably, some single-cell studies have already become closely integrated with clinical trials. For example, Zachary D. Crees et al. performed scRNA-seq analysis on patient samples from an international, multicenter phase 3 clinical trial (NCT03246529). This analysis identified specific hematopoietic stem/progenitor cell (HSPC) populations that drive the success of autologous stem cell transplantation (ASCT).[99] Going a step further, researchers have proposed an emerging discipline—clinical single-cell biomedicine (cscBioMed)—by integrating single-cell biology, single-cell systems biology, and single-cell-based medical approaches.[100] However, significant challenges persist. These include addressing species differences that may limit translational relevance, conducting robust clinical validation in large patient



Scheme 2. Summary of single-cell technology applications in skeletal system scenarios

cohorts, and optimizing drug delivery specifically to skeletal tissues. To overcome these hurdles, integrating multi-omics data with clinical phenotypes will be critical. This integration is poised to accelerate the development of precision medicine strategies, ultimately improving outcomes for patients with skeletal disorders.

Future challenges and directives

The next generation of single-cell technologies in skeletal research must address critical challenges while embracing innovative strategies to unlock their full potential. Foremost among these challenges are high costs and scalability issues[101]: current single-cell sequencing platforms demand substantial investment in specialized equipment and reagents, limiting access for smaller research laboratories. Meanwhile, scaling to analyze large patient cohorts—essential for capturing the full spectrum of skeletal disease heterogeneity—remains logistically complex. These barriers necessitate the development of cost-effective microfluidic systems and automated processing pipelines to reduce per-sample expenses and enable high-throughput analysis across diverse populations.

Concurrently, wider accessibility and standardization of analytical instruments and workflows hold promise for resolving another pivotal obstacle in technology translation: data quality. Current issues including inconsistent data standardization across platforms, algorithmic biases in trajectory and pseudotime inference, and risks of overinterpretation without functional validation create significant hurdles for multi-omics data integration. In some cases, these challenges have even generated misleading "data noise" or even "data garbage" that researchers must painstakingly identify and exclude.[102] Encouragingly, growing recognition of these problems, coupled with the anticipated standardization and broader adoption of analytical instruments and protocols, offers a clear path toward meaningful improvement—and this progress can be further accelerated by the establishment of a "Human Skeletal Cell Atlas" initiative, which is critical to unifying advances through standardized datasets and cross-study integration.[24,38]

This collaborative effort would directly address data standardization across platforms by defining common metrics for cell type annotation, sequencing depth, and clinical metadata—ensuring that scRNA-seq data from femoral head samples, for

instance, can be compared across research centers regardless of the sequencing technology used. To tackle algorithmic biases in trajectory and pseudotime inference, the Atlas would curate diverse reference datasets encompassing samples from different ages, ethnicities, and disease states, laying the groundwork for more generalizable computational tools. Importantly, it would also implement safeguards against the risks of overinterpretation without functional validation by linking transcriptional profiles to orthogonal evidence—such as *in vivo* bone formation capacity or response to therapeutic interventions—through a centralized database of validated findings. Together, these efforts would not only mitigate current data quality issues but also create a robust framework for integrating multi-omics insights, driving skeletal research toward more reliable and translatable discoveries.

Equally pressing are difficulties in validating single-cell-derived targets *in vivo*, stemming from several key factors: the disconnect between *in vitro* transcriptional profiles and *in vivo* functional behavior, the rarity of certain cell subpopulations identified by scRNA-seq, and the complexity of recreating physiological microenvironments in validation models. To address these, researchers are increasingly combining lineage-tracing mouse models with CRISPR-based gene editing to selectively perturb candidate cells in living organisms, while advanced imaging techniques like intravital microscopy allow real-time visualization of cell dynamics during bone formation or fracture healing. Additionally, orthogonal validation using bulk omics and functional assays—such as measuring the mineralization capacity of perturbed cell populations—helps confirm that transcriptional signatures correspond to meaningful biological phenotypes. In particular, when notable discrepancies exist among multi-omics technologies, combined analyses are required to better guide clinical decision-making in the skeletal system (including but not limited to the treatment of osteoarthritis and osteosarcoma, etc. Table S8).[102–104]

AI/ML applications hold tremendous prospect for accelerating skeletal research, with algorithms poised to predict cell fate transitions, identify therapeutic targets, and model disease progression with unprecedented precision. For example, deep learning models trained on single-cell data have already predicted novel miRNA regulators of osteoclastogenesis in osteoporosis,[37,82,83] while graph neural networks are being used to map intercellular communication networks in bone marrow. To ensure these tools deliver reliable insights, rigorous data quality assurance is paramount: this includes implementing standardized preprocessing pipelines to remove technical noise, curating diverse training datasets that reflect demographic variability, and validating model outputs against experimental data. Furthermore, transparent algorithm design and benchmarking against gold-standard methods will help mitigate risks of overfitting to specific datasets.[48]

The synergy between patient-derived organoids (PDOs) and single-cell technologies offers transformative prospect for personalized skeletal research. Skeletal PDOs—3D cultures containing patient-specific osteoblasts, chondrocytes, and stromal cells—recapitulate key features of *in vivo* bone and cartilage microenvironments, while single-cell profiling enables detailed characterization of their cellular composition and dynamic changes. This combination is already driving applications such as modeling rare skeletal dysplasias to identify disease mechanisms, testing patient-specific responses to osteoporosis drugs, and optimizing biomaterial interactions for personalized implant design.[105] By preserving the genetic and epigenetic background of individual patients, these systems bridge the gap between population-level single-cell studies and personalized therapeutic development.

Similarly, capturing real-time cell-cell interactions—an emerging frontier in skeletal single-cell research—can be more effectively achieved using PDOs, offering a pathway to overcome current technical barriers and ultimately realize *in vivo* real-time tracking. Presently, single-cell tracing of real-time intercellular interactions is constrained by two key limitations: imaging duration, as phototoxicity from confocal microscopy, for instance, triggers osteocyte apoptosis during prolonged observation; and resolution, which struggles to capture nanoscale synapse-like contacts. As a result, most existing studies rely on static snapshots combined with computational simulations. However, advancements in technology are bridging this gap. By employing advanced co-culture systems paired with live-cell imaging and single-cell ST, researchers can now directly visualize dynamic processes.[106] It is believed that before long, we will witness the standardized application of this technology in the field of the skeletal system.

After all, advancing skeletal single-cell research requires deep collaboration between biologists, engineers, and clinicians. For example, integrating single-cell data with biomaterial design has led to "osteoimmune intelligent" implants that modulate the microenvironment for improved bone integration.[63] Such collaborations will be critical for translating mechanistic insights—like the role of B cells in fracture healing[7]—into clinical therapies. In summary, single-cell technologies have reshaped skeletal research by providing a bridge between basic science and therapeutic innovation. Overcoming technical hurdles, ensuring rigorous validation, and fostering interdisciplinary collaboration will be essential to unlocking their full potential in precision medicine for skeletal disorders.

Limitations of the study

While this review synthesizes the applications of single-cell technologies in skeletal system research, it has inherent limitations. First, the focus on published studies may introduce bias toward well-characterized skeletal pathologies (e.g., osteoarthritis, osteosarcoma) and underrepresent rare skeletal disorders. Second, technical heterogeneity across single-cell platforms (e.g., scRNA-seq vs. spatial omics) and data analysis tools complicates direct comparison of findings across studies. Third, due to the rapid evolution of single-cell technologies, some emerging methods (e.g., high-resolution spatial multi-omics) and their translational potential could not be fully integrated. Finally, the translation of single-cell-derived insights to clinical practice is still in its early stages, and this review does not fully cover practical challenges such as sample processing standardization and cost-effectiveness in clinical settings.

CONCLUSION

This article systematically reviews how single-cell technologies serve as research tools in key physiological and pathological processes of the skeletal system, including its genesis, development, maintenance, injury, repair, lesions, and degeneration (Scheme 2). It comprehensively analyzes the relationship among various single-cell technologies, bone-related clinical treatments, and bioengineering translation. On one hand, it aims to offer a more comprehensive and updated high-resolution understanding of skeletal system theoretical knowledge. On the other hand, it promotes the in-depth development of single-cell technologies within this systematic field. Finally, by analyzing the advantages and disadvantages of single-cell technologies, it provides feedback for the collaborative advancement between this technology and related disciplines, with the goal of achieving a benign interaction and mutual optimization between disciplinary development and technical tools.

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AUTHOR CONTRIBUTIONS

G.Z., W.L., and L.G. conceived the study and designed the framework. G.Z., W.L., L.G., Y.X., and L.W. conducted literature collection and investigation. Z.L. provided technical guidance and supervision. X.Y. supervised the study, contributed to methodology and conceptualization, and revised the manuscript. All authors drafted the original manuscript, discussed the results, and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that AI tools were solely utilized for non-content-related, technical proofreading (e.g., checking spelling, grammar, and terminology consistency) to ensure linguistic accuracy. Such limited use did not involve any participation in content creation, logical construction, or scientific decision-making, and all final content was reviewed, verified, and approved by the authors.

SUPPLEMENTAL INFORMATION

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