

Review

The Role of Stem Cells in Cartilage and Bone Regeneration: From Mechanistic Insights to Clinical Translation

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Abstract

Stem cells play a central role in cartilage and bone regeneration through mechanisms such as multi-directional differentiation, paracrine regulation, and microenvironment remodeling. Among them, mesenchymal stem cells (MSCs) are particularly important, as they promote the formation of cartilage matrix and bone tissue by activating multiple transcriptional networks and signaling pathways. Additionally, stem cells secrete exosomes and growth factors that facilitate angiogenesis, thereby aiding in the repair of damaged tissue in situ. In recent years, stem cell technology has advanced rapidly. Innovations such as single-cell sequencing (SCS) and organoid models have provided valuable platforms for stem cell research and laid the groundwork for translating basic research into clinical applications. However, the clinical application of stem cell therapy still faces challenges, including low survival rates of transplanted cells and the risk of tumorigenesis. Future research will likely focus on precisely regulating the differentiation microenvironment, enhancing the survival of transplanted cells, and accelerating the transition of regenerative medicine from laboratory studies to clinical practice.

Keywords: Stem cells, chondrogenesis, osteogenesis, organoids.

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Introduction

Osteochondral lesions are implicated in the pathology of a variety of orthopedic disorders, notably osteoarthritis (OA) and fracture non-union [1]. OA exhibits a marked age-related prevalence, and its incidence has increased in line with global demographic aging. Epidemiological studies reveal that approximately 37% of individuals aged 60 years and older in the United States of America (USA) receive a clinical diagnosis of OA [2,3]. Between 1990 and 2017, the global burden of musculoskeletal diseases increased by 58% [4]. OA-associated osteochondral damage has significant socioeconomic impacts worldwide, producing debilitating physical and psychological morbidity in patients and considerable healthcare costs [5,6].

Current therapeutic strategies for OA encompass both pharmacological and non-pharmacological approaches [7]. Pharmacological treatments, however, often show limited efficacy in halting long-term degeneration. By contrast, non-pharmacological approaches—including dietary modification, structured exercise programs, and surgical interventions—are the cornerstone of OA management [3].

Arthroscopic procedures have been effective in alleviating symptoms and facilitating functional rehabilitation of patients unresponsive to conventional therapies [8]. Importantly, regardless of the chosen therapeutic approach, regeneration of functional osteochondral architecture remains a fundamental prerequisite for clinical resolution. Thus, skeletal tissue regeneration is a key therapeutic paradigm [9]. Accordingly, comprehensive elucidation of the mechanisms of bone and cartilage regenerative pathways and the targeted modulation of these processes have become the focus of research aimed at optimizing therapeutic outcomes.

Stem cells are characterized by sustained self-renewal ability and multipotent differentiation potential, which enable them to maintain tissue homeostasis, facilitate repair, and drive regeneration in adult organisms [10]. As such, they are crucial mediators of human organogenesis and post-injury tissue remodeling [11,12]. The core of stem cell therapy is the exploitation of the intrinsic properties of these cells (self-renewal and multilineage differentiation) to restore or replace damaged tissue. In addition to direct differentiation, stem cells exert paracrine effects by secreting cy-

tokines and extracellular vesicles (EVs), thereby modulating immune responses, promoting angiogenesis, and fostering a regenerative microenvironment. These impacts make stem cell therapy foundational to regenerative medicine that emphasizes cellular replacement and tissue regeneration. By leveraging such unique properties, stem cell-based approaches facilitate advanced tissue engineering strategies and have significant potential in therapeutic orthopedic applications, particularly osteochondral regeneration where coordinated repair of cartilage and subchondral bone remains a clinical challenge [13,14].

The regeneration of osseous and cartilaginous tissues is a highly intricate and tightly regulated process involving cellular metabolic reprogramming, lineage commitment, and dynamic immunomodulatory microenvironmental interactions. In this context, immune microenvironment regulation refers to the modulation of inflammatory cells, cytokines, and signaling pathways to maintain local immune homeostasis and support tissue repair [15]. In stem cell-induced cartilage and bone regeneration, MSCs and their EVs facilitate macrophage polarization toward an anti-inflammatory M2 phenotype, enhance regulatory T cell recruitment, and suppress pro-inflammatory cytokine release, thereby creating a regenerative niche conducive to matrix deposition [16]. Thus, precise modulation of the immune microenvironment is a key mechanism underlying stem cell-mediated osteochondral regeneration and an important avenue to enhancing clinical efficacy. This complexity is exemplified by the sophisticated crosstalk between the immune and musculoskeletal systems [17]. During the post-injury repair phase, mobilization of stem cells to a lesion site is essential for niche reconstruction as this enhances cellular proliferation and differentiation and promotes osteochondral regeneration [18]. This framework highlights the indispensable role of stem cells in orchestrating osteogenic cascades and underscores the necessity of in-depth investigations into their functional repertoire and mechanistic contributions to skeletal tissue morphogenesis.

Types and Characteristics of Stem Cells

Stem cells can be classified by developmental potential and cell source, which are fundamental to their biomedical application [19]. Developmental potential encompasses totipotent, pluripotent, multipotent, oligopotent, and unipotent stem cells, with abilities ranging from the generation of all tissues to commitment to a single lineage [20]. Cell source encompasses embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs), and MSCs, as well as tissue-specific populations such as neural stem cells (NSCs) and epidermal stem cells [21]. Adipose-derived mesenchymal stem cells (AT-MSCs), bone marrow-derived MSCs (BM-MSCs), and umbilical cord MSCs (UC-MSCs) are especially important in cartilage and bone regeneration due to their accessibility, immunomodulatory properties, and strong chon-

drogenic/osteogenic potential. ESCs and iPSCs have provided key insights into development and disease modeling, with iPSCs offering patient-specific therapies without ethical concerns [22]. In addition, skeletal stem cells (SSCs) are a lineage-restricted population with self-renewal capacity. They can give rise to bone, cartilage, and stromal cells and show promise in skeletal tissue engineering applications (Fig. 1) [23].

MSCs

MSCs are multipotent stromal cells predominantly located within connective tissues. They are distinguished by their robust self-renewal capacity and multilineage differentiation potential, producing osteoblasts, chondrocytes, adipocytes, and other cell types that contribute directly or indirectly to osseous regeneration. MSCs are broadly classified by their tissue origin into adult-derived (e.g., bone marrow and adipose tissue) and perinatal-derived (e.g., umbilical cord, placenta, amniotic fluid, and synovium) (Fig. 2) [24]. Bone marrow is the earliest identified and most extensively studied reservoir of adult-derived MSCs. *In vitro* investigations demonstrate that BM-MSCs possess superior osteogenic differentiation capacity [13], but their clinical application is hindered by limited cell yield and the invasiveness of harvesting procedures, such as iliac crest aspiration, which can cause patient discomfort and increased risk of infection [25].

By contrast, AT-MSCs are more accessible. Adipose tissue contains an MSC population approximately 500-fold higher than bone marrow, and minimally invasive liposuction procedures facilitate the efficient isolation and expansion of AT-MSCs with markedly reduced patient discomfort [13,26]. Although AT-MSCs exhibit a pronounced tendency toward adipogenic differentiation, increasing evidence supports their application in bone tissue engineering [27].

UC-MSCs are a category of perinatal stem cells [13]. They can be collected via non-invasive methods since the umbilical cord, placenta, and amniotic membrane (rich sources of MSCs) are typically discarded as medical waste following childbirth. Stem cells isolated from these perinatal tissues have substantial potential to differentiate into osteoblasts, adipocytes, and other mesenchymal lineages. They also have the distinct advantages of low immunogenicity and minimal ethical concerns, making them highly attractive for regenerative medicine applications [28].

MSCs have the intrinsic ability to undergo multilineage differentiation, giving rise primarily to osteoblasts, chondrocytes, and adipocytes. Pittenger was the first to demonstrate this multipotent characteristic in 1999, showing that human BM-MSCs could differentiate into distinct mesenchymal lineages [29]. The fate decisions of MSCs are tightly regulated by external cues (including chemical, physical, and biological factors) that activate specific intracellular signaling pathways and transcription factors. These

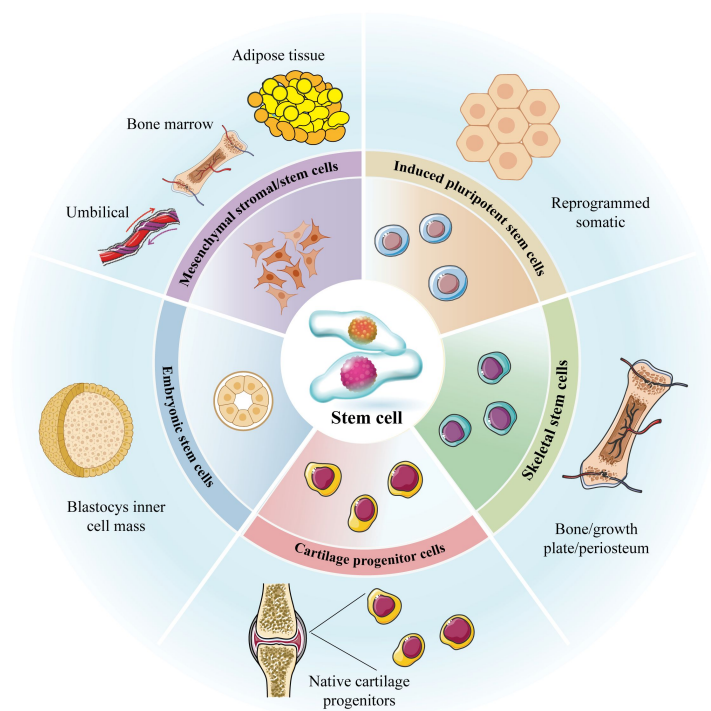


Fig. 1. The sources and types of stem cells. Types of stem cells include ESCs, iPSCs, cartilage progenitor cells (CPCs), MSCs, SSCs, and so on. Drawing software: Adobe Illustrator.

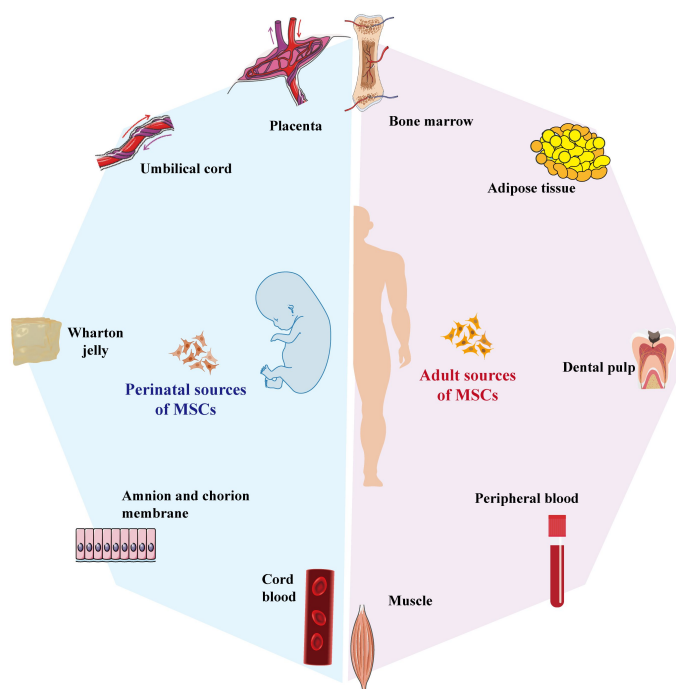


Fig. 2. The source of MSCs. According to the sources, MSCs can be classified into adult sources of MSCs and Perinatal sources of MSCs. In adults, the sources of MSCs include bone marrow, adipose tissue, dental pulp, peripheral blood, and muscle. The sources of MSCs in perinatal infants include placenta, umbilical cord, Wharton's jelly, amnion and chorion membrane, and cord blood. Drawing software: Adobe Illustrator.

mechanisms regulate the balance between osteogenic and adipogenic differentiation, which is essential for skeletal homeostasis and metabolic equilibrium.

The differentiation process of MSCs can be broadly divided into two sequential phases: lineage commitment and maturation. Lineage commitment refers to the transition from multipotent MSCs to lineage-restricted progenitor cells, while maturation describes the progression from committed progenitors to fully differentiated, functional cell types [30]. Several key signaling pathways critically influence lineage commitment, including the transforming growth factor-beta/bone morphogenetic protein (TGF- β /BMP) pathway, the Wnt signaling cascade, the Notch pathway, and fibroblast growth factors (FGFs) [31].

During osteogenic differentiation, TGF- β /BMP ligands engage type I and type II serine/threonine kinase receptors (TGF- β RI and TGF- β RII), triggering downstream canonical Smad-dependent and non-canonical signaling pathways [32,33]. Activation of these pathways upregulates the expression and activity of runt-related transcription factor 2 (Runx2), a master regulator that is essential for mesenchymal progenitor cell commitment toward the osteoblast lineage. Runx2 drives the synthesis of bone matrix proteins and promotes mineralization, thereby facilitating osteogenesis [34].

The Wnt family is a diverse set of secreted glycoproteins that modulate MSC fate through paracrine or autocrine signaling mechanisms [35]. For example, Wnt3a has been shown to activate the transcriptional coactivator with PDZ-binding motif (TAZ) via dephosphorylation mediated by protein phosphatase 1A (PP1A), which in turn promotes osteogenic differentiation [36]. Wnt10b overexpression correlates with increased trabecular bone density, while its deficiency is linked to decreased bone mass and elevated age-related adipogenesis [37].

The Notch signaling pathway exerts complex, context-dependent effects on osteogenesis and is capable of both promoting and inhibiting MSC differentiation into osteoblasts. It can reduce osteogenesis by suppressing Wnt/ β -catenin signaling [33], but also enhances osteogenic differentiation through crosstalk with BMP-2 pathways, revealing its dual functions in bone biology [38].

In adipogenic differentiation, BMP-4 on its own has been shown to promote the adipogenic commitment of MSCs, but a synergistic effect is observed when BMP-2 is combined with the dexamethasone, which markedly enhances adipogenesis [39]. At the transcriptional level, peroxisome proliferator-activated receptor gamma (PPAR γ) is a master regulator of MSC adipogenic differentiation. Upregulation of PPAR γ facilitates the formation of adipocytes, whereas its inhibition significantly suppresses lipid accumulation and fat cell development [40]. PPAR γ exists in two main isoforms—PPAR γ 1 and PPAR γ 2. Both contribute to adipocyte differentiation, but they have distinct regulatory roles. Experimental evidence indicates that

downregulation of CCAAT/enhancer-binding protein alpha (C/EBP α) selectively inhibits PPAR γ 2 expression without significantly affecting PPAR γ 1, suggesting that PPAR γ 2 has a more dominant role in driving MSC adipogenesis [41]. Importantly, osteogenic and adipogenic MSC differentiation pathways are closely connected and dynamically balanced. Perturbation of this bone–fat equilibrium is implicated in various pathological states, including aging-related bone loss, obesity-related metabolic dysfunction, and osteoporosis. Understanding the regulatory mechanisms governing this balance is crucial for developing therapeutic strategies for restoring skeletal and metabolic health.

In 1996, Haynesworth *et al.* [42] were the first to identify the paracrine secretion function of MSCs. This paracrine activity involves the release of soluble bioactive factors as well as EVs (including exosomes and microvesicles) that act as vehicles to transfer miRNAs, mRNAs, proteins, and other signaling molecules from MSCs to recipient target cells. Via these mechanisms, MSCs contribute to a broad spectrum of therapeutic effects such as enhanced angiogenesis, suppression of apoptosis and fibrosis, promotion of neuronal survival and differentiation, stimulation of extracellular matrix remodeling, attenuation of local inflammation, and modulation of immune responses [43]. Key soluble factors secreted by MSCs include vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF), which each have critical roles in angiogenesis and tissue repair processes [44–46]. In addition, MSC-derived exosomes encapsulate diverse miRNAs that regulate proliferation, differentiation, and anti-inflammatory responses in recipient cells. Notably, these exosomal miRNAs have been implicated in promoting osteogenesis and influencing bone regeneration [24].

Immune modulation is another pivotal aspect of MSC paracrine function. For example, MSCs can finely tune the immune microenvironment through exosome-mediated delivery of specific miRNAs, thereby promoting tissue regeneration. BM-MSC-derived exosomes containing miR-223, miR-24-3p, miR-222-3p, miR-146a-5p, miR-34a-5p, and miR-181a-5p facilitate the transition of synovial macrophages from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, reducing levels of IL-1 β , IL-6, and TNF α while increasing IL-10 in the joint fluid—a shift that aids cartilage preservation and slows OA progression [47]. Although EV-transported miRNAs such as miR-223 can promote macrophage polarization toward the M2 phenotype, the role of exosomal miR-223 in MSC-mediated regulation of macrophages within joint tissues, while seemingly plausible, has not yet been thoroughly investigated and warrants further exploration [48]. Chondrocyte-derived exosomes also contribute to immune regulation. Recent data indicate that miR-214-3p contained within chondrocyte exosomes pro-

modulates M2 macrophage polarization, attenuates inflammation, and supports angiogenesis—all of which are conducive to a regenerative microenvironment [49]. Moreover, exosomes from various MSC sources have exhibited widespread immunomodulatory capacity, such as suppressing T helper 1 (Th1) cell-mediated immune responses, including activities of both CD4⁺ and CD8⁺ T cells [50]. They also inhibit B cell activation and natural killer (NK) cell cytotoxic functions, resulting in decreased secretion of pro-inflammatory cytokines and mitigation of local inflammatory environments [51]. Engineered exosome-based strategies for OA therapy that harness these mechanisms have emerged. For example, MSCs overexpressing miR-92a3p produce exosomes that upregulate cartilage matrix markers (e.g., SOX9, COL2A1) while suppressing degradation markers (e.g., Runx2, MMP-13), and demonstrate protection against cartilage degeneration in animal models [52]. Collectively, these findings highlight how exosome-mediated immune cell reprogramming (via miRNA cargos) and engineered enhancement of vesicles can reshape the immune microenvironment to improve regenerative outcomes and the applicability of regenerative therapies.

ESCs

ESCs are a pivotal category of stem cells, typically derived from the inner cell mass of blastocyst-stage embryos. During embryonic development, these cells differentiate into the ectoderm, mesoderm, endoderm, and hypoblast layers, exhibiting robust self-renewal capacity and the potential to differentiate into diverse lineage-specific cell types [53]. Based on their differentiation potential, stem cells are classified into early embryonic, fetal, umbilical cord, and adult stem cells. Early ESCs arise from cleavage-stage embryos prior to blastocyst formation and, along with fetal stem cells, possess extensive pluripotency, enabling differentiation into virtually any cell type within the organism. Umbilical cord stem cells (isolated predominantly from cord blood) exhibit comparatively restricted multipotency, differentiating into a limited array of cell types. Adult stem cells reside within fully developed tissues (such as the nervous system, peripheral blood, and bone marrow) and differentiate into corresponding tissue-specific cells that govern organ growth and maintenance. Notably, adult stem cells exhibit “plasticity”, whereby one type can transdifferentiate into other tissue lineages in response to specific growth or differentiation cues [54].

Based on their source, ESCs can be human (hESCs) or animal model ESCs. hESCs were first isolated in 1998 from the inner cell mass of blastocysts and were capable of being cultured indefinitely *in vitro* while retaining the expression of key markers characteristic of the trophoblast, such as cytokines [55]. hESCs are particularly useful for studying pluripotency and differentiation potential and are valuable tools for disease modeling and investigating human trophoblast biology [56]. They also have considerable

promise for cell replacement therapies for various human diseases.

ESCs have shown certain potential in inducing cartilage and bone regeneration, but they are still rarely applied in clinical settings. In 2004, a classic study by Bielby *et al.* [57] demonstrated that adding ascorbic acid, beta-glycerophosphate, and dexamethasone to the culture medium to induce osteogenic differentiation of ESCs, and implanting these cells through a poly-D, L-lactide (PDLLA) scaffold into SCID mice, mineralized tissues were found within the scaffold after 35 days. Bielby *et al.* [57] also proved that the culture method established for mouse ESC differentiation can be completely transferred to human ESCs.

In 2021, Petrigliano *et al.* [58] transplanted hESCs into porcine knees and observed the repair of the porcine knees six months later. The results showed that after the implantation of hESCs, the structure and joint function of the injured area were better restored. At the same time, compared with the control group, the implantation of hESCs did not trigger a more intense immune response.

In 2025, An *et al.* [59] investigated the effect of mechanical stress on the osteogenic activity of ESC-derived embryoid bodies (EBs). They analyzed the expression of osteogenic genes and signaling pathways under different mechanical stress conditions. They found that tensile stress inhibited osteogenic potency of EBs by downregulating ERK1-mediated signaling and osteogenic gene expression.

However, ethical concerns remain a substantial barrier to the clinical application of hESCs. The core ethical debate revolves around whether it is morally acceptable to destroy early human embryos in pursuit of new therapies [60]. Consequently, policies vary markedly across countries: the United Kingdom permits hESC research but prohibits reproductive and therapeutic nuclear transfer (NT); Italy bans all hESC research; and the USA restricts the generation of new lines requiring embryo destruction, permitting usage only of lines derived before August 9, 2001 [60,61]. Such regulatory disparities not only reflect ethical tensions but also hinder multinational collaboration and harmonized clinical translation.

iPSCs

The emergence of induced iPSCs appears to circumvent many embryo-related ethical issues. First reported by Takahashi and Yamanaka—mouse fibroblasts in 2006 and human somatic cells in 2007—iPSCs are generated by reprogramming with specific transcription factors [62, 63]. They recapitulate many features of ESCs, including pluripotency and long-term self-renewal, but avoid the ethical concerns associated with embryo destruction. Importantly, iPSCs enable patient-specific disease modeling and hold promise for autologous regenerative therapies.

In 2021, Zhou *et al.* [64] obtained the bone marrow samples of patients with ONFH (n = 16) and patients

with the fracture of the femoral neck ($n = 12$) by operation, and isolated BM-MSCs by density gradient centrifugation. These BM-MSCs were reprogrammed to iPSCs and finally differentiated into MSCs (iPSC-MSCs). They found that the immunophenotype of iPSC-MSCs was similar to that of normal BM-MSCs. However, transplantation of iPSC-MSCs could effectively promote bone repair and angiogenesis compared to normal BM-MSCs.

In 2024, Kessler *et al.* [65] implanted PLGA/aCaP scaffolds carrying iPSCs into mice to induce bone differentiation of iPSCs, aiming to repair bone injuries. The study showed that after 9 weeks of implantation, the bone mass in the group using bone-conductive scaffolds carrying iPSCs was significantly higher compared to the group using only bone-conductive scaffolds alone, indicating that the bone-conductive scaffolds carrying iPSCs can improve the regeneration of bone defects in mice.

However, they are not devoid of concerns: oncogenic reprogramming factors (e.g., c-Myc) may raise tumorigenicity risks, and donor-derived genetic materials necessitate stringent informed consent and privacy safeguards [22]. Moreover, inconsistent global regulation may give rise to “iPSC tourism” similar to that observed with unregulated stem cell clinics.

To address such challenges, regulatory agencies have begun issuing guidance for iPSC-derived products—for instance, the FDA and EMA have released frameworks emphasizing safety, characterization, and manufacturing standards for pluripotent stem cell-based therapies [66]. These efforts, together with harmonization initiatives such as the ISCT’s standards (covering MSC definition, potency assays, manufacturing, and clinical-grade production under GMP), aim to reduce regulation fragmentation and promote consistent ethical and safety benchmarks across jurisdictions [67]. Despite lingering ethical and regulatory hurdles, iPSCs remain a versatile and powerful platform for disease modeling, autologous/allogeneic therapy development, and translational research, provided that continued oversight and harmonized regulation keep pace with scientific progress [22].

SSCs

SSCs are a distinct population of lineage-restricted stem cells that govern bone and cartilage development, homeostasis, and regeneration [23]. Unlike the heterogeneous MSC population, SSCs are defined by clear hierarchical organization and lineage potential, capable of giving rise to chondrocytes, osteoblasts, and stromal cells while lacking adipogenic capacity [68]. They have been identified in multiple niches such as the bone marrow, growth plate, and periosteum, with specific subsets exhibiting unique regenerative capacities [69]. Recent advances in single-cell RNA sequencing and lineage tracing have highlighted SSC heterogeneity, including early osteochondral progenitors and perivascular SSCs that have critical roles

in skeletal growth and repair [70].

In a 2017 study, Tevlin *et al.* [71] investigated the role of SSCs in bone regeneration in diabetic mice and the signaling pathways involved. The study found that when *Ihh* was directly antagonized, or a high concentration of *TNF- α* was added to inhibit the *Ihh* signaling pathway, the number of SSCs in the corresponding groups significantly decreased. After local delivery of *Ihh* and *Shh* using hydrogels, the number of SSCs in the bone callus significantly increased, emphasizing the importance of the Hh signal in SSC-dependent bone regeneration.

In 2024, Zhang *et al.* [72] conducted a study on the role of SSC-Exos in promoting meniscal tear healing and ameliorating secondary OA. In this study, SSCs were isolated from bone marrow, and exosomes were extracted via ultracentrifugation. Forty Sprague-Dawley rats and twenty New Zealand White rabbits were randomly divided into two groups after undergoing a full-thickness longitudinal medial meniscus tear. They were then treated with phosphate-buffered saline and SSC-Exos, respectively. The results showed that the SSC-Exos treatment group exhibited higher cell proliferation and tissue regeneration after 8 weeks of treatment, and achieved lower degeneration scores and synovitis scores. At the same time, the researchers also revealed the mechanism of SSC-Exos. SSC-Exos treatment enhanced the expression of key genes related to cell migration, such as the chemokine family, leading to upregulation of extracellular matrix expression, while downregulating the expression of inflammation-related genes such as CD68 and the matrix metalloproteinase family. This indicates a new direction for the application of stem cells in the treatment of OA.

Accumulating evidence supports SSCs as promising candidates for regenerative therapies. For example, Ambrosi *et al.* [73] demonstrated that SSCs transplanted into murine calvarial defects generated robust bone tissue and outperformed heterogeneous MSCs. Similarly, periosteum-derived SSCs generated stable cartilage tissue *in vivo*, providing a potential cell source for OA treatment [68]. In translational contexts, SSC dysfunction has been linked to impaired fracture healing and age-related bone loss, suggesting therapeutic opportunities to restore skeletal homeostasis. Moreover, integration of SSCs with biomaterial scaffolds and bioreactor systems has enhanced their osteochondral differentiation, highlighting their potential in bone defect repair, cartilage regeneration, and degenerative disease therapy [69,74].

Other Types of Stem Cells

Cartilage is a vital connective tissue, playing a crucial role in joint movement and skeletal development. However, it has a particularly limited capacity for self-repair [75]. When the human body experiences trauma or degenerative disease, such as OA, cartilage defects often have difficulty regenerating naturally [5]. However, CPCs have

emerged as a promising solution to this problem due to their unique potential for cartilage regeneration.

CPCs are precursor cells residing within cartilage tissue and are essential in both embryonic cartilage development and adult cartilage homeostasis. They can differentiate into mature chondrocytes; however, their multipotent ability to differentiate into osteogenic and adipogenic lineages is weaker than that of MSCs [76].

CPCs mediate chondrogenesis primarily through the Smad3 and Wnt/ β -catenin signaling pathways. Appropriate activation of these pathways maintains CPC self-renewal and differentiation capacity, but excessive activation can lead to cartilage calcification and may induce osteogenic differentiation [77].

Hypoxia-inducible factors (HIFs) are key regulators that promote chondrogenic differentiation of CPCs. HIF-1 α has been shown to stimulate chondrogenesis, while HIF-2 α is involved in endochondral ossification and cartilage degradation [78]. *In vitro* experiments with microaggregate cultures lacking HIF-1 α showed reduced cartilage formation and decreased expression of key chondrogenic markers SOX9, SOX6, aggrecan, and type II collagen [79].

Given these properties, CPCs are mainly used in autologous transplantation to address cartilage defects. They are typically isolated from non-weight-bearing regions of the patient's joint, cultured *in vitro*, and then implanted into damaged cartilage areas to promote repair [78]. Tissue engineering and organoid models can also be constructed to generate cartilage organoids that simulate the *in vivo* microenvironment, offering useful platforms for drug testing and screening. Using CPCs to create OA disease models mimics the structural and microenvironmental alterations observed in OA *in vivo*, providing a robust *in vitro* system for studying degenerative cartilage diseases [80].

In summary, stem cells have emerged as indispensable players in cartilage and bone regeneration, with each category offering distinct biological properties and translational opportunities (Table 1). MSCs remain the most widely studied owing to their accessibility, multipotent differentiation, and immunomodulatory paracrine effects, yet donor heterogeneity, lineage bias, and limited engraftment capacity continue to restrict reproducible outcomes. iPSCs, including ESCs and induced iPSCs, provide powerful systems for developmental modeling and personalized therapies. However, ESCs are constrained by ethical and regulatory concerns, while iPSCs, despite circumventing these issues, still face challenges such as tumorigenicity, genomic instability, and inconsistent quality control. In parallel, lineage-restricted populations such as SSCs and CPCs are increasingly recognized as promising, more predictable sources for osteochondral repair, with accumulating evidence demonstrating their capacity to restore structural integrity and maintain tissue homeostasis.

Despite encouraging advances, significant barriers remain for clinical translation, including cellular heterogene-

ity, limited delivery efficiency, immune compatibility, and the inability to fully replicate the biomechanical and biochemical complexity of native tissues. Addressing these hurdles will require an integrative approach that combines single-cell and spatial omics for precise stem cell characterization, the development of bioengineered scaffolds and bioreactor systems to refine lineage commitment, and the application of engineered EVs to modulate the immune microenvironment. Collectively, these strategies may accelerate the transition of stem cell-based therapies from experimental studies toward reproducible, safe, and effective clinical interventions for skeletal disorders.

Mechanisms of Stem Cells in the Formation of Bone and Cartilage

Molecular Regulation of Bone and Cartilage Differentiation

Bone and cartilage differentiation refers to the process by which stem cells harness their multipotent differentiation potential to develop into chondrocytes under the influence of various biochemical and biomechanical factors. This is a highly regulated and programmed process involving the coordinated interplay of multiple signaling pathways, transcription factors, and other regulatory molecules that work together to ensure proper chondrogenesis (Fig. 3).

Key Signal Pathway

Stem cells exert their reparative functions in osteochondral tissues through three interrelated mechanisms: multi-directional differentiation, paracrine regulation, and microenvironment remodeling. These processes are orchestrated by canonical signaling pathways—including TGF- β /BMP, Wnt/ β -catenin, Hedgehog (Hh), and Notch—which converge on transcriptional regulators such as SOX9 and Runx2 to direct lineage specification, trophic factor release, and tissue reorganization [34,97–101].

Multi-directional Differentiation

TGF- β /BMP. Stem cells possess the ability to differentiate into chondrocytes, osteoblasts, and other mesenchymal lineages. The TGF- β superfamily (TGF- β isoforms and BMPs) governs skeletal lineage choices via Smad-dependent and Smad-independent (MAPK/ERK/JNK/Rho GTPases) signaling [102,103]. Ligand binding to type I and II receptors phosphorylates R-Smads, which complex with Smad4 and translocate to the nucleus to activate SOX9 and COL2A1 for chondrogenesis, and to induce Runx2 for osteogenesis [34]. Non-Smad arms provide further cross-talk with PTH, FGF, and Wnt/ β -catenin, aligning fate decisions with developmental and repair contexts [88–92]. These signaling cascades ultimately converge on critical transcription factors, notably SOX9 and Runx2, which serve as master regulators of chondrogenesis and osteogenesis, respectively [104,105]. For example, inactivation of TGF- β signaling results in excessive activation of type I PTH recep-

Table 1. Comparative overview of current major stem cell types.

Cell Type	Source	Advantages	Limitations	Clinical Status
MSCs [81–84]	Bone marrow, adipose, umbilical.	Accessible, immunomodulatory, multilineage.	Heterogeneity, survival, standardization.	RCTs in OA; CARTIS-TEM approved.
ESCs [58,85–87]	Blastocyst ICM	True pluripotency, high differentiation.	Ethical/regulatory barriers, tumor risk.	Preclinical & limited trials
iPSCs [58,88–91]	Reprogrammed somatic	Ethical advantage, personalized, versatile.	Tumorigenicity, regulatory complexity.	Early clinical (e.g., TACK-iPS), preclinical.
SSCs [14,68,73,92]	Bone/growth plate/periosteum	Targeted osteochondral lineage	Rare, expandability challenges.	Early preclinical, promising repair efficacy.
CPCs [93–96]	Native cartilage progenitors	Cartilage-specific, migratory.	Low yield, marker ambiguity.	Animal & <i>in vitro</i> models

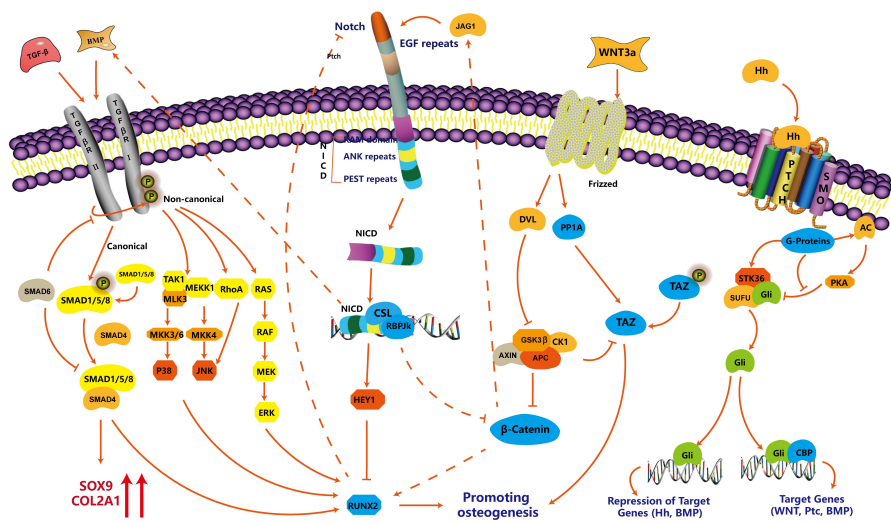


Fig. 3. Mechanisms of stem cells in the formation of bone and cartilage. Stem cells affect downstream genes such as SOX9, COL2A1, and Runx2 through the TGF-β/BMP pathway, Wnt/β-catenin pathway, Hh pathway, and Notch pathway, and finally promote the formation of bone and cartilage in the body. Drawing software: Adobe Illustrator.

tors, leading to bone loss that can be mitigated by PTH injection or PTH receptor knockout [106]. FGF family members (FGF-2, FGF-4, and FGF-6) also modulate skeletal development, enhancing chondrogenesis and osteogenesis in some contexts but inhibiting ALP activity and mineralization in osteoblasts [107]. Importantly, FGF-2 cooperates with BMP-2 during fracture healing, with FGF-2 driving early repair and BMP-2 facilitating mineralization at later stages [108]. TGF-β/BMP signaling further interacts with Wnt/β-catenin pathways, where TGF-β1 activates β-catenin signaling through ALK-5, Smad3, PKA, and PI3K, while Wnt stabilizes β-catenin and activates Runx2 via prostaglandin E2, highlighting the bidirectional regulatory network that is crucial for skeletal homeostasis [34,109].

Wnt/β-catenin. Wingless-like (Wnt) proteins are a family of over 19 secreted compounds. Due to lipid modifications, Wnt family members exhibit poor water solu-

bility, which significantly restricts their capacity to mediate signal transduction across tissues [110]. Based on dependence on β-catenin, Wnt signaling is classified as the canonical pathway, which relies on β-catenin signaling, and the non-canonical pathway, which operates independently of β-catenin [32]. β-catenin in the cytoplasm associates with cadherins on the cell membrane and with actin filaments in the cytoskeleton, contributing to cell adhesion. When phosphorylated by glycogen synthase kinase 3 beta (GSK3β), β-catenin forms a complex with GSK3β and undergoes ubiquitin-mediated proteasomal degradation [111]. In the canonical pathway, Wnt ligands bind to Frizzled (FZD) and low-density lipoprotein receptor-related proteins 5 or 6 (LRP5/6) co-receptors, producing a protein complex that inhibits β-catenin degradation and facilitates its nuclear translocation. Once in the nucleus, β-catenin activates transcription of Wnt target genes [110].

Wnt signaling has a bidirectional regulatory effect on

bone and cartilage formation. Takada *et al.* [112] in 1994 were the first to demonstrate that Wnt3a-deficient embryos exhibit axial skeletal defects, establishing the critical role of Wnt signaling in skeletal morphogenesis. Subsequent research revealed that Wnt3a activates the transcriptional co-activator TAZ via the canonical Wnt pathway, thereby promoting osteogenic differentiation of MSCs [113]. Wnt3a also enhances osteogenic differentiation of human BM-MSCs via the c-Jun N-terminal kinase (JNK) signaling pathway while concurrently inhibiting adipogenic differentiation [114]. More recent studies indicate that Wnt7a facilitates binding of T-cell factor 1 (TCF1) to the Runx2 promoter, upregulating Runx2 expression and promoting osteogenesis in human MSCs [115]. Similarly, Wnt10b fosters osteoblast formation by increasing Runx2 expression and simultaneously suppressing adipogenic transcription factors C/EBP α and PPAR γ , thereby inhibiting adipogenic differentiation of mesenchymal progenitor cells [37,116].

Conversely, low levels of Wnt signaling are essential for cartilage formation. Overexpression of ICAT (a small molecule inhibitor of β -catenin and T-cell factors) in articular chondrocytes suppresses β -catenin activity, thereby inactivating the canonical Wnt pathway. This inhibition results in increased degradation of articular cartilage and elevated chondrocyte apoptosis [117].

Hh. The Hh signaling pathway has an indispensable role in embryonic development, being extensively involved in the differentiation of stem cells into osteogenic and chondrogenic lineages, and in the proliferation of differentiated cells. In mammals, the Hh family has three members: Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) [100,101]. Shh promotes the expression of angiogenic factors in adults, facilitating neovascularization [118]. Ihh is implicated primarily in bone and cartilage development, having three main functions: (i) forming a negative feedback loop with parathyroid hormone-related peptide (PTHrP) to regulate chondrocyte differentiation; (ii) promoting chondrocyte proliferation; and (iii) modulating osteoblastic osteogenic activity [100]. Dhh predominantly regulates spermatogenesis and peripheral nerve sheath formation [119].

The primary receptor for Hh ligands is the Patched protein (Ptch), which associates with two co-receptors: Boc (brother of Cdo) and Gas1 (growth arrest-specific 1). In the absence of Hh, Ptch localizes mainly around the primary cilium and suppresses the activity of the Smoothed receptor (Smo). Upon Hh binding to Ptch on target cells, Ptch is displaced from the cilium, thereby relieving its inhibitory effect on Smo [100]. Downstream transcriptional responses to Hh are mediated by Gli zinc-finger transcription factors (Gli1, Gli2, and Gli3). The Suppressor of Fused (SuFu) protein forms a complex with Gli proteins, sequestering them in the cytoplasm. Without Hh stimulation, Gli2 and Gli3 are phosphorylated by protein kinase A (PKA),

casein kinase 1 α (CKI), and glycogen synthase kinase 3 β (GSK3 β), leading to their proteolytic processing into repressor forms at the base of the cilium. Speckle-type POZ protein (SPOP) acts as a substrate adaptor for ubiquitin E3 ligase, targeting Gli2 and Gli3 for ubiquitination and proteasomal degradation. When Hh is present, activated Smo inhibits the proteolytic processing of Gli2 and Gli3, allowing their activator forms to translocate into the nucleus and induce target gene transcription [120,121]. Given the critical role of Hh signaling in bone and cartilage formation, disruption or absence of this pathway results in various developmental defects, including impaired long bone growth, polydactyly, syndactyly, and ectopic ossification [100,101].

Master Transcription Factors. Among numerous transcription factors, the SRY-related high-mobility group box (SOX) transcription factor family, particularly SOX9, plays a pivotal role in chondrogenesis [122]. SOX9 acts as the master regulator of cartilage formation by orchestrating multiple developmental stages through phase-specific regulation of downstream genes. Working both independently and synergistically with SOX5 and SOX6, SOX9 completes the “chondrogenic SOX trio” essential for skeletal development. SOX5 and SOX6, although unable to initiate cartilage formation independently, enhance SOX9’s DNA-binding affinity by co-binding to cartilage-specific enhancers, thereby potentiating its chondrogenic activity [123,124]. Genetic evidence supports this synergy, as mice deficient in either SOX5 or SOX6 exhibit only mild skeletal abnormalities, whereas simultaneous deletion of both results in severe chondrodysplasia and intrauterine lethality [125]. Functionally, SOX9 directly regulates *COL2A1* and *ACAN*, which encode type II collagen and aggrecan, respectively, that are indispensable for extracellular matrix formation. SOX9 also activates the Ihh and Wnt signaling pathways, thereby controlling chondrocyte proliferation, columnar organization, and growth plate maturation [126]. Ihh, in turn, modulates chondrocyte maturation rates and promotes cortical bone formation in adjacent periosteum [101]. Clinically, diminished SOX9 expression is observed in osteoarthritic cartilage, while aberrant upregulation occurs in cartilage and bone tumorigenesis. Moreover, heterozygous mutations in or near the SOX9 locus cause campomelic dysplasia, a lethal skeletal dysplasia characterized by abnormal cartilage development and multi-organ malformations, including defects of the heart, lungs, kidneys, and pancreas [104].

Similarly, Runx2 is indispensable for osteogenesis. It contains two critical domains: a runt DNA-binding domain and a heterodimerization domain that interacts with core-binding factor β (Cbf β)/polycomb group protein 2 β (PEBP2 β) [127]. Runx2 drives MSCs toward the osteoblast lineage while suppressing adipogenic and chondrogenic fates, thereby committing progenitor cells to bone

formation [128]. Crucially, Runx2 collaborates with Smad proteins to mediate BMP signaling; in the absence of Runx2, cells fail to respond to BMP-2 stimulation, highlighting the indispensable role of the Runx2–Smad complex. Beyond BMP/Smad interactions, Runx2 expression and activity are modulated by additional transcriptional regulators. For instance, HOXB7 upregulates Runx2 mRNA, thereby promoting osteogenesis [129]. During osteogenic differentiation, HOXA2 expression increases, repressing Sox9 and favoring osteogenesis, whereas HOXA9 expression decreases [130]. Conversely, HIF-1 α negatively regulates Runx2 through its downstream effector TWIST [131]. While HIF-1 α overexpression enhances MSC proliferation, survival, and pro-angiogenic gene expression, it simultaneously reduces BMP-2 and Runx2 levels, thereby modulating osteogenic differentiation under hypoxic conditions. These intricate regulatory networks position Runx2 as the principal effector of osteoblast differentiation and highlight the fine balance between chondrogenic and osteogenic pathways mediated by transcription factors and their upstream signaling cascades.

Paracrine Regulation

Beyond direct differentiation, these pathways also regulate the secretome and EVs that amplify regeneration. TGF- β /BMP–Wnt crosstalk modulates the secretion of FGFs, VEGF, IGF-1, and other trophic cues that drive angiogenesis, matrix synthesis, and cell survival [107–109]. For example, during early fracture repair, FGF-2 predominates, whereas BMP-2 facilitates later mineralization, exemplifying time-staged paracrine orchestration within the same network [108]. Shh upregulates angiogenic mediators and supports neovascularization, coupling paracrine vascular cues to chondrogenic/osteogenic programs [118]. These pathways also influence the EV cargo (miRNAs, proteins) released by stem/progenitor cells and chondrocytes, which in turn modulate proliferation, differentiation, and inflammation in recipient cells (e.g., VEGF/IGF-1/HGF and EV-miRNAs impact osteogenesis and immunomodulation) [24,43].

Microenvironment Remodeling

Effective and durable regeneration requires niche repatterning/rebuilding of ECM architecture, normalization of inflammatory signals, and restoration of vascular supply. Wnt/ β -catenin controls ECM turnover (collagens, MMPs) and, when excessively activated, drives chondrocyte hypertrophy via Runx2/MMP-13, which contributes to OA. β -catenin is elevated in OA chondrocytes, and loss of the Wnt antagonist FRZB (females exhibit a predisposition to the common inactivation of FRZB in OA) removes a natural brake on this pathway [32,104,128]. TGF- β integrates skeletal and endocrine cues (e.g., its inactivation hyperactivates PTH1R, causing bone loss that is reversed by PTH injection or receptor knockout) and shapes the inflammatory

milieu toward repair, thereby supporting matrix anabolism and coupling it to bone remodeling [106]. Hh aligns angiogenesis (vascular invasion, nutrient delivery) with osteogenesis to ensure timely callus maturation and endochondral ossification [100,101]. Structurally, the Ptch–Smo–Gli logic (with SuFu/Spop regulation) defines Hh-responsive gene programs that govern growth plate dynamics and cortical bone formation. Disruption at any node produces skeletal malformations such as impaired long-bone growth, polydactyly, and ectopic ossification [100,120,121,132].

Microenvironment and Stem Cell Fate Determination

The microenvironment strongly influences the differentiation of stem cells into bone and cartilage by providing extrinsic cues that complement intrinsic genetic regulation. In cartilage differentiation, the microenvironment directs stem cells toward the chondrocyte lineage through various factors, including mechanical stimulation and the composition and architecture of the extracellular matrix (ECM).

The ECM offers essential physical support to stem cells and serves as a dynamic biochemical signaling reservoir. Its composition and structure tightly regulate stem cell behavior, including proliferation and lineage commitment [133]. The ECM is composed predominantly of two macromolecular classes—proteoglycans and fibrous proteins—which collectively determine its structural characteristics, elasticity, and stiffness [134]. Proteoglycans occupy substantial interstitial space, providing hydration and buffering capacity, while fibrous proteins such as collagen, elastin, fibronectin, and laminin confer tensile strength and elasticity. These fibers also serve as crucial attachment sites for cells, facilitating migration, tissue development, and mechanical energy storage [133].

Mechanical stimulation is a vital extrinsic factor regulating stem cell fate in load-bearing tissues like bone and cartilage. Throughout development and maturation, chondrocytes and their progenitors are constantly exposed to mechanical forces, including deformation, shear stress, fluid flow, electric potentials, hydrostatic pressure, and osmotic pressure (Fig. 4) [135].

Matrix stiffness is a well-characterized determinant of human MSC (hMSC) differentiation. Hadden *et al.* [136] engineered artificial matrices with tunable stiffness by varying chemical cross-linking in order to study hMSC fate in uniform culture conditions. They demonstrated that hMSCs cultured on soft matrices (0.1–1 kPa) preferentially differentiated into neuronal and adipogenic lineages, while on intermediate stiffness matrices (8–17 kPa) myogenic differentiation dominated, peaking near 12 kPa, and on stiff matrices (25–40 kPa) osteogenic differentiation was favored, peaking around 36 kPa [137].

Bone matrix is subjected to considerable mechanical loading due to gravity and muscle contractions, making compressive forces especially important for osteogenic differentiation [138]. Moderate compressive strain (~20%)

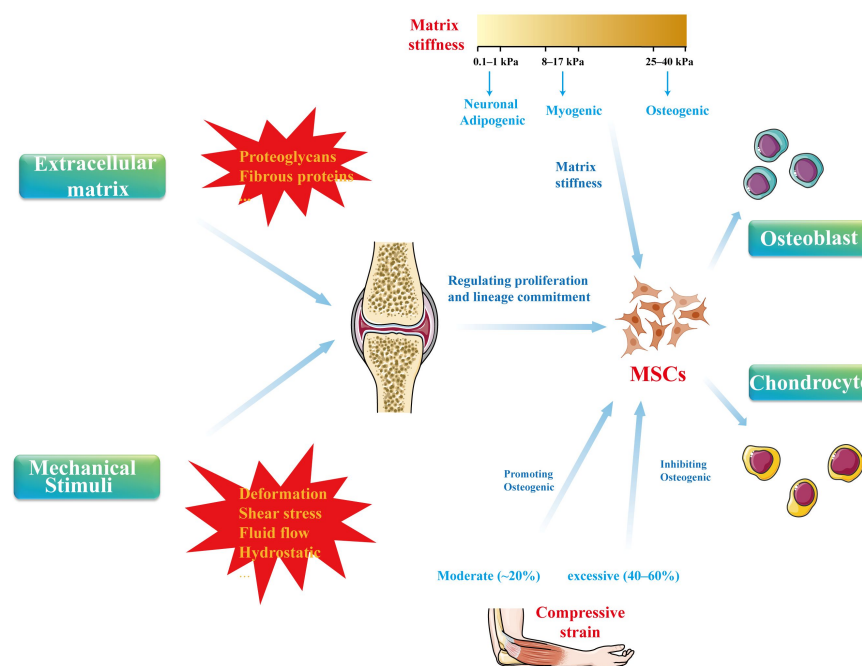


Fig. 4. Microenvironmental regulation of stem cell differentiation into bone and cartilage. The stem cell fate toward bone or cartilage is orchestrated by extracellular matrix composition and mechanical cues, with matrix stiffness and compressive strain acting as key regulators of osteogenic versus chondrogenic differentiation. Drawing software: Adobe Illustrator.

promotes osteogenic differentiation of MSCs, whereas excessive strain (40–60%) inhibits it [139,140]. Baumgartner *et al.* [141] found that under 5% cyclic compressive loading, increased scaffold stiffness correlates with significant upregulation of osteogenic markers such as Runx2 and type I collagen, which promotes osteogenesis. However, other studies have suggested that cyclic compressive stimulation at 5–10% strain does not enhance osteogenic differentiation and may reduce MSC migration [142]. These conflicting reports highlight the complexity of mechanical regulation and underscore the need for further research to elucidate the optimal pressure parameters and underlying mechanisms governing MSC osteogenic differentiation.

Bone and cartilage differentiation is a tightly orchestrated process regulated by signaling pathways, transcription factors, and microenvironmental cues. Key cascades such as TGF- β /BMP, Wnt/ β -catenin, Hh, and Notch converge on master regulators like SOX9 and Runx2, ensuring the balance between chondrogenesis and osteogenesis. These networks not only guide lineage commitment but also regulate paracrine signaling and extracellular vesicle release, which further influence angiogenesis, matrix remodeling, and immune modulation. Equally important, the extracellular matrix and mechanical forces act as decisive determinants of stem cell fate. Variations in stiffness, strain, and compressive loading can shift the balance toward osteogenic or chondrogenic outcomes, yet inconsistent findings highlight the complexity of biomechanical regulation.

Such discrepancies underscore the need for integrative approaches that combine biochemical and mechanical cues.

Despite remarkable progress, clinical translation remains hampered by limited control over signaling dynamics and the inability to fully reproduce the native osteochondral microenvironment. Future directions should prioritize single-cell and spatial omics to decode pathway cross-talk, while advanced biomaterials and bioreactor systems can provide controllable environments that mimic *in vivo* conditions. Together, these strategies may transform our mechanistic understanding into precise and reproducible stem cell-based therapies for cartilage and bone regeneration.

Interaction Between Cartilage and Bone Formation

The interaction of cartilage and bone spans the entire life cycle, from embryonic skeletal development to adult bone remodeling, and maintains skeletal homeostasis via dynamic signaling dialogue and cellular interactions. This interplay is crucial in physiological processes such as growth plate elongation, as well as pathological conditions, including OA and fracture healing.

Ossification of Cartilage Templates

Bone formation begins with the establishment of a cartilaginous precursor. During the embryonic phase, specific lineages of mesenchymal cells migrate to, and aggregate at, sites designated for future skeletal development, grad-

ually differentiating into chondroprogenitor cells. This aggregation forms a hyaline cartilage template, characterized by high cellular density and the secretion of type II collagen and proteoglycans, which serves as the precursor of the future skeleton [143]. Bone formation proceeds within this framework via two main pathways: endochondral ossification, where mesenchymal cells first differentiate into chondrocytes before transforming into osteocytes; and intramembranous ossification, where mesenchymal cells differentiate directly into osteocytes without a cartilaginous intermediate [93]. For example, the growth of cortical bone along the long bone axis typically occurs through intramembranous ossification. Skeletal progenitor cells within the periosteum (marked by PRX1 or CTSK expression) differentiate directly into postnatal periosteal osteoblasts of long bones without passing through a chondrocyte stage [144,145].

The ossification center comprises layers of flattened, rapidly proliferating chondrocytes arranged in a longitudinal columnar structure [146]. These dividing chondrocytes further differentiate into pre-hypertrophic chondrocytes, which then transform into hypertrophic chondrocytes. During this progression, cell and tissue volume increase rapidly, regulated by PTHrP and Ihh signaling pathways. PTHrP inhibits the differentiation of chondrocytes into the hypertrophic stage, maintaining their proliferative state. As the influence of PTHrP wanes, the chondrocytes start to undergo hypertrophy and begin producing Ihh that induces Runx2 expression, thereby enhancing chondrocyte proliferation and promoting osteoblast differentiation in the adjacent perichondrium [147]. Driven by Ihh, the chondrocytes also release VEGF, which recruits endothelial cells to invade the perichondrium, establishing the primary ossification center and attracting osteoclasts and osteoprogenitor cells to the site.

The ends of long bones contain an area rich in stem cells known as the secondary ossification center. The preserved growth plate in this area facilitates continued skeletal elongation through endochondral ossification. Stem cells in the secondary ossification center possess robust self-renewal and multipotent capabilities, playing a crucial role in the growth of long bones during development. Following the rapid growth phase of puberty, the growth plate closes and eventually disappears in late adolescence [93].

Role of Stem Cells in Bone Repair

Bone is one of the most regenerative tissues in the human body, and under pathological conditions, stem cells play a significant role in repairing it.

In the early stages of OA, alterations in collagen and proteoglycan structures, along with degradation of the cartilage matrix, lead to a loss of mechanical buffering capacity of the articular cartilage. This results in abnormal stress being transmitted to the subchondral bone. As cartilage erodes, chondrocytes respond with enhanced proliferation

to increase cartilage matrix synthesis. However, this process simultaneously elevates the production of inflammatory factors such as IL-6 and IL-8 that can activate osteoclasts, which exacerbates cartilage degradation and promotes bone resorption [148]. The final stage of cartilage destruction is marked by chondrocyte apoptosis, which causes an imbalance between the synthesis and degradation of collagen and proteoglycans. Moreover, the release of inflammatory factors induces changes in the synovial tissue and subchondral bone, leading to bone sclerosis and increased thickening of the synovial membrane and joint capsule [149].

Post-fracture bone repair is a rapid and efficient process that parallels developmental bone formation. Repair depends heavily on close interaction between stem cells and the skeletal vascular network, often making it difficult to distinguish newly formed tissue from the original pre-injury bone [150]. Stable fractures primarily heal through intramembranous ossification, where stem cells differentiate directly into osteoblasts without forming cartilage. By contrast, unstable fractures initially form fibrocartilage at the injury site. Healing is achieved when this is subsequently replaced by bone tissue—a process reminiscent of endochondral ossification [93]. Injury causes local vascular disruption that leads to hematoma formation and tissue hypoxia, triggering the release of factors such as HIF-1 α and VEGF that promote angiogenesis. Concurrently, multiple signaling pathways, including TGF- β , Wnt, and Notch, are activated, facilitating bone tissue differentiation [151–153].

Cartilage-bone interplay underlies skeletal growth, remodeling, and repair, integrating progenitor differentiation, vascular invasion, and signaling crosstalk. In OA, cartilage breakdown and inflammatory cascades disrupt this balance, accelerating subchondral remodeling and joint degeneration. Fracture healing, by contrast, reactivates developmental programs where stem cells and angiogenic cues coordinate ossification according to mechanical stability. Although key pathways such as TGF- β , Wnt, and Ihh are well defined, replicating their dynamic integration in engineered systems remains challenging. Future therapies combining stem cells, vascularized biomaterials, and pathway-targeted modulation may enable more faithful osteochondral regeneration and improved treatment outcomes.

Clinical Translation to Application of Stem Cells in Cartilage and Bone Regeneration

As research in cartilage and bone regeneration medicine advances, cutting-edge technologies are rapidly evolving toward precision, intelligence, and individualization, gradually aligning with practical clinical applications. This transformation enhances the efficiency and effectiveness of regenerative medicine and offers patients more personalized treatment options.

Single-cell Sequencing (SCS)

SCS is a high-throughput technology that enables the analysis of genomic, transcriptomic, and epigenomic information at the resolution of individual cells. Unlike traditional bulk sequencing methods, SCS aims to reveal cellular heterogeneity by capturing the unique molecular characteristics of each cell. Since the advent of single-cell RNA sequencing (scRNA-seq) in 2009, SCS technologies have rapidly advanced, with applications extending across genomics, proteomics, and metabolomics. These advances have been widely applied in various research fields, including stem cell biology, allowing researchers to systematically catalog human cell types, elucidate developmental trajectories and inter-individual differences, and uncover cellular associations with diseases [154].

In the context of OA pathogenesis, Ji *et al.* [155] employed scRNA-seq to identify novel chondrocyte subpopulations and associated signaling pathways, thereby revealing the internal states and functional diversity of chondrocytes. They discovered three previously unrecognized chondrocyte clusters: effector chondrocytes (ECs), regulatory chondrocytes (RegCs), and homeostatic chondrocytes (HomCs). ECs, characterized by expression of cytokine-like 1 (CYTL1), contribute to energy supply during chondrocyte development and promote the chondrogenic differentiation of MSCs [156]. Beyond providing mechanistic insights, these subpopulations have translational potential: variations in RegC abundance or signaling activity may serve as predictive biomarkers to stratify OA patients and guide personalized interventions, such as targeted immunomodulatory or regenerative therapies [154]. Moreover, the integration of chondrocyte stem cell technologies with artificial intelligence (AI) offers powerful tools to uncover predictive multiomic signatures, forecast disease trajectories, and define optimal timings for stem cells. Machine learning applied to single-cell transcriptomes has already enabled the identification of diagnostic gene signatures and immune-related pathways that distinguish OA subtypes, supporting precise classification of patients [157]. AI-driven imaging and live-cell prediction models have been used to non-invasively forecast stem cell differentiation outcomes and screen for MSC lines with optimal regenerative capacity [158]. These approaches, when coupled with MSC-derived exosomes or biomaterial-assisted delivery of anti-inflammatory miRNAs, can refine therapeutic timings, improve treatment consistency, and accelerate the translation of stem cell-based strategies into personalized clinical interventions.

Sun *et al.* [159] applied scRNA-seq to analyze healthy human menisci, identifying seven distinct cell types: (i) EC expressing CD93 and CDH5, (ii) CPC expressing CDK1 and BIRC5, (iii) RegC expressing BMP-2 and FOSL1, (iv) fibrocartilage cells (FC) expressing COL1A1, COL3A1, and COL6A1, (v) pre-hypertrophic chondrocytes (PreHTC) expressing MMP1 and TNFAIP6, (vi) fibrocartilage pro-

genitor cells (FCP) co-expressing fibrocartilage markers and MSC markers MCAM and MYLK, and (vii) proliferating fibrocartilage cells (ProFC) expressing COL1A1 alongside growth factors FGF7 and CTGF. They also mapped spatial gene expression patterns in the meniscus, underscoring the power of SCS to reveal cellular heterogeneity in complex tissues.

Despite these advances, the massive amount of data generated by SCS presents significant challenges for translating findings into actionable biological targets suitable for clinical applications. Effective computational tools and integrative analytical frameworks are essential to harness the full potential of SCS in precision medicine. These issues are commonly addressed through anchor-based mapping (e.g., Seurat WNN), latent factor decomposition (MOFA⁺), deep learning frameworks (TotalVI), and spatial integration tools (e.g., Tangram), supplemented by dimensionality reduction, parallel computing, and batch effect correction to enhance efficiency and accuracy [160–164]. Experimentally, cell capture methods (such as droplet-based microfluidics, FACS, and single-nucleus sequencing) often introduce biases, including loss of specific subpopulations and transcriptional stress. These can be mitigated by optimizing tissue dissociation, adopting single-nucleus sequencing, integrating multi-modal validation, and applying computational normalization and correction algorithms, thus improving the reliability of results through both computational and experimental methods [165,166].

Organoids

Organoids are three-dimensional miniature organ models cultured *in vitro*. They are derived from stem cells or primary tissues and are capable of recapitulating the structural and functional characteristics of real organs (Fig. 5) [167]. Organoid technology addresses challenges, such as immunogenic risks and functional integration, encountered in traditional *in vitro* studies, providing a highly biomimetic experimental platform to investigate molecular mechanisms of diseases and develop novel therapeutic strategies. This technology has become an invaluable tool for studying organ development, disease pathogenesis, and drug screening (Table 2) [168].

Chen *et al.* [169] reported the first study of bone organoids in 2022, incorporating the unique features of the bone microenvironment into the organoid concept. They proposed strategies for constructing bone organoids, outlined potential research directions, and discussed applications. *In vitro* three-dimensional culturing combined with directed differentiation was used to co-culture various stem cells (including bone and chondrocyte stem cells) with functional cell types such as osteoblasts, osteoclasts, and endothelial cells. This approach enabled the creation of miniaturized bone and cartilage units rich in vascular networks, which were assembled into bone-like tissues. These constructs serve as useful models to simulate pathological

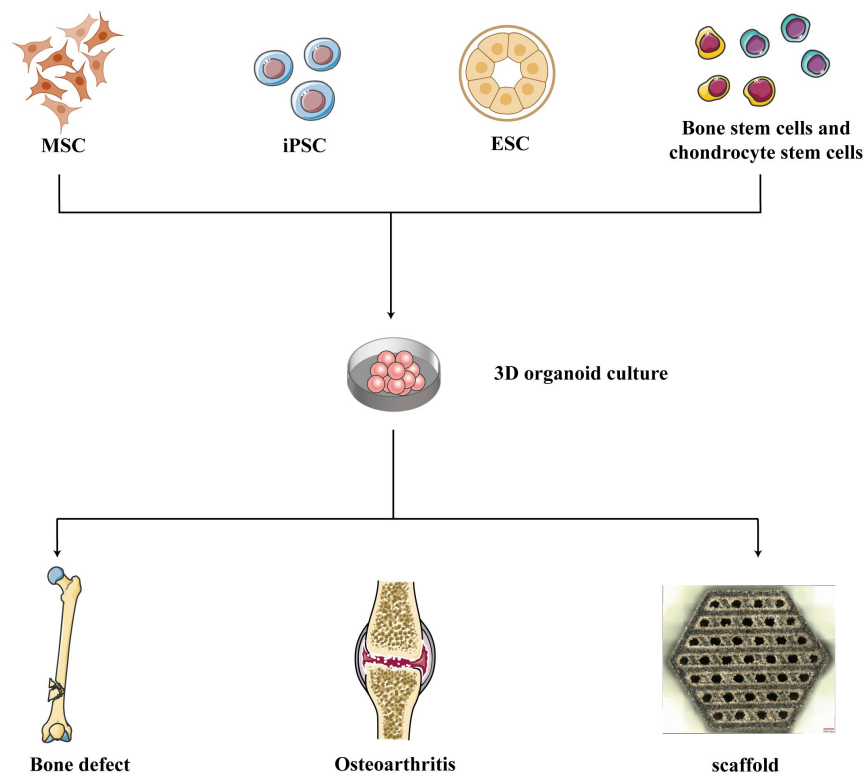


Fig. 5. The origin and application of bone-cartilage organoids. Bone-cartilage organoids are derived from stem cells and are finally capable of recapitulating the structural and functional characteristics of a real organ. Drawing software: Adobe Illustrator.

Table 2. Comparative overview of current bone/cartilage organoid models.

Study/Year	Stem Cell Source	Key Features	Advantages	Limitations
Chen <i>et al.</i> [169], 2022	Bone stem cells, chondrocyte stem cells, co-cultured with osteoblasts, osteoclasts, endothelial cells.	Integrated bone microenvironment with 3D organoid culture; vascularized mini-bone/cartilage units.	Recapitulates cellular heterogeneity of bone tissue; allows modeling of OA pathology.	Limited vascular maturation; insufficient mechanical load-bearing capacity.
Lamandé <i>et al.</i> [172], 2023	iPSCs→chondroprogenitors→chondrocytes→osteoblasts via endochondral ossification	Bone-cartilage organoids mirroring developmental transitions	Reflects dynamic gene expression; useful for genetic disease models.	Mechanically immature; lacks physiological load-bearing.
Other 3D organoid systems [173,174]	MSCs, ESCs, or iPSCs cultured with bioactive scaffolds (e.g., hydrogels, ECM mimetics).	Enhanced matrix organization and 3D structure	Better ECM deposition; customizable microenvironment.	Poor vascularization; limited scalability; integration issues.

processes such as OA, and as platforms for drug testing [170,171].

In 2023, Lamandé *et al.* [172] used iPSCs to generate bone–cartilage organoids. They differentiated the iPSCs into chondroprogenitor cells, which then underwent differentiation into articular chondrocytes or hypertrophic chondrocytes via endochondral ossification pathways, eventually giving rise to osteoblasts. This study elucidated the expression profiles of osteogenesis-related genes during the developmental stages. The constructed bone–cartilage organoids recapitulated various disease states, in-

cluding chondrodysplasia, collagenopathies, and OA, providing a powerful platform for studying disease pathogenesis, screening therapeutics, and optimizing the timing of clinical interventions [172].

Organoids provide a biomimetic platform that bridges the gap between traditional *in vitro* studies and complex *in vivo* biology. Bone and osteochondral organoids derived from stem cells can recapitulate key developmental pathways, disease phenotypes, and therapeutic responses, making them powerful tools for mechanistic research and drug discovery. However, inconsistent differentiation, incom-

plete maturation, and challenges in scaling production remain major barriers to clinical translation. Looking ahead, integration with 3D/4D bioprinting, organ-on-chip systems, and dynamic bioreactors, alongside robust technical standards and ethical oversight, will be essential to realize the full potential of organoid-based regenerative strategies. Looking ahead, integration with 3D/4D bioprinting, organ-on-chip systems, and dynamic bioreactors, alongside robust technical standards and ethical oversight, will be essential to realize the full potential of organoid-based regenerative strategies.

Engineering Strategy: Application of Bioreactors

Advances in stem cell research have enabled their application in both scientific studies and clinical treatments [176,177]. To deepen our understanding of stem cell behavior and expand their therapeutic applications, it is essential to establish specialized *in vitro* culture systems—bioreactors—that can support the controlled expansion and differentiation of specific stem cell types. Dynamic bioreactors offer significant advantages over traditional static culture systems by allowing precise monitoring and regulation of culture conditions. This facilitates large-scale and long-term cultivations while better supporting stem cell proliferation and lineage commitment [178].

However, there are considerable challenges in designing stem cell bioreactors. Different stem cell types and differentiation goals demand tailored environmental parameters such as specific oxygen tensions, pH values, and mechanical stimuli, including compressive forces and shear stress. The ability to precisely replicate these conditions remains a technical hurdle in bioreactor development [179].

Bone and cartilage tissue engineering integrates stem cells (e.g., MSCs, iPSCs) with biomaterial scaffolds and the controlled delivery of bioactive factors (classically TGF- β and BMP-2) to recapitulate osteogenesis and chondrogenesis to repair osteochondral units [180]. Bioreactors have been successfully employed to mimic physiological conditions for bone and cartilage tissue engineering. Luciani *et al.* [181] utilized a dual-axis rotating bioreactor providing continuous liquid perfusion and gas exchange for three-dimensional scaffolds, establishing a dynamic mechanical environment. This setup enhanced nutrient diffusion and metabolic waste removal and generated physiological stimuli (e.g., shear stress) that contributed to the generation of functionally enhanced, engineered cartilage. Similarly, Montorsi *et al.* [182] designed a bioreactor with laminar flow at the top for nutrient delivery and shear stress regulation, coupled with ultrasonic stimulation from below. This system was used to study bone matrix deposition and osteogenic differentiation of SAOS-2 cells under various stimuli.

While *in vitro* stem cell bioreactors provide more reliable and physiologically relevant data, current tissue-engineered cartilage still falls short of fully replicating the

biomechanical and functional properties of native cartilage [181]. Given this limited ability to reproduce native cartilage/bone mechanics, 3D bio-printing integrated with dynamic-loading chambers is gaining traction [183]. Custom compression bioreactors applied directly to bio-printed osteochondral/meniscal constructs enable long-term cyclic loading in culture, improving matrix deposition and construct stiffness while maintaining cell viability. Recently reported systems exhibit sustained compression of printed tissues and computer-controlled mechanical conditioning of bio-printed menisci *in vitro*. Lai *et al.* [184] propose 4D bio-printing and stimuli-responsive inks as routes to dynamically tune construct mechanics during culture.

Microfluidic organ-on-chip (OoC) platforms recreate the osteochondral microenvironment under precisely controlled perfusion, shear, and cyclic strains, overcoming diffusion limits and facilitating physiological load patterns that are difficult to maintain in bulk bioreactors [185,186]. Bone/joint-on-chip systems also now model osteochondral crosstalk and OA-like inflammation under controlled flow conditions. Importantly, several designs incorporate embedded sensors (impedance, strain, electrochemical) for real-time readout of mechanical stress and tissue responses, enabling closed-loop optimization of loading regimens, which together point to a practical path for mimicking native mechanics while capturing relevant human biology data [187].

Bioreactors provide dynamic and controllable environments that support stem cell proliferation, differentiation, and tissue maturation, offering clear advantages over static culture systems. Recent advances, including perfusion-based designs, 3D/4D bio-printing with mechanical conditioning, and organ-on-chip platforms with embedded sensors, have enhanced osteochondral tissue engineering by better replicating physiological cues. However, current constructs still lack the full mechanical complexity of native bone and cartilage, and stem cell heterogeneity complicates universal design. Future integration of smart biomaterials, real-time monitoring, and personalized bioreactor parameters holds promise to achieve more reproducible and clinically relevant regenerative therapies.

Combined Treatment Strategy

Immunotherapy activates the body's immune system to combat disease through enhanced immune cell function, in contrast to traditional chemotherapy and radiotherapy that directly target abnormal cells. Significant clinical progress has been seen with this approach in oncology, autoimmune diseases, and infectious conditions [188]. While immunotherapy has recently been applied to malignancies such as osteosarcoma, it also has potential in regenerative contexts, particularly for OA (Fig. 6).

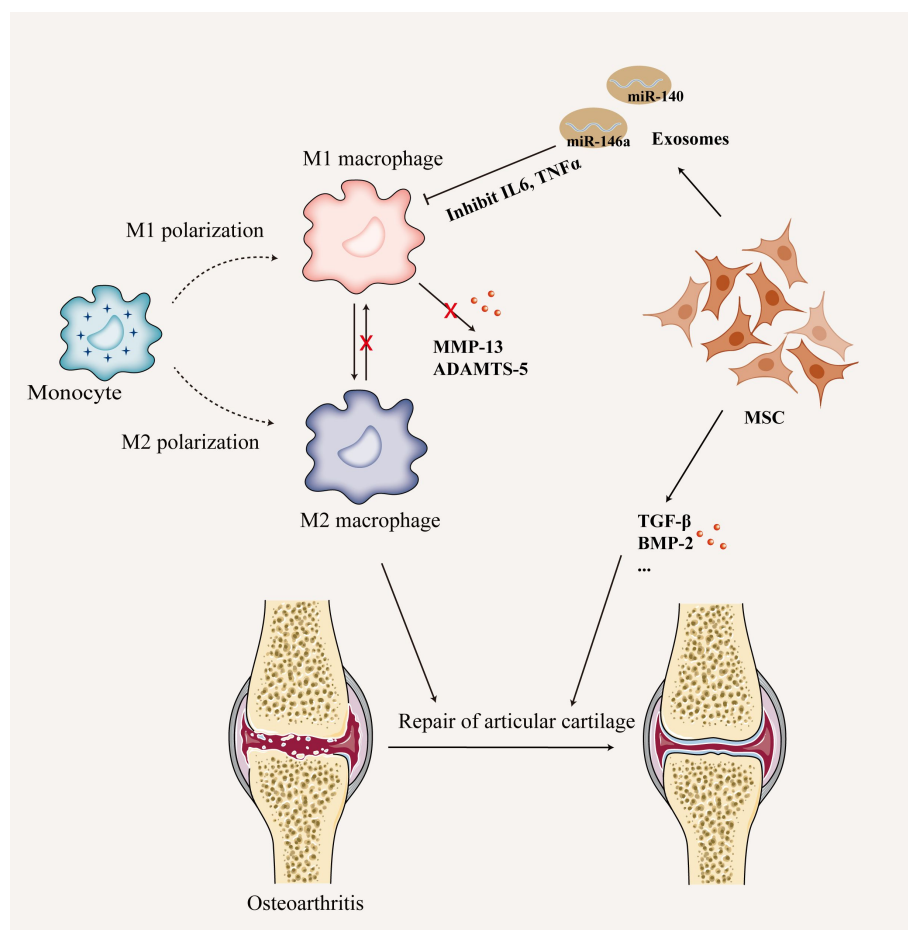


Fig. 6. The immune regulatory mechanism of stem cells in bone and cartilage regeneration. The EVs and cytokines produced by MSCs can regulate the functions of immune cells and promote the repair of articular cartilage. Drawing software: Adobe Illustrator.

MSC-derived Exosomes for Immunomodulation in OA

MSC-derived exosomes have gained attention as effective cell-free immunomodulators to treat OA. Preclinical studies show they can alleviate synovial inflammation, inhibit cartilage degradation, and restore matrix homeostasis in both knee and temporomandibular joint models of OA [174]. Exosomes engineered to carry miR-146a or miR-140 can suppress pro-inflammatory mediators (e.g., IL-6, TNF- α), induce macrophage polarization toward an anti-inflammatory M2 phenotype, and inhibit matrix-degrading enzymes such as MMP-13 and ADAMTS-5—thereby supporting cartilage preservation and repair [189].

Biomaterials for Targeted miRNA Delivery

To overcome the problem of rapid clearance from the joint, biomaterial-based carriers such as injectable hydrogels offer controlled, localized delivery of anti-inflammatory miRNAs. For example, delivering miR-140 using chondrocyte-targeting exosomes or embedding it in hydrogels can directly inhibit MMP-13/ADAMTS-5 activity and counter synovial IL-6-driven inflammation, thereby promoting matrix deposition and cartilage regeneration [189]. By combining MSC-derived exosomal im-

munomodulation with biomaterial-mediated miRNA delivery, a comprehensive therapeutic strategy emerges—one that simultaneously attenuates inflammation and fosters cartilage/bone regeneration through modulation of the immune microenvironment and direct preservation of extracellular matrix. This integrative “exosomemiRNAbiomaterial” platform embodies a translational approach that bridges the gap between immunotherapy and regenerative medicine in the treatment of OA.

Immunomodulatory approaches are increasingly recognized as integral to cartilage and bone regeneration, complementing conventional regenerative strategies. MSC-derived exosomes act as potent cell-free therapies that alleviate synovial inflammation, promote M2 macrophage polarization, and suppress catabolic enzymes such as MMP-13 and ADAMTS-5. When engineered to deliver specific miRNAs (e.g., miR-146a, miR-140), they further enhance matrix preservation and immune balance [190]. Biomaterial carriers, particularly injectable hydrogels, enable localized and sustained delivery, reducing clearance and amplifying therapeutic effects. Together, the integration of exosomes, miRNA modulation, and biomaterial scaffolds represents a promising translational platform that bridges im-

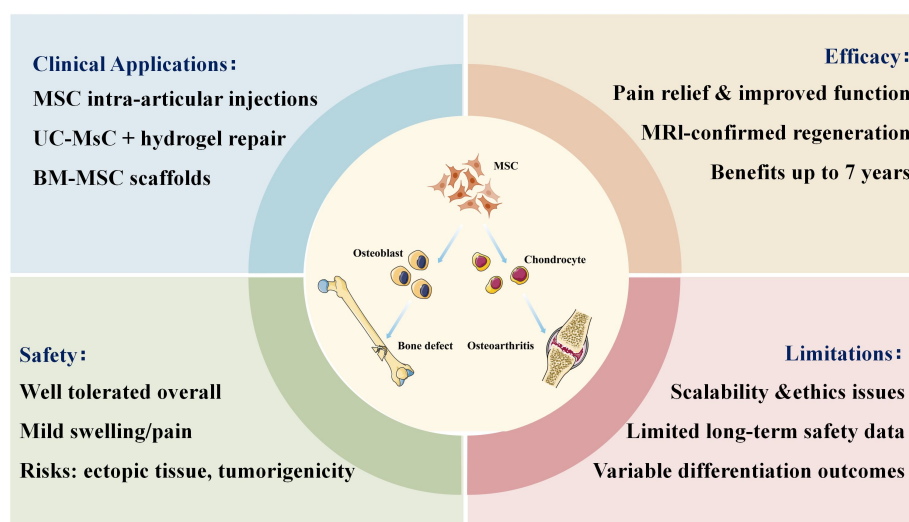


Fig. 7. Graphical illustration of the clinical translation pathway for stem cell-based therapies in cartilage and bone regeneration. Clinical applications of stem cell-based therapies in cartilage and bone regeneration include intra-articular injection of MSCs to relieve joint pain and improve function. The therapies own advantages, with well tolerated and mild pain, while also having risks of ectopic tissue and tumorigenicity. While limitations concentrate on ethics and long-term safety issues. Drawing software: Adobe Illustrator.

munotherapy with regenerative medicine in OA.

Clinical Efficacy and Safety Evidence in Cartilage and Bone Regeneration

Preclinical studies have provided crucial insights into the mechanisms of stem cell-based therapies for cartilage and bone regeneration. For example, Kaczanowska *et al.* [191] demonstrated the utility of genetically engineered mesenchymal-like cells (GEMys) in murine models. These studies highlight proof-of-concept mechanisms but should be clearly distinguished from clinical translation.

Clinical data provide cautious optimism. Multiple clinical trials have demonstrated promising results for stem cell-based therapies in cartilage and bone regeneration. For instance, Phase I/II trials using intra-articular injections of AD-MSCs or hUCB-MSc have reported significant improvements in joint pain, function scores, and cartilage quality (evaluated via MRI), with minimal adverse events such as mild injection-site pain or transient swelling [192,193]. In osteochondral defect repairs, autologous bone marrow-derived MSCs embedded in biomaterial scaffolds have shown durable defect filling and improved clinical scores up to 5 years post-surgery, with no evidence of ectopic bone formation or tumorigenicity [194].

The absence of late-onset safety issues in ongoing iPSC and hUCB-MSc studies is encouraging, yet larger, controlled Phase III trials with standardized protocols are urgently needed. Ultimately, combining immune regulation with regenerative repair may establish a safer and more

effective paradigm for osteochondral therapy.

Challenges and Prospects

Barriers to Clinical Translation

Clinical translation of stem cell therapy faces numerous challenges, notably ethical concerns and safety risks. A 2016 survey identified 351 companies operating across various states in the USA providing “stem cell therapy” services directly to consumers through 570 clinics. Many of these services are characterized by unverified efficacy and unapproved procedures and have given rise to “stem cell tourism” where regulatory scrutiny is circumvented [195]. This phenomenon highlights the substantial ethical barriers that continue to impede the widespread application of stem cell therapies. As previously noted, the primary ethical controversy surrounding the use of hMSCs arises from their procurement, which frequently involves the destruction of embryos. Consequently, countries including the United Kingdom, the USA, and Italy have implemented varying restrictions on research and therapeutic use of hMSCs [60].

Safety concerns arise predominantly from the inherent multipotent differentiation potential of stem cells, which poses challenges to controlling their fate post-transplantation. For example, hESCs possess the capacity to differentiate into hundreds of distinct cell types, complicating efforts to direct their differentiation upon transplantation into living organisms [196]. The reported incidence of teratoma formation following hESC trans-

plantation in immunodeficient mice varies widely—from 33% to 100%—depending on factors such as implantation site, cell maturity, purity, and transplantation technique [197]. Moreover, although stem cell therapies have shown promise in treating retinal diseases, their long-term efficacy remains uncertain [198]. Given patients' expectations of absolute safety and favorable outcomes, these reports amplify concerns regarding the clinical safety of stem cell therapies.

Overcoming these barriers requires targeted strategies. To improve post-transplantation cell survival, biomaterial scaffolds such as hydrogels can provide a three-dimensional microenvironment that enhances cell retention, protects against anoikis, and allows sustained release of trophic factors [199]. Similarly, hypoxic preconditioning (1–5% O₂) has been shown to increase MSC resistance to stress, augment their paracrine secretome, and improve bone and cartilage repair efficacy [200]. Co-transplantation with endothelial progenitor cells (EPCs) also enhances neovascularization, thereby supporting graft survival and functional integration into osteochondral defects [201].

Equally important are approaches to mitigate tumorigenic risks. Genetic safety switches, such as the inducible caspase-9 (iCasp9) system, can be engineered into transplanted cells to enable rapid elimination if uncontrolled proliferation or malignant transformation occurs [202]. Advances in CRISPR technology facilitate precise insertion of such constructs, providing an additional layer of safety [203]. Removal of senescent cells—from within stem cell preparations or host tissue—has also emerged as another promising approach. Senolytic agents (e.g., dasatinib plus quercetin [204], or navitoclax [205]) and experimental peptides (e.g., FOXO4-DRI [206]) selectively clear senescent cells, reducing their pro-inflammatory secretome and lowering the risk of tumor initiation [207].

Future Research Directions

To address these challenges and facilitate the translation of stem cell therapies from bench to bedside, future research must prioritize enhancing the precision of microenvironmental regulation and improving cell viability. Controlling the differentiation microenvironment is critical: extensive investigation is required to determine optimal parameters (including matrix stiffness and mechanical stimuli such as compressive and shear forces) that provide suitable conditions for directed stem cell differentiation, thereby maximizing the fidelity of lineage commitment [133].

Ensuring the survival of transplanted cells is paramount. Studies on stem cell therapies for peripheral arterial disease have demonstrated that co-transplantation of stem cells with EPCs can enhance regional blood perfusion and improve the survival of transplanted stem cells, ultimately improving therapeutic efficacy [208].

The clinical translation of stem cell therapies faces persistent ethical and safety challenges, including unreg-

ulated practices, risks of uncontrolled differentiation, and tumorigenicity (Fig. 7) [209]. Emerging strategies—such as biomaterial scaffolds, hypoxic preconditioning, co-transplantation with endothelial progenitor cells, and encapsulation techniques—are improving cell survival and functional integration. Genetic safety switches and senolytic agents offer additional safeguards against malignant transformation and inflammatory risks. Future progress will rely on precise control of the differentiation microenvironment and standardized regulatory frameworks. Together, these advances may transform stem cell therapies into safe, reproducible, and ethically sustainable treatments for degenerative and regenerative disorders.

Conclusions

Stem cells are irreplaceably valuable in the field of cartilage and bone formation and regeneration due to their multipotent differentiation potential, paracrine effects, and ability to regulate microenvironments.

They can differentiate directly into chondrocytes or osteoblasts and can repair bone defects. Under specific induction conditions, MSCs activate key transcription factors such as SOX9 and Runx2, driving the production of functional cartilage matrix and bone tissue. This differentiation lays the structural foundation for bone regeneration and contributes to the restoration of normal biomechanical function.

The paracrine effects of stem cells also play a vital role in tissue regeneration. The cells secrete a variety of growth factors (including VEGF, IGF-1, and HGF) that stimulate angiogenesis and recruit host cells to the injury site. Moreover, EVs secreted by stem cells carry bioactive molecules (such as miRNAs, mRNAs, and proteins) that suppress inflammation and retard cartilage degeneration. Stem cells modulate the immune microenvironment by fostering anti-inflammatory and tissue-repair conditions that create a favorable milieu for regeneration.

SCS has emerged as a powerful technological tool for analyzing gene expression profiles at the individual cell level. It can reveal cellular heterogeneity and precisely identify disease-related subpopulations, providing a foundation for the development of targeted therapies. Meanwhile, organoid technology leverages stem cells to construct three-dimensional *in vitro* functional tissue models that simulate organ development and pathological processes. Such models enable personalized drug screening and disease modeling.

Combined stem cell therapies harness both regenerative and immunomodulatory capabilities. For example, MSCs can serve as delivery vehicles for CAR-T cells that cross the blood–brain barrier to treat brain tumors, or they can be integrated into biomaterial scaffolds to repair bone defects. These strategies enhance therapeutic efficacy while minimizing side effects. Such interdisciplinary approaches—from cellular-level analysis and model con-

struction to clinical interventions—constitute a closed-loop innovation system that accelerates breakthroughs in precision medicine and regenerative therapies.

Looking forward, the integration of SCS and intelligent biomaterials is poised to steer stem cell therapies toward greater precision and personalization and has the potential to reshape the field of regenerative medicine, offering more effective and tailored treatment options for a wide range of skeletal and soft tissue diseases.

Fundamental integration of basic stem cell research and clinical translation is the driving force behind the development of regenerative medicine. Basic research establishes a theoretical foundation for the precise manipulation of cell fate by analyzing molecular regulatory mechanisms, epigenetic characteristics, and microenvironment interactions with stem cells—for example, SCS reveals the heterogeneity of MSC subpopulations, while organoid models simulate disease microenvironments.

Clinical translation, meanwhile, directly addresses real-world challenges by ensuring cell viability through standardized production systems and iterating foundational designs based on feedback from clinical trials. Synergies between basic research and clinical translation can accelerate successful technological applications in clinical settings. Stronger interdisciplinary research collaborations are needed to build a comprehensive innovation system encompassing molecular mechanisms, engineered modifications, and real-world evidence to achieve the leap from theory to practice.

List of Abbreviations

OA, osteoarthritis; MSCs, mesenchymal stem cells; SCS, single-cell sequencing; BM-MSCs, bone marrow-derived MSCs; AT-MSCs, adipose-derived mesenchymal stem cells; FGFs, fibroblast growth factors; Runx2, runt-related transcription factor 2; TGF- β /BMP, transforming growth factor-beta/bone morphogenetic protein; PP1A, protein phosphatase 1A; PPAR γ , proliferator-activated receptor gamma; C/EBP α , CCAAT/enhancer-binding protein alpha; EVs, extracellular vesicles; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor-1; HGF, hepatocyte growth factor; Th1, T helper 1; NK, natural killer; ESCs, Embryonic stem cells; NT, nuclear transfer; iPSCs, pluripotent stem cells; CPCs, cartilage progenitor cells; HIFs, Hypoxia-inducible factors; MAPK, mitogen-activated protein kinase; ALP, alkaline phosphatase; GSK3 β , glycogen synthase kinase 3 β ; FZD, Frizzled; LRP5/6, lipoprotein receptor-related proteins 5 or 6; JNK, c-Jun N-terminal kinase; TCF1, T-cell factor 1; MMP-13, matrix metalloproteinase 13; FRZB, frizzled-related protein; Hh, Hedgehog; PTHrP, parathyroid hormone-related peptide; PKA, protein kinase A; CKI, casein kinase 1 α ; SPOP, Speckle-type POZ protein; SOX, SRY-related high-mobility group box; Cbf β , core binding factor β ; PEBP2 β , polycomb group protein 2 β ; ECM, ex-

tracellular matrix; ECs, effector chondrocytes; RegCs, regulatory chondrocytes; HomCs, homeostatic chondrocytes; ProFC, proliferating fibrocartilage cells; GEMys, genetically engineered bone marrow cells; EPCs, endothelial progenitor cells.

Availability of Data and Materials

Not applicable.

Author Contributions

SJC, YQZ and DW designed the review; XXL provided help and advice on the framework of the thesis; YQZ, ZCT, and DW jointly wrote the manuscript; DW and ZXL completed the drawing of the pictures; YQZ, QD, CQC and GQL jointly completed the translation of the article. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgments

We gratefully acknowledge the assistance and instruction from Professor Yi Peng of the Third Xiangya Hospital of Central South University.

Funding

This research was funded by the National Natural Science Foundation of China (Grant Nos. 82373046, 82172594) and the Wisdom Accumulation and Talent Cultivation Project of the Third XiangYa Hospital of Central South University (YX202001).

Conflict of Interest

The authors declare no conflict of interest.

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Editor's note: The Scientific Editors responsible for this paper were Chris Evans and Martin Stoddart.

Received: 1st May 2025; **Accepted:** 11th April 2026;
Published: 30th October 2026