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Nanomaterial-Assisted Stem Cell-Based Therapy for Primary Osteoporosis: Challenges and Future Trends

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Abstract: Osteoporosis is a global health crisis affecting millions of people worldwide, and its incidence increases as a consequence of the increase in the percentage of aged people within the population. Osteoporosis-related fractures are the most prominent reasons for disability and mortality. Bone is continually remodeled to sustain its structural integrity and mineral content. Any imbalance in bone remodeling activity could lead to many bone disorders. Current therapeutic interventions in osteoporosis target resorptive and anabolic events. Although these treatment modalities aim to reduce the intensity of bone remodeling, they fail to reverse the structural damage. PubMed, Google Scholar, and ScienceDirect were searched for all relevant English-language publications. This review synthesizes findings from 135 studies published between 2000 and 2025 that investigate nanomaterial-based differentiation of mesenchymal stem cells (MSCs). MSCs have been recognized as an excellent cell source for regenerative medicine due to their remarkable characteristics. Accumulating evidence suggests that integrating stem cells with nanomaterials opens a new avenue for tissue repair and regeneration and could significantly influence stem cell fate. Integrating stem cells with nanomaterials introduces a paradigm shift from passive cell carriers to bio-instructive systems, in which the nanostructure directly activates osteogenic signaling pathways, leading to enhanced osteogenesis independent of osteogenic factors. Moreover, it discusses the therapeutic potential of osteoblast transplantation in both preclinical and clinical trials for managing bone defects, including osteoporosis. Osteoblast therapy could offer new insights into the future direction of osteoporosis therapy owing to its specificity and minimal side effects compared to current medication. Despite the promising outcomes in pre-clinical settings, further studies are required to establish the optimal safe dosage and to elucidate the underlying mechanism of action. If proven effective, osteoblast transplantation could be eligible for clinical translation.

Keywords: bone remodeling; nanomaterials; osteoblasts; osteogenic differentiation; osteoporosis; stem cells.

1. Introduction

Osteoporosis is a chronic health issue affecting millions of people worldwide, and its prevalence rate is rising as the number of aged people increases [1]. It is defined by the overall

bone loss, which causes a decline in bone mass and increases the susceptibility to fractures. Osteoporosis-related fractures are the most prominent reasons for disability and mortality. The mortality rate is raised following a hip fracture. There are usually no symptoms of osteoporosis until a fracture occurs; hence, it is known as a silent disease [2]. It was estimated to affect 500 million individuals worldwide by 2025 [3].

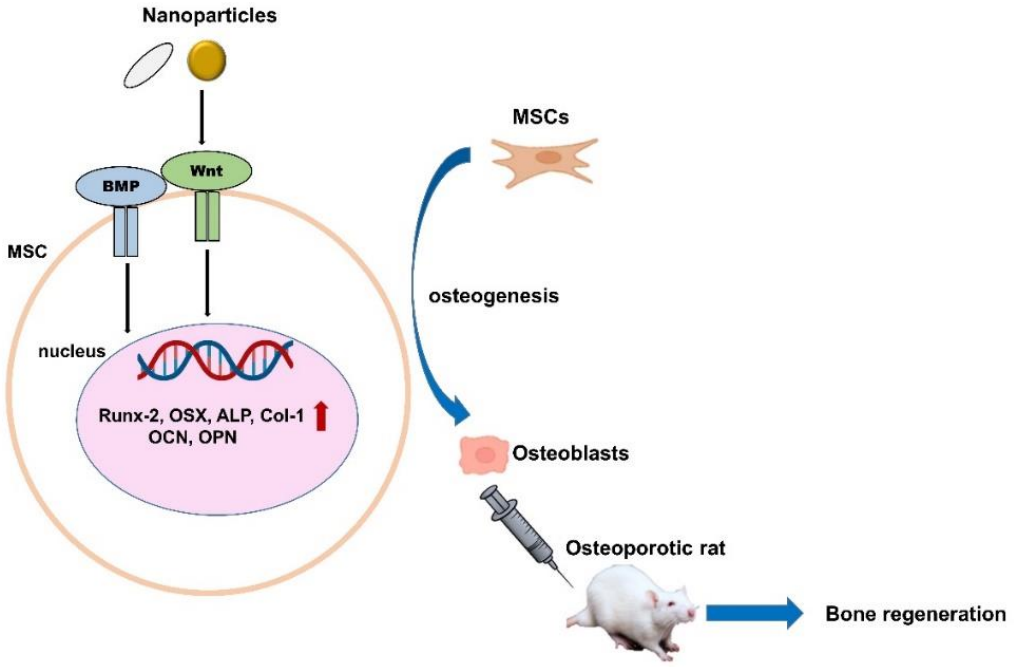
Bone is continually remodeled and repaired to maintain skeletal integrity and mineral content [4]. The bone remodeling process depends on an equilibrium between bone resorption by osteoclasts and bone formation by osteoblasts, ensuring the replacement of the old bone matrix. While the osteoclasts degrade the old bone matrix, osteoblasts deposit a new one [5]. Any imbalance in this process, favoring osteoclast or osteoblast activity, causes many clinical diseases, such as osteopenia and osteoporosis [6]. Primary osteoporosis usually affects postmenopausal women due to estrogen deficiency. It is estimated that osteoporosis affects approximately one in three women and one in five men over the age of 50. More than one-third of adult women can develop one or more osteoporotic fractures [7].

Current treatments for osteoporosis are focused either on suppressing bone resorption or stimulating bone formation [8]. Although such treatment modalities aim to reduce the intensity of bone remodeling and the risk of further fractures, they have certain limitations, including limited efficacy, long-term safety concerns, and the inability to reverse structural damage [9]. Bone fractures in osteoporotic individuals, along with other large bone defects caused by trauma, tumor removal, or congenital issues, often require therapeutic interventions for repair. While autologous bone grafting is regarded as the gold standard in such cases, it involves major invasive surgeries, which can raise the risk of complications, especially in older patients [10].

MSCs are multipotent cells that can be isolated from many tissues, including bone marrow, adipose tissues, dermal tissue, dental tissue, amniotic fluid, and the umbilical cord. MSCs can proliferate to self-regenerate themselves and can also differentiate into multiple types of tissues [11]. MSC-based therapy has gained attention as a promising approach to enhance bone regeneration for mitigating osteoporotic defects [12]. Systemic infusion of MSCs has been shown to alleviate bone loss caused by gonadectomy in mice [13]. A growing body of evidence indicates that the integration of cells and nanomaterials offers a promising approach for tissue injury repair and regeneration. Many studies have demonstrated that combining MSCs with bone extracellular matrix (ECM) components significantly affects the fate and function of stem cells [14]. Tissue engineering strategies aim to mimic the natural bone matrix for bone regeneration, using natural polymers such as collagen, which are combined with hydroxyapatite (HA) or other calcium phosphate ceramics to stimulate osteoblast differentiation [15].

This review highlights the potential of nanomaterials to stimulate specific signaling pathways in stem cells, thereby stimulating their differentiation into osteogenic lineages. The nanomaterial-induced osteogenic differentiation of MSCs into functional osteoblasts will offer a promising therapeutic option for primary osteoporosis. Owing to its specificity and safety, this approach has the potential to overcome the drawbacks of current osteoporosis therapies and reshape the future of bone regeneration therapies. Scheme 1 illustrates the osteogenic effects of nanomaterials on MSC differentiation into functional osteoblasts, as well as the therapeutic potential of transplanting these osteoblasts into osteoporotic rats, resulting in accelerated bone healing.

This review discusses the stem cell-based therapy for osteoporosis, highlighting its current limitations. It also emphasizes the osteoinductive potential of nanomaterials of different natures in stimulating the differentiation of stem cells into functional osteoblasts, owing to their nanoscale dimensions and surface chemistry, which closely mimic the natural nanoscale structure of bone ECM. Moreover, the review addresses the toxicity, biocompatibility, and challenges facing the clinical translation of nanomaterials. Finally, it provides recent insights into the clinical potential of osteoblasts, paving the way for the development of new regenerative therapies for bone defect repair.



Scheme 1. Schematic representation of the osteogenic effects of nanomaterials on MSCs and the possible underlying mechanisms. Nanomaterials can activate the BMP or Wnt/β-catenin signaling pathways to promote osteogenic differentiation of MSCs into functional osteoblasts *via* upregulating the downstream osteoblast-specific genes (Runx-2, OSX, ALP, Col-1, OPN, OCN). Transplantation of resultant osteoblasts in osteoporotic rats accelerates bone regeneration. MSCs, mesenchymal stem cells; BMP, bone morphogenetic protein; Wnt, wingless-type; **Runx-2, runt-related transcription factor 2;** Osterix, OSX; Alkaline phosphatase, ALP; Col-1, type I collagen; OPN, Osteopontin; OCN, Osteocalcin**. ↑: upregulated**.

1.1. Ultrastructure of bone.

1.1.1. Bone matrix.

Bone is a connective tissue consisting of cells surrounded by a mineralized ECM, containing a mixture of organic and inorganic components. The organic components are mainly constituted of collagenous proteins, predominantly type I collagen (Col-I), synthesized by osteoblasts. After being secreted, the procollagen propeptides, including the N-terminal and the C-terminal propeptides, undergo proteolytic cleavage, followed by accumulation in the bone matrix. The remainder of the proteins are non-collagenous proteins, including osteocalcin (OCN), osteopontin (OPN), bone sialoprotein (BSP), and osteonectin (ON), which contribute to strengthening the collagen structure and regulating its mineralization. The inorganic components consist of mineral salts, predominantly calcium HA [$Ca_{10}(PO_4)_6(OH)_2$], which are found associated with the collagen fibers. This variation in bone matrix composition is responsible for bone stiffness, flexibility, and mechanical stability [16].

1.1.2. Cellular elements.

The major cellular content of bone is constituted by osteoblasts, osteoclasts, and osteocytes (Figure 1).

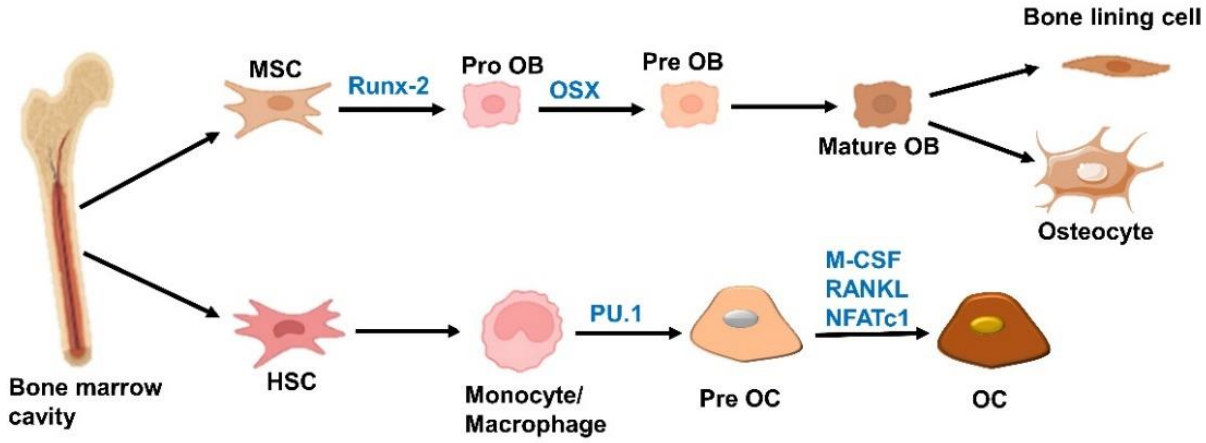


Figure 1. Bone cell formation. (1) mesenchymal stem cells (MSCs) differentiate into osteoblast progenitors (Pro OB), which in turn are converted to pre-osteoblasts (Pre OB) and then to mature osteoblasts (OB). Mature osteoblasts can either differentiate into bone lining cells or osteocytes. The main transcription factors involved in osteoblast differentiation include Runx-2 and OSX. (2) Hematopoietic stem cells (HSCs) are transformed into macrophage/monocyte, which are later converted to osteoclastogenic progenitors, pre-mature osteoclasts (Pre OC), and osteoclasts (OC). The key transcriptional modulators of this cascade comprise PU box-binding-1 (PU.1), M-CSF, nuclear factor of activated T cells 1 (NFATc1), and RANKL.

1.1.2.1. Osteoblasts.

Osteoblasts are bone-forming cells derived from MSCs, involved in bone matrix mineralization and bone remodeling, and in regulating the metabolic activities of other bone cells. Osteoblast differentiation undergoes four maturational stages, including pre-osteoblasts, mature osteoblasts, osteocytes, and bone lining cells. Upon exposure to specific stimuli, MSCs differentiate into pre-osteoblasts, which later develop into mature osteoblasts that produce Col-I and other non-collagenous proteins, as well as alkaline phosphatase (ALP), which is necessary for bone matrix mineralization [17]. After bone formation, osteoblasts can either undergo apoptosis, differentiate into osteocytes, or become quiescent bone-lining cells [18]. These bone-lining cells remain on the bone surface to regulate mineral diffusion and can transdifferentiate into osteoblasts in response to specific stimuli, such as hormonal signals or mechanical stress [19]. The canonical wingless-related integration site (Wnt) signal pathway is one of the most essential pathways regulating osteoblastogenesis. It is regulated by various transcription factors, including runt-related transcription factor 2 (Runx-2) and osterix (OSX). The Wnt pathway is initiated by the binding of the Wnt ligand to a specific receptor complex composed of frizzled receptor and low-density lipoprotein (LDL) receptor-related protein (LRP) 5 or LRP-6. This results in Dishevelled activation, which in turn leads to glycogen synthase kinase 3 β (GSK3β) inhibition and β-catenin translocation to the nucleus to regulate osteogenesis-related transcription factors. Other important signaling pathways, including transforming growth factor beta (TGF-β) and bone morphogenetic proteins (BMPs) pathways, are involved in osteoblast differentiation [20].

1.1.2.2. Osteoclasts.

They are multinucleated cells responsible for bone resorption arising from the hematopoietic progenitors. Hematopoietic stem cells (HSCs) differentiate to produce osteoclast precursors with the aid of osteoclast differentiation factors, including macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kB ligand (RANKL), secreted by osteoblasts. Such factors are necessary for osteoclastogenesis [21]. Osteoclastogenesis is induced upon binding of RANKL to its receptor (receptor activator of nuclear factor-kB; RANK), found on the surface of osteoclast precursors, resulting in triggering intracellular signaling cascades initiated by binding of the tumor necrosis factor (TNF) - receptor-associated factors (TRAF6) to the RANK receptor. Then, TRAF6 phosphorylates inhibitory-κB kinase (IKK), leading to proteasomal degradation of inhibitory-κB (IκB). Thus, nuclear factor kappa B (NF-κB) can be released and translocated to the nucleus, where it initiates the transcription of osteoclast-related genes such as tartrate-resistant acid phosphatase (TRAP). Pre-osteoclasts fuse, forming giant multinucleated cells that express additional osteoclast markers, such as calcitonin receptor and cathepsin K. Osteoclastogenesis is inhibited by osteoprotegerin (OPG), a decoy receptor that binds RANKL, preventing its interaction with RANK and, in turn, inhibiting osteoclastogenesis and, hence, bone resorption. The RANKL signal transmission is controlled by the RANKL/ OPG ratio, which determines the bone mass [19].

1.1.2.3. Osteocytes.

Osteocytes function as mechanosensors, detecting mechanical loading during locomotion or microdamage in bone, and initiating the remodeling process by regulating the activity of osteoblasts and osteoclasts. Moreover, they play an essential role in maintaining mineral homeostasis, particularly phosphate regulation, *via* secreting fibroblast growth factor 23. Additionally, they regulate bone formation by secreting sclerostin and Dickkopf-related protein I, which are negative regulators of the Wnt pathway [22].

1.2. Bone remodeling.

Bone remodeling continuously occurs at specific sites called basic multicellular units, which are composed of osteoclasts, osteoblasts, and a capillary blood supply, to maintain bone mass and mineral homeostasis, repair microdamage in the skeleton, and replace old bone with new bone through sequential osteoclastic resorption and osteoblastic bone formation. The bone remodeling process is made up of 5 stages: activation, resorption, reversal, formation, and termination (Figure 2). (a) Activation: During the activation phase, the bone lining cells detach from the bone surface. Then, osteoclast precursors are recruited from the circulation and get activated. Bone-marrow mononuclear cells, derived from the monocyte-macrophage lineage, differentiate into multinucleated cells that fuse to form active osteoclasts, which in turn bind to the bone surface to initiate the resorption process. (b) Resorption: Osteoclasts secrete hydrogen ions to create an acidic environment, essential for mobilizing minerals, and release proteolytic enzymes, including cathepsin K and matrix metalloproteinase (MMPs), to degrade the collagen-rich bone matrix, creating cavities within the resorption lacuna. This phase is terminated by osteoclast apoptosis, preventing further bone resorption. (c) Reversal: The freshly resorbed bone is now prepared for the deposition of new matrix. Osteoblast precursors proliferate and differentiate into mature osteoblasts, which migrate to the resorption lacuna.

Although the exact signals that couple the resorption phase to the subsequent formation phase are not fully understood, it is assumed that osteoclasts secrete cytokines such as interleukin 6 (IL-6) that act as a coupling factor. (d) Formation: Osteoblasts deposit new collagenous matrix (osteoid) to fill the resorption lacuna, followed by its mineralization, where HA crystals are deposited within the collagen fibrils. (e) Termination: Once mineralization is accomplished, osteoblasts either undergo apoptosis, differentiate into osteocytes, or become bone-lining cells that cover the newly formed bone surface [5,22].

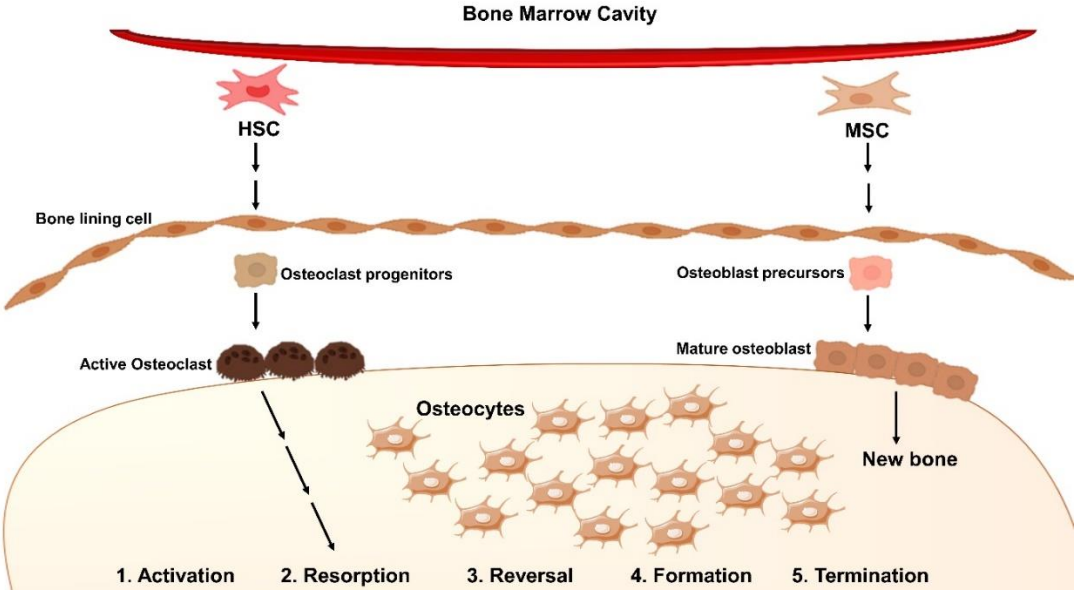


Figure 2. The bone remodeling cycle consists of five phases: (**1**) Activation phase, in which the osteoclast progenitors, derived from HSCs, are activated and differentiated into mature osteoclasts, (**2**) Resorption phase, where mature osteoclasts initiate bone resorption by secreting digestive enzymes. After resorption, they detach from the bone surface and undergo apoptosis. (**3**) Reversal phase; where osteoblast precursors, derived from MSCs, differentiate into mature osteoblasts, (**4**) Formation phase; where mature osteoblasts are recruited to the resorption site and start depositing the collagenous bone matrix (osteoid). (**5**) Termination: after the formation phase, the osteoid gets mineralized, and the bone surface returns to the resting phase, covered by bone-lining cells. HSC, Hematopoietic stem cell; MSC, Mesenchymal stem cell.

1.3. Role of estrogen in the regulation of the bone remodeling cycle.

Estrogen is essential for maintaining bone integrity. Estrogen decline after menopause in women stimulates bone resorption, causing osteoporosis [23]. It has been assumed that bone loss is accelerated during the first 3-5 years after menopause. Estrogen acts through estrogen receptors-alpha (ER-α), widely expressed on osteoblasts and osteoclasts, to regulate bone metabolism. It suppresses the activation of the bone remodeling cycle and bone resorption by decreasing the activity of osteoclasts. In addition, it regulates osteoblastic cell differentiation and prevents their apoptosis, thereby maintaining bone formation [24]. Estrogen inhibits osteoclastogenesis by suppressing M-CSF expression, thereby decreasing RANKL activity [25]. Moreover, Eghbali-Fatourechi *et al*. [26] indicated that estrogen can inhibit RANKL expression in bone marrow stromal cells by inhibiting the activity of RANKL-stimulating cytokines such as interleukin 1 (IL-1), IL-6, and TNF-α. Estrogen may also mediate OPG expression in bone lining cells [27]. Estrogen deficiency leads to a spontaneous increase in cytokine levels, including IL-1, IL-6, and TNF-α, which, in turn, elevates RANKL expression and reduces OPG expression, thereby enhancing osteoclastogenesis and decreasing osteoclast apoptosis [24,25].

1.4. Osteoporosis.

In healthy bone, bone resorption and bone formation are tightly connected. Consequently, metabolic bone diseases, such as osteoporosis, lack this balance. Osteoporosis, the most common skeletal disease, is identified by bone loss and micro-architectural deterioration [28]. It has been estimated that approximately 9 million osteoporosis-related fractures occur globally each year, including 1.6 million hip fractures [29]. In Egypt, it is expected that approximately 10,802,554 million men and 11,841,133 million women over the age of 50 years will develop osteoporosis by the end of 2030, leading to an increased risk of bone fractures [30]. Primary osteoporosis is the most predominant form of osteoporosis, including postmenopausal and age-related osteoporosis. It may arise from glucocorticoid use or from immobilization that reduces mechanical loading on the skeleton, ultimately leading to structural deterioration and a loss of bone mass [22]. Major risk factors for osteoporosis include aging, female sex, inadequate calcium intake, reduced physical activity, the early loss of gonadal function, long-term exposure to glucocorticoids, and possibly some genetic factors [31].

1.5. Current osteoporosis treatments.

The main goal of osteoporosis treatment is to prevent fractures by enhancing bone mass and strength. Pharmacological therapies are prescribed to osteoporosis patients at high risk of osteoporotic fracture. FDA-approved pharmacological treatments are classified into two groups: anti-resorptive agents, which suppress bone resorption, and anabolic agents, which induce bone formation, such as teriparatide. Anti-resorptive agents include bisphosphonates (alendronate, zoledronate, risedronate, and ibandronate), RANKL inhibitors (romosozumab, denosumab), and selective estrogen receptor modulators (SERMs) (bazedoxifene, raloxifene) [32]. Bisphosphonates are widely prescribed and have shown efficacy in reducing the risk of vertebral, non-vertebral, and hip fractures. They act by reducing osteoclastic activity *by inhibiting farnesyl diphosphate synthase, which mediates the post-translational modification of guanosine triphosphate (GTP)- binding proteins*. Such protein is essential for osteoclastic activity; hence, its blocking leads to the suppression of osteoclastic activity and, consequently, bone resorption. However, their long-term use is associated with potential adverse effects such as gastrointestinal discomfort, osteonecrosis of the jaw, and atypical femur fractures. SERMs are thought to inhibit osteoclast recruitment by mimicking estrogen activity on a bone. Despite their effectiveness in certain populations, their use is restricted by the accompanying serious side effects upon long-term usage, such as an increased risk of breast cancer development, cardiovascular diseases, and thromboembolism [33]. Calcitonin, produced by the parafollicular cells of the thyroid gland, acts by inhibiting bone resorption *via* the calcitonin receptor on osteoclasts by reducing osteoclast activity, inducing their detachment from bone, and suppressing the proteolytic enzyme secretion from osteoclasts. It may also prevent osteoblast and osteocyte apoptosis. It is widely used for osteoporosis treatment in the USA; however, it is not a very powerful anti-resorptive agent since its long-term usage is associated with an increased risk of cancer. Teriparatide (recombinant human parathyroid hormone analog) is an osteoanabolic drug that stimulates bone formation. However, treatment with teriparatide is restricted to two years due to its associated side effects and potential risk of neoplasia. Strontium ranelate, known to have double actions on bone *via* enhancing bone formation and reducing bone resorption, was previously used for osteoporosis treatment. Now, it has been

withdrawn from the market due to serious risks for cardiovascular, cutaneous, and hepatic diseases. Biological and immunological drugs have also been developed for osteoporosis, such as RANKL Monoclonal Antibody. It is the first biological anti-resorptive agent targeting osteoclastogenic cytokines, thereby preventing osteoclast recruitment [34]. Considering the costs and disadvantages of long-term treatment with drugs and hormones in osteoporosis patients, cell therapy may be a perfect alternative therapeutic modality to mitigate osteoporosis [35].

2. Methods

Literature searches were conducted on PubMed, Google Scholar, and ScienceDirect on 15 April 2025 using the keywords “osteogenic differentiation”, “mesenchymal stem cells”, “nanomaterials”, and “osteoblasts”. Articles published in English over the last 25 years (2000 –2025) that discussed various types of nanomaterials and their roles in the osteogenic differentiation of MSCs were included. Studies irrelevant to the aim of this review, as well as letters, commentaries, and conference abstracts, were excluded. Relevant data were extracted and descriptively presented in the text and tables. In addition, a keyword search for “osteoblast transplantation” and “bone defects” in the ClinicalTrials.gov database yielded only a few results during the last 20 years.

3. Results and Discussion

3.1. Stem cell-based therapy for osteoporosis.

Cell therapy has gained great attention for treating many diseases for decades. Stem cells are an excellent source for treating bone diseases owing to their unique characteristics, which enable the repair of damaged tissues. Stem cells have various types, including embryonic stem cells (ES), induced pluripotent stem cells (iPS), and somatic stem cells such as MSCs. The usage of ES and iPS cells is restricted due to ethical issues and virus-based derivation methods [36]. Thus, MSCs have been used instead to overcome these limitations. MSCs have become dramatically interesting for osteoporosis treatment. MSCs are characterized by self-renewal and the ability to differentiate into various tissues, such as bone, cartilage, and adipose tissue. Human MSCs are defined by the expression of a cluster of surface markers, including CD105, CD73, and CD90, whereas they display negative expression of hematopoietic markers, such as CD34, CD45, and CD14 [37]. MSCs exhibit immunogenicity owing to their minimal expression of major histocompatibility molecules, enabling them to evade allogeneic rejection *via* modulating the T cell phenotype [38,39]. Moreover, osteogenic cells derived from MSCs exhibit immunoprivileged and immunomodulatory properties, similar to those of their parental MSCs [40]. The migration of MSCs to the injury site *in vivo* is motivated by chemokines and inflammatory cytokines secreted upon injury. Such factors stimulate the activation and expression of selectins and integrins on the surface of stem cells, resulting in their adherence and transmigration across the endothelium to the target sites. MSCs migration is regulated by the binding of stromal cell-derived factor-1a (SDF-1a) and its chemotactic receptor, CXC chemokine receptor type 4 (CXCR4). Moreover, inflammatory cytokines such as TGF-β1, IL-1β, and TNF-α promote the overexpression of MMPs in MSCs, thereby inducing their chemotactic migration through the ECM [41]. MSC transplantation could enhance new bone formation and increase bone strength, improving bone integrity and reducing fracture risk in osteoporosis. After transplantation, MSCs induce bone formation in two possible ways: (1)

MSCs' homing to the degenerated site with subsequent differentiation into osteoblasts to repair the damaged tissue, and (2) MSCs can secrete certain growth factors through a paracrine action, modifying the environment and recruiting resident cells to repair the damaged tissue [42,43].

3.1.1. Bone marrow-derived mesenchymal stem cells.

Bone marrow is the most attractive source for adult MSCs. Bone marrow-derived MSCs (BM-MSCs) have been widely utilized in bone regeneration and repair due to their great osteogenic efficiency [44]. Ichioka *et al*. [45] showed that allogeneic BM-MSCs could stimulate the trabecular bone formation and reduce the loss of bone mineral density (BMD) after being infused into the bone marrow cavity of an irradiated P6 substrain of senescence-accelerated mice (SAMP6). Also, autologous BM-MSC transplantation was demonstrated to enhance bone formation and repair osteoporotic bone in an ovariectomized rabbit model of osteoporosis [46]. Moreover, Niu *et al*. [47] have reported that the transplantation of BM-MSCs combined with fibrin glue stimulated bone regeneration and repair of the socket defect of maxillary alveolar bone in osteoporotic rats. However, the use of autologous BM-MSCs for osteoporosis treatment in aged patients is restricted owing to the decreased overall BM-MSC population with aging [48]. Additionally, the isolation procedure of bone marrow aspirate is highly invasive, painful, and entails complete anesthesia [49].

3.1.2. Adipose tissue-derived MSCs.

Adipose tissue offers an ideal source of MSCs that have been extensively used in various stem cell applications. Adipose tissue-derived MSCs (AD-MSCs) are harvested from white adipose tissues *via* a minimally invasive approach and can be propagated and differentiated into multiple lineages, including adipogenesis, osteogenesis, and chondrogenesis. AD-MSCs are more abundant since they provide higher yields than BM-MSCs and can be more easily isolated [50]. However, the yield of AD-MSCs, as well as their proliferation and differentiation capacities, differ according to the tissue harvesting site and donor age [35]. AD-MSCs represent an effective autologous cell-based therapy for osteoporosis. SAMP6 osteoporosis mice displayed significant enhancement in many parameters of trabecular bone after transplantation of AD-MSCs [51]. Moreover, Ye *et al*. [43] revealed that autologous transplantation of AD-MSCs stimulates bone regeneration in ovariectomized rabbit models of osteoporosis, not only through their osteogenic differentiation but also through their inhibition of adipogenesis by activating the BMP-2/BMP receptor type IB signaling pathway.

3.1.3. Dental-derived stem cells.

Dental-derived stem cells (DSCs) are attractive cell sources due to the ease of their isolation, greater growth capacity, and low immunogenicity. DSCs comprise various types of populations, including dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), gingival mesenchymal stem cells, dental follicle stem cells (DFSCs), stem cells from human exfoliated deciduous teeth, and stem cells from apical papilla [52]. In particular, DPSCs demonstrated an exceptional differentiation potential owing to their neural crest origin, making them a promising candidate for nerve and bone regeneration [53]. Local injection of DPSCs was found to promote bone regeneration and attenuate the progression of temporomandibular joint arthritis in a rat model by hindering the STAT1 signaling cascade

[54]. However, the direct transplantation of DSCs faces many challenges owing to their poor proliferation and differentiation into functional cells and low survival rates due to local inflammation. Therefore, combining biomaterials with DSCs regulates their differentiation and behavior, and provides a supportive environment for DSCs till they can be incorporated into the surrounding tissue [52]. Lee *et al*. [55] reported that the implantation of DPSCs seeded on Bio-Oss elicited an elevated expression of osteogenesis-related proteins and stimulated bone regeneration at the defect site in a rabbit cranial defect model.

3.1.4. Perinatal-derived MSCs.

Although BM- and AD-MSCs are excellent cell sources, the therapeutic efficacy of these adult MSCs depends on the donor's lifestyle and age. Perinatal tissues are alternative sources of MSCs that have attracted great attention in bone regenerative medicine. Since these cells are younger than adult MSCs, they can be easily isolated with a non-invasive procedure and without any risk to the donor. MSCs can be harvested from different perinatal tissues, including the umbilical cord, umbilical cord blood, and amniotic fluid. Such tissues have been reported to exhibit characteristics similar to those of BM-MSCs, including phenotypes, growth features, differentiation potential, secretory protein profiles, and low immunogenicity [56]. However, these stem cell sources remain limited due to their lower differentiation capacity compared to BM-MSCs and AD-MSCs [57].

3.1.5. Placenta-derived MSCs.

The placenta represents an easily accessible, plentiful source of perinatal MSCs. Placenta-derived MSCs (PL-MSCs) have been found to express the same MSC markers and exert adipogenic, osteogenic, and neurogenic differentiation capacities [58]. PL-MSCs have been reported to induce bone formation by differentiating into osteoblasts, producing bone matrix, and enhancing matrix mineralization. They also release bioactive molecules that stimulate osteogenesis by recruiting and activating endogenous stem cells, supporting angiogenesis, and establishing an anti-inflammatory environment essential for bone regeneration. These characteristics render PL-MSCs a promising candidate for treating bone diseases, fractures, and osteoporosis [59]. Sanvoranart *et al*. [60] reported that PL-MSCs responded to bortezomib, a chemotherapeutic agent that improves osteolytic lesions in multiple myeloma, *via* stimulating osteogenic differentiation in a similar way to BM-MSCs. This finding suggests the potency of PL-MSCs as a therapeutic option in osteopenia and osteoporosis.

3.1.6. Umbilical cord-derived MSCs.

Umbilical cord-derived MSCs (UC-MSCs) offer several advantages over BM-MSCs, including easier and non-invasive collection and lower immunogenicity. They also show superior differentiation abilities, without raising ethical concerns, making them a promising option for regenerative therapies. However, the application of UC-MSCs faces some limitations, including slower engraftment compared to BM-MSCs and reduced efficacy of autologous donations due to hereditary disorders [61]. It was reported that human UC-MSCs could improve bone formation markers and attenuate bone loss in ovariectomized mice [62]. Moreover, Hong *et al*. [63] demonstrated that UC-MSCs stimulated bone regeneration in an

osteoporotic rat model, indicating that they can offer a promising alternative stem cell therapy for osteoporosis.

3.1.7. Wharton's jelly-derived MSCs.

Wharton's jelly, a gelatinous connective tissue surrounding the umbilical cord vein, plays an essential role in protecting the umbilical cord vessels from any pressure [64]. Human Wharton's jelly-derived MSCs (WJ-MSCs) were reported to exhibit the most significant inhibitory effects on T cell proliferation and the lowest expression of major histocompatibility complex molecules class II and other human leukocyte antigens, as compared to BM-, AD-, and PL-MSCs [65]. These immunomodulatory and immunosuppressive characteristics of WJ-MSCs make them more attractive for clinical application as a cell therapy. Kang *et al*. [66] demonstrated that canine WJ-MSCs could enhance new bone formation in recipients with bone defects after orthotopic implantation of beta-tricalcium phosphate (β-TCP) for 20 weeks. The osteogenic ability of WJ-MSCs *in vitro* and *in vivo*, confirmed by new bone formation, resembles that of canine BM-MSCs, AD-MSCs, and UC-MSCs. Hence, WJ-MSCs can be employed in the treatment of bone defect diseases.

3.2. Trends in stem cell-based therapy for osteoporosis.

The major obstacles facing the utilization of stem cells in osteoporosis treatment are short-term engraftment and poor homing following transplantation. In addition, the action of stem cells might be regulated through paracrine mechanisms rather than through continuous engraftment in injured tissues [67,68]. The senescence of MSCs is one of the main factors hindering their expansion *in vitro*, which could affect the cells' survival after transplantation [69]. Many attempts now focus on achieving effective MSC-based therapy for osteoporosis by improving in vitro MSC cultures to increase survival and engraftment rates, possibly through modifying MSCs with specific factors and refining in vitro culture and differentiation procedures. MSC culture conditions, such as in vitro hypoxia preconditioning, have been adjusted to stimulate proliferation and differentiation and enhance the mobilization and homing of MSCs upon transplantation [70,71].

Genetically modified MSCs have emerged to improve their homing, differentiation capability, survival, and long-term engraftment at the damaged sites of recipients. Immortalization of MSCs has been achieved through p53 knockdown (a cell cycle regulator), in parallel with stimulating the expression of human telomerase reverse transcriptase, leading to telomere elongation, which can induce proliferation and increase survival while maintaining the cells' differentiation potential [72]. Transplantation of MSCs, retrovirally co-transfected with RANK-Fc (inhibitor of RANKL) or CXCR4, in the osteoporotic mice was reported to enhance the cell engraftment into the bone tissue and attenuate bone loss [73]. Akbar *et al*. [74] indicated that the implantation of AD-MSCs, transfected with lentiviral vectors expressing alpha-1 antitrypsin, attenuated bone loss in osteoporotic mice. Surprisingly, the engraftment of the transplanted cells into the bone tissue stimulated the secretion of alpha-1 antitrypsin and significantly reduced the serum levels of IL-6 and IL-1β, as well as the gene expression level of RANK in the bone tissue. Moreover, Wan *et al*. [75] reported that the transplantation of OPG-expressing AD-MSCs in osteoporotic rats inhibited bone resorption and induced bone formation, thereby facilitating the maxilla bone repair.

3.3. Trends in osteoinductive activity of nanomaterials.

Nanomaterials have attracted significant interest in bone tissue engineering owing to their superior mechanical strength, biodegradability, and biocompatibility compared with micro- or macro-scale structures [76]. Surface properties, such as surface area, charge, and topography, depend on the particle size of a material. Hence, cell response to the nanoparticles (NPs) is significantly different from that to bulk materials [77,78]. Moreover, nanomaterials provide a similarity to native bone architecture as compared to micron-sized materials [79]. About 70% of the natural bone matrix is composed of HA crystals of a rod shape in the nanoscale, in addition to other proteins of the bone ECM that are also in the nanometer dimension [80]. Such nanostructured bone matrix affects the adhesion, proliferation, and differentiation of mesenchymal stem cells, osteoblasts, osteoclasts, and fibroblasts [81]. Thus, it is necessary to fabricate biomaterials that resemble those present in bone to be employed in bone repair and regeneration [79].

3.3.1. Integration between nanomaterials properties and MSCs osteogenesis-related mechanisms.

Several studies reported that interactions between BM-MSCs and the ECM can significantly control the fate and behavior of stem cells [14,82]. Upon culturing NPs with stem cells, they interact with cell membranes, are internalized, encapsulated into vesicles, and then transported into the cells. During these processes, the interaction of NPs with cell membrane receptors or intracellular biomolecules may trigger cellular signaling pathways to induce the specific differentiation of stem cells. In addition, the surface protein adsorption by NPs may play a substantial role in directing the fate of stem cells [83]. It was reported that the adsorption and bioactivity of proteins that mediate specific osteoblast adhesion, such as fibronectin and vitronectin, are enhanced upon combination with nanoscale materials. Recent studies have investigated the role of nanostructured surfaces and scaffolds in stimulating stem cell proliferation, migration, and differentiation into the osteogenic lineage. Factors such as surface topography, stiffness, and chemical composition of nanomaterials have been found to influence stem cell differentiation [84]. The size of NPs significantly affects their osteoinductive activity. For instance, Li *et al*. [85] reported that 20 nm gold nanoparticles (Au-NPs) were more effective than 40 nm particles in promoting osteogenesis. Whereas smaller Au-NPs (<10 nm) suppressed the expression of osteogenesis-related genes in BM-MSCs [86]. Similarly, Shen *et al*. [87] demonstrated that larger titanium NPs (80 nm) enhanced MSC proliferation and differentiation compared with smaller ones. The morphology of NPs also influences the osteogenic differentiation. Spherical (40 nm, 70 nm) and rod-shaped (70 nm) Au-NPs were found to promote osteogenic differentiation, while rod-shaped 40 nm Au-NPs inhibited it. Larger Au-NPs (star-shaped and spherical, 110 nm) exhibited minimal osteogenic effects [85]. Moreover, the mechanical stress of NPs affects the differentiation potential of stem cells. Intracellular accumulation of NPs can affect cytoskeleton assembly and cell mechanics, and evidence suggests that enhanced mechanical properties favor osteogenic differentiation while inhibiting adipogenesis [88]. Additionally, modulating the stiffness and elasticity of nanomaterials can direct lineage commitment of stem cells. Wang *et al*. [89] demonstrated that increasing the hardness of gradient nanostructured titanium materials improved the efficiency of osteogenic differentiation. The antioxidant capacity of NPs is another important factor, as excessive reactive oxygen species (ROS) can hinder the osteogenic differentiation of MSCs.

Tian *et al*. [90] described a novel approach using manganese-substituted Co_3O_4 nanocrystals with enhanced ROS-scavenging ability, which effectively protected human MSCs from oxidative damage, reversed apoptosis, and restored essential cellular functions, including adhesion, proliferation, and osteogenic differentiation. Table 1 summarizes the different types of nanomaterials and their osteogenic effects on MSCs and pre-osteoblasts, along with the associated underlying mechanisms.

Table 1. Summary of the osteogenic effect of different nanomaterials and implicated molecular pathway.

Nanomaterials	Cell type and source	Surface modification/combination	Cellular outcome and molecular signaling	Ref.
Natural polymer				
Chitosan	Human AD-MSCs	$CaCO_3$/MgO/Chitosan/BMP-2	Enhanced matrix mineralization, elevated ALP activity, and osteoblast-specific gene levels *via* activating ERK1/2 and AKT pathways	[91]
	Human BM-MSCs	Non-thermal biocompatible plasma -modified chitosan scaffold	Enhanced osteogenic differentiation as reflected by increased ALP activity	[92]
Collagen I	BM-MSCs	-	Enhanced osteogenesis *via* activating the ERK and Akt pathways	[93]
	Human BM-MSCs	Collagen/bioactive glass NPs coatings and electrical field (EF) stimulation regimes.	Enhanced proliferation, osteogenesis, and calcium deposition *via* the Wnt/β-Catenin signaling pathway were activated by mechanotransduction cues upon exposure to the consecutive (12/12) EF regime. While exposure to the disrupted (4/4) EF regime resulted in activation of BMP/Smad4 pathways	[94]
Hyaluronic acid	Human DPSCs	-	Enhanced osteogenesis and matrix mineralization, as evidenced by upregulated levels of bone-related markers, e.g., OC, OPN, and BSP gene and protein expression *via* activating the YAP/TAZ pathway	[95]
Synthetic polymer				
PLA	Human AD-MSCs	$NiFe_2O_4$/ZnO-coated PLA nanofibrous scaffold	Enhanced osteogenesis and matrix mineralization as reflected by increased ALP activity and osteogenic gene expression (ALP, ON, OCN, Col-I, and Runx-2)	[96]
PCL	Human MSCs (BM-MSCs, AD-MSCs, and UC-MSCs)	-	Enhanced adhesion, osteogenesis, matrix mineralization, and upregulated expression levels of osteoblast-specific genes (Runx-2, BMP-2, Col-I, and ALP) *via* activating Wnt/β-catenin and Smad3 signaling pathways	[97]
	Rat BM-MSCs	Azide-pegylated PCL nanofibers functionalized with dibenzocyclooctyne-modified nanocapsules containing growth factor (BMP-2).	Improved stem cell adhesion, proliferation, and osteogenesis as evidenced by the augmented ALP staining and calcium deposition	[98]
PLGA	Mouse pre-osteoblast cell (MC3T3-E1)	poly-L-lysine surface-modified PLGA/ graphene oxide hybrid fiber matrix	Enhanced osteogenesis and calcium deposition, documented by elevated ALP activity and Runx-2 and OPN gene expression	[99]
Inorganic nanoparticles				
HA	Human BM-MSCs and UC-MSCs	Octacalcium phosphate -coated 3D-printed nano-HA scaffolds	Enhanced proliferation and osteogenic differentiation, as evidenced by elevated ALP activity and upregulated levels of osteogenic genes (Runx-2, OSX, OCN)	[44]
Au-NPs	Human BM-MSCs	Silica-coated Au-NPs	Improved cell adhesion, proliferation, differentiation, mineralization, and expression of pro-osteogenic cellular proteins	[100]
	Human BM-MSCs	Au-NPs-loaded HA composites	Enhanced osteogenesis *via* activation of the Wnt/β-catenin signaling pathway	[101]
	MC3T3-E1 cells, human and rat BM-MSCs	PEGylated Au-NPs	Enhanced osteogenesis and matrix mineralization, as evidenced by elevated ALP activity and upregulated levels of osteogenesis markers *via* activation of the Wnt/β-catenin signaling pathway	[102]

Nanomaterials	Cell type and source	Surface modification/combination	Cellular outcome and molecular signaling	Ref.
	PDLSCs	-	Enhanced proliferation and osteogenesis *via* modulating PTEN-induced putative kinase 1 (PINK1) dependent mitophagy	[103]
Pt-NPs	human DFSCs	-	Enhanced *in vitro* osteogenic differentiation *via* activation of the PI3K/AKT pathway and scavenging ROS	[104]
AgNPs	Human MSCs	-	Enhanced osteogenesis and matrix mineralization *via* activation of autophagy, as evidenced by upregulated levels of autophagy-related proteins (LC3-II and p62)	[105]
Titanium	Rat osteoblasts	Titanium implants coated with nano-HA/chitosan composite.	Upregulated Runx-2 and osterix, enhanced osteogenesis *via* the activation of the focal adhesion kinase (FAK), which in turn activates the integrin/BMP-2/Smad pathway	[106]
	BM-MSCs	Sodium bicarbonate – surface modified Titanium Disc	Enhanced stem cells adhesion, proliferation, spreading, and osteogenesis *via* the activation of the integrin/ FAK/ALP signaling pathway	[107]
ZnONPs	Rat bone marrow-derived pericytes (BM-PCs)	Zinc-modified calcium silicate coatings	Enhanced matrix mineralization and osteoblastic differentiation *via* regulating the TGF-β/Smad signaling pathway	[108]
	MC3T3-E1 cells	Polydopamine-modified ZnONP incorporated chitosan-gelatin hydrogel.	Promoted osteoblast differentiation, proliferation, and adhesions, as evidenced by increased ALP activity and calcium nodule formation	[109]
Fe_2O_3 NPs	Human BM-MSCs	-	Enhanced osteogenesis as documented by up-regulated osteogenic markers such as ALP, Runx-2, BMP-2, as well as osteomodulin, forkhead box O1 (FOXO1), and activating transcription factor 4 (ATF4), *via* activating the MAPK pathway	[110]
	Human DPSCs	Fe_2O_3 NPs/calcium phosphate cement scaffolds	Stem cell osteogenic differentiation through Wnt/β-catenin signaling activation	[111]
	Human BM-MSCs	-	Stem cell osteogenic differentiation *via* activating the BMP/SMAD pathway through upregulation of long noncoding RNA INZEB2	[112]

3.3.2. Toxicity, biocompatibility, and clinical translation barrier of nanomaterials.

Nanomaterials can induce toxicity *via* multiple mechanisms, including oxidative stress, inflammatory responses, DNA damage, and different forms of cell death, such as apoptosis, autophagy, and necrosis. The physicochemical properties of nanomaterials, such as size, shape, and surface charge, as well as the dose and type of NPs, can affect their biochemical behavior, cellular uptake, and potential cytotoxicity [113]. Many pathways regulate the cellular uptake of NPs depending on particle size, including endocytosis, phagocytosis, and pinocytosis. The size and surface area of nanomaterials significantly affect their cell-penetrating ability, as the larger surface area enables more effective adsorption of NPs. Specifically, as NP size decreases, surface area increases; thus, small NPs are more reactive in the biological environment and exhibit larger catalytic surfaces for chemical reactions. NPs of (1 to 100) nm size can be easily taken up by cells because of the similarity of their size to the thickness of cell membranes (10 nm). For example, Au-NPs of (10–16) nm in size have been accumulated in the cytoplasm, whereas those smaller than 6 nm can penetrate cellular and nuclear membranes, leading to genotoxicity and systemic exposure, as smaller NPs bypass renal filtration and accumulate in non-target tissues. Thus, smaller NPs can be more toxic. Overall, NPs of optimum size (50 nm) are more efficiently internalized. However, several studies have reported that smaller NPs (15–30 nm) or larger NPs (70–240 nm) display lower cellular uptake rates [114]. The shape of NPs can also affect their cytotoxicity and cellular uptake. It has been shown that NPs of spherical morphology are more readily taken up by cells than rod-, cubic-, or star-shaped NPs, as they encounter a lower membrane-bending energy barrier [115].

Additionally, spherical nanoparticles stimulate the least cytotoxicity by preventing the cell membrane disruption caused by direct diffusion. NPs with angular or sharp edges also tend to cause more damage to cell membranes than spherical or rounded ones. Moreover, surface charge plays a crucial role in the toxicity of NPs. Positively charged NPs interact strongly with the negatively charged components of cell membranes, resulting in cytotoxicity and cell death. Conversely, neutral or slightly negatively charged particles generally exhibit fewer nonspecific interactions, thereby enhancing their biocompatibility. Surface modifications, such as coating with polyethylene glycol (PEG), improve colloidal stability, reduce protein adsorption, and prolong circulation time. However, repeated exposure may stimulate the formation of anti-PEG antibodies, leading to faster clearance from the body. The material composition determines degradation products and potential toxicity. For example, zinc oxide nanoparticles (ZnONPs) can release Zn^{2+} ions that disturb cellular homeostasis, whereas titanium dioxide (TiO_2) NPs are less reactive but can produce ROS under UV exposure. NPs that contain heavy metals, such as quantum dots, pose a risk of ion leaching, which can cause cytotoxicity. In contrast, biodegradable polymers and lipid-based NPs are generally safer alternatives, though they may still provoke immune reactions if they accumulate within tissues [116]. There are several challenges in incorporating metal NPs into scaffolds for bone tissue regeneration. One limitation is their potential toxicity since the metal ions can leach from the scaffold, triggering toxicity and immune reactions. The nanoscale size and large surface area of NPs make them highly reactive, leading to elevated ROS levels, causing cell damage and ultimately compromising the regeneration process. Long-term *in vivo* studies are still lacking to fully assess the safety and potential accumulation of NPs in organs such as the liver, spleen, kidneys, and lungs. Understanding their metabolism and clearance is essential for long-term safety. Another major challenge is achieving targeted delivery to bone defects while avoiding non-specific accumulation in other tissues. Hence, more biocompatible NPs should be incorporated into the scaffolds to provide suitable mechanical strength, enable efficient bone replacement, and avoid the release of toxic degradation products. To overcome these limitations, scaffolds can be designed with resorbable nanostructures, including nanostructured ceramics (e.g., β-TCP, biphasic calcium phosphate, and HA) and natural polymers, which can be effectively degraded by cellular metabolism and enzymes. Additionally, biogenic metal NPs synthesized using bacteria, algae, or medicinal plants can enhance mechanical strength and improve biocompatibility while minimizing toxicity [117].

3.3.3. Types of Nanomaterials based on chemical structure.

Nanomaterials are classified into two categories based on their chemical structure: inorganic NPs, including ceramics and metals such as HA, silica, gold, and silver, and organic nanomaterials, such as polymers.

3.3.3.1. Organic polymer.

(i) Natural polymers

Naturally sourced polymers, including chitosan, collagen, and hyaluronic acid, have been extensively utilized in various tissue engineering applications. Due to their resemblance to the natural ECM, these polymers exhibit excellent biocompatibility for *in vivo* use. Furthermore, they offer a diverse array of ligands and peptides that enhance cell-material interactions, promote osteogenesis, and minimize immunogenicity [118].

Chitosan

Chitosan is a polysaccharide obtained by the alkaline deacetylation of chitin, a natural biopolymer existing in crustacean shells [119]. It consists of β-(1-4)-linked D-glucosamine residues with a variable number of randomly located N-acetyl-glucosamine units [120]. The ability of chitosan to elicit favorable cellular responses is attributed to its structural resemblance to glycosaminoglycans in the natural ECM [121]. This biomimetic characteristic enables chitosan to interact with various cell surface receptors, thereby activating specific signaling pathways that regulate cell attachment, proliferation, and differentiation, providing a suitable microenvironment crucial for tissue regeneration [122]. Chitosan, a natural polymer, offers superior bioactivity, biodegradability, non-immunogenic characteristics, cell ingrowth capacity, and biocompatibility, compared to conventional synthetic materials [119,123]. However, its application is limited by inadequate stability, mechanical strength, and osteoconductivity. To overcome these challenges, chitosan is often combined with metals, ceramics, natural and synthetic polymers, and other materials to improve its overall performance in biomedical applications [124]. Chitosan/HA nanocomposites have been shown to imitate both organic and inorganic portions of natural bone. Chitosan/HA nanocomposites have been widely investigated for bone tissue engineering since they have been reported to stimulate osteogenesis and induce new bone formation in vivo [125,126]. It is well known that integrating chitosan with HA could minimize the degree of HA-NPs agglomeration, which is accountable for the cell toxicity [127]. Demirtaş *et al*. [128] successfully demonstrated the bioprinting of pre-osteoblast cells using chitosan and a chitosan-nano-HA hydrogel, which exhibited high cell viability, proliferation, and osteogenic differentiation. However, the signaling pathways, or specific interactions between the hydrogel components and pre-osteoblasts that drive osteogenesis, were not elucidated. A recent study by Mahmoud *et al*. [129] demonstrated the osteoinductive effect of the chitosan/HA nanocomposite, which stimulated the differentiation of rat BM-MSCs into osteoblasts, as evidenced by high cell viability, enhanced matrix mineralization, and upregulated levels of osteoblast-related genes such as Runx-2 and BMP-2. Additionally, chitosan/gelatin/nano-HA scaffolds supported the viability, proliferation, odontogenic differentiation, and *in vitro* biomineralization of dental pulp stem cells, confirmed by pronounced upregulation of Runx-2, OSX, BMP-2, and ALP, without the inclusion of any osteoinductive factors [130]. However, the long-term biocompatibility, degradation, and mechanical stability of the scaffold remain untested, and its relevance to broader bone regeneration applications was not evaluated. Recently, Di Stefano *et al*. [131] have highlighted the osteoinductive potential of chitosan/HA nanocomposite in driving the differentiation of AD-MSCs spheroids towards osteoblasts, as evidenced by the remarkable expression of osteopontin. However, this study provides limited evidence of the hydrogel's osteogenic efficacy, as long-term differentiation, matrix mineralization, and late-stage osteogenic markers were not fully assessed. The underlying mechanisms of osteogenic induction remain unclear, and the translational potential of the derived osteoblasts has not been evaluated *in vivo*.

Collagen

Collagen is the most abundant protein in connective tissues such as tendons and bone, with type I collagen being the most abundant protein of the ECM [132]. It plays a critical role in bone growth and remodeling and is widely used as a scaffolding material in bone tissue engineering due to its excellent biocompatibility and biodegradability. However, its low mechanical strength and rapid degradation by collagenase restrict its effectiveness in bone

regeneration applications. Therefore, incorporating extra-polymeric elements is essential to increase the strength and durability of collagen-based gels [133]. It has been found that culturing BM-MSCs with collagen I induced the osteogenic differentiation of stem cells into osteoblasts, as indicated by enhanced expression of osteogenic genes and increased ALP activity [134,135]. Biocomposite scaffolds composed of collagen (Col) reinforced with HA are an attractive option for bone tissue engineering due to their composition closely mimicking natural bone [136]. Hayrapetyan *et al*. [137] reported that AD-MSCs co-cultured with Col/HA nanocomposite displayed higher proliferation, osteogenic, and mineralization capacities compared to BM-MSCs, regardless of the concentration of nano HA in the composite, as demonstrated by high ALP activity, elevated gene expression levels of Runx-2, BMP-2, and Col-I, as well as upregulated OCN mRNA and protein expressions over an extended culture period. Although this study confirmed the sustained osteoinductive potential of the hydrogel, which provides a 3D microenvironment that better mimics the *in vivo* microenvironment for stem cells than traditional 2D cultures, the underlying molecular mechanisms driving osteogenesis were not investigated. Furthermore, the lack of *in vivo* validation of the bone-regenerative efficacy of the resultant differentiated osteoblasts limits the translational feasibility of the study. Recent studies have demonstrated that co-culturing of AD-MSCs or BM-MSCs with Col/HA scaffolds resulted in improved cytocompatibility and enhanced osteogenesis as indicated by the over-expression of genes implicated in ossification such as SP7, SMAD3, BMP-2/3, TGF-β3, NOG, and SPP1, essential for skeletal development, such as MMP9 and MMP10 as well as increased matrix mineralization and elevated expression of OCN and OPN proteins [138,139]. Moreover, the study of Zuo *et al*. [140] indicated that human AD-MSCs cultured on a mineralized collagen scaffold composed of 45% Col-I and 55% nano-sized HA predominantly differentiated into osteoblasts *in vitro* as confirmed by increased calcium deposition, and ALP activity, as well as notable upregulation of osteogenic genes, including ALP, Runx-2, and BMP-2. While these findings confirm early osteogenic differentiation, late-stage osteogenic markers or long-term matrix mineralization over an extended culture period have not been assessed, which limits the evaluation of sustained osteoinductive potential. In addition, the underlying molecular mechanisms driving the scaffold's osteogenic effect were not investigated.

Hyaluronic acid

Hyaluronic acid is a biodegradable and highly biocompatible polysaccharide found in the ECM of cartilage. It plays a crucial role in regulating cell mobility, cell-matrix adhesion, and cell-cell interactions [141]. Liu *et al*. [142] indicated that Simvastatin-loaded and HA-modified zeolitic imidazolate framework-8 (ZIF-8) particles/dopamine-hyaluronic acid /tannic nanocomposites showed improved mechanical strength, stability, excellent tissue adhesion, and antibacterial characteristics. This nanocomposite also stimulated the osteogenic differentiation of osteoblast cell line (MC3T3-E1) as evidenced by upregulated mRNA levels of ALP, OCN, and Runx-2 and enhanced matrix mineralization. Further, it induced bone regeneration and mitigated bone remodeling in the bone defect rat model. The release of Zn^{2+} and Ca^{2+} ions from the SP particles provides additional osteogenic cues. However, it remains unclear whether the enhanced osteogenesis primarily results from simvastatin release, HA incorporation, ion release, or their synergistic effect, due to the lack of mechanistic evidence. Moreover, while these findings confirm early osteogenic differentiation, late-stage osteogenic markers and long-term matrix mineralization over an extended culture period have not been assessed. In a more recent study by Kasi *et al*. [143], the gelatin/hyaluronic acid/HA scaffolds

incorporated with poly(3,4-ethylenedioxythiophene) nanoparticles (PEDOT NPs) showed enhanced cell proliferation, viability, and osteogenic differentiation of human foetal osteoblastic 1.19 cells, and human BM-MSCs, as evidenced by higher ALP activity, upregulated expression of OPN, and OCN. Although incorporating PEDOT NPs enhances the scaffold's conductivity, biocompatibility, and bioactivity, its translational relevance is constrained by a lack of *in vivo* evidence and limited mechanistic insight.

(ii) Synthetic polymers

Synthetic polymers have gained significant attention in bone grafting due to their low immunogenicity, high water resistance, controlled degradation rates, ease of fabrication, affordability, and ability to provide tunable mechanical properties [144]. Synthetic polymers such as aliphatic polyesters, including polycaprolactone, polylactic acid, poly(glycolic acid), and their copolymers, poly(lactic-co-glycolic acid), are favorable in orthopedic applications due to their high tensile strength and rigidity [145]. However, since synthetic polymers often exhibit limited cellular interactions, they are often blended with natural polymers and other bioactive materials to enhance their biocompatibility and cellular response [146].

Polylactide

Polylactide (PLA) is an aliphatic polyester synthesized from lactic acid, which can be derived from renewable sources such as corn, sugar, and biomass. Due to its favorable mechanical and thermoplastic properties, along with its biocompatibility and biodegradability, PLA has been widely used for bone implants. However, the hydrophobic properties of PLA limit its use in tissue regeneration. Thus, bioactive coatings of the PLA surface can enhance its hydrophilicity, thereby improving cell adhesion and supporting cellular functions [147]. Kim *et al*. [148] demonstrated that bioactive glass-embedded PLA nanocomposite scaffold showed improved cytocompatibility, bioactivity, and enhanced osteogenic potential, as compared to pure PLA scaffold, upon culturing with BM-MSCs, as indicated by significant elevation of ALP activity and osteogenic-associated gene expression levels. The enhanced osteogenic effect is attributed to the release of bioactive ions, such as calcium, phosphate, and silicon, from the glass nanocomponent. However, the biocompatibility data remain insufficient, as no cell viability assay was conducted to evaluate the potential cytotoxic effects of the nanocomposite on BMSCs. Calcium silicate nanoadditive (CSN)/PLA nanocomposite has been shown to stimulate the cell adhesion, proliferation, and osteogenesis of rat BM-MSCs as indicated by elevated expression of osteogenic genes, including Col I, OPN, OCN, and ALP, in addition to the overexpression of osteopontin protein as shown by western blot analysis [149]. However, the underlying mechanism by which this nanocomposite enhances osteogenesis was not fully elucidated, and the absence of *in vivo* data limits its clinical applicability. PLA/HA nanofibers scaffold co-cultured with pre-osteoblasts in the presence of osteogenic supplements showed improved cytocompatibility, viability, and proliferation up to 21 days, suggesting their potential for supporting the growth of osteoblasts and hence guided bone regeneration [150]. In HA/PLA composites, PLA could provide a substrate for supporting cell adhesion, whereas the HA could stimulate cell proliferation and osteoinduction. The incorporation of HA was also found to improve the mechanical strength, fiber diameter, and pore size of PLA nanofibers. Notably, 3D-printed PLA/HA scaffolds successfully induced osteogenic differentiation of MSC even in the absence of osteogenic supplements [151]. Recently, Dawood and Mahdee [152] reported that a 3D-printed PLA/nano-HA/naringin nanoscaffold stimulated the osteogenic differentiation of DPSCs, as confirmed by elevated protein expression of ALP and

dentine sialophosphoprotein (DSPP) and enhanced calcium deposition. The synergistic action of naringin and nHA enhances the osteoinductive potential of this scaffold, as the antioxidant properties of naringin can regulate pH, increasing HA solubility and enabling sustained calcium ion release, which, in turn, stimulates osteoblast differentiation over time. However, the nanocomposite's biocompatibility remains uncertain, as no cell viability assays were performed to assess potential cytotoxicity in DPSCs.

Polycaprolactone

Polycaprolactone (PCL) is a synthetic polyester compound known for its biocompatibility, biodegradability, and good permeability. Its unique characteristics, including thermal sensitivity, drug encapsulation, and mechanical properties, ease of fabrication, make PCL an attractive biomaterial for tissue engineering applications [153]. Recently, PCL/Col-I/iron oxide (Fe_3O_4) nanocomposite scaffolds have been reported to maintain cell viability, cell adhesion, and stimulate osteogenic differentiation of rat AD-MSCs even in the absence of osteogenic supplements, as evidenced by elevated ALP activity, calcium-mineralization, as well as upregulated osteogenic-related genes or proteins expression [154]. However, the lack of mechanistic evidence and *in vivo* validation limits its translational feasibility. Moreover, a study by Azaryan *et al*. [155] showed that PCL/nano-HA (P/nHAEA) with an average diameter of 191.8 ± 43.1 nm, green synthesized from Elaeagnus angustifolia (EA) extract, stimulates the cell adhesion, osteogenic/odontogenic differentiation of DPSCs as confirmed by enhanced ALP activity, calcium deposition, and over-expression of osteogenic genes such as Runx-2, BMP-2, and DSPP. The enhanced hydrophilicity of P/nHAEA *versus* standard nano-HA likely improves cell attachment and differentiation; however, it remains unclear whether this effect is primarily driven by hydrophilicity, rod morphology, or Ca^{2+}/PO_4^{3-} ion release. While early osteogenic differentiation is evident, late-stage osteogenesis over an extended culture period was not evaluated. The lack of mechanistic insight and *in vivo* validation further restrains its translational potential**.**

Poly (lactic-co-glycolic acid)

Poly (lactic-co-glycolic acid) (PLGA) is a synthetic copolymer made from glycolic acid and lactic acid, forming an aliphatic biodegradable polyester. Its biocompatibility, customizable degradation rate, and overall biological safety have led to its widespread use in bone tissue engineering. However, its relatively poor mechanical properties and limited osteoconductivity limit its applications in clinical settings. To overcome these limitations and enhance its bone regeneration ability, PLGA is often combined with other biomaterials [156]. Moreover, Wang *et al*. [157] demonstrated that the electrospun PLGA/PCL membranes doped with octacalcium phosphate (OCP) nanofibers possess osteoinductive properties. This was evidenced by increased ALP activity, mineral nodule formation, and upregulation of osteoblast-specific gene markers. Electrospinning and incorporation of OCP into the PCL matrix increased the surface area to volume ratios, generating ECM-like architecture and improving the mechanical properties. Despite the pronounced mechanical stability, biocompatibility, and osteoinductive activity of the scaffold, late-stage osteogenesis over prolonged culture duration and underlying molecular mechanisms have not been investigated. Also, the translational feasibility is limited by the lack of *in vivo* study validation. Recently, Zhou *et al*. [158] indicated that a PLGA/silicon nitride (Si_3N_4) (2 wt.%) nanofiber scaffold supports the cell adhesion, proliferation, and elicits the osteogenic commitment of MSCs as evidenced by elevated gene expression of osteogenic markers and enhanced calcium

deposition. However, long-term *in vitro* osteogenic differentiation, mechanistic insight, and *in vivo* validation have not been evaluated.

3.3.3.2. Inorganic nanoparticles.

(i) Ceramics

Hydroxyapatite

HA is a calcium phosphate ceramic that has been extensively used for bone tissue engineering due to its chemical composition similar to that of natural bone, excellent biocompatibility, biodegradability, and osteoconductivity. The favorable characteristics of HA nanoparticles (nano-HA), including surface grain size, pore size, and wettability, compared to their microscale counterparts, influence protein interactions and thereby guide cellular responses. Therefore, nano-HA exhibits greater bioactivity, enabling osteoblast adhesion, proliferation, differentiation, osteointegration, and calcium deposition on its surface, resulting in rapid healing of degenerate tissue [15]. Multiple studies have demonstrated that nano-HA is internalized by cells via endocytosis [159] and subsequently digested in lysosomes, leading to elevated cytoplasmic Ca^{2+} levels. This rise in Ca^{2+} concentration is believed to be essential for osteoblast proliferation and differentiation [160]. Lu *et al*. [161] demonstrated that the rod-shaped nano-HA-incorporated PCL films, which imitate the native bone, exert the most osteoinductive effect on human osteoblasts differentiation, when compared with spherical nano-HA-incorporated PCL or PCL alone, *via* significantly up-regulating Runx-2, BSP, and OCN gene expression levels. In addition, Huang *et al*. [82] mentioned that rat BM-MSCs incubated with osteogenic medium containing 200 μg/ml nano-HA displayed a significant over-expression of the osteoblast-related genes, when compared with micro-sized HA. This could be ascribed to the fact that nano-HA alters the cell culture microenvironment, greatly influencing MSCs' differentiation [162]. Since nano-HA acts to adsorb the serum proteins, a new matrix is formed, thus motivating osteogenesis. Moreover, the study by Kim et al. [163] demonstrated that incubating rat BM-MSCs with poly(propylene fumarate) (PPF)/HA nanocomposite scaffolds greatly promoted their differentiation towards the osteoblast lineage by activating the upregulation of BMP-2 and Runx-2. These findings indicate that low cell seeding density combined with high HA content may be optimal for enhancing osteogenic differentiation. While the study suggests mechanistic involvement *via* upregulated FGF-2 and TGF-β1 gene expression, protein-level confirmation is lacking. Additionally, late-stage osteogenesis has not been assessed, limiting insights into the scaffold's long-term osteogenic potential. Recently, Mahmoud *et al*. [129] reported the osteogenic effect of nano-HA on rat BM-MSCs, which was confirmed by enhanced calcium deposition and upregulated levels of osteogenic markers. Moreover, Li *et al*. [164] reported that the nano-HA/resveratrol/chitosan composite enhanced the adhesion, proliferation, and osteogenic differentiation of BM-MSCs, as evidenced by increased mineralization and upregulation of osteogenic markers. Furthermore, when these composites were implanted in osteoporotic rat femoral condyles, they induced bone regeneration, highlighting their potential as bone fillers for osteoporotic defects. Despite the promising findings of this study, however, its translational feasibility is limited by a relatively short *in vivo* duration and limited mechanistic investigation.

(ii) Metal nanoparticles

Noble metal NPs, such as Au, Ag, and platinum (Pt), display exceptional properties that make them extensively employed in catalysis, cosmetics, biotechnology, and electronics [165]. Different metal ions and metal NPs have been reported to regulate the proliferation and differentiation of osteoblasts, as well as bone ECM mineralization. In addition, it has been demonstrated that various metal ions are essential components of bone tissue, contributing to the physiological cellular environment and playing a role in the bone healing process [117].

Gold

Au-NPs have been considered attractive materials for biomedical and regenerative medicine applications owing to their unique physical and chemical characteristics. Various forms of Au-NPs have been shown to affect the differentiation of human MSCs into different lineages. Many studies have proposed that Au-NPs stimulate the osteogenic differentiation of human MSCs [166]. Moreover, Liu *et al*. [167] reported that Au-NPs of 20 and 40 nm size promote the osteogenic differentiation of MC3T3-E1 osteoblast-like cells. Furthermore, Au-NP-loaded mesoporous silica nanoparticles (Au-MSNs) have been shown to stimulate the osteogenic differentiation in pre-osteoblastic MC3T3 cells, as confirmed by the over-expression of osteogenic markers, ALP production, and calcium deposition. This study further performed an *in vivo* study in a critical-sized rat cranial defect model, showing that the Au-MSNs accelerated new bone formation, suggesting their therapeutic potential for bone tissue repair and regeneration [168]. While this nanostructure demonstrated enhanced immunomodulatory and osteogenic activity, the underlying mechanistic pathways were not explored. Similarly, Mahmoud *et al*. [129] have reported that Au-NPs and Gold/HA nanocomposites (Au/HA-NPs) motivated the osteogenic differentiation of BM-MSCs into functional osteoblasts as evidenced by a prominent over-expression of osteoblast-related genes such as Runx-2 and BMP-2 genes, as well as enhanced matrix mineralization. These authors further investigated the bone regenerating efficacy of the osteoblasts, derived from the co-culture of rat BM-MSCs with Au-NPs and Au/HA-NPs *in vitro*. They indicated that osteoblast infusion in osteoporotic rats enhanced bone repair by restoring the balance of bone remodeling [169]. Recently, Hung *et al*. [170] have shown that fibronectin-gold and collagen-gold nanocomposites stimulated the osteoblastic differentiation of human WJ-MSCs as evidenced by overexpression of Runx-2 and enhanced calcium deposition. Although this study demonstrated the osteogenic potential of these nanocomposites, it provides only preliminary osteogenic data and lacks a comprehensive evaluation of late-stage osteogenic maturation and underlying mechanistic insights.

Platinum

Platinum nanoparticles (Pt-NPs) have gathered great attention owing to their unique characteristics, including great antimicrobial properties [165]. Moreover, Pt-NPs display anti-oxidative activities, making them promising candidates for treating oxidative stress-related diseases [171]. In addition, Pt-NPs have been shown to ameliorate bone loss by inhibiting osteoclastogenesis [172]. Aglan *et al*. [173] have demonstrated that Pt-NPs and platinum-HA (Pt-HA) nanocomposites stimulated the osteogenic differentiation of rat BM-MSCs into functional osteoblasts, which displayed upregulated expression levels of osteogenic genes as well as enhanced matrix mineralization. The injection of the generated osteoblasts, resulting from the co-culture of BM-MSCs with Pt-NPs or Pt-HA nanocomposites, successfully attenuated bone loss in the osteoporotic rat model. Although this study performed *in vivo*

validation of the cultured osteoblasts, the underlying mechanisms have not been investigated. Moreover, it has been reported that Pt-NPs could stimulate the osteogenic differentiation of human AD-MSCs *in vitro*. Furthermore, the transplantation of AD-MSCs combined with Pt-NPs could accelerate the healing of tibial fractures in rats [174]. Despite the promising outcomes of AD-MSCs and Pt-NPs combination therapy in enhancing bone repair, the translational feasibility of this approach is limited by the small sample size, lack of mechanistic elucidation, undefined optimal dosing and delivery route, and the absence of long-term safety and biodistribution assessments.

Silver

Silver NPs (AgNPs) have attracted considerable attention due to their outstanding antimicrobial properties and osteogenic potential. Additionally, AgNPs have demonstrated toxic effects on bone cancer cells [175]. However, they displayed trivial toxicity on osteoblasts and stem cells. Zhang *et al*. [176] reported that AgNPs induced the osteogenic differentiation of mouse MSCs, as confirmed by enhanced calcium deposition, elevated ALP activity, and upregulated Runx-2 expression level, compared to the control. Further, these investigators indicated that an AgNPs-encapsulated collagen scaffold, alone or accompanied by MSCs, promotes healing of a mouse femoral bone fracture upon implantation at the fracture site. However, the data from this study were preliminary, as they did not provide a full characterization of AgNPs, including kinetic release and zeta potential. Moreover, translational feasibility is limited because the optimal dosing regimen is unclear, and long-term toxicity, biodistribution, *in vivo* clearance, or immunological responses have not been assessed. Moreover, Vaidhyanathan *et al*. [177] have shown that AgNPs incorporated into chitosan scaffolds stimulated the osteogenic differentiation of MG-63 osteoblastic cells and rat BM-MSCs by upregulating Runx-2, Col-I, ALP activity, and the level of secreted OCN. However, cytotoxicity studies to determine a safe scaffold concentration for stimulating osteoblastic differentiation, as well as investigations into the underlying mechanisms, have not been conducted. In addition, late-stage osteogenesis, including prolonged culture periods and in vivo validation, has not been evaluated. Furthermore, it has been indicated that AgNPs alone or combined with Calcium Hydroxide stimulate the proliferation and osteogenic differentiation of human MSCs *via* upregulating the osteogenesis-related genes [178]. Despite the promising findings of this study, a detailed characterization of the nanoparticles, including surface charge and release kinetics, was not performed. Furthermore, late-stage osteogenesis, such as matrix mineralization, was not assessed, limiting conclusions regarding the functional maturation of osteoblasts. Recently, Mira *et al*. [179] showed that 3D-printed PCL/AgNPs scaffolds, prepared using a facile green synthesis approach, promoted the osteogenic differentiation of human WJ-MSCs, as evidenced by enhanced Col-I, OPN, and ALP gene expression levels and immunostaining over 21 days of osteogenesis. Despite the promising finding that the scaffold enhances MSC osteogenesis at low concentrations, this approach is limited by the lack of mechanistic insight and *in vivo* validation.

Titanium

Titanium is considered the gold standard for prosthetic materials due to its excellent mechanical and chemical properties. When exposed to oxygen, titanium forms a biocompatible oxide called anatase, which promotes osseointegration. It has been demonstrated that titanium nanotubes can stimulate the osteogenic differentiation of AD-MSCs and DPSCs, as evidenced by the upregulation of bone-related genes Runx-2, FOSL1, and SPP1 [180]. Ahmadi *et al*. [181] declared that TiO_2 NPs and metformin-co-embedded PCL/gelatin nanofibers stimulated

the cell adhesion, proliferation, and osteoblastic differentiation of human AD-MSCs *via* enhanced mineralization and elevated expression levels of osteoblast-related markers. Despite the scaffold's pronounced osteoinductive activity, testing different concentrations of TiO2 NPs to achieve optimal mechanical and bioactive properties was not conducted. Moreover, the lack of mechanistic insight and *in vivo* validation restricts its advancement toward clinical bone repair applications. It has been reported that incorporating TiO_2 NPs into the β titanium alloy Ti–35Nb–2Ta–3Zr (TNTZ) surface remarkably improved the adhesion, proliferation, and osteogenic differentiation of rat BM-MSCs, elevated the expression of osteogenic genes, and promoted the protein expression levels of ALP and OCN [182]. Recently, Liu *et al*. [183] reported that TiO2/Polyvinylidene fluoride (PVDF) nanocomposites could provide an electromechanical microenvironment that facilitates the viability, attachment, and osteogenesis of rat BM-MSCs. TiO_2@ PVDF nanostructure remarkably enhanced ALP staining and matrix mineralization, in addition to upregulated expression levels of Runx-2, OCN, and OPN. However, the lack of mechanistic elucidation and *in vivo* validation limited its translational relevance.

Zinc

ZnONPs have been shown to attenuate oxidative stress by scavenging detrimental free radicals. Functionalized ZnONPs and their alloys have displayed potential antioxidant, antidiabetic, and antibacterial activities. Hence, ZnONPs are promising NPs that can be incorporated into scaffolds to enhance bone regeneration, while also scavenging reactive oxygen species (ROS) and preventing bacterial infections associated with implants [117]. Recently, Song *et al*. [184] reported the influence of zinc silicate/nano-HA/collagen-based scaffolds on bone regeneration and angiogenesis. The sustained release of zinc ions and silicon was biocompatible with BM-MSCs and enhanced bone regeneration in a rat model of bone defects. Further, the overexpression of osteogenesis-related genes BMP-2 and OSX was associated with the *in vivo* bone repair process. The composite scaffolds upregulated the expression of osteogenic genes such as OSX and BMP-2, angiogenesis-related genes such as VEGF-α and CD31, and activated the p38 signaling pathway in monocytes, resulting in stimulating their differentiation into TRAP+ cells expressing elevated levels of SDF-1, TGF-β1, VEGF-α, and PDGF-BB, which recruited BM-MSCs and endothelial cells to the defect site to enhance tissue repair. Tang *et al*. [185] reported that ZnONPs synthesized via a green method using S. baicalensis extract displayed minimal toxicity, accompanied by enhanced proliferation, osteogenic differentiation, and mineralization in human osteoblast-like MG-63 cells. However, the translational feasibility of this study is limited by the lack of mechanistic insight and *in vivo* validation. Moreover, zinc-doped nano-HA incorporated into gelatin nanofibers stimulated the osteogenic differentiation of MSCs and enhanced angiogenesis, as evidenced by elevated expression of iNOS and CD31 [186]. Although this scaffold demonstrated enhanced osteogenic differentiation of MSCs without the aid of an osteogenic medium, the study did not fully address late-stage osteogenesis/mineralization. In addition, detailed characterization of Zn^{2+} ion release kinetics and assessment of potential cytotoxicity were not performed.

Furthermore, Ugli AK *et al*. [187] showed that rod-shaped ZnONPs (50 nm) did not affect the viability of BM-MSCs at concentrations up to 25 μg/mL. ZnONPs stimulated osteogenesis in rat BM-MSCs in a dose-dependent manner, as reflected by enhanced calcium deposition, increased ALP activity, and upregulation of osteogenic markers. Although this study has demonstrated promising osteogenic effects of ZnONPs on MSCs, its conclusions are

limited by the lack of *in vivo* validation and the absence of data on the long-term fate and biodegradation of ZnONPs, which are crucial for assessing their safety and biocompatibility in translational applications.

Iron oxide

Iron oxide (Fe_2O_3) NPs are widely utilized in many biomedical applications, including *in vivo* imaging, drug delivery, and cell tracking. It has been reported that γ-Fe_2O_3/nano-HA/PLA scaffold enhanced the osteogenesis of MC3T3-E1 [188]. Cai *et al*. [189] also reported enhanced cell adhesion and osteogenic differentiation of MC3T3-E1 cells upon treatment with PLA/Fe_2O_3 nanocomposite under static magnetic field exposure, as evidenced by increased ALP activity and calcium deposition. However, crucial gaps remain, including a lack of full characterization data of the scaffold, including *in vitro* degradation behavior and kinetic release, as well as a lack of mechanistic insight and *in vivo* validation. Addressing these issues is essential to advance this approach toward clinical bone repair applications. Recently, Zhao *et al*. [190] demonstrated that superparamagnetic Fe_2O_3 NPs encapsulated into PLGA microspheres enhanced the adhesion and the osteogenesis of BM-MSCs, as indicated by over-expression of ALP, Col–I, OPN, and OCN. Further, these microspheres improved trabecular thickness and BMD following implantation into the femoral rat bone defect model. In addition, the magnetic microspheres stimulated macrophage polarization, transitioning from an inflammatory to a regeneration-promoting phenotype, thereby creating a favorable osteo-immune microenvironment during the later stages of bone repair.

3.4. Osteoblast-based therapy for bone regeneration

Bone regeneration using autologous MSC transplantation is challenging due to their poor homing to the target site and their inability to differentiate into functional osteoblasts at the damaged bone surface [191]. It was reported that MSCs circulate throughout the body after systemic injection, with a significant portion initially accumulating in non-target organs, including the lung, liver, and spleen. After a few days, they may redistribute and migrate to the injury site, but the number of cells reaching the target site is often lower than the initial number injected [192]. A previous study reported that MSCs were detected in the lung, liver, intestine, skin, and bone marrow 48h after intravenous injection in a mouse model, and about (5-10) % of the injected cells get trapped in the spleen [193]. The therapeutic approach involving the injection of autologous cultured osteoblasts is based on the theory that bone marrow osteoprogenitor cells stimulate and maintain bone formation. Autologous injection of cultured osteoblasts successfully induced bone formation at the bone defect site *in vivo* with the least possibility of tissue injury and impaired blood circulation. On the contrary, surgical intervention required for the transplantation of both autologous and allogenic bone grafts may impair the blood supply [194]. Okabe *et al*. [195] demonstrated that bone marrow-derived osteoblast-like cells, when infused into a rat model of a bone defect, survive, reside at the defect site, and contribute to the healing process. The investigators confirmed the safety of osteoblast infusion, demonstrating that nearly all infused cells localized to the defect site with negligible distribution to non-target organs. A minimal proportion of cells (0.03%) was detected in the lungs; however, these were rapidly cleared within 3 days after injection. Moreover, these researchers stated that no histological alterations, such as vascular occlusion, invasion, or division of implanted cells, were observed in any organs as evidenced by histological examination. Moreover, a study conducted by Duek *et al*. [196] registered the successful detection of implanted PKH-26-labeled osteoblasts, seeded with PLA scaffold, at the site of

the central bony defect of the rat calvaria and the edge of the newly formed bone, suggesting their contribution to tissue repair in this area. The study of Kim *et al*. [194] demonstrated that autologous transplantation of osteoblasts stimulates bone formation and mitigates the long tubular bone defect in a rabbit model. However, the mechanism by which the implanted osteoblasts migrate *via* the bloodstream to the bone defect site and contribute to the osteogenesis process remains undefined and warrants further investigation. Sadat-Ali *et al*. [197] reported that the intravenous transplantation of BM-MSCs-derived osteoblasts in the osteoporotic rats enhances osteogenesis in the injured distal femur and lumbar spine and increases the trabecular thickness, suggesting that this beneficial action may be attributed to the homing of the transplanted osteoblasts to the bone defect site rather than any other tissues. Moreover, Liao *et al*. [198] explained that local transplantation of porcine pluripotent stem cell-derived osteoblast-like cells improves the trabecular bone volume, thickness, and porosity of the tibia of Lanyu pigs. Recently, a study by Mahmoud *et al*. [169] demonstrated that the infusion of osteoblasts, derived from culturing BM-MSCs in an osteogenic medium supplemented with Au-NPs or Au/HA nanomaterials, ameliorated bone resorption and improved the bone histoarchitecture in osteoporotic female rats as evidenced by significant downregulation of bone resorption markers, including RANKL and Cathepsin K, and histological investigation of bone tissue. Moreover, it has been reported that transplantation of osteoblasts, derived from the cultivation of BM-MSCs in an osteogenic medium supplied with nano-HA or chitosan/HA nanocomposite nanocomposite, in ovariectomized rats successfully restored bone remodeling balance as evidenced by downregulated RANKL/OPG ratio and downregulation of bone resorption markers as well as upregulating osteogenic markers such as Runx-2 and BMP-2 in the bone tissue resulting in mitigating postmenopausal osteoporosis induced in female rats [199].

3.4.1. Clinical trials of osteoblast transplantation.

Some clinical studies have evaluated the translational potential of osteoblast-based therapies for bone regeneration. Kim *et al*. [200] conducted a multicenter, open-label, randomized clinical trial to assess the efficacy and safety of autologous cultured osteoblast injections derived from BM-MSCs in patients with long-bone fractures showing delayed callus formation, noted 6 weeks after surgical fixation. Sixty-four patients aged 15–65 years were enrolled and assigned to either the osteoblast injection group (n = 31) or the control group (n = 33), which received no additional treatment beyond standard fixation. The osteoblast-treated group demonstrated a significantly greater increase in callus formation scores and accelerated fracture healing compared with the control group, whereas adverse event rates were similar and no treatment-related complications occurred, indicating that the procedure is safe and well-tolerated. The study's limitations include its non-blinded design and a short follow-up period (two months), which was insufficient to evaluate long-term outcomes such as complete bone union or functional recovery. These authors concluded that autologous cultured osteoblast injection appears to be a safe and potentially effective therapeutic option to accelerate fracture healing, warranting further large-scale, masked, long-term studies. More recently, Baek *et al*. [201] initiated a Phase I clinical trial to evaluate the safety and preliminary efficacy of human umbilical cord-derived osteoblasts (hUC-Os), derived from UC-MSCs through osteogenic differentiation, in patients with early-stage osteonecrosis of the femoral head (ONFH). Nine patients aged 19–70 years with Association Research Circulation Osseous (ARCO) stage I or II disease were assigned to low- (1×10^7 cells, n = 3), medium- (2×10^7 cells, n = 3), and high-

dose (4×10^7 cells, n = 3) groups. Safety was monitored over 12 weeks via adverse event reporting, laboratory and antibody testing, vital signs, physical examination, and electrocardiography. The study's key limitations include its open-label design, small sample size, and short follow-up period, which restrict statistical power and prevent definitive conclusions regarding efficacy or rare adverse events. Nonetheless, demonstrating the safety and potential benefit of hUC-Os could represent a promising joint-preserving strategy for patients with ONFH. The available clinical trials on osteoblast transplantation retrieved from ClinicalTrials.gov are summarized in Table 2.

Table 2. Clinical trials related to long bone fractures and osteoblast therapy, cited in trial registries, and completed.

Title	Trial registration number	Sponsor	Study phase	Condition	Treatment	Status	Ref.
Treatment of Refractory Non-union Fractures by Pre-osteoblast Cells Grafting: a Pilot Study.	NCT00916981	Jean Philippe Hauzeur	1–2	Long bone nonunion	autologous Preosteoblast transplantation	Completed 2009	[202]
A Pivotal Phase 2b/3, Multicentre, Randomised, Open, Controlled Study on the Efficacy and Safety of Autologous Osteoblastic Cells (PREOB®) Implantation in Non-Infected Hypotrophic Non-Union Fractures.	NCT01756326	Bone Therapeutics S.A	2b/3	Long bone nonunion	Autologous osteoblastic cells transplantation	Completed 2012	[203]
A Pilot Phase 1/2a, Multicentre, Open Proof-of-concept Study on the Efficacy and Safety of Allogeneic Osteoblastic Cells (ALLOB®) Implantation in Non-infected Delayed-Union Fractures	NCT02020590	Bone Therapeutics S.A	1-2	Long Bone Delayed-Union Fracture	Allogeneic osteoblastic cells transplantation	Completed 2013	[204]

4. Limitations

The use of metal NPs in bone scaffolds faces several challenges, including potential toxicity, oxidative stress, and immune responses, as well as insufficient long-term data on systemic accumulation and clearance. Targeted delivery to bone defects remains challenging, with a potential risk for non-specific organ accumulation. Similarly, MSC transplantation is limited by poor homing, uncontrolled differentiation, and possible immune rejection, which may require immunosuppression. Osteoblast transplantation, derived from the osteogenic differentiation of stem cells induced by nanomaterials, can overcome these limitations by enhancing targeted regeneration, promoting controlled differentiation, and reducing toxicity. Nevertheless, larger-scale, masked studies are needed to evaluate long-term safety and immunogenicity.

5. Conclusions and Future Perspectives

Nanostructured materials have attracted considerable attention in bone tissue engineering due to their superior mechanical strength, biodegradability, and biocompatibility. Simultaneously, MSCs, renowned for their outstanding characteristics, have been widely applied in regenerative medicine. This article has discussed the limitations of MSC-based therapy for osteoporosis. Moreover, it has highlighted the favorable impact of integrating MSCs with nanomaterials of different structures, including organic and inorganic nanomaterials, in enhancing osteogenic differentiation of MSCs into osteoblasts. This is accomplished by regulating specific signaling pathways and modulating stem cell proliferation and adhesion. This review clarifies how the unique physicochemical properties of

nanomaterials influence the biological processes that govern stem cell fate. This review further demonstrates the advantages of osteoblast-based therapy over MSC therapy and conventional therapies, suggesting that MSC-derived osteoblast transplantation may offer a promising strategy for counteracting osteoporotic bone defects. Building on this, the osteogenic differentiation of MSCs, supported by nanomaterials, into functional osteoblasts presents a new therapeutic strategy for osteoblast-based therapy against postmenopausal osteoporosis in women, owing to its specificity and safety. This approach has the potential to reshape the future of osteoporosis treatment by overcoming the drawbacks of currently available treatments. While the integration of MSCs and nanomaterials holds tremendous potential for advancing osteoporosis treatment, progress in standardization, regulatory oversight, and interdisciplinary collaboration between expertise in materials science, stem cell biology, bioengineering, and clinical medicine will be crucial to facilitate the safe and efficient translation of this therapeutic approach from the laboratory to the clinic. Nevertheless, further preclinical studies are required to elucidate the mechanisms underlying osteoblast-mediated bone regeneration, evaluate long-term safety and immune responses, and establish optimal safe dosages. If validated, osteoblast transplantation could become a viable option in clinical practice. Future research should prioritize clinical trials to validate these findings in humans and to investigate the safety and specificity of osteoblast transplantation.

Author Contributions

Conceptualization, N.M.; investigation, N.M. and H.A.; writing—original draft preparation, N.M.; writing—review and editing, H.H. and H.A.; supervision, H.H., M.M., H.A., and M.A.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

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