Minireview *Highlight article*

Modern approaches on stem cells and scaffolding technology for osteogenic differentiation and regeneration

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Impact statement

Stem cell therapy plays a major role in diminishing the disadvantages faced by surgical graft implantation and helps to develop a non-invasive futuristic approach for the regeneration of bones. Bone regeneration in patients with bone disorders can be improved through modification of stem cells with several osteogenic factors or using stem cells as carriers for osteogenic factors. This review has been focused on the role of various stem cell therapies and the molecular mechanisms during bone regeneration and the *in vivo* factors affecting bone regeneration.

Abstract

The process of bone repair has always been a natural mystery. Although bones do repair themselves, supplemental treatment is required for the initiation of the self-regeneration process. Predominantly, surgical procedures are employed for bone regeneration. Recently, cell-based therapy for bone regeneration has proven to be more effective than traditional methods, as it eliminates the immune risk and painful surgeries. In clinical trials, various stem cells, especially mesenchymal stem cells, have shown to be more efficient for the treatment of several bone-related diseases, such as non-union fracture, osteogenesis imperfecta, osteosarcoma, and osteoporosis. Furthermore, the stem cells grown in a suitable three-dimensional scaffold support were found to be more efficient for osteogenesis. It has been shown that the three-dimensional bioscaffolds support and simulate an *in vivo* environment, which helps in differentiation of stem cells into bone cells. Bone regeneration in patients with bone disorders can be improved through modification of stem cells with several osteogenic factors or using stem cells as carriers for osteogenic factors. In this review, we focused on the various types of stem cells and scaffolds that are being used for bone regeneration. In addition, the molecular mechanisms of various transcription factors, signaling pathways that support bone regeneration and the senescence of the stem cells, which limits bone regeneration, have been discussed.

Keywords: Stem cells, osteogenic differentiation, scaffolds, bone regeneration

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Introduction

Osteogenesis is an intricate process of bone formation. Although bones do repair themselves, there are difficulties in the self-regeneration process that require additional support through treatment. The newly formed bone will be identical to the rest of the unimpaired ones.[1] Bone healing can occur through two osteogenic pathways, intramembranous ossification and endochondral ossification.[2,3] In intramembranous ossification, the regeneration of bone occurs directly from sheets of mesenchymal connective tissue, whereas in endochondral ossification, the regeneration of bone occurs through replacement of hyaline cartilage. Supportive treatment facilitating both osteogenic pathways is necessary for the regeneration of bones.[4] The repair or remodeling of bone takes place in a series of steps (Figure 1). At first, inflammatory cells release growth factors and cytokines leading to clotting of blood (hematoma formation).[4,5] Then, the degranulating platelets release transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF) leading to chemotaxis, angiogenesis, and subsequent differentiation of mesenchymal stem cells (MSCs).[6] MSCs are of multipotent

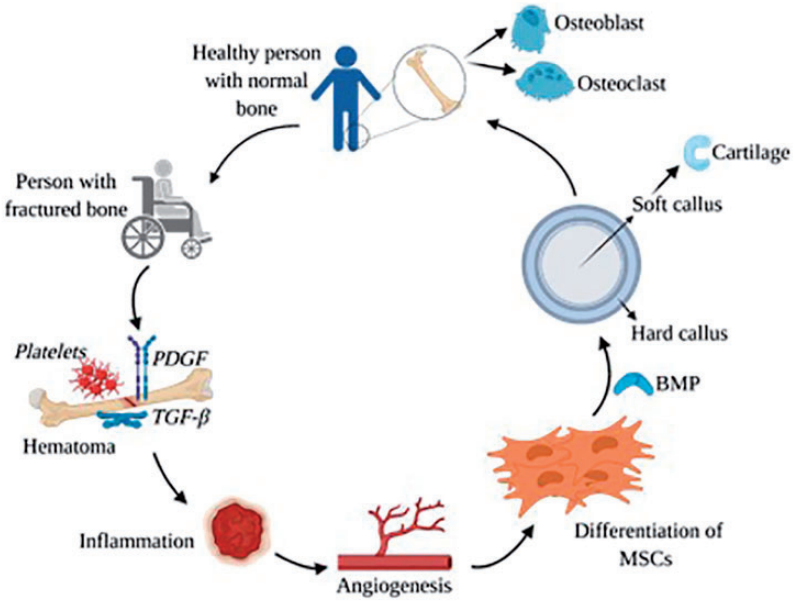

Figure 1. Sequential steps in the process of bone remodeling. The main stages in the repair of a broken bone include the formation of hematoma at the break, leading to inflammation and recruitment of stem cells to the site of injury and their stimulation and differentiation towards the formation of bony callus for the remodeling of bone. (A color version of this figure is available in the online journal.)

progenitors which play a major role in osteogenic differentiation. Some of the proangiogenic factors that are expressed during this process include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and Angiopoietin 1 and 2.[7] The MSCs that produce bone morphogenetic proteins (BMPs) play a major role in the initiation of the ossification process.[8]

The differentiation of MSCs into chondroblasts occurs in endochondral ossification. The chondroblasts form a soft callus, along with a mechanical support, which produces the cartilage.[3,9] The cortex of osteoblasts forms a hard callus.[6] The hard callus determines the intramembranous ossification. The combination of hard callus and soft callus together results in bone remodeling.[5] When the osteoblast deposits into the new lamellar bone, the osteoclasts perform resorption of mineralized bone in hard and soft callus.[8,9] The mineralization of cartilage occurs on encountering with the vasculature through removal of chondroblasts leading to bone formation.[3,9] Bone grafting has been a standard primary therapeutic procedure for bone regeneration. The bones are either taken from iliac crest or intramedullary canal from the own or donor sites. The bone regeneration through grafting occurs in three different mechanisms, such as osteogenic, osteoconductive, and osteoinductive. The grafted material initiates new bone formation (osteogenic), provides mechanical support (osteoconductive), and produces factors that induce the growth of the bone (osteoinductive).[10] Several adverse reactions associated with the grafting procedures include: the occurrence of complications such as infection, hematoma/seroma, fracture, nerve and vascular injuries, chronic pain at the donor site, hernias, unsightly scars, and poor cosmetic outcome.[11] A proper form of treatment that can satisfy the needs of patients has not been developed yet. However, several alternative methods are available as a substitute for autologous grafts.

Recently, studies have been underway to use stem cells as a substitute for bone graft in bone regeneration. Stem cells are of unspecialized cells that can self-renew and develop into specialized cells during regeneration.[9] Osteoconductive grafts can be replaced with different types of natural and artificial scaffolds, which provide a temporary three-dimensional (3D) support where the stem cells can adhere and synthesize extracellular matrix (ECM).[12] The scaffold would be easy to manufacture, store, and handle.[13,14] Growth and transcription factors can be directly supplemented as inductive signals. It is important to understand the role of growth factors and cytokines on various types of stem cell populations. Stem cell therapy plays a major role in diminishing the disadvantages faced by surgical graft implantation and helps to develop a non-invasive futuristic approach for the regeneration of bones. This review has been focused on the role of various stem cell therapies for bone regeneration and the *in vivo* factors affecting bone regeneration.

Bone regeneration using different types of stem cells

Different types of MSCs obtained from various tissues like bone marrow, skin, umbilical cord, and placenta are now under investigation for bone regeneration.[15] Many types of adult stem cells such as skeletal stem progenitor cells (SSCs),[9] dental pulp stem cells (DPSCs), adipose-derived stem cells (ADSCs) that are functionally similar to MSCs have been used for bone regeneration. Recently, somatic cells have been reprogrammed into induced pluripotent stem cells (iPSCs), which are pondered into investigation to produce bone cells.[16] Various stem cells grown in a serum-containing media supplemented with ascorbic

acid, β-glycerophosphate, and dexamethasone are capable of forming osteogenic cells. These cells can also be provided with cytokines, growth factors, chemicals, and a solid 3D supportive structure to enhance their quality of growth (Figure 2).

Embryonic stem cells

The embryonic stem cells (ESCs) are a type of stem cells originating from the inner cell mass of the blastocyst. They can be differentiated into the three germ layers: endoderm, mesoderm, and ectoderm. Bone cells are derived from the mesoderm layer and can be produced by culturing the ESCs in the appropriate *in vitro* cell culture conditions. Murine ESCs had been differentiated into bone cells, which were characterized by the formation of discrete mineralized bone nodules that consisted of 50–100 cells within an ECM. Co-culturing of ESCs with fetal murine osteoblasts increased the nodule number by fivefold.[17] The inducers such as ascorbic acid, β-glycerophosphate, and dexamethasone appeared to be much more effective when added after 14 days. This suggests that the extents of the ESCs differentiating into bone cells were dependent on the type of combination of stimuli and their timing used.[17] Moreover, the growth of ESCs (CHA3-hESC line) co-cultured with primary bone-derived cells in the presence of 3D porous poly(DL-lactic-co-glycolic acid)/hydroxyapatite composite scaffold added with BMP-2 successfully regenerated bone tissue when implanted into subcutaneous space of immunodeficient mice.[18]

ESCs can be differentiated into MSCs, which can further be differentiated into osteoprogenitor cells. The ESC-MSCs cultured in a three-dimensional scaffold produced more effective bone cells. For instance, the human ESC-derived MSCs were cultured in the appropriate medium and differentiated into adipocytes, osteocytes, and chondrocytes.[19] When the ESC-derived MSCs were cultured in the presence of collagen composite scaffolds, their osteogenic differentiation was enhanced and their bone regeneration capacity was successfully tested through *in vivo* transplantation.[19] Another study has shown that the growth of bone from human ESC-derived-MSCs seeded on calcium phosphate cement as a scaffold yielded a much higher osteogenic lineage with high alkaline phosphatase activity, osteocalcin expression, and effective mineralization. In addition, the use of human platelet concentrates enhanced the formation of bone with blood vessel formation.[20] Although ESCs are pluripotent in nature and can be differentiated into almost any type of cell with a high proliferative capacity, the usage of human ESCs is considered unethical and to pose a severe threat to humanity. Also, there is a high risk for the development of immunogenic incompatibilities and for the formation of teratomas after the transplantation of ESC-derived cells. These controversial characteristics have confined the use of ESCs.[21,22]

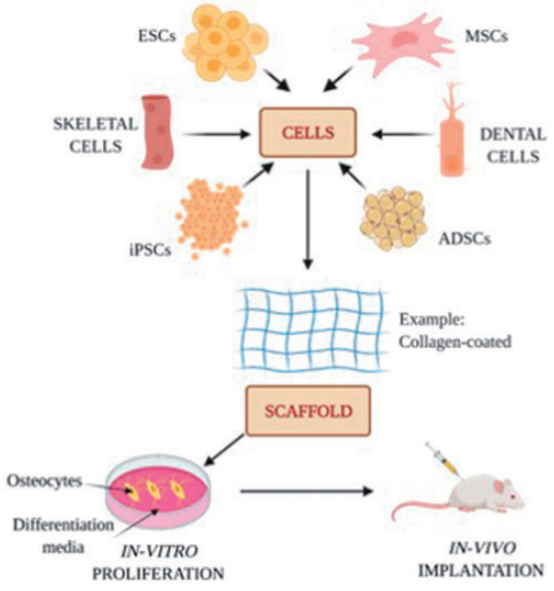

Figure 2. Diagram showing the types of various cells involved in osteocyte differentiation both *in vitro* and *in vivo*. Various cell types such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and adipose stem cells (ADSCs), etc. can be cultured *in vitro* in a three-dimensional scaffold, which facilitate the osteogenic differentiation leading to an effective *in-vivo* transplantation. (A color version of this figure is available in the online journal.)

Adult stem cells

Skeletal stem cells

Skeletal stem cells reside in postnatal bone marrow and give rise to bone, cartilage, and other cells. They have been shown to be effective for bone regeneration through both, intramembranous ossification and endochondral ossification.[23] The mouse skeletal stem cells were differentiated into cartilage, bone and marrow stroma through BMP, and VEGF.[9] However, the mechanism of their differentiation process is not fully understood, and there is limited knowledge of the fate of skeletal stem cells, its immune-phenotype and selection criteria, which restricted the widespread clinical application of these cells.[24]

Dental pulp stem cells

It is easy to isolate dental pulp stem cells (DPSCs) either from extracted wisdom teeth or during pulpectomy.[25] DPSCs are said to have MSC-like properties and they were shown to be self-renewable; they can also be differentiated into several lineages of cells.[15,25,26] The DPSCs tend to express mesenchymal markers STRO-1, CD13, CD29, CD44, and CD73 and osteogenic markers alkaline phosphatase, Runx2, and osteocalcin.[25,27] Studies have shown that culturing rat DPSCs in collagen gel scaffolds benefitted bone regeneration.[28] Implantation of DPSCs in Wistar male rats with critical-size calvarial defect, resulted in an increased expression of alkaline phosphatase and tartrate-resistant acid phosphatase. The bone mineral density and bone micro-architectural parameters were also increased upon treatment with DPSCs.[28] Paduano et al. have tested the osteogenic capacity of DPSCs comparing DPSCs cultured on hydrogel-scaffolds derived from decellularized bone ECM with DPSCs cultured on a collagen hydrogel-scaffold. Furthermore, the DPSCs were grown in three different culture medium such as basal medium, osteogenic medium, or medium supplemented with growth factors.

Their results demonstrated that the decellularized bone ECM hydrogel-grown DPSCs in osteogenic or growth factor-supplemented medium showed a higher expression of osteocyte-specific markers.[29] Studies have shown that the DPSCs can be differentiated into both dental as well as bone tissues.[15,30] Tatullo et al. have shown that though DPSCs help in bone regeneration, they have been directed more towards dentinogenesis than osteogenesis. Moreover, human periapical cyst-MSCs and human exfoliated deciduous teeth-derived stem cells were shown to be an alternative source of cells for bone regeneration.[30] *In vivo* experiments using DPSCs in different types of animal models with actual bone defects have not been studied well; however, when it is done, DPSCs may prove as a fundamental source for bone regeneration.[31]

Adipose stem cells

Adipose stem cells (ADSCs) can be isolated from surgical fat specimens; these cells can be utilized for osteogenic and chondrogenic differentiation. Gruber et al. have studied the effect of long-term passaging, doubling time, and senescence of ADSCs. They have demonstrated that when the donor age was increased, the doubling time of ADSCs was longer. The senescence of ADSCs and their doubling time were increased significantly with each passage.[32] Yoon et al. have cultured ADSCs in osteogenic media layered over polylactide-co-glycolic acid. They were then implanted in a mouse model with a critical calvarial defect. After the implantation, the bone filling was found to be about 72% in 14 days.[33] In another study, dental implant consisted of ADSCs cultured with tissue-engineered construct of β-tricalcium phosphate granules and recombinant BMP-2 was implanted into a 55-year-old patient with parasymphyseal defect. This implantation resurrected the original anatomy with viable cells.[34] Treatment of rats with photobiomodulation using polychromatic light in the near infrared region (600–1200 nm) showed enhanced *in vivo* bone regeneration and the osteogenic differentiation potential of ADSCs encapsulated in methacrylated gelatin hydrogels.[35] Han et al. compared the effect of bone regeneration between bone marrow-derived MSCs and ADSCs. They have shown that although the ability of bone remodeling was less in ADSCs when compared to BM-MSCs, the ADSCs can still be considered as a potential source for bone regeneration.[36]

Mesenchymal stem cells

MSCs are the most utilized cells for bone regeneration. This is mainly because the MSCs are multipotent in nature and can directly produce osteoprogenitor and osteo precursor cells. There is a wide range of literature that describe the usage of MSCs as a potential source for bone remodeling. A few of them are discussed here. MSCs cultured in media containing dexamethasone, ascorbic acid, and β-glycerophosphate seeded on a porous ceramic layer and implantation into the subcutaneous tissue resulted in the production of vascularized bone within the material. The resulted bone tissue expressed a high alkaline phosphatase activity as well as increased hydroxyapatite deposition on the ECM.[37] In 2006, the ideas of MSCs as a source for bone regeneration were improved by implementing three strategies. (i) Including MSCs from various sources and not limiting to the MSCs aspirated from bone marrow alone. (ii) Autologous therapy that involves the collection of patient's own stem cells to expand, create new bone tissues and transfer back to the patient using appropriate carriers. (iii) A therapy that avoids cell culture but uses autogenous stem cells from large bone marrow aspirates. The aspirates are possibly concentrated to produce a bone graft substitute.[38] Later in 2009, it was shown that the delivery of required genes and proteins by genetically modifying autologous MSCs *ex vivo* could be feasible. This would eventually reduce the number of MSCs required for implantation and avoid *in vitro* culture and expansion.[22] MSCs can also be used as vehicles for bone regeneration therapy, where genetically modified MSCs act as recombinant cellular carriers providing a sustained supply of osteogenic factors.[39]

A comparative study evaluated bone regeneration using the MSCs derived from ovine bone marrow and adipose tissue. After expansion and implantation of these cells into the sheep tibia with a critical size defect, it was found that higher amount of bone growth was found with bone marrow-derived MSCs than adipose tissue-derived MSCs.[40] The surface markers that have been described for MSCs since late 1990s include CD73 and CD105. But these markers were also found to be expressed by fibroblasts. So, currently markers such as Stro-1 and CD271 were identified as specific to MSCs. Also, native BM MSCs tend to express markers such as GD2, SSEA4, and CD146.[41] Interestingly, MSCs were found to have the ability to repair an injured bone after the latter produce signals. The repair mechanism involving MSCs could be attributed either by direct differentiation into bone tissues or by secreting factors that provide antiapoptotic effects, immunoregulatory function, and/or stimulation of endothelial progenitor cell proliferation.[39,42] Tian et al. have shown that decellularized cartilage ECM scaffold stimulated the polarization of BM-derived macrophages, which promoted BM-MSCs invasion, migration, proliferation, and chondrogenic differentiation. Furthermore, when this scaffold was combined with early-stage intra-articular injection of IL-4, it improved the wound-healing microenvironment and cartilage regeneration in a rat model of knee osteochondral defect.[43]

Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are the reprogrammed cells generated from adult somatic cells. Several studies have shown that iPSC-derived cells have effectively promoted bone repair and angiogenesis.[44–47] Human iPSC-derived osteoblasts and osteoclasts were co-cultured with macrophages on hydroxyapatite-coated poly lactic-co-glycolic acid/poly L-lactic acid scaffold.[16] Subsequent *in vivo* implantation of co-cultured osteoblasts and osteoclasts in rodents showed a mature bone-like growth. It was also shown that the coupling activity of osteoblasts and osteoclasts moderated the expression of inflammatory molecules especially in *in vitro* bone formation.[16] Human iPSCs cultured on plasma-treated polymeric nanofibrous

polyethersulfone scaffolds showed extensive bone reconstruction after implantation of these cells *in vivo* on critical-size calvarial defect rats.[48] Although iPSCs are pluripotent in nature and can be differentiated into any type of somatic cell, it has the risk of teratoma formation after transplantation.[21]

Molecular mechanism of the cells

Transcription factors

The commitment towards osteoblasts, and their further differentiation and function are governed by many transcription factors. The primary transcription factor which helps in the osteoblast differentiation from MSCs is Runt-related transcription factor 2 (Runx2).[14] It acts as a scaffolding protein that helps in both endochondral and intramembranous ossification.[49] Runx2 binds with the Runx consensus sequence which are known as osteoblast-specific element2 (OSE2), which promotes bone sialoprotein and osteocalcin expression. Homeobox proteins act as repressors/activators of Runx2 and regulate the expression of bone sialoprotein, osteocalcin, and alkaline phosphatase. On binding with CCAAT/enhancer-binding proteins (C/EBPs), Runx2 regulates bone-specific genes expression and increases the amount of Smad ubiquitination regulatory factor 1 (Smurf1) or peroxisome proliferator-activated receptor 2 (PPARγ-2), resulting in the inhibition of Runx expression.[14] Disruption of Runx2 leads to inhibition of bone formation altogether. Runx2 has bipotential characteristics, as it can induce chondrogenic as well as osteogenic genes. The second important transcription factor is Osterix (Osx), which is also known as specificity protein-7. It is a zinc finger transcription factor expressed in osteoblasts. The specific domains of the Osx help in the activation of osteocalcin and collagen type 1 alpha 1 (COLLA1) genes. Runx2 was shown to be dependent on Osx during the differentiation process.[49] Runx2 and Osx contribute together for the maturation of osteoblasts from pre-osteoblasts.[14,15] But, Osx can function without the presence of Runx2 also.[49] The Osx, along with nuclear factor of activated T-cells (NFAT), activates COLLA1 and osteocalcin promoters, resulting in osteoblast differentiation and bone formation through stimulation of Wnt/β-catenin pathway.[14,49] The third important osteogenic transcription factor is activating transcription factor 4/cAMP response element binding protein 2 (ATF4/CREB2), which on binding with Runx2 increase the production of bone sialoprotein and osteocalcin.[14,49] ATF4 gets phosphorylated by ribosomal S6 kinase 2 (Rsk2), which helps in controlling amino acid transport.[14] ATF4 is also known to induce terminal differentiation of the osteoblasts.[49] Some other transcription factors involved in bone regeneration include AP1, helix-loop-helix (HLH) proteins, PPARγ2, C/EBPs, and SRY-box transcription factor (Sox) proteins. HLH is upregulated during the proliferation of osteoblasts. PPARγ-2 plays the role of lineage determination. Increased expression of the protein PPARγ-2 indicates adipocyte differentiation. C/EBPβ and C/EBPδ activates osteocalcin gene transcription. Sox proteins play a vital role in chondrogenesis. The main Sox proteins include Sox9, Sox5, and Sox6, which are responsible for the expression of collagen IX, alpha1, aggrecan, and other co-factors.[14]

Signaling pathways governing the differentiation of stem cells to osteogenic cells

During the early stage of osteogenic differentiation, most of the up-regulated genes were related to cell proliferation, whereas in later stages, the expression of genes relevant to osteogenic growth factor-signaling pathways. Several important pathways have been identified to play vital roles during the osteogenic differentiation of MSC include TGF-β, FGF, insulin-like growth factors (IGF), PDGF, etc.[49] They bind to their receptors and translocate to the nucleus in order to activate their respective transcription factors as shown in Figure 3. This occurs via both smad as well as non-smad pathways through interaction with BMPs and BMP signaling components.[15,50] TGF-β signaling helps in the growth and differentiation of cells. TGF-β superfamily consists of 34 members, including BMP. Certain BMPs like BMP-4, BMP-2, BMP-7, BMP-6, and BMP-9 upregulate osteogenesis, whereas BMP-3 alone inhibits the differentiation.[49,51] BMP-2 and BMP-7 belong to two closely related subclasses, namely BMP-2/4 and BMP-5/6/7, respectively.[14] The BMP-TGF-β signaling pathway has two types of serine/threonine kinase receptors, such as type-I and type-II receptors. The type-I receptor includes BMPR-1A/ALK-3, BMPR-1B/ALK-6 and ALK-2. The type II receptors include BMPR-2 and activin receptors such as ActR-2 and ActR-2B. The type-I and type-II receptors combine with the ligand to form a complex. This complex come in contact with transcriptional modulators called the Smad proteins. The Smad proteins are of three types: Receptor-regulated Smad (R-Smad) (Smad 1, 2, 3, 5, 8), Co-Smad (Smad 4), and Inhibitory Smad (Smad 6 and 7).[52] The BMP and TGF-β activates the R-Smad. The phosphorylated R-Smad forms a complex with the Co-Smad (Smad 4). The complex is translocated to the nucleus, where it regulates the transcription of their respective genes.[53] There is an another possibility of non-Smad pathway, involving BMP-2 along with mitogen-activated protein kinases (MAPK) signaling.[49] The MAPK signaling contains a series of signaling cascade called MAP Kinase, MAP kinase (MAP2K) and MAP3K.[54] The growth factors activate these signals in order to trigger the transcription factors.[49]

IGF system consists of two ligands IGF1 and IGF2 and two cell-surface receptors IGF1R and IGF2R. It also constitutes six high affinity-binding proteins (IGFBP 1–6). IGF2R helps mainly in the differentiation of MSCs into osteoblasts and cell proliferation.[55] The IGF1R is a ligand-activated tyrosine kinase receptor. IGF1R and IGF2R together promote osteoblast function and bone matrix deposition. The IGF-1 uses insulin receptor-substrate proteins insulin receptor substrate 1 (IRS1) and IRS2.[56] They also make use of P13K and influence the activation of Akt and the MAPK pathway which in turn activates p38, Jun-N-Terminal kinases and ERK1/2. The type of pathway for activation depends on culture conditions and the stage of differentiation.[49] The Wnt is the ligand with its membrane

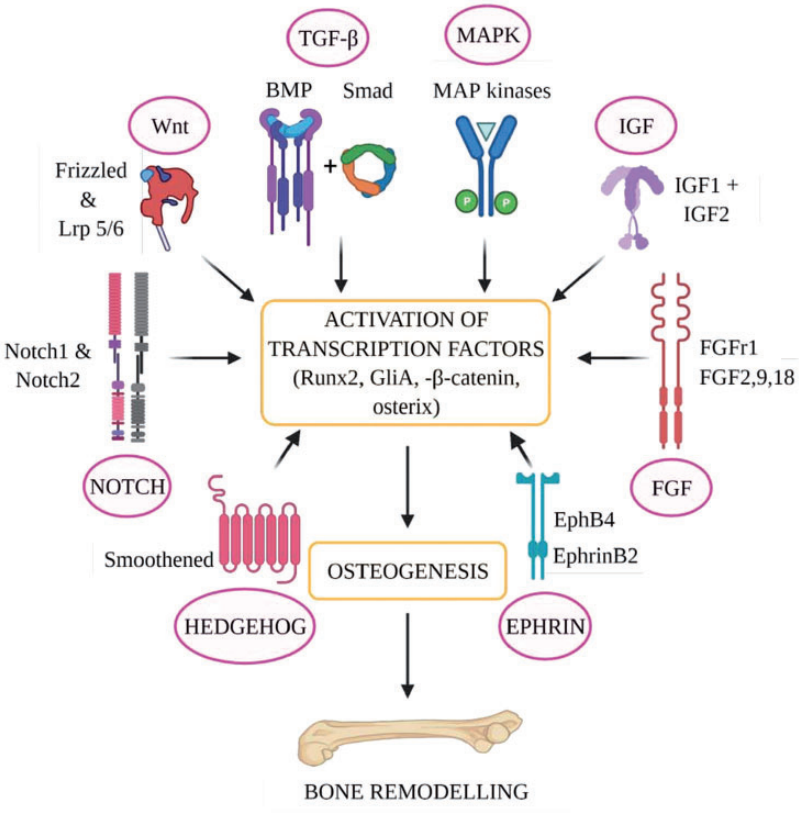

Figure 3. The involvement of various signaling pathways during osteogenesis and bone remodeling process. Bone remodeling includes the activation of various signaling pathways such as Wnt, TGF-β, MAPK and IGF-1, FGF, Ephrin, Notch, and Hedgehog leading to the activation of several transcription factors like Runx2, GliA, β-catenin, and osterix, towards osteogenesis and bone repair. TGF-β: transforming growth factor-β; MAPK: mitogen-activated protein kinase; IGF-1: Insulin-like growth factor-1; FGF: fibroblast growth factor. (A color version of this figure is available in the online journal.)

spanning Frizzled receptor. Wnt and its receptor together can create two types of pathways: canonical and non-canonical pathways. The canonical pathway forms a complex including Wnt protein, frizzled receptor, and low-density Lrp5/6 receptors.[57] This complex activates the Dishevelled (Dsh) and creates a signal that inhibits the production of glycogen synthase kinase-3 (Gsk-3). The silencing of Gsk-3 blocks the phosphorylation of β-catenin, and thus the degradation of β-catenin through ubiquitin-pathway in the cytoplasm is avoided. The β-catenin translocate to the nucleus and undergo transcription of T-cell factor (Tcf/Lcf family) and help in the activation of Runx2.[58] Thus, it plays a critical role in osteogenesis and its growth, differentiation, maturation, and death. A decreased amount of β-catenin indicates the process of chondrogenesis.[59] It also has a bipotential feature that has the ability to produce osteocytes as well as chondrocytes.[49]

Notch pathway plays a main part in determining the fate of the cell. Notch receptor and its ligands δ1, 3, 4 and jagged 1, 2 are transmembrane proteins that initiate cell-cell interaction and signal transduction.[60] The levels of Notch 1 and 2 are increased during osteoblast production, whereas Notch 3 and 4 are found in the subsets of the lineage.[49] This signal can upregulate the proliferation of immature osteoblasts and inhibit the transactivation function of Runx2. The proliferation activity may lead to osteosclerosis.[61] The Hedgehog signaling pathway is proved to assist in the formation of bone and cartilage. The pre-hypertrophic chondrocytes produce Indian Hedgehog, whose signal transduction act on the perichondrium osteoblast progenitors.[62] The Hedgehog protein binds with the Patched receptor and activates Smoothened (Smo), which is a transmembrane protein responsible for the transcription of GliA in the nucleus thereby aiding in stem cell proliferation and activation of the target genes.[49] This pathway is known to have limited effect on the early stage of osteoblast commitment.[63] The FGF gene family consists of 22 members.[14] The gene family combines with FGF tyrosine receptor isoforms in order to produce a signal. Usually, FGFs 2, 9, and 18 were involved in osteogenesis. When FGFs 9 and 18 are expressed in periosteum, FGF2 is involved in both periosteum and osteoblast production.[64] FGFR1 receptor stimulates the differentiation as well as arrest the maturation of osteoblasts.[62] The FGFs 2, 9, 18 binds with FGFR1 to activate the transcription of their respective target genes. This signaling increase the bone density as well.[49]

Ephrin pathway, best known for its bi-directional signaling, consists of classes A and B. Class A includes GPI-anchored EphA receptors (A1-A5), while Class B includes EphB1-6 tyrosine kinase receptors (B1-B3).[65] Bidirectional signaling occurs from receptors to ligand and vice-versa.[49] The signaling from Ephrin ligand EphB4 to the receptor ephrin B2 leads to osteoclast differentiation. And their reverse signaling activates osteogenic transcription factor resulting in bone remodeling.[66] The PDGF is a dimeric

molecule that exerts extracellular signaling and it is formed by two polypeptide chains, PDGF-A and PDGF-B. The dimerization of both the polypeptides can lead to three different isoforms ($\alpha\alpha$, $\alpha\beta$, and $\beta\beta$).[66] The $\beta\beta$ dimer reduces the osteogenic differentiation and alkaline phosphatase activity, whereas its inhibition reduces the mitogenic and migratory responses,[49] Angiogenesis plays a very important role in bone formation. VEGF is considered as a mediator of osteo-inductive factors and enhances other signaling pathways such as TGF-β1, IGF and FGF-2.[67] VEGF-mediated activation helps with the transportation of endothelial cells to ECM. This type of mediation is not suitable for implanted bone constructs as the endochondral route undergo its own vasculature instead of exogenous angiogenic factors.[14,68]

Increasing evidence indicates that non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) play a pivotal role in the chondrogenic and osteogenic differentiation of MSCs.[69,70] A panel of miRNAs that target RUNX2 such as miR-23a, miR-23b, miR-30b, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-143, miR-203, miR-204, miR-205, miR-217, miR-218, miR-221, and miR-338 have been reported to regulate osteoblast differentiation.[71,72] Among the miRNAs that regulate chondrogenesis include miR-140, which targets Sp1 and SOX9.[73,74] Currently, different lncRNA/miRNA axes have been found to have a positive regulation on the osteogenesis in bone marrow MSCs include lncH19/miR-138, lncH19/miR-188, lncH19/miR-675, KCNQ1OT1/miR-320a, NEAT1/miR-29b-3p, MALA-T1/miR-143, etc.[70]

Senescence of cells

Major stem cells responsible for bone remodeling are MSCs, but their function reduces as they age through senescence. Aging is induced through several factors such as hormonal, nutritional, and other ageing-factors. Common reasons for cellular senescence include telomere shortening, genomic damage, epigenomic damage, and oxidative stress.[75] It is explained that both the donor age and the number of passages play a major role in determining the osteogenic and chondrogenic potential of murine bone marrow-derived MSCs. MSCs from a younger donor have a better adherence capacity as well as good proliferation rate compared to cells from an older donor.[76] Cheng et al. have demonstrated that the upregulation of senescent associated genes such as p16, p21, and p53 predicts osteogenic and adipogenic differentiation.[77] All three senescence-associated genes were expressed in bone marrow-derived MSCs upon prolonged cell culture. However, there was a gradual increase of only p21 in umbilical cord-MSCs (UC-MSCs). P16 and p53 were reduced during early stage, whereas increased during later stages in UC-MSCs. The expression of transcription factors like C/EBPα and PPARγ was decreased in senescent cells. However, there was an exception in UC-MSCs, where an increased level of C/EBPα was found during the late stage of growth. The mean percentage of senescence was increased to 3.4% at passage 13 in adult adipose-derived MSCs.[32]

There are a few studies aimed at finding an alternative method to reduce or avoid the ageing of cells. Khan et al. have employed fat pad-derived stem cells obtained from patients who were 55 years older and above. Although the growth was not identical to bone marrow-derived stem cells, the proliferation rate was similar. It was shown that fat pad-derived stem cells did not decline with ageing, and increased expression of alkaline phosphatase and osteocalcin genes and enhanced calcium phosphate deposition.[78] Addition of a lower level of calcium, antioxidant N-acetyl-L-cysteine and L-ascorbic acid-2-phosphate in growth medium enhanced the lifespan of the adipose stem cells.[79] Ascorbic acid has been portrayed as a major co-factor in the differentiation of MSCs as well as its proliferation. Induction with ascorbic acid can help in the growth of periodontal ligament cells.[80] Interestingly, micro RNAs such as miR-195 when silenced, increased telomerase reverse transcriptase (Tert); they also helped in the phosphorylation of AKT and FOXO3 expression in old MSCs. This led to telomere re-lengthening and reduction of the expression of senescence-associated β-galactosidase. It also restored anti-ageing factors like Tert and Sirt1.[81] When the culture media was supplemented with growth factors like FGF-2, PDGF-BB, epidermal growth factor, they increased the cell proliferation and the number of cell doublings before attaining senescence.[79,82] Additionally, the nuclear factor-erythroid 2-related factor (Nrf2) has also been shown to delay senescence through regulation of antioxidant genes.[83,84]

Scaffolds

The scaffolds provide a temporary 3D support that will be removed through reabsorption during the formation of functional tissue.[14] The newly formed bone tissue adheres to the scaffold and synthesize ECM. Once the remodeling of the bone is done, the newly formed bone replaces the scaffold with its own tissues.[14] Ideally, a scaffold should possess characteristics, such as (i) biocompatible and non-toxic, (ii) bond with the host bone without formation of scar tissue, (iii) yield sufficient bone growth and provide proper attachment to the cell, (iv) allow growth of the bone in 3D interface as well, (v) produce equal amounts of bone tissues that are degraded, (vi) be able to excrete degraded non-toxic products easily, (vii) promote mechanical properties similar to the existing bone even after *in vivo* implantation, (viii) be able to mold into any shape according to the type of the bone defect, and (ix) have the ability to be produced commercially and to be sterilized for clinical use.[8,85] Scaffolds can be of different types based on the material they are made of. Some of the different types of materials that can be used as scaffolds are explained below (Figure 4).

Bioactive glass

Bioactive glasses are amorphous silicate-based materials. They have the potential to bond with the host bone and stimulate the growth of new tissues. Eventually, they dissolve overtime. Bioactive glasses are produced by two different methods, such as melt-derived and sol–gel method. The first ever bioactive glass used for the production of bone was Bioactive glass 45S5, which was implanted in a

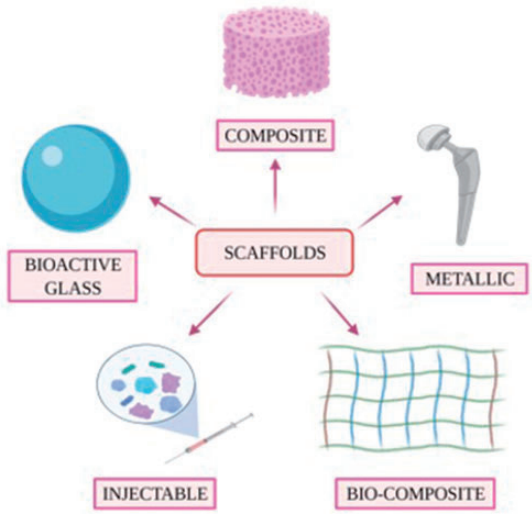

Figure 4. Different types of scaffolds that are generally used for *in vitro* osteogenesis. Preparation of three-dimensional scaffolds with different types of materials including bioactive glass, composite, bio-composite, metallic, and injectable preparations that enhance the differentiation potentials of stem cells in it. (A color version of this figure is available in the online journal.)

mouse.[85] It was prepared using melt-derived method by melting high-purity oxides in platinum crucibles at 1370°C. Sol–gel process is performed by hydrolyzing alkoxide precursors to produce a colloidal liquid (sol). Currently, sol–gel process is preferred especially for *in-vitro* cultures because of their ability to produce porous scaffolds with macropores. NovaBone® is the most recently developed bioactive glass scaffold, which was widely used in orthopedic applications mostly as bone filling material.[85]

Recent studies have improvised the use of glass scaffolds by modifying the existing scaffolds. For example, San Miguel et al. have created a surface-modified porous bioactive glass scaffold composed of BG1, a type of melt-derived scaffold. They treated the scaffold with simulated body fluid and calcium-deficient carbonated hydroxyapatite. Thus, modified scaffolds showed enhanced osteogenesis of MC3T3-E1 pre-osteoblasts, when compared to untreated scaffolds.[86] Melt-derived bioactive glass, ICIE 16, is porous in nature; it was prepared using the gel-cast foaming technique. Gel-cast foaming is a process of formation of gel by in situ polymerization of organic monomers through sintering or frottage technique. This technique was previously used to produce dense, porous ceramic structures. It was shown that this scaffold was more suitable for bone regeneration and stimulated bone growth rapidly.[87] Inter-connective pores are known to improve the characteristics of glass-like mesoporous bioactive glass (MBG), which is usually brittle. The binding the MBG with polyvinyl alcohol (PVA) increases the mechanical strength of the scaffold and provides an enhanced architecture and mineralization potential. Thus, this type of 3D printed scaffold assists in a flawless bone regeneration.[88]

Composite

A composite scaffold can be made with mixing of components like polymers and inorganic components extending the ability to release a drug over a sustained period of time.[89] The effect of the addition of osteoinductive factor with the scaffold was studied on a BMP-2 loaded with poly (D,L-lactide-co-glycolide) (PLGA)/Hydroxyapatite (HAp) scaffold. The addition of BMP-2 improved the growth of bone tissue.[90] Polycaprolactone is a type of polymer extensively used in the scaffold for bone regeneration; 10% of Strontium-substituted bioactive glass is incorporated into polycaprolacton by melt electrospinning technique. The produced scaffold is convinced to act as an ideal scaffold with a porous structure and provided an increased deposition of calcium phosphate layer using MC3T3-E1 cells.[91] It is interesting to know that composite scaffolds are also suitable for dental applications. For instance, Brown et al. have experimented with porous metallic magnesium/PLGA scaffolds using solvent casting and salt leaching method. PLGA is a polymer that produces acidic by-products during its degradation. This composite scaffold showed lower inflammation than the traditional PLGA scaffolds, with an improved osteogenesis, as it contains a porous environment, which helped in increasing the bone stromal cell population *in vitro*. These composite scaffolds showed a promise for dental socket preservation.[92]

Bio-composite

Bio-composite is a type of composite scaffold constituting biological materials. Such materials include fibrin, collagen, chitosan, etc. The bio-composite scaffold composed of PLGA and collagen matrix derived from a porcine bladder submucosa matrix was evaluated. This scaffold possesses porous structures and promoted cellular interactions and maintained structural integrity of human ESCs and bovine osteoblasts.[93] Another biodegradable scaffold made with chitosan/nanocrystalline calcium phosphate composite increased the fibronectin adsorption and osteoblast proliferation.[94] In another study, 3D printing of composite calcium phosphate and collagen scaffolds offered osteoconductive new bone formation *in vivo* using murine models with a femoral defect.[94] It was also shown that the phosphoric acid based binder solution, when complemented with Tween 80 and collagen, significantly improved the mechanical and flexural strength, and cell viability.[95] Fangfang et al. have studied the combination of nanocrystalline HAp and various types of bioactive polymers. They have described several preparation methods of HAp/polymer composite scaffolds using solvent/solution casting method, thermally induced phase separation (freeze-drying method), electrospinning technique, in-situ mineralization of HAp in polymers, electrodeposition, and 3D microstructures.[96]

Metallic

Porous metallic scaffolds are often used to restore the damaged bones' functionality. They help to maintain the structure and shape of the repaired bone. Also, they provide interfacial porosity and permanent structural framework. They were made by different methods like powder metallurgy, decomposition of foaming agents, replication, rapid prototyping technologies, etc. Metallic scaffolds seem to be

useful in load-bearing applications. Metallic scaffolds have also been used as a composite with several other polymers such as RGD-peptide, vitronectin, and fibronectin.[97] Examples of metallic scaffolds include porous tantalum, magnesium, titanium and titanium alloys, and nickel-titanium alloy. They are biocompatible, durable, and highly corrosion resistant.[98] Calcium-phosphate has been used as a potential scaffold for a very long time because of its identical properties to carbonate hydroxyapatite, which is the matrix of the bone. Strontium is another metallic scaffold, which helps in bone formation. [99]

Injectable

Injectable scaffolds are another type of scaffolds widely used for research and considered as a non-invasive method of producing scaffolds creating a 3D network and assisting in the formation of bone. Some of the examples include injection of calcium phosphate foam by mixing α-tricalcium phosphate powder with a foamed polysorbate 80 solution. The paste should be injected immediately after mixing to develop a porous structure.[100] Another study developed an injectable calcium phosphate cement for delivery of osteogenic cells. The scaffolds were developed using absorbable fibers, biopolymer chitosan, and mannitol porogen with MC3T3-E1 osteoblast-like cells. It was observed that the cell attachment and proliferation were markedly good.[101] Moreover, the usage of stimuli-responsive gels containing hydroxyapatite and carbon nanotubes have been developed[102]. The nanotubes helped in improving the mechanical properties, activity, and prolonged drug release. The scaffold thus formed is thermosensitive and required less gelation time.[102]

Another study has shown that the use of PuraMatrix (PM), a peptide nano-material with dog MSCs (dMSCs) and platelet-rich-plasma (PRP) enhanced bone regeneration.[103]This scaffold was implanted into the teeth extracted from an adult hybrid dog. After eight weeks of implantation, the bone generation was found high in PM/dMSCs/PRP with $58.43 \pm 5.06\%$ followed by $50.07 \pm 3.97\%$ of bone produced in PM/dMSCs.[103] In another study, gellan xanthan gels were used as matrix as well as carriers for growth factors. This gel, along with chitosan nanoparticles, basic FGF and BMP7 increased the proliferation and production of the human fetal osteoblasts.[104] The differentiated cells showed a high alkaline phosphatase activity and calcium deposition. Additionally, the scaffold also showed an enhanced anti-bacterial effect against bacteria generally occurring during implantation.[104] Injectable composite hydrogel promoted spinal fusion through improving the osteogenic and angiogenic potentials of BM-MSCs.[105]

Cell therapy for different bone disorders

Traditional drug treatments for certain bone defects tend to produce severe side effects. Currently, cell-based therapy attracted researchers for treating several bone disorders like fracture, osteogenesis imperfecta, and osteoporosis.

Non-union fracture

Fracture healing is usually classified into two types: primary cortical fracture healing and secondary fracture healing. Primary healing has no callus formation, no periosteal response, but establishes a new harversian system. Secondary healing, on the other hand, undergoes hematoma formation and construction/de-construction of the wounded area. This type of healing consists of intramembranous and endochondral formation, thus following a series of steps, primarily leading to bone remodeling.[9] Treatment with autologous bone-marrow cell grafting was found to be safe and efficient for the treatment of non-union fracture.[106,107] These study results show that bone marrow aspirates constitute of both osteogenic and osteoinductive characteristics.

Delivery of human adipose derived pericytes and MSCs to the fracture gap prevented the failure of healing atrophic non-union fracture in a rat model.[108] In the same study, 80% animals showed healing of bone in eight weeks with good quality.[108] Another interesting study showed that non-union stromal cells obtained from atrophic non-union fracture tissue have exhibited a reduced osteogenesis, increased cell senescence, and an increased secretion of Dickkopf-1, an important inhibitor of Wnt signaling during osteogenesis when compared to bone marrow mesenchymal stromal cells.[109]

Osteogenesis imperfecta

Osteogenesis imperfecta (OI) is a type of genetic disorder which produces brittle bones. It is caused due to mutations in the *COLIA1* and *COLIA2* collagen genes resulted in abnormal assembly of collagen fibrils.[110] People with severe disease are said to be suffering from a type III OI.[111] The defect in collagen may lead to multiple fractures, short stature, and severe bone deformities. A clinical study has shown that engraftment of MSCs in three children with OI resulted in high-density bone formation after three months. In this study, a total increase in the mineral content was estimated to be around 21 to 29 g.[112] A similar study employing bone-marrow transplantation in six children with severe OI showed an improvement in the growth of children from 60% to 94%.[113]

MSCs have proven to be an integral part of treatment for OI and this was proved from the following studies. Chamberlain et al. have demonstrated a successful gene targeting in adult human stem cells using adeno-associated viral vectors that disrupted the mutated *COLIA1* and *COLIA2* genes.[114] In another study, transplantation with human leukocyte antigen (HLA)-MSC into a female fetus with OI in 32nd week of gestation was performed. At two years of age, the motor development of the baby was found to be normal and the growth was persistent from then on.[110]

Osteoporosis

Osteoporosis is a type of bone disorder with low bone mineral density. This degrades the structure of the bone and thus leads to fracture.[115,116] Three patients aged between 35

and 45 with systemic mastocytosis, a stem cell disorder with an increase in number of mast cells in the skin has been effectively treated for severe osteoporosis by using interferon alpha-2b.[117] It has been postulated that osteoporosis can be caused due to oxidative damage. Therefore, MC3T3-E1 osteoblastic cells were induced with hydrogen peroxide (H_2O_2) to create an oxidative damage. When the cells were treated with hydrogen sulfide (H_2S), the transcription level was found to be increased for alkaline phosphatase, osteocalcin, bone matrix protein, and collagen through activation of MAPK pathway.[118]

Osteoporosis may occur due to an increased resorption of bone by osteoclasts or by rapid death of osteoblasts and osteocytes. Yamaza et al. have shown that aspirin increased osteogenesis of bone marrow MSCs and inhibited osteoclast activity in the ovariectomy-induced osteoporosis mouse model.[119] In this study, osteoporosis-induced mice treated with aspirin improved osteoporosis with an elevation of osteoblasts and a reduction of osteoclasts.[119] Furthermore, a flavonoid obtained from *Herba epimedii* called icarrin is found to be effective for the treatment of osteoporosis. In an *in vitro* co-culture model of mouse bone marrow-MSCs with mouse pre-osteoclastic RAW264.7 and rat ovarian follicular granulosa cells, it was shown that icarrin increased alkaline phosphatase activity and estradiol production while it decreased tartrate-resistant acid phosphatase levels.[120]

Advantages and future perspectives

Cell-based therapy for bone regeneration has proven to be more effective than traditional methods, as it eliminates the risk of an immune response and painful surgeries. It has been clearly demonstrated that various stem cells, especially MSCs are more effective for osteogenesis. Furthermore, the cells grown in a suitable 3D scaffold support were found to be more efficient for osteogenesis, as the 3D support simulates an *in vivo* environment, which helps in the differentiation of stem cells into bone cells. The iPSC technology also helps to produce a highly proliferating MSCs for bone regeneration. Even though there are many studies related to the production of osteocytes from adult MSCs, their production from iPSCs can be easily applied for future autologous cell therapy. Currently, several cell-based clinical trials have shown to be more effective in treating patients with bone fracture or osteoporosis. Bone regeneration in patients with bone disorders can be enhanced through modification of stem cells with several osteogenic factors or using stem cells as carriers for osteogenic factors. Millions of road traffic accidents related bone fractures, especially for the young patients, can be easily healed through the application of cell-based therapies. CRISPR-Cas9 technology can be applied for correcting the mutation of genes in the stem cells obtained from patients with osteogenesis imperfecta, and can be applied for autologous cell therapy. Particular attention needs to be given to the process of obtaining and manufacturing clinical grade stem cells, and modification of them for the possible enhancement of their osteogenic and angiogenic potentials.

Limitations of stem cells-based therapy for bone regeneration

The source of MSCs from donor and the donor's age, sex, and health conditions play a very important role in regenerative therapy. Moreover, MSCs may pose minor immunological rejection associated with the treatment. Although MSCs are attractive candidates for bone regeneration, their performance on large bone defects and defining the best approaches to be used in clinical practice is yet to be defined. Furthermore, obtaining a good manufacturing practice-grade effective stem cells, including their isolation, characterization, expansion, and selecting homogeneous population of MSCs to deliver at the site of bone injury are other challenges associated during MSC therapy. Partly, the osteogenic and angiogenic potentials of MSCs can be improved by employing several osteoinductive and osteoconductive biomaterials which provide a 3D environment for MSCs at the site of bone injury. Currently, a series of issues needs to be addressed including developing a more efficient scaffold delivery system, improving biological stability, specific differentiation capacity of stem cells, and reducing their off-target effects during stem cell therapy.

Conclusions

Osteogenic cells can be derived from a wide array of cell sources from our body including iPSCs. These cells have the regenerative potential and also possess the immunomodulatory characteristics to repair the injured tissues. In addition, the availability of modern techniques may facilitate the ease of scaling up the cells along with the scaffolding technology which can offer a powerful therapeutic tool for regenerative medicine.

AUTHORS' CONTRIBUTIONS

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REFERENCES

1. Hutchison C, Pilote M, Roy S. The axolotl limb: a model for bone development, regeneration and fracture healing. *Bone* 2007;**40**:45–56
2. Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? *Mech Dev* 1999;**87**:57–66
3. Tsiridis E, Upadhyay N, Giannoudis P. Molecular aspects of fracture healing: which are the important molecules? *Injury* 2007;**38**: S11–25
4. Deschaseaux F, Sensébé L, Heymann D. Mechanisms of bone repair and regeneration. *Trends Mol Med* 2009;**15**:417–29
5. Phillips AM. Overview of the fracture healing cascade. *Injury* 2005;**36**: S5–7
6. Einhorn TA. The cell and molecular biology of fracture healing. *Clin Orthop Relat Res* 1998;**355 Suppl**:S7–21.
7. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury* 2005;**36**:1392–404
8. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: the cellular picture. *Semin Cell Dev Biol* 2008;**19**:459–66
9. Walmsley GG, Ransom RC, Zielins ER, Leavitt T, Flacco JS, Hu MS, Lee AS, Longaker MT, Wan DC. Stem cells in bone regeneration. *Stem Cell Rev Rep* 2016;**12**:524–9
10. Dimitriou R, Mataliotakis GI, Angoules AG, Kanakaris NK, Giannoudis PV. Complications following autologous bone graft harvesting from the iliac crest and using the RIA: a systematic review. *Injury* 2011;**42**: S3–15
11. Van Heest A, Swiontkowski M. Bone-graft substitutes. *Lancet* 1999;**353**: Si28–9
12. Stevens MM, George JH. Exploring and engineering the cell surface interface. *Science* 2005;**310**:1135–8
13. Bonzani IC, George JH, Stevens MM. Novel materials for bone and cartilage regeneration. *Curr Opin Chem Biol* 2006;**10**:568–75
14. Ivkovic A, Marijanovic I, Hudetz D, Porter RM, Pecina M, Evans CH. Regenerative medicine and tissue engineering in orthopaedic surgery. *Front Biosci (Elite Ed)* 2011;**3**:923–44
15. Jimi E, Hirata S, Osawa K, Terashita M, Kitamura C, Fukushima H. The current and future therapies of bone regeneration to repair bone defects. *Int J Dent* 2012;**2012**:148261
16. Jeon OH, Panicker LM, Lu Q, Chae JJ, Feldman RA, Elisseeff JH. Human iPSC-derived osteoblasts and osteoclasts together promote bone regeneration in 3D biomaterials. *Sci Rep* 2016;**6**:26761
17. Buttery LD, Bourne S, Xynos JD, Wood H, Hughes FJ, Hughes SP, Episkopou V, Polak JM. Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. *Tissue Eng* 2001;**7**:89–99
18. Kim S, Kim SS, Lee SH, Eun Ahn S, Gwak SJ, Song JH, Kim BS, Chung HM. In vivo bone formation from human embryonic stem cell-derived osteogenic cells in poly(d,l-lactic-co-glycolic acid)/hydroxyapatite composite scaffolds. *Biomaterials* 2008;**29**:1043–53
19. Arpornmaeklong P, Brown SE, Wang Z, Krebsbach PH. Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells. *Stem Cells Dev* 2009;**18**:955–68
20. Liu X, Wang P, Chen W, Weir MD, Bao C, Xu HH. Human Embryonic stem cells and macroporous calcium phosphate construct for bone regeneration in cranial defects in rats. *Acta Biomater* 2014;**10**:4484–93
21. Kim HJ, Park JS. Usage of human mesenchymal stem cells in cell-based therapy: advantages and disadvantages. *Dev Reprod* 2017;**21**:1–10
22. Undale AH, Westendorf JJ, Yaszemski MJ, Khosla S. Mesenchymal stem cells for bone repair and metabolic bone diseases. *Mayo Clin Proc* 2009;**84**:893–902
23. Bianco P, Robey PG. Skeletal stem cells. *Development* 2015;**142**:1023–7
24. Dawson JI, Kanczler J, Tare R, Kassem M, Oreffo RO. Concise review: bridging the gap: bone regeneration using skeletal stem cell-based strategies – where are we now? *Stem Cells (Cells* 2014;**32**:35–44
25. Graziano A, d'Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. *Stem Cell Rev* 2008;**4**:21–6
26. Mattei V, Martellucci S, Pulcini F, Santilli F, Sorice M, Delle Monache S. Regenerative potential of DPSCs and revascularization: direct, paracrine or autocrine effect? *Stem Cell Rev Rep*. Epub ahead of print 7 Apr 2021. doi: 10.1007/s12015-021-10162-6.
27. Yamada Y, Nakamura S, Ito K, Sugito T, Yoshimi R, Nagasaka T, Ueda M. A feasibility of useful cell-based therapy by bone regeneration with deciduous tooth stem cells, dental pulp stem cells, or bone-marrow-derived mesenchymal stem cells for clinical study using tissue engineering technology. *Tissue Eng Part A* 2010;**16**:1891–900
28. Chamieh F, Collignon AM, Coyac BR, Lesieur J, Ribes S, Sadoine J, Llorens A, Nicoletti A, Letourneur D, Colombier ML, Nazhat SN, Bouchard P, Chaussain C, Rochefort GY. Accelerated craniofacial bone regeneration through dense collagen gel scaffolds seeded with dental pulp stem cells. *Sci Rep* 2016;**6**:38814
29. Paduano F, Marrelli M, Alom N, Amer M, White LJ, Shakesheff KM, Tatullo M. Decellularized bone extracellular matrix and human dental pulp stem cells as a construct for bone regeneration. *J Biomater Sci Polym Ed* 2017;**28**:730–48
30. Tatullo M, Falisi G, Amantea M, Rastelli C, Paduano F, Marrelli M. Dental pulp stem cells and human periapical cyst mesenchymal stem cells in bone tissue regeneration: comparison of basal and osteogenic differentiated gene expression of a newly discovered mesenchymal stem cell lineage. *J Biol Regul Homeost Agents* 2015;**29**:713–8
31. Morad G, Kheiri L, Khojasteh A. Dental pulp stem cells for in vivo bone regeneration: a systematic review of literature. *Arch Oral Biol* 2013;**58**:1818–27
32. Gruber HE, Somayaji S, Riley F, Hoelscher GL, Norton HJ, Ingram J, Hanley EN, Jr. Human adipose-derived mesenchymal stem cells: serial passaging, doubling time and cell senescence. *Biotech Histochem* 2012;**87**:303–11
33. Yoon E, Dhar S, Chun DE, Gharibjanian NA, Evans GR. In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model. *Tissue Eng* 2007;**13**:619–27
34. Sándor GK, Tuovinen VJ, Wolff J, Patrikoski M, Jokinen J, Nieminen E, Mannerström B, Lappalainen OP, Seppänen R, Miettinen S. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: a case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. *J Oral Maxillofac Surg* 2013;**71**:938–50
35. Calis M, Irmak G, Demirtaş TT, Kara M, Üstün GG, Gümüşderelioğlu M, Türkkanı A, Çakar AN, Özgür F. Photobiomodulation combined with adipose-derived stem cells encapsulated in methacrylated gelatin hydrogels enhances in vivo bone regeneration. *Lasers Med Sci*. Epub ahead of print 11 Apr 2021. doi: 10.1007/s10103-021-03308-y.
36. Han DS, Chang HK, Kim KR, Woo SM. Consideration of bone regeneration effect of stem cells: comparison of bone regeneration between bone marrow stem cells and adipose-derived stem cells. *J Craniofac Surg* 2014;**25**:196–201
37. Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S. Mesenchymal stem cells in osteobiology and applied bone regeneration. *Clin Orthop Relat Res* 1998;S247–56
38. Kraus KH, Kirker-Head C. Mesenchymal stem cells and bone regeneration. *Vet Surg* 2006;**35**:232–42
39. Zomorodian E, Baghaban Eslaminejad M. Mesenchymal stem cells as a potent cell source for bone regeneration. *Stem Cells Int* 2012;**2012**:980353
40. Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, Pearce S, Kasten P. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. *Biomaterials* 2010;**31**:3572–9
41. Jones E, Yang X. Mesenchymal stem cells and bone regeneration: current status. *Injury* 2011;**42**:562–8
42. Granero-Moltó F, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, Longobardi L, Jansen ED, Mortlock DP, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. *Stem Cells (Cells)* 2009;**27**:1887–98

43. Tian G, Jiang S, Li J, Wei F, Li X, Ding Y, Yang Z, Sun Z, Zha K, Wang F, Huang B, Peng L, Wang Q, Tian Z, Yang X, Wang Z, Guo Q, Guo W, Liu S. Cell-free decellularized cartilage extracellular matrix scaffolds combined with interleukin 4 promote osteochondral repair through immunomodulatory macrophages: in vitro and in vivo preclinical study. *Acta Biomater* 2021;**127**:131–45
44. Zhou M, Xi J, Cheng Y, Sun D, Shu P, Chi S, Tian S, Ye S. Reprogrammed mesenchymal stem cells derived from iPSCs promote bone repair in steroid-associated osteonecrosis of the femoral head. *Stem Cell Res Ther* 2021;**12**:175
45. Li J, Lin Q, Lin Y, Lai R, Zhang W. Effects of DLX3 on the osteogenic differentiation of induced pluripotent stem cell–derived mesenchymal stem cells. *Mol Med Rep* 2021;**23**:232
46. Song B, Fu H, Liu J, Ren K, Weir MD, Schneider A, Wang P, Song Y, Zhao L, Xu H. Bioactive small molecules in calcium phosphate scaffold enhanced osteogenic differentiation of human induced pluripotent stem cells. *Dent Mater J* 2021;**40**:615–24
47. Hou Y, Yan Z, Wu Z. Concise review; the recent methods that enhance the osteogenic differentiation of human induced pluripotent stem cells. *Curr Stem Cell Res Ther* 2021;**16**:949–57
48. Ardeshirylajimi A, Dinarvand P, Seyedjafari E, Langroudi L, Adegani FJ, Soleimani M. Enhanced reconstruction of rat calvarial defects achieved by plasma-treated electrospun scaffolds and induced pluripotent stem cells. *Cell Tissue Res* 2013;**354**:849–60
49. Arvidson K, Abdallah BM, Applegate LA, Baldini N, Cenni E, Gomez-Barrena E, Granchi D, Kassem M, Konttinen YT, Mustafa K, Pioletti DP, Sillat T, Finne-Wistrand A. Bone regeneration and stem cells. *J Cell Mol Med* 2011;**15**:718–46
50. Liu Z, Lin Y, Fang X, Yang J, Chen Z. Epigallocatechin-3-Gallate promotes osteo-/odontogenic differentiation of stem cells from the apical papilla through activating the BMP-Smad signaling pathway. *Molecules* 2021;**26**:1580
51. Ryoo HM, Lee MH, Kim YJ. Critical molecular switches involved in BMP-2-induced osteogenic differentiation of mesenchymal cells. *Gene* 2006;**366**:51–7
52. Lai CF, Cheng SL. Signal transductions induced by bone morphogenetic protein-2 and transforming growth factor-beta in normal human osteoblastic cells. *J Biol Chem* 2002;**277**:15514–22
53. Wan M, Cao X. BMP signaling in skeletal development. *Biochem Biophys Res Commun* 2005;**328**:651–7
54. Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature* 2001;**410**:37–40
55. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev* 2008;**29**:535–59
56. Nakae J, Kido Y, Accili D. Distinct and overlapping functions of insulin and IGF-I receptors. *Endocr Rev* 2001;**22**:818–35
57. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene* 2004;**341**:19–39
58. Fujita K, Janz S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. *Mol Cancer* 2007;**6**:71
59. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell* 2005;**8**:739–50
60. Canalis E. Notch signaling in osteoblasts. *Sci Signal* 2008;**1**:pe17
61. Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM, Chen Y, Wang L, Zheng H, Sutton RE, Boyce BF, Lee B. Dimorphic effects of notch signaling in bone homeostasis. *Nat Med* 2008;**14**:299–305
62. Chung UI, Schipani E, McMahon AP, Kronenberg HM. Indian hedgehog couples chondrogenesis to osteogenesis in endochondral bone development. *J Clin Invest* 2001;**107**:295–304
63. Day TF, Yang Y. Wnt and hedgehog signaling pathways in bone development. *J Bone Joint Surg Am* 2008;**90**:19–24
64. Jacob AL, Smith C, Partanen J, Ornitz DM. Fibroblast growth factor receptor 1 signaling in the osteo-chondrogenic cell lineage regulates sequential steps of osteoblast maturation. *Dev Biol* 2006;**296**:315–28
65. Mundy GR, Elefteriou F. Boning up on ephrin signaling. *Cell* 2006;**126**:441–3
66. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006;**4**:111–21
67. Hsiong SX, Mooney DJ. Regeneration of vascularized bone. *Periodontol 2000* 2006;**41**:109–22
68. Scotti C, Tonnarelli B, Papadimitropoulos A, Scherberich A, Schaeren S, Schauerte A, Lopez-Rios J, Zeller R, Barbero A, Martin I. Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering. *Proc Natl Acad Sci U S A* 2010;**107**:7251–6
69. Iaquinta MR, Lanzillotti C, Mazziotta C, Bononi I, Frontini F, Mazzoni E, Oton-Gonzalez L, Rotondo JC, Torreggiani E, Tognon M, Martini F. The role of microRNAs in the osteogenic and chondrogenic differentiation of mesenchymal stem cells and bone pathologies. *Theranostics* 2021;**11**:6573–91
70. Lanzillotti C, De Mattei M, Mazziotta C, Taraballi F, Rotondo JC, Tognon M. Martini F. Long non-coding RNAs and MicroRNAs interplay in osteogenic differentiation of mesenchymal stem cells. *Front Cell Dev Biol* 2021;**9**:646032
71. Zhang Y, Xie RL, Croce CM, Stein JL, Lian JB, van Wijnen AJ, Stein GS. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. *Proc Natl Acad Sci U S A* 2011;**108**:9863–8
72. Zeng HC, Bae Y, Dawson BC, Chen Y, Bertin T, Munivez E, Campeau PM, Tao J, Chen R, Lee BH. MicroRNA miR-23a cluster promotes osteocyte differentiation by regulating TGF-beta signalling in osteoblasts. *Nat Commun* 2017;**8**:15000
73. Yang J, Qin S, Yi C, Ma G, Zhu H, Zhou W, Xiong Y, Zhu X, Wang Y, He L, Guo X. MiR-140 is co-expressed with Wwp2-C transcript and activated by Sox9 to target Sp1 in maintaining the chondrocyte proliferation. *FEBS Lett* 2011;**585**:2992–7
74. Budd E, Waddell S, de Andres MC, Oreffo ROC. The potential of microRNAs for stem cell-based therapy for degenerative skeletal diseases. *Curr Mol Biol Rep* 2017;**3**:263–75
75. Zhang Q, Nettleship I, Schmelzer E, Gerlach J, Zhang X, Wang J, Liu C. Tissue engineering and regenerative medicine therapies for cell senescence in bone and cartilage. *Tissue Eng Part B Rev* 2020;**26**:64–78
76. Kretlow JD, Jin YQ, Liu W, Zhang WJ, Hong TH, Zhou G, Baggett LS, Mikos AG, Cao Y. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. *BMC Cell Biol* 2008;**9**:60
77. Cheng H, Qiu L, Ma J, Zhang H, Cheng M, Li W, Zhao X, Liu K. Replicative senescence of human bone marrow and umbilical cord derived mesenchymal stem cells and their differentiation to adipocytes and osteoblasts. *Mol Biol Rep* 2011;**38**:5161–8
78. Khan WS, Adesida AB, Tew SR, Andrew JG, Hardingham TE. The epitope characterisation and the osteogenic differentiation potential of human fat pad-derived stem cells is maintained with ageing in later life. *Injury* 2009;**40**:150–7
79. Lin TM, Tsai JL, Lin SD, Lai CS, Chang CC. Accelerated growth and prolonged lifespan of adipose tissue-derived human mesenchymal stem cells in a medium using reduced calcium and antioxidants. *Stem Cells Dev* 2005;**14**:92–102
80. Choi KM, Seo YK, Yoon HH, Song KY, Kwon SY, Lee HS, Park JK. Effect of ascorbic acid on bone marrow-derived mesenchymal stem cell proliferation and differentiation. *J Biosci Bioeng* 2008;**105**:586–94
81. Okada M, Kim HW, Matsu-Ura K, Wang YG, Xu M, Ashraf M. Abrogation of age-induced microRNA-195 rejuvenates the senescent mesenchymal stem cells by reactivating telomerase. *Stem Cells (Cells)* 2016;**34**:148–59
82. Gharibi B, Hughes FJ. Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells. *Stem Cells Transl Med* 2012;**1**:771–82
83. Hun Lee J, Shu L, Fuentes F, Su ZY, Tony Kong AN. Cancer chemoprevention by traditional Chinese herbal medicine and dietary phytochemicals: targeting nrf2-mediated oxidative stress/anti-inflammatory responses, epigenetics, and cancer stem cells. *J Tradit Complement Med* 2013;**3**:69–79

84. Li Y, Wu Q, Wang Y, Li L, Bu H, Bao J. Senescence of mesenchymal stem cells (review). *Int J Mol Med* 2017;**39**:775–82
85. Jones JR, Lin S, Yue S, Lee PD, Hanna JV, Smith ME, Newport RJ. Bioactive glass scaffolds for bone regeneration and their hierarchical characterisation. *Proc Inst Mech Eng H* 2010;**224**:1373–87
86. San Miguel B, Kriauciunas R, Tosatti S, Ehrbar M, Ghayor C, Textor M, Weber FE. Enhanced osteoblastic activity and bone regeneration using surface-modified porous bioactive glass scaffolds. *J Biomed Mater Res A* 2010;**94**:1023–33
87. Wu ZY, Hill RG, Yue S, Nightingale D, Lee PD, Jones JR. Melt-derived bioactive glass scaffolds produced by a gel-cast foaming technique. *Acta Biomater* 2011;**7**:1807–16
88. Zhang J, Zhao S, Zhu Y, Huang Y, Zhu M, Tao C, Zhang C. Three-dimensional printing of strontium-containing mesoporous bioactive glass scaffolds for bone regeneration. *Acta Biomater* 2014;**10**:2269–81
89. Romagnoli C, D'Asta F, Brandi ML. Drug delivery using composite scaffolds in the context of bone tissue engineering. *Clin Cases Miner Bone Metab* 2013;**10**:155–61
90. Kreuter J. Nanoparticles and microparticles for drug and vaccine delivery. *J Anat* 1996;**189**: 503–5
91. Ren J, Blackwood KA, Doustgani A, Poh PP, Steck R, Stevens MM, Woodruff MA. Melt-electrospun polycaprolactone strontium-substituted bioactive glass scaffolds for bone regeneration. *J Biomed Mater Res A* 2014;**102**:3140–53
92. Brown A, Zaky S, Ray H, Jr, Sfeir C. Porous magnesium/PLGA composite scaffolds for enhanced bone regeneration following tooth extraction. *Acta Biomater* 2015;**11**:543–53
93. Lee SJ, Lim GJ, Lee JW, Atala A, Yoo JJ. In vitro evaluation of a poly (lactide-co-glycolide)-collagen composite scaffold for bone regeneration. *Biomaterials* 2006;**27**:3466–72
94. Chesnutt BM, Viano AM, Yuan Y, Yang Y, Guda T, Appleford MR, Ong JL, Haggard WO, Bumgardner JD. Design and characterization of a novel chitosan/nanocrystalline calcium phosphate composite scaffold for bone regeneration. *J Biomed Mater Res A* 2009;**88**:491–502
95. Inzana JA, Olvera D, Fuller SM, Kelly JP, Graeve OA, Schwarz EM, Kates SL, Awad HA. 3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration. *Biomaterials* 2014;**35**:4026–34
96. Sun F, Zhou H, Lee J. Various preparation methods of highly porous hydroxyapatite/polymer nanoscale biocomposites for bone regeneration. *Acta Biomater* 2011;**7**:3813–28
97. Marei MK, Saad MM, El-Ashwah AM, El-Backly RM, Al-Khodary MA. Experimental formation of periodontal structure around titanium implants utilizing bone marrow mesenchymal stem cells: a pilot study. *J Oral Implantol* 2009;**35**:106–29
98. Civinini R, De Biase P, Carulli C, Matassi F, Nistri L, Capanna R, Innocenti M. The use of an injectable calcium sulphate/calcium phosphate bioceramic in the treatment of osteonecrosis of the femoral head. *Int Orthop* 2012;**36**:1583–8
99. Billström GH, Blom AW, Larsson S, Beswick AD. Application of scaffolds for bone regeneration strategies: current trends and future directions. *Injury* 2013;**44**: S28–33
100. Montufar EB, Traykova T, Gil C, Harr I, Almirall A, Aguirre A, Engel E, Planell JA, Ginebra MP. Foamed surfactant solution as a template for self-setting injectable hydroxyapatite scaffolds for bone regeneration. *Acta Biomater* 2010;**6**:876–85
101. Xu HH, Weir MD, Simon CG. Injectable and strong nano-apatite scaffolds for cell/growth factor delivery and bone regeneration. *Dent Mater* 2008;**24**:1212–22
102. Yasmeen S, Lo MK, Bajracharya S, Roldo M. Injectable scaffolds for bone regeneration. *Langmuir* 2014;**30**:12977–85
103. Yoshimi R, Yamada Y, Ito K, Nakamura S, Abe A, Nagasaka T, Okabe K, Kohgo T, Baba S, Ueda M. Self-assembling peptide nanofiber scaffolds, platelet-rich plasma, and mesenchymal stem cells for injectable bone regeneration with tissue engineering. *J Craniofac Surg* 2009;**20**:1523–30
104. Dyondi D, Webster TJ, Banerjee R. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. *Int J Nanomedicine* 2013;**8**:47–59
105. Zhang B, Huang J, Liu J, Lin F, Ding Z, Xu J. Injectable composite hydrogel promotes osteogenesis and angiogenesis in spinal fusion by optimizing the bone marrow mesenchymal stem cell microenvironment and exosomes secretion. *Mater Sci Eng C Mater Biol Appl* 2021;**123**:111782
106. Homma Y, Zimmermann G, Hernigou P. Cellular therapies for the treatment of non-union: the past, present and future. *Injury* 2013;**44**: S46–9.
107. Sampson S, Botto-van Bemden A, Aufiero D. Stem cell therapies for treatment of cartilage and bone disorders: osteoarthritis, avascular necrosis, and non-union fractures. *PM R* 2015;**7**:S26–s32
108. Tawonsawatruk T, West CC, Murray IR, Soo C, Péault B, Simpson AH. Adipose derived pericytes rescue fractures from a failure of healing – non-union. *Sci Rep* 2016;**6**:22779
109. Bajada S, Marshall MJ, Wright KT, Richardson JB, Johnson WE. Decreased osteogenesis, increased cell senescence and elevated dickkopf-1 secretion in human fracture non union stromal cells. *Bone* 2009;**45**:726–35
110. Le Blanc K, Götherström C, Ringdén O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Nordén-Lindeberg S, Jansson M, Dalton A, Aström E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 2005;**79**:1607–14
111. Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. *Nat Rev Endocrinol* 2011;**7**:540–57
112. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999;**5**:309–13
113. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci U S A* 2002;**99**:8932–7
114. Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD, Pace JM, Underwood RA, Song KM, Sussman M, Byers PH, Russell DW. Gene targeting in stem cells from individuals with osteogenesis imperfecta. *Science (Science)* 2004;**303**:1198–201
115. Antebi B, Pelled G, Gazit D. Stem cell therapy for osteoporosis. *Curr Osteoporos Rep* 2014;**12**:41–7
116. Hu L, Yin C, Zhao F, Ali A, Ma J, Qian A. Mesenchymal stem cells: cell fate decision to osteoblast or adipocyte and application in osteoporosis treatment. *IJMS* 2018;**19**:360
117. Weide R, Ehlenz K, Lorenz W, Walthers E, Klausmann M, Pflüger KH. Successful treatment of osteoporosis in systemic mastocytosis with interferon alpha-2b. *Ann Hematol* 1996;**72**:41–3
118. Xu ZS, Wang XY, Xiao DM, Hu LF, Lu M, Wu ZY, Bian JS. Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H_2O_2-induced oxidative damage-implications for the treatment of osteoporosis. *Free Radic Biol Med* 2011;**50**:1314–23
119. Yamaza T, Miura Y, Bi Y, Liu Y, Akiyama K, Sonoyama W, Patel V, Gutkind S, Young M, Gronthos S, Le A, Wang CY, Chen W, Shi S. Pharmacologic stem cell based intervention as a new approach to osteoporosis treatment in rodents. *PloS One* 2008;**3**:e2615
120. Liu YQ, Han XF, Liu T, Cheng MC, Xiao HB. A cell-based model of bone remodeling for identifying activity of icarrin in the treatment of osteoporosis. *Biotechnol Lett* 2015;**37**:219–26