Cell-Scaffold Constructs for Bone Regeneration Therapy

Subjects: Allergy

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Bone tissue engineering (BTE) is a process of combining live osteoblast progenitors with a biocompatible scaffold to produce a biological substitute that can integrate into host bone tissue and recover its function. Mesenchymal stem cells (MSCs) are the most researched post-natal stem cells because they have self-renewal properties and a multi-differentiation capacity that can give rise to various cell lineages, including osteoblasts. BTE technology utilizes a combination of MSCs and biodegradable scaffold material, which provides a suitable environment for functional bone recovery and has been developed as a therapeutic approach to bone regeneration.

bone tissue engineering MSCs osteoblasts scaffolds

1. Introduction

Continuous research is ongoing in bone tissue regeneration technologies related to orthopedics and dentistry. Vast challenges remain, however, in the application of these modalities to reconstituting damaged skeletal structures. Bone grafting has been widely utilized as a regenerative therapy for critical size bone defects (CSDs), and various bone grafting and prosthetic bone materials have been developed in this regard. There is no one standard definition of CSDs. In general, a “critically-sized” defect is regarded as one that would not heal spontaneously within a patient’s lifetime and would require surgical stabilization and further surgical intervention [1][2]. Currently, bone grafting materials are classified as autogenous, allogeneic, or heterogeneous and artificial bone substitutes such as hydroxyapatite (HA), β-TCP (beta-tricalcium phosphate), bioactive glass, and calcium sulfate. Autologous bone has no particular disadvantages other than restrictions on the collection amount and collection site and is recognized as a good prosthetic material with new bone formation capacity. It is thus considered the current gold standard for the regeneration of bone defects but has been most widely used in clinics to treat only small-sized bone defects [3][4][5].

To overcome the limitations of current bone graft therapies, such as autologous bone graft and artificial bone substitutes, many researchers have attempted to develop BTE to regenerate and restore lost bone tissue using MSCs, growth factors, and scaffolds [6][7][8][9][10]. MSCs are referred to as multipotential progenitor cell populations that can differentiate into osteoblast progenitors in vitro under specific conditions, and these cells are most commonly used for bone regeneration [11]. In addition, MSCs are immune tolerant and are used for immunosuppressive therapy via allogenic applications to accelerate bone healing [12]. The use of a scaffold can provide the space needed to deliver and confine MSCs to the bone target site, provide an environment suitable for the migration, proliferation, and differentiation of the stem cells, enable diffusion of nutrients and eventually create

early osteoid tissue at the site of the defect which is subsequently mineralized to form new bone. This combination of MSCs and scaffolds has been developed as a BTE therapy. Clinical trials for recovering bone defects have already commenced and reported the accelerated bone healing ability of these approaches. The current bone regenerating ability of the BTE approach is therefore successful but cannot as of yet recover the functional loss caused by large bone defects, such as those resulting from inflammatory diseases.

Osteoblasts are bone-forming cells derived from multipotent mesenchymal stem cells. During skeletal development, multipotent mesenchymal stem cells differentiate into osteoblast progenitor cells and undergo a commitment to form immature osteoblasts that are capable of proliferating before becoming mature osteoblasts. Although mature osteoblasts can synthesize and deposit bone extracellular matrix components, their ability to proliferate is significantly reduced [13]. Thus, recapitulating immature osteoblast differentiation has been suggested as a potential approach to bone regeneration therapy [14]. Currently, osteoblast progenitor cells can be isolated from adult human tissue and are good alternatives to MSCs for bone regeneration. BTE using immature osteoblast and bioscaffolds is, therefore an alternative tissue-engineered construct for recovering large bone defects.

2. MSCs Derived from Embryonic Stem Cells and Induced Pluripotent Stem Cells for Bone Tissue Regeneration

Functional bone tissue engineering generally involves the use of osteoprogenitors derived from MSCs and seeded onto a scaffold to predictably restore the lost architecture and function of bone tissue. MSCs have been isolated from adult tissues such as adipose tissue, bone marrow, and dental tissues, are widely used in regenerative medicine, including BTE, and, thus, have both research and clinical applications. However, MSCs cannot be isolated from patients with systemic disorders such as cardiovascular disease, diabetes, inflammatory bone disease, or advanced aging-related issues. Embryonic stem cell (ESCs)- or Induced pluripotent stem cell (iPSCs)-derived MSCs may be potential cell sources for the clinical trial of BTE [15]. A better understanding of cell fate decisions and differentiation processes during osteoblast development may help to generate functional progenitor cells for tissue restoration. Over the years, technologies involving the osteoblast differentiation of ESCs and iPSCs have been significantly improved, and several studies have demonstrated the successful production of MSCs derived from ESCs/iPSCs for use in BTE therapies [16][17][18] (**Figure 1**).

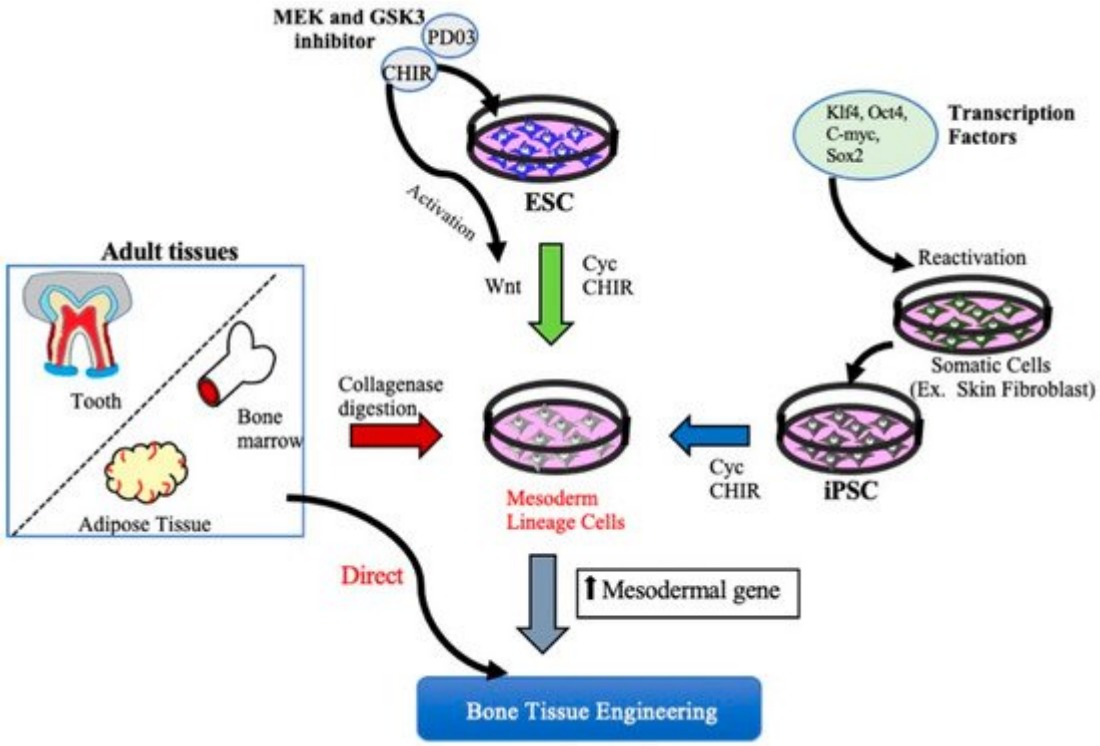

Figure 1. Schematic diagram of different approaches to obtain Mesenchymal stem cells. MSCs can be derived from either iPSCs, ESCs, or adult mesenchymal tissue. MSCs can be obtained by ESCs and iPSCs using small molecules such as mitogen-activated protein kinase (MEK) inhibitor, (MEK) inhibitor, PD0325901, glycogen synthase kinase 3 (GSK3) inhibitor, and CHIR99021 (CHIR). MSCs are also be derived from various connective tissues such as bone marrow, adipose tissue, and dental tissues by collagenase digestion or aspirates from bone marrow and adipose tissue directly used for BTE therapeutics. KLf4: Kruppel Like Factor 4, Oct4: Octamer-binding transcription factor 4, C-myc: Cellular-Myelocytomatosis, Sox-2: sex-determining region Y-box 2.

3. MSCs for Bone Regeneration

To develop MSCs that have clinical utility for BTE, a standard protocol for the characterization, osteoblast differentiation, and transplantation of these cells in combination with a biodegradable scaffold is required. Various types of MSCs are currently available with osteoblastic lineage differentiation potential; however, their origin and development are not clearly understood. There have been few reports on MSCs being successfully derived from neural crest cells during the development of vertebrates, which is seen as transient embryonic tissue [19]. Most studies on MSCs to date have reported their derivation from perivascular cells, the pericytes. These cells reside in specific niches, which are commonly found in bone marrow, adipose tissue, and various fetal and other adult tissues [20][21]. This has been the primary cell source of the MSCs used in BTE to date.

3.1. Characterization of MSCs

Surface markers are currently being used to identify MSCs for quality control assurance prior to cell preparation, based on ‘good manufacturing practice,’ which is required for investor-mediated clinical developments. Hence, the

characterization of MSCs based on surface marker analysis is an essential criterion for the clinical application of BTE methodologies. According to the International Society of Cell Therapy (ISCT) criteria, MSCs express a cluster of differentiation (CD) surface markers such as CD90, CD105, and CD73, but do not express CD11b, CD14, CD19, CD34, CD45, or human leukocyte antigen (HLA)-DR [22][23][24].

3.2. Clinical Translation of MSC-Based Bone Regeneration

The basic concept behind a scaffold is to mimic the structure and function of the extracellular matrix (ECM) in tissues. The ECM provides both structural and mechanical stability and regulates some of the core cellular functions [25][26][27]. The basic role of scaffolds in BTE is to mimic the ECM of the native bone tissue and provide a functional three-dimensional space for the adhesion, migration, proliferation, and differentiation of osteoblast progenitors in which bone growth can occur [28][29][30][31]. An ideal scaffold for BTE should substitute for both the structure and function of the ECM and thus be capable of regenerating the lost bone tissue when seeded in conjunction with osteoblast progenitors. BTE innovations have led to the development of new biomaterials that resemble the 3D bone structure, in terms of mechanical properties as well as osteoconductive, osteoinductive, and osteogenic features [32][33]. Traditional bone repair approaches mainly focus on the use of bone grafts from autologous, allogeneic, and xenogeneic sources; however, complications such as donor-site morbidity and host immune rejection limit the application of these tissues [34]. The promise of BTE has principally involved overcoming these problems. The aims of BTE are to regenerate and restore the function of lost bone tissue using combinations of osteoblast progenitors and synthetic biomaterial scaffolds. Over the past decade, the use of synthetic biomaterials to enhance bone regeneration has significantly developed because of their capacity to mimic the natural environment of the extracellular matrix. The synthetic scaffold biomaterials predominantly used in BTE include calcium phosphate ceramics, biodegradable polymers, and composites, and the combination of ceramics and polymer scaffolds aims to utilize the properties of both materials [31][34][35].

3.2.2. Preclinical Studies of BTE in a Large Animal Model Using MSC/Scaffold Combinations

To translate the clinical use of MSCs combined with scaffolds for BTE, large animal model systems that closely resemble human physiology are required. A number of preclinical studies conducted using MSCs with varying combinations of biomaterials in critical bone defect models are listed in **Table 1**.

Table 1. Pre-clinical experiments of MSCs-combined with biomaterial for bone regeneration in large animal bone defect models.

Author	Experiment Animal	Type and Size of Defect	Experimental Transplant Groups	Post-Transplant Follow up Period	Outcome
Probst et al., 2020 [34]	Mini pigs	Critical mandibular defect (3 × 1 × 2 cm)	3D TCP-PLGA scaffold seeded with osteogenic differentiated Porcine ADSCs (pADSCs).	12 weeks	pADSCs seeded TCP-PLGA scaffold constructs significantly

Author	Experiment Animal	Type and Size of Defect	Experimental Transplant Groups	Post-Transplant Follow up Period	Outcome
					improved bone regenerations compared to empty scaffold.
Wang et al., 2019 [36]	Rhesus Monkeys	Critical alveolar bone defect (10 × 10 × 5 mm)	3D-Bioactive glass (BG) + BMP/chitosan (CS) + BMMSCs	12 weeks	BMP/CS nanoparticles loaded on 3D-BG scaffold promoted bone regeneration ability in vivo, and preload of BMMSCs promote this ability further.
Hsieh et al., 2019 [37]	domestic Ds-Red pigs	Calvarias defect (8 mm in diameter and 2 mm in depth)	Hemostatic gelatin sponge scaffold seeded with EGFP pig BMMSCs	1, 2, 3 and 4 weeks	Osteoid formation in the scaffolds transplanted with seeded BMMSCs was significantly higher than the control group.
Shi et al., 2019 [38]	Minipigs	Maxillary Intraosseous circular defects (12 mm in diameter and 5 mm in depth)	Bio-Oss/autogenous (Pig Gingival MSCs) pGMSCs (2 × 10^6)/SB431542 (TGF-β signalling inhibitor).	8 weeks	pGMSCs treated with a TGF- β signaling inhibitor successfully repair minipig severe maxillofacial bone defects.
Qiu et al., 2018 [39]	Minipigs	Lateral femoral condyle defect (8 mm in diameter and 10 mm in depth)	Calcium phosphate cement (CPC) scaffold seeded with autologous BMMSCs plus autologous PRP (CPC-BMSC-PRP, 1 × 10^6 cells/scaffold)	6 and 12 weeks	CPC scaffold co-delivered BMMSCs-PRP promoted scaffold resorption and doubled bone regeneration in large defects than control groups
Zhang et al., 2017 [40]	Minipigs	Non-healing full thickness cranial defects (2 cm width × 3 cm length × 0.5 cm depth)	IMC (intrafibrillarly-mineralized collagen) scaffold seeded with 1 × 10^6 PDLSCs cells	12 weeks	Compared with HA, IMC-seeded PDLSCs achieved a significantly higher extent of new bone formation, with the normal architecture

Author	Experiment Animal	Type and Size of Defect	Experimental Transplant Groups	Post-Transplant Follow up Period	Outcome
					of natural bones and blood vessels.
Scarano et al., 2017 [41]	Minipigs	Critical-size circular defects (5 mm diameter; 5 mm thickness) in the mandibular body	Bone porcine block (BPB) scaffold seeded with 100 ul cell suspension of BMMSCs	12 weeks	BPB when used as a scaffold induce bone regeneration and further benefit from the addition of BMMSCs in the tissue-engineered constructs.
Lin et al., 2015 [42]	Minipigs	Massive segmental bone defects (30 mm in length) at the mid-diaphysis of femora	Transduced pig ADSCs loaded onto PLGA scaffold	2, 4, 8 and 12 weeks	ADSCs/scaffold constructs successfully healed massive segmental bone defects at the mid-diaphysis of femora in minipigs significantly than control group.
Cao et al., 2015 [43]	Mini pigs	Calvarial bone defects (3 cm × 1.8 cm oval defect)	BMMSCs pretreated with 75 μg/mL aspirin for 24 h seeded onto hydroxyapatite/tricalcium phosphate (HA/TCP)	6 months	BMMSCs pretreated with aspirin have a greater capacity to repair calvarial bone defects in a mini swine model [45]
Fan et al., 2014 [44]	Rhesus monkeys [45][46]	Segmental tibial defects (20 mm in length)	Autologous prevascularized BMMSCs (5 × 10^6)-β-TCP constructs	4, 8 and 12 weeks	Significantly higher amount of neo-vascularization and radiographic grading score in prevascularized BMMSCs-β-TCP constructs

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demonstrate high transfection efficiency with immunogenicity and toxicity, raising an issue of safety. In contrast, non-viral vectors usually consist of plasmid or related DNA, which are non-immunogenic and high safety but with low transfection efficiency [46][47]. Another promising approach is the sequential delivery of exogenous genes to promote the osteogenesis of stem cells. For example, genes that are expressed early and in the final stages of osteogenesis are different. Hence, delivering required osteogenic genes at specific time intervals into target cells induces efficient osteogenic differentiation. A recent study by Kim et al. demonstrated an effective sequential delivery of runt-related transcription factor 2 (RUNX2) and osterix genes induced conversion of human MSCs into pre-osteoblasts and subsequent delivery of activating transcription factor 4 (ATF4) gene triggered further osteogenesis. Differentiation of MSCs into desired mature cells can be regulated by the delivery time of specific osteogenic genes mimicking the natural process of bone remodeling [47][48].

3.2.4. Clinical Trials of MSCs for BTE

Over the past decade, a greater understanding has emerged with regard to the capabilities of MSCs to promote bone tissue regeneration, with numerous preclinical and clinical studies now underway. To identify the current potential combination of cell-scaffold constructs or tissue-engineered substitutes for bone tissue regeneration, we found twenty clinical trials. Nine are published (**Table 2**), and others are listed in the ClinicalTrails.gov database (**Table 3**). These trials have highlighted the importance of using cell-based therapy with various scaffolds to treat bone tissue regeneration in a real clinical setting. From the twenty identified clinical studies listed in **Table 2** and **Table 3**, the majority report the use of BMMSCs, reflecting the fact that they are the most accepted cell source and the current gold standard in most clinical trials for treating bone disease, including nonunion fractures of long bones and craniofacial bone defects. However, in a few clinical trials, researchers have used umbilical cord (UC)- MSCs [49], BMMSCs [50], and adipose-derived MSCs as allogeneic cell sources to prepare the tissue-engineered constructs for regeneration of critical bone defects (NCT02307). Ceramic-based scaffolds are the primary choice in the majority of clinical trials, indicating their high clinical relevance. From the clinical trials listed in **Table 2** and **Table 3**, most studies used a combination of BMMSCs with calcium-phosphate ceramics such as hydroxyapatite [49][51][52], β-TCP [50][53] (NCT02803177, NCT02153372), biphasic calcium phosphate, a combination of hydroxyapatite and β-TCP [54] (NCT04297813, NCT03325504, NCT01842477). Although most of these clinical trials used a simple combination of calcium-phosphate ceramics with BMMSCs, in a few studies, however, additional factors were included to facilitate enhanced bone regeneration. For example, Dilogo et al. added growth factor BMP2 along with cell scaffold constructs to enhance bone regeneration [49][52]. Similarly, researchers used BMMSCs mixed with BMP2 and loaded them on to 3-dimensional tissue-engineered collagen scaffold (NCT01958502) in another clinical trial. However, a clinical study by Baba et al. used polylactic scaffold seeded BMMSCs mixed with platelet-rich plasma solution and an additional 5000 units of human thrombin dissolved in 10% calcium chloride [55].

Table 2. Completed and published clinical studies using MSCs combined with biomaterials for bone tissue regeneration.

Author	Type and Size of Defect	Transplant Groups	Origin of Cell Source	Pre-Transplant Incubation	Outcome
Dilogo et al., 2020 [49]	Nonunion fractures of Humerus/tibia with critical size bone defects	Combination of HA Bongros®-HA, Daewoong), BMP2, UC-MSCs with demineralized bone matrix	Allogeneic Umbilical Cord MSCs (UC-MSCs)	None	Allogeneic UC-MSCs can be used safely to treat the critical sized bone defects of long bones.
Dilogo et al., 2019 [52]	Humerus, Tibia and Femur Critical sized defects	Combination of HA granules (Bongros®-HA, Bioalpha, Seungnam, Korea), BMP2 and BMMSCs	Autologous Bone marrow harvested from	None	Dramatic improvement of bone regeneration compared to

Author	Type and Size of Defect	Transplant Groups	Origin of Cell Source	Pre-Transplant Incubation	Outcome
		mixed with Plasma solution.	posterior Iliac crestal bone		preoperative radiographs.
Gjerde et al., 2018 [54]	Severe mandibular ridge resorption.	Expanded, autologous MSCs with biphasic calcium phosphate (MBCP^{+}TM; Biomatlante, France)	Bone marrow cells from the posterior iliac crest	None	MSCs successfully induce significant new bone formation
Baba et al., 2016 [55]	Intrabony Periodontal defect. Probing depth >4 mm	The mixture of BMMSCs and PRP, combined with human thrombin dissolved in 10% calcium chloride perfused in a 3D woven-fabric composed of poly-L-lactic acid resin fibers (MSCs/PRP-3D woven Fabric)	Autologous Bone marrow harvested from posterior Iliac crestal bone	Induced under Osteogenic Medium	BMMSCs/PRP-3D woven Fabric constructs showed efficient regeneration of the periodontal tissue including alveolar bone.
Morrison et al., 2018 [50]	Cranial defects with less than 80 mm diameter	Allogeneic mesenchymal stromal cells (MSCs) on a ceramic carrier (ChronOS granules, synthes, and polymer scaffold,	Allogenic BMMSCs from 18–25 years aged donors	None	Allogeneic MSCs can be safely used for bone regeneration.
Kaigler et al., 2015 [53]	Severe Bone Atrophy of upper Jaw	Combination of BMMSCs and β-TCP (Cerasorb, Curasan AG, Germany)	Autologous Bone marrow harvested from posterior Iliac crestal bone	None	Higher density of regenerated bone with MSCs+ β-TCP group was observed than control group.
Marcacci et al., 2007 [51]	Humerus, Tibia and ulnar Critical sized defects	Combination of invitro expanded BMMSCs seeded with porous hydroxy apatite scaffolds (Finblock, FinCeramica Srl, Faenza, Italy)	Autologous Bone marrow harvested from posterior Iliac crestal bone	None	Significant healing of the CSDs. Attained long term durability of bone regeneration.
Bajada et al., 2007 [56]	Tibial non-union	Combination of invitro expanded BMMSCs seeded with calcium sulphate pellets (Stimulan,	Autologous Bone marrow harvested from	None	Clinical and radiological healing of nonunion was observed

Author	Type and Size of Defect	Transplant Groups	Origin of Cell Source	Pre-Transplant Incubation	Outcome
		Biocomposites Ltd.,	posterior Iliac		

NCT Number	Brief Title	Phase	Conditions	Interventions
NCT04297813	Efficacy in Alveolar Bone Regeneration With Autologous MSCs and Biomaterial in Comparison to Autologous Bone Grafting	Phase I	• Alveolar Bone Atrophy	Autologous MSCs and a biomaterial, biphasic Calcium Phosphate (BCP).
NCT03325504	A Comparative Study of 2 Doses of BM Autologous H-MSC+Biomaterial vs. Iliac Crest AutoGraft for Bone Healing in Non-Union	Phase III	• Non Union Fracture	Culture-expanded autologous BMMSC combined with biphasic calcium phosphate (BCP) biomaterial granules
NCT02803177	Cell Therapy by Autologous BMC for Large Bone Defect Repair	Phase II	• Humerus Fracture Displaced Proximal	Autologous Bone Marrow-derived Mononuclear Cells (BMC) seeded onto ß-TCP
NCT02307435	Allogenic Mesenchymal Stem Cell for Bone Defect or Non Union Fracture	Early Phase I	• Non Union Fracture, Metaphyseal Fibrous Defect	Allogeneic MSCs from umbilical cord/bone marrow/adipose combined and HA-CaSo4
NCT02153372	Cell Therapy by Bone Marrow-derived Mononuclear Cells (BMC) for Large Bone Defect Repair: Phase-I Clinical Trial	Phase I	• Humerus Fracture Displaced Proximal	Autologous Bone Marrow-derived Mononuclear Cells (BMC) seeded onto ß-TCP
NCT01958502	Evaluation the Treatment of Nonunion of Long Bone Fracture of Lower Extremities (Femur and Tibia) Using Mononuclear Stem Cells from the Iliac Wing Within a 3-D Tissue Engineered Scaffold	Phase II	• Nonunion of Fracture	BMMSCs with BMP2 within a 3-D tissue engineered collagen scaffold
NCT01842477	Evaluation of Efficacy and Safety of Autologous MSCs Combined to Biomaterials to Enhance Bone Healing	Phase I/II	• Delayed Union After Fracture of Humerus, Tibial or Femur	BMMScs mixed with biphasic calciulm granules
NCT00250302	Autologous Implantation of Mesenchymal Stem Cells for	Phase I/II	• Tibial Fracture	BMMSCs loaded onto a carrier and implanted locally at the defect site

NCT Number	Brief Title	Phase	Conditions	Interventions
	the Treatment of Distal Tibial Fractures			
NCT00557635	Osseous Setting Improvement With Co-implantation of Osseous Matrix and Mesenchymal Progenitors Cells From Autologous Bone Marrow	Phase II	• Tibia or Femur Pseudo-arthrosis	Injection of an osseous matrix (osteopure) combined with MSC progenitors from autologous bone marrow.
NCT02177565	Autologous Stem Cell Therapy for Fracture Non-union Healing	Not available	• Non-union of Fractures	Autologous BMSCs combined with carrier material
NCT01435434	Mononucleotide Autologous Stem Cells and Demineralized Bone Matrix in the Treatment of Non Union/Delayed Fractures	Not available	• Non Union/Delayed Fractures	Injection of Autologous Stem Cells and Demineralized Bone Matrix

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4. Osteoblast-Based Bone Tissue Regeneration

In addition to the efforts to increase the bone-forming ability of MSCs as a cell source for bone tissue engineering, the use of osteoblasts that are capable of proliferating before maturing, and that can synthesize and deposit bone extracellular matrix components such as osteocalcin (OCN) and bone sialoprotein (BSP), provides a potential alternative BTE cell source for the treatment of large bone defects. However, since BTE is generally approached using a combination of osteoblasts induced from MSCs on biodegradable scaffolds, the resulting bone forming efficacy will be dependent on the differentiation potential of MSCs into osteoblasts. This could hamper the progress of BTE for treating large bone defects. There are two major mechanisms underlying skeletal development, intramembranous and endochondral ossification. In intramembranous ossification, osteoblast lineage cells, i.e., immature osteoblasts, are formed directly from condensed mesenchymal tissue. Endochondral ossification, by contrast, involves the production of osteochondral progenitors from MSCs that give rise to hyperchondrocytes which activate perichondrial cells to differentiate into immature osteoblasts. From the perspective of BTE, the formation of immature osteoblasts is the convergence point for both types of ossification.

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5. Conclusions

Critical bone defects that cannot self-heal without a surgical intervention pose a significant challenge in the field of BTE. Compared to the traditional gold standard approach of using autogenous bone, regenerative methods will typically use either exogenous MSCs or immature osteoblasts seeded onto a bioactive scaffold placed at the defect area to regenerate functional bone. Adult MSCs from bone marrow and adipose tissue have often been used in various clinical studies for bone regeneration. The available data from both preclinical studies and clinical trials have shown promising results when BMMSCs are used as a cell source for bone tissue regeneration. Many clinical

studies have also shown the beneficial effects of MSCs administration on bone healing. Although encouraging clinical results have been obtained by transplanting MSCs-scaffold constructs, the exact dosage and route of application remain to be optimized, and the fate of transplanted cells and their mechanisms of action need to be better monitored in more extensive future clinical trials. The development of an alternative immature osteoblast source combined with more effective scaffolds is also anticipated in the future. Immature osteoblasts have the ability to become a potential alternative cell source to adult MSCs, as they are osteogenic lineage-committed cells that enhance the efficacy of bone regeneration.

Immature osteoblasts can be obtained from bone tissue samples during bone regeneration procedures from mandibular, maxillary alveolar bone or surgeries involving long bones from the femur or tibia. The advantage of immature osteoblasts over MSCs is their spontaneous matrix formation upon transplantation without promoting osteogenic differentiation. Immature osteoblasts can directly secrete bone collagen matrix and release various factors such as M-CSF, RANKL, and VEGF, enhancing bone remodeling and bone regeneration in various BTE applications. The combination of an immature osteoblast culture system that possesses robust osteogenic activity and an appropriate biodegradable scaffold is an expected future BTE therapeutic option. This approach will facilitate the establishment of better clinical protocols for regeneration therapy in cases of large bone defects, as a treatment for orthopedic conditions such as back pain resulting from stenosis and lumbar spondylolisthesis osteosarcoma, and for horizontal alveolar bone defects in the dental field.

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