Suitability and limitations of mesenchymal stem cells to elucidate human bone illness

Izaskun Mitxitorena, Arantza Infante, Blanca Gener, Clara I Rodríguez

Abstract

Functional impairment of mesenchymal stem cells (MSCs), osteoblast progenitor cells, has been proposed to be a pathological mechanism contributing to bone disorders, such as osteoporosis (the most common bone disease) and other rare inherited skeletal dysplasias. Pathological bone loss can be caused not only by an enhanced bone resorption activity but also by hampered osteogenic differentiation of MSCs. The majority of the current treatment options counteract bone loss, and therefore bone fragility by blocking bone resorption. These so-called antiresorptive treatments, in spite of being effective at reducing fracture risk, cannot be administered for extended periods due to security concerns. Therefore, there is a real need to develop osteoanabolic therapies to promote bone formation. Human MSCs emerge as a suitable tool to study the etiology of bone disorders at the cellular level as well as to be used for cell therapy purposes for bone diseases. This review will focus on the most relevant findings using human MSCs as an in vitro cell model to unravel pathological bone mechanisms and the application and outcomes of human MSCs in cell therapy clinical trials for bone disease.

Key words: Mesenchymal stem cells; Bone illness; Osteoporosis; Osteogenesis; Osteoanabolic therapies; In vitro cell models; Cell therapy

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Core tip: Human mesenchymal stem cells (hMSCs) have emerged as an encouraging therapeutic strategy for the treatment of bone diseases. Moreover, certain limitations of
animal models for the study of bone disorders highlight the suitability and benefits of hMSCs for the elucidation of these pathologies. The current review explains the available strategies based on hMSCs for bone illness, new treatment development, and future directions in the field for more accurate knowledge of the cause underlying these human pathologies.

INTRODUCTION

In humans, the structural maintenance of the skeleton during adulthood is ensured by the continuous self-regeneration of bone tissue in a process called bone remodeling. The entire skeleton is renewed approximately every 10 years by a sequentially coordinated action of two coupled processes performed in bone remodeling units at distinct locations all throughout the skeleton: Bone resorption and bone formation. Bone resorption, in which old and damaged bone is removed by osteoclasts, is a relatively fast process that can last 4-6 wk; whereas, new bone formation orchestrated by osteoblasts, which produce collagen and mineralized bone matrix, takes approximately 4-5 mo. Osteoclasts and osteoblasts are differentiated cells originating from two separate cell lineages: Osteoclasts differentiate from hematopoietic precursors, and osteoblasts are cells of mesenchymal origin. Thus, osteogenic differentiation and the generation of new osteoblasts are driven by a sequential cascade of processes performed by mesenchymal stem cells (MSCs). First by the recruitment of MSCs to bone remodeling sites and subsequent proliferation, then lineage commitment with expression of lineage-specific markers, and finally with collagen secretion and extracellular matrix (ECM) mineralization.

Bone remodeling is a continuous process throughout life; however, the balance between bone formation and bone resorption is age-dependent. Thus, bone formation predominates for the first three decades until peak bone mass occurs. Thereafter, when the growth period is complete in adulthood, there is a remodeling balance in which the previously achieved bone mass is maintained, and the amount of resorbed bone equals that which is subsequently formed. Later, in aging, the bone loss common to this period of life is due to an imbalance between bone resorption and bone formation: Accelerated osteoclastic bone resorption occurs compared to the amount of new bone formed by osteoblasts. Moreover, aged MSCs show a shift of lineage commitment to adipogenesis at the expense of osteogenesis and a concomitant reduction in self-renewal capacity. This dysfunction of MSCs, which contributes to the remodeling imbalance, lies at the root of bone loss due to aging. As a consequence, bone aging is the leading risk factor for primary osteoporosis, a progressive systemic skeletal disease characterized by a reduction in bone mineral density, predisposing the elderly population to an increased risk of fractures. In this scenario, the use of MSCs (osteoblast progenitor cells) for bone disease modeling emerges as a suitable approach to perform mechanistic studies, devise drug discovery by high throughput screenings, and test cell-based therapies. This review will focus on the current benefits and limitations of MSCs for two different goals related to bone illness: As in vitro disease models to study the pathogenic mechanisms of bone disease in order to screen and/or develop new therapeutic drugs, and as treatments based on cell therapies.

THE SOCIO-ECONOMIC IMPACT OF BONE DISEASES

Age-related osteoporosis is the most prevalent bone disease, especially among postmenopausal women and older men, affecting over 200 million worldwide and causing more than 9 million fractures per year. Improvements in socioeconomic and health-related factors have resulted in an increase in population life expectancy making osteoporosis a global and growing public health challenge. Osteoporotic fractures cause a 20% increase in mortality within 1 year of the broken bone and also result in poor quality of life, functional impairment, and loss of independence leading to further health and economic burdens.
to an increased financial burden in health care systems\textsuperscript{[8]}. In addition to osteoporosis, more than 450 skeletal dysplasias have been described that affect primarily bone and cartilage, most of them with limited treatment options\textsuperscript{[9]}. Abnormal bone formation directed by osteoblasts, abnormal bone resorption by osteoclasts, or both may be among the underlying pathological cellular mechanisms of these heritable diseases. Studying these rare genetic bone disorders is clinically highly relevant, and although individually they affect a small percentage of the population, their overall frequency is high: Two to five per 10000 live births\textsuperscript{[10]}. Importantly, many of these diseases become apparent early in life and are present throughout the patient’s entire life implying tremendous burdens in disability and suffering and requiring extensive medical and surgical treatments. Research focusing on these genetic skeletal disorders is not only beneficial for future treatment of patients but significantly contributes to the knowledge of key concepts of bone biology.

Pharmacologic therapies for osteoporosis can be categorized as either antiresorptive or anabolic; both strategies focus on reducing the risk of fractures\textsuperscript{[11]}. Current treatments are mainly based on antiresorptive agents, including estrogen, selective estrogen receptor modulator, bisphosphonates, and a monoclonal antibody to receptor activator of NF-κB ligand (RANKL) (denosumab)\textsuperscript{[12]}. These therapies decrease the generation, function, and survival of osteoclasts thereby reducing the rate of bone resorption. However, because bone resorption and formation are coupled processes, this inhibition in bone resorption also results in lower bone formation. Although antiresorptive drugs are effective in reducing fracture risk\textsuperscript{[13]}, there are concerns about side effects accompanying their continued use, such as increased cardiovascular events, increased breast cancer risk due to estrogen use\textsuperscript{[14]}, and more rare side effects, such as atypical femur fractures\textsuperscript{[15]} and osteonecrosis of the jaw\textsuperscript{[16]}. Moreover, bisphosphonates are known to accumulate in the skeleton and continue to be released for long periods of time following treatment\textsuperscript{[17]}. Given that osteoporosis is a chronic disease, treatments for osteoporosis should theoretically be administered throughout the patient’s life. However, due to the aforementioned side effects of antiresorptive drugs, they are generally not administered for more than 5 years. Taking into account both increased life expectancies and these limitations regarding the continued use of antiresorptive agents, there is an urgent need to develop new drugs for osteoporosis focused on osteoanabolic goals (to increase bone formation).

Currently, the two available anabolic drugs are teriparatide and abaloparatide, both recombinant human parathyroid hormone (PTH) analogs, which have been demonstrated to increase bone formation when given intermittently in small doses\textsuperscript{[18]}. However, there were initial concerns regarding the long-term administration of these therapies as well because extended exposure to analogs of PTH in preclinical (animal) studies was associated with a higher risk of osteosarcoma\textsuperscript{[19]}. However, a later long-term surveillance study of adult cases of osteosarcoma did not show an increased risk of osteosarcoma associated with teriparatide treatment\textsuperscript{[20]}. Overall, these observations evidence that the range of current anabolic treatment is quite limited, making it imperative to identify, characterize, and develop novel effective and safe osteoanabolic therapies.

### ADVANTAGES AND FLAWS OF ANIMAL MODELS OF BONE DISEASES

Several animal models have been developed in order to study the different molecular mechanisms underlying bone-related diseases and to serve as fundamental tools in which to test and develop new therapeutic strategies. The biggest challenge when choosing the appropriate animal model is not knowing the exact cause of the disease. Rodents are the most commonly used animal model for research, despite the fact that large animals show bone development resembling the human process more accurately than rodents\textsuperscript{[21]}. We will briefly summarize the advantages and disadvantages of different animal models used for the study of various bone diseases, and we will focus here on the success and failures of murine models to mimic different types of the bone disorder called osteogenesis imperfecta (OI).

Some rodent models successfully resemble the human characteristics of several bone-related diseases. A mouse model of Paget’s disease in which the normal bone recycling process is affected shows increased bone resorption and bone formation and increased numbers of osteoclasts that are larger and multinucleated, a finding similar to human patients suffering from this disease\textsuperscript{[22,23]}. Osteoporosis is distinguished by low bone mass and structural deterioration of bone tissue, occasioning bone fragility, and increased risk of fractures\textsuperscript{[24]}. Osteoporosis has been studied in different animal models; however, none of these models...
satisfactorily resembles the characteristics of the disease in humans\(^{[23]}\). The most extensively used model is the ovariectomized rodents (mouse or rat). This process induces a loss of bone mass and strength due to the reduction of estrogen, similar to the loss of estrogen in postmenopausal women. Despite the low costs and easy handling, rodents lack the Harvesian canal system of the cortical bone present in humans\(^{[25]}\). This is the initial animal system for identifying possible therapies. Potential drugs or treatments are subsequently replicated and tested on larger animals, such as primates, rabbits, sheep, and pigs\(^{[23,24]}\).

Hypophosphatasia (HPP), or deficiency of the alkaline phosphatase (ALP) enzyme\(^{[23]}\), has been investigated in various murine models. ALP knock-out mice have been largely used to identify mechanisms underlying the disease since affected mice adequately mimic the phenotype of children with HPP\(^{[23,24]}\).

OI is a genetic disease with high heterogeneity at both the genotypic and phenotypic levels\(^{[26-29]}\). OI patients are classified into different OI types according to their phenotype and genetic mutation causing the disease. The majority of the mutations are autosomal dominant and are located in the COL1A1 and COL1A2 genes (Type I-IV), while some less frequent mutations are recessive and located in different genes involved in the osteogenic process (IFTM5, CRTAP, LEPRE1, SERPINF1, PPIB, and FKBP10 among others)\(^{[30-32]}\). As the genetic causes of the OI phenotype are so diverse, several different models have been described for the study of the different OI types. Various models have been useful for the elucidation of OI pathology, while some models have shown effects opposite to those observed in OI patients. Here we present some of the murine models and their effectiveness in reproducing human OI phenotypes/symptoms (Table 1).

The low prevalence of certain types of OI (IX\(^{[33,34]}\), XII\(^{[35,36]}\), XIII\(^{[37,38]}\), XIV\(^{[39,40]}\), XV\(^{[41]}\), and XVI\(^{[42]}\)) makes it difficult to develop an exact diagnosis of symptoms and causes of these types of OI. Therefore, it is difficult to assess the suitability of the models even though such models could be a useful tool for gaining basic knowledge of these OI types. In contrast, several OI types have been successfully described for which the suitability of the animal models can be evaluated. Murine models for OI types I\(^{[25,26]}\), II\(^{[43]}\), III\(^{[44,45]}\), IV\(^{[46]}\), V\(^{[47]}\), VII\(^{[48-51]}\), and XI\(^{[52-54]}\) positively mimic human phenotypes. Models developed for OI type VI \(^{[55,56]}\) or atypical \(^{[57]}\), VIII\(^{[58-61]}\), IX\(^{[62]}\), and XI\(^{[63]}\) show differences in the mechanisms underlying those mutations with diverse grades of severity when compared to humans and different signaling pathways involved in the process.

Despite murine models being the most utilized animal models for the study of human bone-related diseases, mice and humans diverge at some stages of the skeletal regulatory process\(^{[27]}\). More than half of the signaling pathways and bone development-related genes are expressed in both species. These include BMP, Hedgehog, FGF, and Notch and transcriptional regulators of osteogenesis like RUNX2 and SOX9\(^{[73]}\). On the other hand, divergent genes comprise various members of the WNT signaling pathway, such as SOST, CXXC4, and deoxyribonucleic acid (DNA)\(B6^{[71]}\). This fact should be kept in mind when trying to extrapolate results from murine models to patients.

In summary, animal models are a useful and necessary tool when elucidating the molecular mechanisms underlying disease with low prevalence, but are not sufficient to properly understand the human pathophysiology of the disease.

**MSCs AS EXPERIMENTAL HUMAN DISEASE MODELS**

**In vitro bone disease modeling by primary MSCs**

The failure of some animal models to resemble the features of many human diseases led to the development of a field focused on the creation of *in vitro* cell models using primary cells isolated from patients and healthy cohorts. These disease-relevant cell types recapitulate the majority of the pathological phenotypes observed in patients, providing new opportunities to study the cell biology and pathophysiology of the disease.

An example of such models focusing on prematurely aging cells is based on either human MSCs (hMSCs)\(^{[67]}\) or induced pluripotent stem cells (iPSCs)\(^{[68-70]}\). MSCs are characterized by multipotency, self-renewal capacity, and the ability to differentiate into different cell lineages, *e.g.*, an osteogenic lineage\(^{[71-73]}\). The osteogenic potential of MSCs has been demonstrated in MSCs expanded culture\(^{[70]}\) making them a perfect cell type for the study of molecular mechanisms regulating bone disorders, especially those disorders caused by osteoblast alterations\(^{[74-76]}\). Thus, MSCs, which are the context-related cell type for modeling diseases with mesenchymal defects, have emerged as an essential tool to unravel the molecular and cellular mechanisms.
| OI type | Mutations at gene | Human phenotype | Ref. | Mouse model | Mouse phenotype | Effectiveness | Ref. |
|---------|------------------|-----------------|------|-------------|----------------|--------------|------|
| I       | COL1A1/2         | α1 chain collagen haplo-insufficiency; vertebral compression fractures; short height; low lumbar spine bone mineral density | [32,33] | Col1a1+/Mov13 | Decreased type I collagen in mineralized tissue, weakened bone strength; abnormal shape of long bones; alterations of the mechanical properties of long bones | + | [44-46] |
| II      | COL1A1/2         | Perinatal lethal | [32,33] | BrtlII; Aga2/b | Perinatal lethal | + | [44,47] |
| III     | COL1A1/2         | High bone turnover; decreased mineralization; increased osteoclastic activity; small size; fractures; osteopenia; bone deformities | [32,33] | COL1A2 KO | Increased bone formation rate; fractures; reduced size; osteopenia; decreased mineralization; abnormal bone shape | + | [44,48-51] |
| IV      | COL1A1/2         | Increased bone fragility; growth deficiency; weak bone geometry; impaired bone remodeling; decreased bone volume | [32,33] | 349G->C COL1A1 | Decreases in severity with age; increased bone brittleness; reduced bone size; abnormal bone shape; impaired bone remodeling | + | [52-56] |
| V       | IFITM5           | Increased mineralization; increased osteoblast markers; decreased COL1A1 expression, secretion and deposition in the matrix; hyperplastic callus; calcification of the forearm interosseous membrane; radial-head dislocation; subphyseal metaphyseal radiodense band | [32,33] | 14C->T IFITM5 | Severe skeletal defects; perinatal lethality; decreased mineralization; reduced expression of osteoblast markers | - | [64-66] |
| VI      | Atypical IFITM5  | Decreased levels of PEDF; decreased mineralization | [32,33] | IFITM5 Knock-Down | Reduced skeletal size less extreme in adults; no abnormal osteoclastogenesis; no abnormal osteoblastogenesis | - | [67] |
| VI      | SERPINF1         | Decreased mineralization; decreased trabecular bone | [32,33] | PEDF KO | Decreased ECM mineralization; reduced trabecular bone volume | + | [57] |
| VII  | CRTAP | Growth delay; osteopenia; decreased bone formation; decreased mineralization; multiple fractures | CRTAP KO | Growth underdevelopment; osteopenia; decreased osteoblastogenesis; decreased mineralization; no spontaneous fractures |
|------|-------|-------------------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------|
| VIII | LEPRE1 | Lethal; severe growth deficiency; bone fragility; poorly mineralized skull; scoliosis; decreased mineralization | LEPRE1 Knock-Down | No lethality; abnormal collagen fibril ultrastructure in bone, tendon and skin |
| IX   | PPIB  | Lethal; severe bone mass reduction; extreme bone strength reduction | PPIB KO | Bone mass reduction; bone strength reduction |
| X    | SERPINH1 | Embryonic lethality; delayed type I collagen secretion; collagen accumulation in Golgi apparatus; osteopenia; dentinogenesis imperfecta; thin bones | HSP47 KO | Delayed type I collagen secretion; collagen accumulation in the endoplasmic reticulum |
| XI   | FKBP10 | Growth delay; neonatal lethality; bone fragility | FKBP10 KO | Bone brittleness; underdeveloped growth; lethality |
| XII  | OSX   | Skeletal deformities; fractures; osteoporosis | Onx KO | No bone formation; decreased mineralization |
| XIII | BMP1  | Skull defects; reduced bone mass; reduced bone strength | BMP1 KO | Reduced ossification of certain skull bones |
| XIV  | Tric-b | Reduced bone mass | Tric-b | No incorporation of collagen in the matrix; matrix insufficiency |
| XV   | WNT1  | Reduced bone mass; reduced bone strength; fractures; increased ductility | sw/sw | Bone fragility; low bone mass |
| XVI  | CREB3L1 | Reduced bone mass and fractures | CREB3L1 KO | Severe osteopenia; reduced type I collagen |

*+/- stand for positive mimicry of the OI type symptoms in humans (+) or negative mimicry of OI type symptoms in humans (-). OI: Osteogenesis Imperfecta; KO: Knock-out; ECM: Extracellular matrix.*

Physiological aging is known to be accompanied by a switch of MSCs differentiation to the adipogenic lineage at the expense of osteogenesis, which leads to osteoporosis. MSCs used as in vitro disease models of aging have been essential to elucidate various mechanisms that account for the osteogenic differentiation impairment exhibited in the context of aging, such as dysregulation of transcription factors and microRNAs, autophagy impairment, alterations of the nuclear lamina, and epigenetic modifications of DNA.

MSCs isolated from patients with particular bone disorders have also been essential in deciphering the underlying molecular mechanisms of the associated bone diseases.
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HPP: MSCs isolated from pediatric patients suffering from HPP showed a premature entry into senescence and a differentiation switch to adipogenesis at the expense of osteogenesis, both of which are typical features of aging MSCs. These results indicated that the ALPL gene contributes to controlling MSC lineage differentiation and prevents cell senescence[83].

Hutchinson-Gilford progeria syndrome (HGPS): Also known as progeria, is a devastating rare genetic disorder characterized by dramatic premature aging in children, and the disease primarily affects tissues of mesenchymal origin[84]. Skeletal defects are among the HGPS phenotypes, including abnormalities in bone morphology and alterations in bone structure, which result in a unique skeletal dysplasia[85]. MSCs differentiated from patients iPSCs recapitulate some aspects of the syndrome, including abnormal nuclear architecture, progerin expression, defects in the DNA repair process, and premature differentiation into the osteoblast lineage[84].

Recently, two simultaneous works based on a high throughput drug screening in progeria-MSCs showed the usefulness of this cell model to decipher the functional effects of drugs that are currently used in HGPS patients and to identify new potential pharmacological drugs to treat the disease[86]. Both works evaluated the capacity of already known and new screened drugs to restore the impaired osteoblastic differentiation exhibited by progeria-MSCs. Moreover, paracrine signaling appears to be impaired in aged MSCs, a hypothesis supported by results in which an in vitro aged hMSCs model has a secretome enriched in osteogenesis-related proteins that can trigger accelerated early osteogenesis in normal MSCs[87]. Among the increased secreted factors, insulin-like growth factor binding protein 7 (IGFBP7) was identified. Subsequent experiments silencing IGFBP7 expression revealed an essential and unknown role for IGFBP7 to maintain the viability of MSCs during the first steps of osteogenesis in which MSCs and pre-osteoblasts proliferate actively. Moreover, sheets of hMSCs overexpressing IGFBP7 improved bone healing in a rat tibial osteotomy model[86].

2D versus 3D culture of MSCs

Although experimental modeling of human bone disorders represents a breakthrough to provide outstanding insight into the cellular and molecular mechanisms involved in bone pathologies, there are several drawbacks regarding the use of MSCs models that must be taken into account. The limited availability and extreme heterogeneity of MSCs from patients as well as limited proliferation capacity and loss of functionality are among the most common pitfalls when using MSCs in vitro.

In addition, the main cell culture approach used in research is 2D cell cultures in which cells adhere to the culture dish forming monolayers, a situation that does not reflect the in vivo cellular display where cells grow in a complex 3D disposition[89]. The conditions of the natural environment in vivo are poorly mimicked by 2D cell cultures since they do not preserve normal physiological shape and function. In other words, the morphology and physiology of 2D cultured cells highly diverge from in vivo grown cells[89,90]. Due to the complex architecture of bone tissue, the use of 2D cell cultures does not adequately mimic the actual mechanisms involved in bone tissue development and repair[90] making it a limited approach to the study of bone-related diseases. Furthermore, bone remodeling consists of a highly regulated balance between bone resorption and bone formation mediated by osteoclasts and osteoblasts, respectively. Osteoclasts are phagocytic cells derived from circulating macrophages in charge of bone degradation while osteoblasts differentiate from MSCs and are in charge of bone formation[91,92]. Osteoblast and osteoclast activity is tightly coupled positively influencing the osteogenic differentiation and matrix deposition in the same way as osteoclast development[91,92]. Therefore, osteoblast and osteoclast activity is directly regulated by the crosstalk between both cell types leading to an increased matrix deposition in osteoblast and osteoclast co-culture experiments[91,92]. Moreover, conventional 2D cultures have limited cell-cell and cell-matrix interactions, which are especially relevant in bone tissue such as the direct crosstalk between osteoblasts and osteoclasts, highlighting the need for more realistic 3D in vitro models of bone disease[89]. 3D cultures have been proposed as a bridge between 2D cell cultures and in vivo models, and therefore have been used in the study of bone diseases[90] as a consequence of their higher structural complexity and cellular homeostasis, which is more closely comparable to that of tissues and organs[89].

Due to the importance of the ECM in bone microarchitecture, a wide range of scaffolds have been developed for 3D culture of bone tissue[90]. The purpose of these scaffolds is to serve as extracellular support for adhesion of growing cells in a 3D structure[90]. Scaffolds used for bone tissue culture can be formed by different materials, such as collagen, bioceramics, titanium, gelatin, chitosan, polymers, hydrogels, and others[91-94]. The ideal scaffold should have similar mechanical
MSCs AS THERAPEUTIC TOOLS FOR BONE DISEASES

MSCs are fibroblast-like cells that exist in almost all tissues, including bone marrow, fat, and the umbilical cord among others. They comprise a heterogeneous population of cells with differentiation and self-renewal ability ensuring a replacement mechanism for cells that die due to normal aging, injury, or disease\[110\]. Three criteria were proposed by the International Society for Cellular Therapy to define hMSCs as a cell type: (1) Plastic adherence when grown in standard culture conditions; (2) Expression of the cell surface molecules CD73, CD90, and CD105 and lack expression of hematopoietic markers CD34, CD45, CD14, CD19, CD11b, and HLA-DR; and (3) Expression of the cell surface markers CD34, CD45, CD14, CD19, CD11b, and HLA-DR. Several studies have described the possibility of 3D-bioprinted bone substitutes for tissue regeneration\[102-105\]. In these studies, osteogenic differentiation of MSCs is possible allowing successful bone repair processes in vitro and in vivo\[102,106,107\]. Vascularization of the tissue is a crucial limitation\[107\]. Bioprinting of MSCs along with a functionalized vascular endothelial growth factor allows vascularization of the tissue leading to a successful proliferation, differentiation, and generation of the mineralized ECM in vitro\[108\].

The classical methods for osteogenic differentiation of MSCs in culture are based on the addition of chemical and growth factors although environmental properties influence the in vitro osteogenic differentiation of MSCs. Osteogenic differentiation of 3D-bioprinted MSCs could be performed by the classical addition of chemical and growth factors or by the use of the appropriate bioink containing these stimuli. In fact, environmental effects can be mimicked through 3D bioprinting by the addition of soluble factors and additives into the chosen bioink\[109\]. Accessibility of nutrients and osteogenic stimuli are problems recognized in 3D cultures on scaffolds. Thus, stimulation of the cells through components of the bioink allows for a homogeneous distribution of the osteogenic stimulus reaching all the seeded cells\[109\].

On the other hand, 3D co-cultures of osteoblasts and osteoclasts have been described in which cells are able to deposit mineral matrix\[91,92,103\]. Most of the literature describing 3D co-cultures of bone cells is based on human and murine cell lines, which are barely exportable to human primary cells lines. However, recently a 3D co-culture system has been successfully described using patient MSCs for the study of jawbone osteonecrosis\[110\], which would be exportable to other bone-related diseases. This system means advancement in the elucidation of the pathogenic mechanisms and the discovery of novel therapeutic strategies for the treatment of bone-related diseases\[110\].
On account of the aforementioned features of MSCs, they are thought to be ideal candidates for cell therapy purposes. However, from a clinical point of view, it must be considered that MSCs show huge variability in terms of functional capacities depending on different factors: Donors, tissue sources, clonal subpopulations, and even at the single-cell level\textsuperscript{[114]}. When focusing on bone diseases, it would be recommended to screen those MSCs with a higher osteogenic potential to enhance the efficacy of cell therapy applications. With this regard, a striking paper recently described the identification of a human skeletal stem cell population that gave rise to the progenitors of bone and cartilage by the differential expression of four surface markers: PDGN, CD146, CD73 and CD164\textsuperscript{[9]}.

Importantly, these human skeletal stem cells were also shown to be locally amplified in response to skeletal injury. We anticipate that further characterization, isolation and amplification of human skeletal stem cells would be of special interest to obtain better outcomes in the treatment of skeletal disorders by future cell therapy approaches\textsuperscript{[2]}.

For most clinical indications, hMSCs are administered intravenously despite a post-infusion febrile reaction, which is a unique adverse effect associated with their use\textsuperscript{[115]}. It was initially thought that, upon administration, the cells would home to the sites of injury, engraft, and differentiate into functional cells and then replace affected tissues. However, after administration, especially if they are systemically infused, MSCs engraftment levels are low, and their numbers decrease rapidly with time. The greater cell size of MSCs relative to the pulmonary microvasculature causes the vast majority of infused MSCs to be transiently trapped in the lungs upon the first pass through the circulation; the cells then become undetectable within hours\textsuperscript{[116]}. This low survival and homing capacity of exogenous MSCs after administration raised the question of the underlying mechanisms responsible for the reported therapeutic benefits of MSCs therapy. Currently, there is growing evidence suggesting that the beneficial effects of MSCs come mainly from their paracrine properties. MSCs are known to secrete a wide range of bioactive factors and extracellular vesicles (exosomes and/or microvesicles) containing proteins, microRNAs, and hormones in response to the local environment, which affects the biology of nearby and distant responder cells and tissues\textsuperscript{[117]}. Whether the observed beneficial effects of MSCs infusions are directly induced by their secreted factors, or if these factors initiate a cascade of signaling in the resident cell population, which then perform tissue repair, is currently under intense investigation.

**MSCs-based therapies for skeletal dysplasias**

MSCs infusion has already been tested in clinical trials for two types of skeletal dysplasias, OI and HPP.

OI, or brittle bone disease, is a highly heterogeneous group of genetic disorders mainly caused by autosomal dominant mutations in one of the two genes (COL1A1 or COLIA2) that encode type I collagen. These mutations can affect collagen structure (more severe phenotypes) or collagen quantity (milder phenotypes)\textsuperscript{[118]}. In addition, severe additional non-collagenous genes have been described recently that cause severe forms of OI, including genes involved in post-translational modification, bone matrix mineralization, and osteoblast differentiation and function\textsuperscript{[12]}. At this time, there is no cure for OI, and current treatments are focused on inhibiting bone resorption in these patients thus preventing bone loss.

The first proof of principle with allogenic MSCs infusions in the context of human OI was performed in 2002 by Horwitz et al\textsuperscript{[119]}. They based their approach on a previous preclinical study that showed successful MSC engraftment into a murine model of OI, which produced a small but appreciable improvement in the disease phenotype\textsuperscript{[24]}. Horwitz’s study included six children, who had received bone marrow transplantation in a previous clinical trial that were given two infusions of adult MSCs. Although MSC engraftment was minimal (<1% in osteoblasts), an increase in linear growth velocities was observed. Thus, it was established that allogeneic MSC infusion was not only safe in those pediatric patients affected by OI but also resulted in an increase in growth velocity albeit for a limited period of time\textsuperscript{[199]}. A later investigation from this group of children indicated that the observed benefits could not be attributed to the direct differentiation of surviving infused MSCs into osteoblasts. The authors showed that infusion of MSCs conditioned medium in a mouse model stimulated chondrocyte proliferation suggesting that the secreted factors from MSCs could be responsible for the observed benefits in patients\textsuperscript{[12]}.

Gotherstrom and collaborators demonstrated the safety and efficacy of prenatal transplantation of human fetal MSCs in two fetuses affected by OI, with the premise that the administration of MSCs before birth would be more effective in alleviating OI symptoms\textsuperscript{[22]}. However, both studies showed that the benefits from a single transplant of MSCs, regardless of the stage of life at administration, are transient, and subsequent infusions with the same donor-MSCs are needed to maintain the
beneficial effects.

HPP is a rare metabolic disorder resulting from a loss-of-function mutation in the ALPL gene that codes for the tissue-nonspecific ALP (TNSALP). There is no curative therapy for the disorder\[123\]. Impaired function of TNSALP leads to increased concentration of inorganic pyrophosphate in bone ECM; the deposition of this pyrophosphate hampers mineralization of bone and teeth and leads to pathological fractures. Due to the fact that current therapies for HPP have shown limited clinical improvements, hMSCs transplantation offers an attractive therapeutic option for these patients since MSCs, as well as osteoblasts, express high levels of TNSALP in their cell membrane, where it functions as an ectoenzyme\[124\].

Two studies have been carried out in which hMSC therapy has been administered to children suffering HPP showing improvements in bone mineralization in patients. In both of these studies, an hMSC infusion was given after previous transplantation of allogeneic bone marrow\[125\]. Moreover, chimerism analysis of the ALPL gene in the latest study revealed both the expression of wild type and mutant ALPL gene products suggesting that donor-derived MSCs were engrafted\[126\].

**MSCs-based therapies for delayed fracture healing**

Non-unions are complications that imply a permanent failure of healing 6 mo after the fracture\[127\]. *In vitro* studies showed a decreased functionality of the pool of hMSCs in patients affected by nonunions likely due to a decreased serum expression level of chemokines and growth factors required for their recruitment and proliferation\[128\]. However, there was no impairment in the osteogenic capacity of these hMSCs once they were committed to osteogenic differentiation. Taking into account these previous results, a very recent prospective study described the treatment of fracture non-unions in patients with autologous culture expanded bone marrow-derived MSCs. A total of 35 patients received cell therapy, and fracture union was observed in 21 patients. Interestingly MSCs doubling time as well as age, diabetes, and multiple surgeries arose as significant predictors for the outcome of fracture unions\[129\].

**Cell-free therapies based on the secretome of MSCs**

A concentrated secretome of MSCs, *i.e.* the paracrine factors secreted by MSCs mixed with beta-tricalcium phosphate scaffold have been used as a treatment in a recent clinical study for alveolar bone regeneration with encouraging outcomes. In this clinical study, authors showed an enhancement in vascularization, and early bone formation in patients treated with grafts impregnated with MSCs conditioned medium when compared to control patients, which were treated only with beta-tricalcium phosphate scaffolds. Moreover, the presence of MSCs conditioned medium shortened the time needed for degradation and replacement of beta-tricalcium phosphate scaffolds\[130\].

**CONCLUSION**

In summary, primary MSCs isolated from patients in comparison with established cell lines efficiently resemble the pathological mechanisms of bone disease *in vivo*. Secondly, co-cultures offer a greater opportunity to mimic the *in vivo* intercellular crosstalk occurring in patients affected by bone diseases. Lastly, 2D cultures are easier to handle but are quite limited in mimicking the 3D architecture of bone *in vivo*; therefore, 3D cultures are more appropriate to resemble the *in vivo* cellular phenotype in the pathological conditions.

Moreover, MSCs are demonstrating their potential as human experimental models, as essential tools to develop new pharmacological and cell-based treatment strategies, and specifically as a therapeutic modality for bone disorders. Still, there are many questions to be elucidated regarding MSCs therapeutic effects and action mode on human pathologies. A better characterization of the pro-osteogenic MSCs will enable the development of more efficient cell therapies focused on the skeletal disorder.

The advances in using MSCs for therapeutic purposes indicate the extreme relevance of MSC in addressing bone disorders, and the unanswered challenges also suggest many opportunities for further research in this intensive field.

**REFERENCES**

1. Manolagas SC, Parfitt AM. What old means to bone. *Trends Endocrinol Metab* 2010; 21: 369-374 [PMID: 20223679 DOI: 10.1016/j.tem.2010.01.010]
2. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. *Lancet Diabetes Endocrinol* 2017; 5: 908-923 [PMID: 28889768 DOI: 10.1016/S2213-8587(17)30184-5]
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3 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 2009; 27: 1887-1898 [PMID: 19544445 DOI: 10.1002/stem.103]

4 Berger C, Goltzman D, Langstroth L, Joseph L, Jackson S, Kreiger N, Tenenhouse A, Davison KS, Josse RG, Prior JC, Hanley DA; CaMos Research Group. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. J Bone Miner Res 2010; 25: 1948-1957 [PMID: 20499378 DOI: 10.1001/jbmr.2010.95]

5 Coïpeau P, Rosset P, Langonne A, Gaillard J, Delorme B, Rico A, Domenech J, Charpont P, Sensebe L. Impaired differentiation potential of human trabecular bone mesenchymal stromal cells from elderly patients. Cytotherapy 2009; 11: 584-594 [PMID: 19626496 DOI: 10.1016/j.cytog.2009.07.038]

6 Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 2003; 33: 919-926 [PMID: 14678851 DOI: 10.1016/j.bone.2003.07.005]

7 Register JY, Burlet N. Osteoporosis: a still increasing prevalence. Bone 2006; 38: S4-S9 [PMID: 16455317 DOI: 10.1016/j.bone.2005.11.024]

8 Pham HM, Nguyen SC, Ho-Le TP, Center JR, Eismann JA, Nguyen TV. Association of Muscle Weakness With Post-Fracture Mortality in Older Men and Women: A 25-Year Prospective Study. J Bone Miner Res 2017; 32: 698-707 [PMID: 27962226 DOI: 10.1002/jbmr.3037]

9 Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, Mortlock D, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Expert Panel. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A 2011; 155A: 943-968 [PMID: 21438135 DOI: 10.1002/ajmg.a.33999]

10 Krakow D, Lachman RS, Rimoin DL. Guidelines for the prenatal diagnosis of fetal skeletal dysplasias. Genet Med 2009; 11: 127-133 [PMID: 19265753 DOI: 10.1016/j.gene.2009.05.001]

11 Cosman F. Anabolic and antiresorptive therapy for osteoporosis: combination and sequential approaches. Curr Osteoporos Rep 2012; 14: 385-395 [PMID: 23541476 DOI: 10.1007/s11914-014-0237-9]

12 Khosla S, Hofbauer LC. Osteoporosis treatment: recent developments and ongoing challenges. Lancet Diabetes Endocrinol 2017; 5: 898-907 [PMID: 28513207 DOI: 10.1016/S2215-1779(17)30082-2]

13 Eriksson EF, Diaz-Perez A, Boonen S. Update on long-term treatment with bisphosphonates for postmenopausal osteoporosis: a systematic review. Bone 2014; 58: 126-135 [PMID: 24120384 DOI: 10.1016/j.bone.2013.09.023]

14 Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002; 288: 321-333 [PMID: 12117397]

15 Shane E, Burr D, Abrahamson B, Adler RA, Brown TD, Cheung AM, Cosman F, Curtis JR, Dell R, Dempster DW, Ebeling PR, Einhorn TA, Genant HK, Greenspan A, Recker R, Rennard S, Rubin G, Slatopolsky E, Smolen JS, Slatopolsky E, Smolen JS, Slatopolsky E, Smolen JS. Effect of estradiol and bone density in postmenopausal women with osteoporosis. N Engl J Med 2001; 344: 1434-1441 [PMID: 11348608 DOI: 10.1056/NEJM200105103441904]

16 Vahle JL, Long GG, Sandusky G, Westmore M, Ma YL, Sato M. Bone neoplasms in F344 rats given 1,25(OH)2D3 and 19-nortestosterone: a 2-year study. Bone 2006; 38: S4-S9 [PMID: 15204966 DOI: 10.1016/S8756-3282(05)00927-0]

17 Papapoulos SE, Cremers SC. Prolonged bisphosphonate release after treatment in children. N Engl J Med 2007; 356: 1075-1076 [PMID: 17347467 DOI: 10.1056/NEJMoa062792]

18 Neer RM, Arnaud CD, Zanchetta JR, Ebeling PR, Eriksson EF, Ish-Shalom S, Genant HK, Wang Q, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 2001; 344: 1434-1441 [PMID: 11348608 DOI: 10.1056/NEJM200105103441904]

19 Vahlle JL, Long GG, Sandusky G, Westmore M, Ma YL, Sato M. Bone neoplasms in F344 rats given 1,25(OH)2D3 and 19-nortestosterone: a 2-year study. Bone 2006; 38: 126-135 [PMID: 24120384 DOI: 10.1016/j.bone.2013.09.023]

20 Andrews EB, Gilsenan AW, Midkiff K, Sherrill B, Wu Y, Mann BH, Masica D. The US postmarketing surveillance study of adult osteosarcoma and paraneoplastic syndromes: study design and findings from the first 7 years. J Bone Miner Res 2012; 27: 2429-2437 [PMID: 22991313 DOI: 10.1002/jbmr.1768]

21 Paukte C, Kreuzer K, Weitz J, Knödler M, Müntel D, Wessel G, Otto S, Hafipelmier A, Stürenbaum S, Tischer T. Bisphosphonate related osteonecrosis of the jaw: A minipig large animal model. Bone 2012; 51: 592-599 [PMID: 22575441 DOI: 10.1016/j.bone.2012.04.020]

22 Cundy T. Paget's disease of bone. Metabolism 2018; 80: 5-14 [PMID: 28780255 DOI: 10.1016/j.metabol.2017.06.010]

23 Daroszewska A, van ’t Hof RJ, Rajas JA, Layfield R, Landaan-Baasonga E, Rose L, Rose K, Ralston SH. A point mutation in the ubiquitin-associated domain of SQSTM1 is sufficient to cause a Paget’s disease-like disorder in mice. Hum Mol Genet 2011; 20: 2734-2744 [PMID: 21515589 DOI: 10.1093/hmg/ddr172]

24 Yedavalli-Yellayi S, Ho AM, PatilPuringham EM. Update on Osteoporosis. Prim Care 2019; 46: 175-190 [PMID: 30704657 DOI: 10.1016/j.pop.2018.10.014]

25 Paschalis EP, Gamsjäger S, Condon K, Klaushofer K, Burr D. Estrogen depletion alters mineralization regulation mechanisms in an ovariectomized monkey animal model. Bone 2019; 120: 279-284 [PMID: 30414509 DOI: 10.1016/j.bone.2018.11.004]

26 Merlotti D, Materozzi M, Picchioni T, Biancardi S, Alessandri M, Nuti R, Gennari L. Recent advances in models for screening potential osteoporosis drugs. Expert Opin Drug Discov 2018; 13: 741-752 [PMID: 29869573 DOI: 10.1080/17460441.2018.1480699]

27 Mornet E. Hypophosphatemia. Metabolism 2018; 82: 142-155 [PMID: 28939177 DOI: 10.1016/j.metabol.2017.08.013]

28 Liu J, Nam HK, Campbell C, Gasque KC, Millán JL, Hatch NE. Tissue-nonspecific alkaline phosphatase deficiency causes abnormal craniofacial bone development in the Alp(-/-) mouse model of infantile hypophosphatemia. Bone 2014; 67: 81-94 [PMID: 25034884 DOI: 10.1016/j.bone.2014.06.040]
Sinder BP, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM. Rapidly growing Brtl/+ mouse model of osteogenesis imperfecta demonstrates anabolic response to sclerostin antibody treatment with increased bone mass and strength. J Osteoporos Int 2014; 25: 1717-1727 [PMID: 24968150 DOI: 10.1007/s00198-014-2737-y]

Marini JC, Forlino A, Blücher HG, Bishop NJ, Byers PH, Paape A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. Nat Rev Dis Primers 2017; 3: 17052 [PMID: 28820180 DOI: 10.1038/nrd.2017.52]

Morello R. Osteogenesis imperfecta and therapeutics. Matrix Biol 2018; 71-72: 294-312 [PMID: 29540309 DOI: 10.1016/j.matbio.2018.03.010]

Choi JW, Sutor SL, Lindquist L, Evans GL, Madden BI, Bergen HR, Heffleran TE, Yasenzemki MJ, Bram RJ. Severe osteogenesis imperfecta in cyclophilin B-deficient mice. PLoS Genet 2009; 5: e0007570 [PMID: 19997487 DOI: 10.1371/journal.pgen.1000750]

Cabral WA, Perdívora I, Weis M, Terajima M, Blissett AR, Chang W, Perosky JE, Makareeva EN, Mertz EL, Leikin S, Tomer KB, Kozloff KM, Eyre DR, Yamauchi M, Marini JC. Abnormal type I collagen post-translational modification and crosslinking in a cyclophilin B KO mouse model of recessive osteogenesis imperfecta. PLoS Genet 2016; 12: e1006156 [PMID: 27441836 DOI: 10.1371/journal.pgen.1006156]

Zhou X, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 109: 17-29 [PMID: 11792318 DOI: 10.1016/S0092-8674(01)00622-5]

Elhossini R, Amr K, Vilaboa N, Ruiz-Perez VL. Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. Am J Hum Genet 2010; 87: 110-114 [PMID: 20796260 DOI: 10.1016/j.ajhg.2010.05.016]

Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 109: 17-29 [PMID: 11792318 DOI: 10.1016/S0092-8674(01)00622-5]

Suzuki N, Labosky PA, Futura Y, Hargett L, Dunn R, Fogo AB, Takahara K, Peters DM, Greenspan DS, Hogan BL. Failure of ventral body wall closure in mouse embryos lacking the intracellular cation channel TMEM38B/TRIC-B. J Cell Sci 2006; 119: 3587-3595 [PMID: 16884723 DOI: 10.1212/jcm.aa005]

Cabral WA, Ishikawa M, Gurtman M, Makareeva EN, Sargent BM, Weis M, Barnes AM, Webb EA, Shaw NJ, Ala-Kokko L, Lachewian PL, Högner W, Leikin S, Blank Y, Yamam S, Marini JC, Yamauchi M, Marini JC. Absence of the ER Cation Channel TMEM38B/TRIC-B Impairs Intracellular Calcium Homeostasis and Dys regulates Collagen Synthesis in Recessive Osteogenesis Imperfecta. JBone Miner Metab 2009; 27: 211-218 [PMID: 17593490 DOI: 10.1007/s00774-007-0750-3]

Zhao C, Li H, Yoneda T, Kou I, Furuichi T, Ikegawa S, Ikawa M, Okabe M, Okawa R, Carleton SM, Kettle AD, Melander J, Phillips CL, Wang Y. Gender-dependence of bone structure and properties in adult osteogenesis imperfecta murine model. Ann Biomed Eng 2008; 36: 4035-4042 [PMID: 18934583 DOI: 10.1007/hmp-012-9969-x]

Hartung S, Jaenisch R, Breindl M. Retrovirus insertion inactivates mouse alpha 1(I) collagen gene by blocking initiation of transcription. Nature 1994; 365: 367-367 [PMID: 18109211 DOI: 10.1016/S0092-8674(01)00622-5]

Kang H, Aryan AC, S, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone development. Transl Res 2017; 181: 27-48 [PMID: 27914223 DOI: 10.1016/j.trsl.2016.11.005]

Kang H, Aryan AC, S, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone development. Transl Res 2017; 181: 27-48 [PMID: 27914223 DOI: 10.1016/j.trsl.2016.11.005]

Yoshinaga K, Saitoh M, Nishimura R, Yoneda T, Kou I, Furuichi T, Ikegawa S, Ikawa M, Okabe M, Okawa R, Carleton SM, Kettle AD, Melander J, Phillips CL, Wang Y. Gender-dependence of bone structure and properties in adult osteogenesis imperfecta murine model. Ann Biomed Eng 2008; 36: 4035-4042 [PMID: 18934583 DOI: 10.1007/hmp-012-9969-x]

Hartung S, Jaenisch R, Breindl M. Retrovirus insertion inactivates mouse alpha 1(I) collagen gene by blocking initiation of transcription. Nature 1994; 365: 367-367 [PMID: 18109211 DOI: 10.1016/S0092-8674(01)00622-5]

Kang H, Aryan AC, S, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone development. Transl Res 2017; 181: 27-48 [PMID: 27914223 DOI: 10.1016/j.trsl.2016.11.005]

Kang H, Aryan AC, S, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone development. Transl Res 2017; 181: 27-48 [PMID: 27914223 DOI: 10.1016/j.trsl.2016.11.005]
Mitoxitorena I et al. MSCs applications for bone disease

Bone 2015; 71: 115-123 [PMID: 25445450 DOI: 10.1016/j.bone.2014.10.012]

Uveges TE, Collin-Osdoby P, Cabral WA, Legard G, Goldberg I, Bergwitz C, Forlino A, Osdoby P, Gromowicz G, Marin J. Cellular mechanism of decreased bone in Brtl mouse model of OI: imbalance of decreased osteoblast function and increased osteoclasts and their precursors. J Bone Miner Res 2008; 23: 1983-1994 [PMID: 18684089 DOI: 10.1359/jbmr.080804]

Forlino A, Porter FD, Lee EJ, Westphal H, Marin J. Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an alpha(I) G349C substitution. Variability in phenotype in BrtlIV mice. J Biol Chem 1999; 274: 37923-37931 [PMID: 10608859 DOI: 10.1074/jbc.274.53.37923]

Kozloff KM, Carden A, Bergwitz C, Forlino A, Uveges TE, Morris MD, Marin J, Goldstein SA. Brittle IV mouse model for osteogenesis imperfecta IV demonstrates postpubertal adaptations to improve whole bone strength. J Bone Miner Res 2004; 19: 614-622 [PMID: 15005849 DOI: 10.1359/JBMR.040111]

Bogan R, Riddle RC, Li Z, Kumar S, Nandula A, Faugere MC, Boskey A, Crawford SE, Clemens T. A mouse model for human osteogenesis imperfecta type VI. J Bone Miner Res 2013; 28: 1531-1536 [PMID: 23431416 DOI: 10.1002/jbmr.1892]

Bi X, Grafe I, Ding H, Flores R, Munivez E, Jiang MM, Dawson B, Lee B, Ambrose CG. Correlations Between Bone Mechanical Properties and Bone Composition Parameters in Mouse Models of Dominant and Recessive Osteogenesis Imperfecta and the Response to Anti-TGF-β Treatment. J Bone Miner Res 2017; 32: 347-359 [PMID: 27649409 DOI: 10.1002/jbmr.2997]

Fratzl-Zelman N, Morello R, Lee B, Rauch F, Glorieux FH, Misof BM, Klaushofer K, Roschger P. CRTC deficiency leads to abnormally high bone matrix mineralization in a murine model and in children with osteogenesis imperfecta type VII. Bone 2010; 46: 820-826 [PMID: 19895918 DOI: 10.1016/j.bone.2009.10.037]

Baldridge D, Schwarze U, Morello R, Lenington J, Bertin TK, Pacie JPM, Mepin MG, Weis M, Eyre DR, Walsh J, Lambert D, Green A, Robinson H, Michelson M, Hougé G, Lindman C, Martin J, Ward J, Lemyre E, Mitchell JJ, Krakow D, Rimmol DL, Cohn DH, Byers PH, Lee B. CRTC and LEPRE1 mutations in recessive osteogenesis imperfecta. Hum Mutat 2008; 29: 1435-1442 [PMID: 18566967 DOI: 10.1002/humu.20799]

Tauer JT, Abdullah S, Rauch F. Effect of Anti-TGF-β Treatment in a Mouse Model of Severe Osteogenesis Imperfecta. J Bone Miner Res 2019; 34: 207-214 [PMID: 30357929 DOI: 10.1002/jbmr.3617]

Lietman CD, Rajagopal A, Hornan EP, Munivez E, Jiang MM, Bertin TK, Chen Y, Hicks J, Weis M, Eyre D, Lee B, Krakow D. Connective tissue alterations in FlpN1-/- mice. Mol Hum Genet 2014; 23: 4822-4831 [PMID: 24777810 DOI: 10.1038/hmgdda197]

Schwarze U, Cundy T, Pyott SM, Christiansen HE, Hegde MR, Bank RA, Pals G, Ankala A, Conneely K, Seaver L, Yandow SM, Raneey E, Babovic-Vuckovac D, Stoler J, Ben-Neriah Z, Segel R, Lieberman S, Siderius L, Al-Aqel A, Hannah M, Hudgins L, McPherson E, Clemens M, Sussman MD, Steiner RD, Mahan J, Smith R, Anyane-Yeboa K, Wynn J, Chong K, Uster T, Affifos S, Sutton VR, Davis EC, Kim LS, Weis MA, Eyre D, Byers PH. Mutations in FKBP10, which result in Bruck syndrome and recessive osteogenesis imperfecta, impair the hydroxylation of telopeptide lysines in bone collagen. Hum Mol Genet 2013; 22: 1-17 [PMID: 22949511 DOI: 10.1093/hmg/ddl371]

Lietman CD, Aramon R, Munivez E, Bertin KJ, Jiang MM, Chen Y, Dawson B, Weis MA, Eyre D, Lee B. A transgenic mouse model of OI type V supports a neomorphic mechanism of the IFITM5 mutation. J Bone Miner Res 2015; 30: 489-498 [PMID: 25251575 DOI: 10.1002/jbmr.2363]

Rauch F, Gieng Y, Lamplugh L, Hekmatnejad B, Gaumond MH, Penney J, Yamanaoka Y, Moffatt P. Crisp-Cas9 engineered osteogenesis imperfecta type V leads to severe skeletal deformities and perinatal lethality in mice. Bone 2018; 107: 131-142 [PMID: 29174560 DOI: 10.1016/j.bone.2017.11.013]

Reich A, Bae AS, Barnes AM, Cabral WA, Hinek A, Stimec J, Hill SC, Chitayat D, Marini JC. Type V OI primary osteoblasts display increased mineralization despite decreased COL1A1 expression. J Clin Endocrinol Metab 2015; 100: E235-E332 [PMID: 25387264 DOI: 10.1210/jc.2014-3082]

Farber CR, Reich A, Barnes AM, Becerra P, Rauch F, Cabral WA, Bae A, Qinlan A, Glorieux FH, Clemens TL, Marini JC. A novel IFITM5 mutation in severe atypical type V OI impairs osteoblast production of pigment epithelium-derived factor. J Bone Miner Res 2014; 29: 1402-1411 [PMID: 24519609 DOI: 10.1002/jbmr.2173]

Vranka JA, Pokidyshova E, Hayashi L, Zientek K, Mizuno K, Ishikawa Y, Maddox K, Tufa S, Keene DR, Klein R, Bächinger HP. Prolyl 3-hydroxylation 1 null mice display abnormalities in fibrillogen collagen-rich tissues such as tendons, skin, and bones. J Biol Chem 2010; 285: 17253-17262 [PMID: 20363794 DOI: 10.1074/jbc.M110.112228]

Marini JC, Cabral WA, Barnes AM. Null mutations in LEPRE1 and CRTAP cause severe recessive osteogenesis imperfecta. Cell Tissue Res 2010; 339: 59-70 [PMID: 19982557 DOI: 10.1007/s00441-009-0872-0]

Lindert U, Weis MA, Rai J, Seeliger F, Haussler I, Leeb T, Eyre D, Rohrbach M, Giunta C. Molecular Consequences of the SERPINH1/HSP47 Mutation in the Dachshund Natural Model of Osteogenesis Imperfecta. J Biol Chem 2015; 290: 17679-17689 [PMID: 26004778 DOI: 10.1074/jbc.M115.661025]

Nagai N, Hosokawa M, Iiura S, Adachi E, Matsushita T, Hosokawa N, Nagata K. Embryonic lethality of molecular chaperone hsp47 knockout mice is associated with defects in collagen biosynthesis. J Cell Biol 2000; 150: 1499-1506 [PMID: 10995453 DOI: 10.1083/jcb.150.6.1499]

Chat CKF, Guliati GS, Sinha R, Tomkins JV, Lopez M, Carter AC, Ransom RC, Reinisch A, Weardt T, Murphy M, Brewer RE, Koepcke LS, Marecic O, Mananjath A, Seo EY, Leavitt T, Lu WJ, Nguyen A, Conley SD, Salhotra A, Ambrosi TH, Borrelli MR, Siebel T, Chan K, Schallmoser K, Maijetti R, Wan GD, Goodman S, Weissman IS, Longaker MT. Identification of the Human Skeletal Stem Cell. Cell 2017; 185: 43-56.e21 [PMID: 30241615 DOI: 10.1016/j.cell.2018.07.029]

Ruiz de Eguino G, Infante A, Schlengen K, Aranayam AM, Fullaondo A, Soriano M, Garcia-Verdugo JM, Martin AG, Rodriguez CI. Sp1 transcription factor interaction with accumulated prelamin a impairs adipose lineage differentiation in human mesenchymal stem cells: essential role of sp1 in the integrity of lipid vesicles. Stem Cells Transl Med 2012; 1: 309-321 [PMID: 23197810 DOI: 10.5966/sctm.2011-0010]

Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal PK, Vera E, Shim JW, Kriks S, Taldone T, Fusaki N, Tomishima MJ, Krainc D, Milner TA, Rossi DJ, Studer L. Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell 2013; 13: 691-705 [PMID: 23815443 DOI: 10.1016/j.stem.2013.11.006]
Gibon E, Lu L, Goodman SB. Aging, inflammation, stem cells, and bone healing. Stem Cell Res Ther 2016; 7: 44 [PMID: 27006971 DOI: 10.1186/s13287-016-0300-9]

Kraus KH, Kirker-Heard C. Mesenchymal stem cells and bone regeneration. Vet Surg 2006; 35: 232-242 [PMID: 16635002 DOI: 10.1111/j.1532-9505.2006.00142.x]

Angello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. Hum Gene Ther 2010; 21: 1226-1238 [PMID: 20804388 DOI: 10.1089/hum.2010.173]

Beyh S, Schroeder J, Liebergall M. Stem cells in bone diseases: current clinical practice. Br Med Bull 2011; 99: 199-210 [PMID: 21813557 DOI: 10.1093/bmbld/ldr035]

Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S. Mesenchymal stem cells in stem cell biology and applied bone regeneration. Clin Orthop Relat Res 1998; S247-S256 [PMID: 9917644 DOI: 10.1007/0-387-98196-1_0025]

Oryan A, Kamali A, Moshiri A, Baghban Es laminate M. Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? Cells Tissues Organs 2017; 204: 59-83 [PMID: 2867733 DOI: 10.1159/000469704]

Moerman EJ, Teng K, Lipsitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging Cell 2004; 3: 379-389 [PMID: 15569355 DOI: 10.1111/j.1474-9728.2004.00127.x]

Infante A, Rodriguez CI. Osteogenesis and aging: lessons from mesenchymal stem cells. Stem Cell Res Ther 2018; 9: 244 [PMID: 30257716 DOI: 10.1186/s13287-018-0995-x]

Liu W, Zhang L, Xuan K, Hu C, Liu S, Liao L, Li B, Jin F, Shi S, Jin Y. <i>β</i>Ap/β<i>i</i> prevents bone aging sensitivity by specifically regulating senescence and differentiation in mesenchymal stem cells. Bioresour Technol 2018; 6: 27 [PMID: 30210899 DOI: 10.1016/j.biortech.2018.09.029]

Merideth MA, Gordon LB, Sachs C, Svehlik V, Smith AC, Perry MB, Brewer CC, Zalewski C, Kim HJ, Solomon B, Brooks GP, Berger LH, Turner ML, Domingo DL, Hart TC, Graj J, Reynolds JC, Groppman A, Yanovsky JA, Gerhard-Herman M, Collins FS, Nabel EG, Cannon RO, Gahl WA, Intone JW. Phenotype and course of Huntington-Gilford progeria syndrome. N Engl J Med 2008; 358: 592-604 [PMID: 18256394 DOI: 10.1056/NEJMoa0708898]

Gordon CM, Gordon LB, Snyder BD, Nazarian A, Quinn N, Huh S, Gibb-Purie-Huber A, Neuberg D, Cleveland R, Kleinman M, Miller DT, Kieran MW. Huntington-Gilford progeria is a skeletal dysplasia. J Bone Miner Res 2011; 26: 1670-1679 [PMID: 21449982 DOI: 10.1002/jbmr.392]

Lo Cicero A, Jaskowiak AL, Egesipe AL, Tourounis J, Brinon P, Pitrez PR, Ferreira L, de-Sandre-Giovannoli A, Levy N, Nissan X. A High Throughput Transcriptomic Screening Reveals compounds that counteract premature osteogenic differentiation of HGPS iPSC-derived mesenchymal stem cells. Sci Rep 2016; 6: 34798 [PMID: 27739443 DOI: 10.1038/srep34798]

Infante A, Rodriguez CI. Secretome analysis of in vitro aged human mesenchymal stem cells reveals IGFBP7 as a putative factor for promoting osteogenesis. Sci Rep 2018; 8: 4632 [PMID: 29545581 DOI: 10.1038/s41598-018-2285-z]

Zhang W, Chen E, Chen M, Ye C, Qi Y, Ding Q, Li H, Xue D, Gao X, Pan Z. IGFBP7 regulates the osteogenic differentiation of bone marrow-derived mesenchymal stem cells via Wnt/β-catenin signaling pathway. FASEB J 2018; 32: 2280-2291 [PMID: 29242275 DOI: 10.1096/fj.201700999R]

Hess MW, Pfälzer K, Ehrler HL, Beer B, Hekl D, Seppi T. 3D versus 2D cell culture implications for electron microscopy. Methods Cell Biol 2010; 96: 649-670 [PMID: 20869542 DOI: 10.1007/20069275-7]

Zhu S, Ehntert S, Roull M, Häussling V, Aspera-Werz RH, Chen T, Nussler AK. From the Clinical Problem to the Basic Research-Co-Culture Models of Osteoblasts and Osteoclasts. Int J Mol Sci 2018; 19 [PMID: 30081223 DOI: 10.3390/ijms19082284]

Bongio R, Lopa S, Giardini P, Bersini S, Moretti M. A 3D vascularized bone remodeling model combining osteoblasts and osteoclasts in a CaP nanoparticle-enriched matrix. Nanomedicine (Lond) 2016; 11: 1073-1091 [PMID: 27078856 DOI: 10.2217/nmn-2015-0021]

Hayden RS, Fortin JP, Harwood B, Subramanian B, Quinn KP, Georgakoudi I, Kopin AS, Kaplan DL. Cell-tethered ligands modulate bone remodeling by osteoblasts and osteoclasts. Adv Funct Mater 2014; 24: 472-479 [PMID: 25419210 DOI: 10.1002/adfm.201302210]

Vanderburgh J, Sterling JA, Guelcher SA. 3D Printing of Tissue Engineered Constructs for In Vitro Modeling of Disease Progression and Drug Screening. Ann Biomed Eng 2017; 45: 164-179 [PMID: 27169904 DOI: 10.1007/s10439-016-1640-4]

Tortelli F, Cancetta R. Three-dimensional cultures of osteogenic and chondrogenic cells: a tissue engineering approach to mimic bone and cartilage in vitro. Eur Cell Mater 2009; 17: 1-14 [PMID: 19579210 DOI: 10.22233/eCM.v017a01]

Li J, Wang Q, Gu Y, Zhu Y, Chen L, Chen Y. Production of Composite Scaffold Containing Silk Fibroin, Chitosan, and Gelatin for 3D Cell Culture and Bone Tissue Regeneration. Med Sci Moni 2017; 23: 531-5320 [PMID: 29114098 DOI: 10.12699/MSM.905085]

Turnbull G, Clarke J, Picard F, Riches P, Jia L, Han F, Li B, Shu W. 3D bioactive composite scaffolds for bone tissue engineering. Bioact Mater 2017; 3: 278-314 [PMID: 29744467 DOI: 10.1016/j.bioactmat.2017.10.001]

LaL H, Patrakheek MK. 3D printing and its applications in orthopaedic trauma: A technological marvel. J Clin Orthop Trauma 2018; 9: 260-268 [PMID: 3020159 DOI: 10.1016/j.jcot.2018.07.022]

Tatullo M, Marrelli M, Paduano F. The regenerative medicine in oral and maxillofacial surgery: the most important innovations in the clinical application of mesenchymal stem cells. Int J Med Sci 2015; 12: 72-77 [PMID: 2552921 DOI: 10.7150/ijms.10709]

Andrews S, Cheng A, Stevens H, Logun MT, Webb R, Jordan E, Xia B, Karumbiaiah L, Goldberg RE, Stice S. Chondroitin Sulfate Glycosaminoglycan Scaffolds for Cell and Recombinant Protein-Based Bone Regeneration. Stem Cells Transl Med 2019; 8: 575-585 [PMID: 30666821 DOI: 10.1002/sctm.18-0141]

Ma Y, Hu N, Liu J, Zhai X, Wu M, Hu C, Li L, Lai Y, Pan H, Lu WW, Zhang X, Luo Y, Ruan C. Three-Dimensional Printing of Biodegradable Piperazine-Based Polycarbonate-Urea Scaffolds with Enhanced Osteogenesis for Bone Regeneration. ACS Appl Mater Interfaces 2019; 11: 9415-9424 [PMID: 30968946 DOI: 10.1021/acsami.8b20323]

Zhang K, Wang S, Zhou C, Cheng L, Gao X, Xie X, Sun J, Wang H, Weir MD, Reynolds MA, Zhang N, Bai Y, Xu HHK. Advanced smart biomaterials and constructs for hard tissue engineering and regeneration. Bone Res 2018; 6: 31 [PMID: 30374416 DOI: 10.1038/s41414-018-0102-z]

Wang XF, Song Y, Liu YS, Sun YC, Wang YG, Wang Y, Lyu PJ. Osteogenic Differentiation of Three-
Dimensional Bioprinted Constructs Consisting of Human Adipose-Derived Stem Cells In Vitro and In Vivo. *PLoS One* 2016; 11: e0157214 [PMID: 27332814 DOI: 10.1371/journal.pone.0157214]

Dhanaw A, Kennedy PM, Rizk EB, Ozboldt IT. Three-dimensional Bioprinting for Bone and Cartilage Restoration in Orthopaedic Surgery. *J Am Acad Orthop Surg* 2019; 27: e215-e226 [PMID: 30371527 DOI: 10.5435/JAAOS-D-17-00632]

Mandrycky C, Wang Z, Kim K, Kim DH. 3D bioprinting for engineering complex tissues. *Biotechnol Adv* 2016; 34: 422-434 [DOI: 10.1016/j.biotechadv.2015.12.011]

Daly AC, Freeman FE, Gonzalez-Fernandez T, Critchley, SE, Nulty, J, Kelly DJ. 3D Bioprinting for Cartilage and Osteochondral Tissue Engineering. *Adv Healthc Mater* 2017; 6 [PMID: 28304984 DOI: 10.1002/adhm.201700298]

Zhai X, Ruan C, Ma Y, Cheng D, Wu M, Liu W, Zhao X, Pan H, Lu WW. 3D-Bioprinted Osteoblast-Laden Nanocomposite Hydrogel Constructs with Induced Microenvironments Promote Cell Viability, Differentiation, and Osteogenesis both In Vitro and In Vivo. *Adv Sci (Weinh)* 2017; 4: 1708550 [PMID: 29599358 DOI: 10.1002/advs.201700550]

Wu Q, Tang J, Li Y, Li L, Wang Y, Bao J, Bu H. Hepatic differentiation of mouse bone marrow-derived mesenchymal stem cells using a novel 3D culture system. *Mol Med Rep* 2016; 17: 9473-9479 [PMID: 29125655 DOI: 10.3892/mmr.2017.77818]

Byambaa B, Aabati N, Yue K, Trujillo-de Santiago G, Alvarez MM, Jia W, Kazemzadeh-Narbat M, Shin SR, Tamayo A, Khademhosseini A. Bioprinted Osteogenic and Vasculogenic Patterns for Engineering 3D Bone Tissue. *Adv Healthc Mater* 2017; 6 [PMID: 28524375 DOI: 10.1002/adhm.201700015]

Irvin SA, Venkatraman SS. Bioprinting and Differentiation of Stem Cells. *Molecules* 2016; 21 [PMID: 27617991 DOI: 10.3390/molecules21091883]

Pensolazzi L, Lolli A, Sardelli L, Angelozzi M, Lamberti E, Trombelli L, Ciarpella F, Vecchiari R, Piva R. Establishment of a 3D-dynamic osteoblast-osteoclast co-culture model to simulate the jawbone microenvironment in vitro. *Life Sci* 2016; 152: 82-93 [PMID: 27015789 DOI: 10.1016/j.lfs.2016.03.035]

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moore MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143-147 [PMID: 10102814 DOI: 10.1126/science.284.5411.145]

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-317 [PMID: 16923408 DOI: 10.1016/j.cyto.2006.08.003]

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; 2: 141-150 [PMID: 18371435 DOI: 10.1016/j.stem.2007.11.014]

McLeod CM, Mauck RL. On the origin and impact of mesenchymal stem cell heterogeneity: new insights and emerging tools for single cell analysis. *Eur Cell Mater* 2017; 24: 217-231 [PMID: 29076514 DOI: 10.22203/cem.v04i14]

Lalu MM, Mcntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One* 2012; 7: e47559 [PMID: 23133515DOI: 10.1371/journal.pone.0047559]

Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SL, Iane GA, Cox CS. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev* 2009; 18: 683-692 [PMID: 19099374 DOI: 10.1089/scd.2008.0253]

Heldring N, Müller I, Wood MJ, Le Blanc K, Andaloussi SE. Therapeutic Potential of Multipotent Mesenchymal Stromal Cells and Their Extracellular Vesicles. *Hum Gene Ther* 2015; 26: 506-517 [PMID: 26153722 DOI: 10.1089/hum.2015.0727]

Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. *Nat Rev Endocrinol* 2011; 7: 540-557 [PMID: 21670757 DOI: 10.1038/nrendo.2011.81]

Hovitz EW, 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: 8932-8937 [PMID: 12084934 DOI: 10.1073/pnas.132252399]

Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K, Prockop DJ. Marrow stem cells as a source of progenitor cells for nonosteopetrous tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci U S A* 1998; 95: 1142-1147 [PMID: 9448289 DOI: 10.1073/pnas.95.3.1142]

Otsuru S, Desbourdes L, Guess AJ, Hofmann TJ, Relation T, Kaito T, Dominici M, Iwamoto M, Horwitz EM. Extracellular vesicles released from mesenchymal stromal cells stimulate bone growth in osteogenesis imperfecta. *Cytotherapy* 2018; 20: 62-73 [PMID: 29107738 DOI: 10.1017/ cyt.2017.09.012]

Le Blanc K, Götherström C, Ringdén O, Hassan M, McMahon R, Horwitz E, Agerberth B, Axelsson O, Alén EM. Extracellular vesicles released from mesenchymal stromal cells stimulate bone growth in osteogenesis imperfecta. *Cytotherapy* 2009; 14: 173-184 [PMID: 19066333 DOI: 10.3124/haematol.13740]

Whyte MP, Kervinberg J, McAlister WH, Mumm S, Podgornik MN, Coburn SP, Ryan LM, Miller CR, Gottlesman GS, Smith AK, Douville J, Waters-Pick B, Armstrong RD, Martin PL. Marrow cell transplantation for infantile hypophosphatasia. *J Bone Miner Res* 2003; 18: 624-636 [PMID: 12674323 DOI: 10.1359/jbmr.2003.18.4.624]

Taketani T, Oyama C, Mihara A, Tanabe Y, Abe M, Hiraide T, Yamamoto S, Bo R, Kanai R, Tadenuma T, Michibata Y, Yamamoto S, Hattori M, Katsube Y, Ohnishi H, Sasao M, Oda Y, Hattori K, Yuba S, Ogahishi H, Yamaguchi S. Ex Vivo Expanded Allogeneic Mesenchymal Stem Cells With Bone Marrow Transplantation Improved Osteogenesis in Infants With Severe Hypophosphatasia. *Cell Transplant* 2015; 24: 1931-1943 [PMID: 25396326 DOI: 10.3727/096368915X685410]
Frölke JP, Patka P. Definition and classification of fracture non-unions. *Injury* 2007; 38 Suppl 2: S19-S22 [PMID: 17920413 DOI: 10.1016/S0020-1383(07)80005-2]

Mathieu M, Rigutto S, Ingels A, Spruyt D, Stricwaut N, Kharrouri I, Albarani V, Jayankuru M, Rasschaert J, Bastianelli E, Gangji V. Decreased pool of mesenchymal stem cells is associated with altered chemokines serum levels in atrophic nonunion fractures. *Bone* 2013; 53: 391-398 [PMID: 23318974 DOI: 10.1016/j.bone.2013.01.005]

Bhattacharjee A, Kuiper JH, Roberts S, Harrison PE, Cassar-Pullicino VN, Tins B, Bajada S, Richardson JB. Predictors of fracture healing in patients with recalcitrant nonunions treated with autologous culture expanded bone marrow-derived mesenchymal stromal cells. *J Orthop Res* 2019; 37: 1303-1309 [PMID: 30474883 DOI: 10.1002/jor.24184]

Katagiri W, Watanabe J, Toyama N, Osugi M, Sakaguchi K, Hibi H. Clinical Study of Bone Regeneration by Conditioned Medium From Mesenchymal Stem Cells After Maxillary Sinus Floor Elevation. *Implant Dent* 2017; 26: 607-612 [PMID: 28727618 DOI: 10.1097/ID.0000000000000618]
