Chapter 12

Regenerative Medicine: A New Paradigm in Bone Regeneration

Orlando Chaparro and Itali Linero

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62523

Abstract

Bone defects are the cause of functional disability and the restoration of skeletal function remains an important challenge on orthopedics, neurosurgery and oral and maxillofacial surgery. Because of the limitations of the currently used techniques for the reconstruction of bone defects and the difficulties for the implementation of new therapeutic strategies, a new paradigm in the field of reconstructive surgery has arisen, leading to tissue engineering and regenerative medicine. Mesenchymal stem cells (MSC) have emerged as a promising alternative for the treatment of bone lesions. It was postulated that the therapeutic action was the result of proliferation and differentiation of MSCs, replacing injured tissue. However, recent studies have shown that MSCs secrete a number of trophic factors that have a strong effect during repair and tissue regeneration. This represents a shift from a paradigm centered on MSC proliferation and differentiation to a new paradigm in which the MSCs exert their beneficial effect by the secretion of paracrine factors that induce endogenous repair mechanisms. This chapter will bring together basic and clinical aspects, focused on novel findings on MSC paracrine effect and the development of new therapeutic strategies based on growth factors, cytokines and signaling molecules involved in bone regeneration.

Keywords: mesenchymal stem cells, paracrine effect, bone regeneration, growth factors, cytokines

1. Introduction

The regeneration of bone tissue remains an important challenge in the field of orthopedic and maxillofacial surgery. Bone defects produced by trauma, tumors, infectious diseases, biochem-

ical disorders, congenital disorders or abnormal skeletal development are the major causes of functional disability, and esthetic and psychological trauma for patients.

One of the goals of treating a bone defect is to restore the normal morphology and function of the affected structure. Specific surgical techniques such as distraction osteogenesis, implantation of biomaterials (bone substitutes) and implants of bone grafting have been developed to reach bone regeneration [1, 2]. Demand for bone grafts is considerable and represents the second most common procedure after blood transplants, with more than 2.2 million bone grafts performed annually worldwide in orthopedics and dentistry [3].

Despite advances in bone regeneration and the availability of many treatments, most clinicians and researchers continue to come to the same conclusion: autologous bone grafting remains the "gold standard," compared to other reconstructive procedures [4–9]. Bone from the same patient lacks immunogenicity and contains all the elements necessary to effectively induce tissue regeneration. It has osteoprogenitor cells which go directly to the implant site, cytokines and extracellular matrix [5], providing the three classic elements of an ideal bone graft: osteogenesis, osteoconduction and osteoinduction [5–7, 9, 10]. However, autologous bone grafts have several important limitations, including high risk of morbidity in the donor site [5, 6, 11], with disadvantages in terms of costs, time of surgical procedure, discomfort for the patient and possible complications.

Additionally, many times the volume of tissue available for the procedure is not sufficient to fill or cover a defect, given the limited availability of autologous tissue [4, 10], and the quality of the autograft is highly variable and is influenced by age and metabolic abnormalities of the patient [7]. To overcome these limitations, a variety of exogenous substitutes, including allografts, xenografts and alloplastic materials, have been introduced into clinical practice in the past three decades [4]. However, these substitutes have less osteogenic and osteoinductive properties [6, 12] and a greater possibility of transmission of infectious diseases [6, 8], restricting their use [8].

In order to successfully overcome the shortcomings of current approaches for bone regeneration, tissue engineering emerged as a discipline that provides the necessary tools for bone regeneration and restoration. The presence of cell populations that orchestrate the release of growth factors, the maintenance of a stable matrix and the stimulation of angiogenesis are key factors to successful regeneration of bone tissue, because they play a decisive role in the healing process [13, 14]. The technologies developed recently based on tissue engineering, such as gene therapy, stem cell therapy and the application of osteoinductive growth factors, looking for the control of the dynamics of these elements to enable more predictable bone regeneration surge as a significant promise in clinical practice [15].

Cell-based therapy for the regeneration of bone tissue has been extensively investigated. Several cell types have been used as alternatives for the reconstruction of bone tissue, including osteoblasts, embryonic stem cells, periostium derived-progenitor cells (a specialized cell type that covers bone surfaces and have the potential to differentiate into multiple mesenchymal tissues, including bone) and mesenchymal stem cells, also known as multipotential stromal cells (MSC) [16].

http://dx.doi.org/10.5772/62523

MSC has become one of the best alternatives in cell therapy and specifically in bone regeneration. MSCs can be isolated from virtually all vascularized tissue and they are able to differentiate into various mesenchymal tissues such as bone, cartilage, muscle, tendon, adipose tissue and hematopoiesis-supporting stroma. However, a growing number of recent reports in the literature have revealed that even if a therapeutic effect can be documented, the implanted MSC cells do not differentiate and do not survive for a long time [17, 18].

The use of MSCs in the treatment of musculoskeletal injuries was initially based on their ability to differentiate into various cell types [1, 7, 8]. The rationale was that after implantation or MSC injection, the cells would be able to colonize the injured site and differentiate into the appropriate lineages. This mechanism has now been challenged by a new paradigm to extend it to an alternative mechanism called **paracrine effect**, where MSCs secrete biologically active molecules which have beneficial effects on the injured tissues [9] by inhibiting fibrosis, apoptosis and inflammation [10, 11] and promoting angiogenesis and tissue regeneration [19–21].

2. Physiological process of bone regeneration

For the development of new therapeutic tools for restoring bone defects exceeding the critical size, it is necessary to look at the prototype model of physiological bone regeneration. This process, involving a coordinated interaction of cells, growth factors and extracellular matrix, consists of multiple and well-orchestrated stages that start immediately after the injury occurs, with a local inflammatory response followed by the mobilization of hematopoietic and mesenchymal stem cells to the site of injury to form new vascular networks, soft tissue matrix, cartilage and/or bone and finally inducing mature bone formation [22–24]. All four components involved in the site of injury, including cortical bone, periosteum, bone marrow and external soft tissue, contribute to a different extent in the healing process, depending on various parameters such as growth factors, hormones and nutrients, pH, oxygen tension, the electrical environment and mechanical stability [25].

Immediately after bone trauma, damage of the local vasculature at the site of injury is responsible for producing a blood clot or hematoma [24, 26, 27]. This hematoma is a localized collection of blood products, including platelets, leukocytes, macrophages, fibrin, soluble growth factors and cytokines, which in turn provides a matrix that allows the migration of inflammatory cells, endothelial cells and fibroblasts [24] (**Figure 1**).

This first stage of fracture healing is the beginning of the so-called **inflammatory phase**, which begins within the first 12 to 14 hours, has its peak during the first 24 hours and is completed around 7 days after the injury. It is characterized by a destructive phase, with a local acute inflammatory response and hypoxia. The first cells to arrive at the site of injury are neutrophils, and subsequently macrophages and lymphocytes. Macrophages not only phagocyte necrotic tissue but also release a number of growth factors and cytokines that initiate the healing process of bone wound [26] (**Figure 1**).

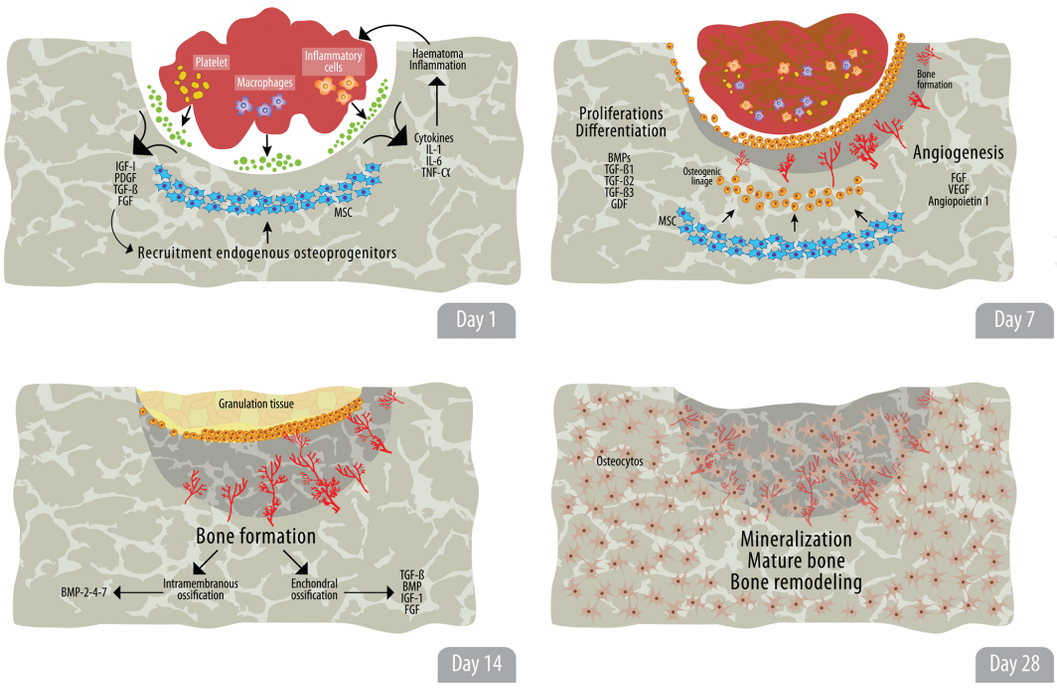

Figure 1. Temporal progression of bone healing. The healing response to bone injury is characterized by overlapping biological processes: immediately after bone injury, hematoma formation and inflammatory response permits the release of pro-inflammatory cytokines and growth factors that initiate the process of wound healing. Between days 1–7, MSCs proliferate and differentiate into the osteogenic or chondrogenic lineages and increase the production of blood vessels from pre-existing vessels. New bone formation occurs through intramembranous or endochondral ossification that is finally mineralized, forming a mature bone that is continuously remodeled through the rest of his life.

The factors secreted by platelets, macrophages and bone cells include transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), interleukins 1 and 6 (IL-1 and IL-6), tumor necrosis growth factor alpha (TNF-α), bone morphogenetic proteins (BMPs) [26, 27], fibroblast growth factor (FGF) and insulin-like growth factors I and II (IGF-I and IGF-II) [26]. These factors stimulate the migration of multipotent stem cells, probably originated from the periosteum, bone marrow, blood vessels and the surrounding soft tissue and induce the differentiation of cells to different mesenchymal cell types including angioblasts, fibroblasts, chondroblasts and osteoblasts [26].

During the following days, the **construction phase** starts. This phase is characterized by the formation of new blood vessels [17], and the thrombus reorganization into granulation tissue, which is then condensed in a soft callus providing an osteoid and/or cartilage scaffold, which acts as a stabilization structure and a template for subsequent mineralization [26] (**Figure 1**).

Depending on the type of bone, the type of bone lesion, the morphology and structure of the tissue and the fixation method, bone healing can take two forms: primary healing, where osteoblasts secrete an osteoid matrix for future mineralization (intramembranous ossification); and secondary healing, which occurs through the formation of a cartilage matrix produced by chondrocytes, which is then replaced by an osteoid matrix with subsequent mineralization (endochondral ossification) [24–27]. Most common growth factors related to bone healing,

osteoinduction and osteoconduction are: PDGF, BMPs [15, 28, 29], IGFs [28, 30], TGF-β [15, 28], FGF [24, 29] and VEGF [15, 24, 29].

Local vascularization at the site of injury has been identified as one of the most important parameters that influence the healing process [14, 31, 32]. Bone formation can only proceed successfully if the tissue is adequately vascularized [15]; therefore, angiogenesis is a key component in bone repair. The new blood vessels carry oxygen and nutrients to the metabolically active callus, allowing gas exchange and the output of waste products and serve as a route for inflammatory cells, and cartilage and bone precursor cells [33, 34], and also provide the gateway of systemically circulating factors that can modify the bone healing process [34]. Vascularization is needed for both the formation of intramembranous and endochondral bone. During the formation of endochondral bone, cartilage avascular environment is invaded by blood vessels that allow the osteoblastic, chondroblastic and progenitor cells, to deposit new bone on the surface of the islands of cartilage. During intramembranous ossification, vascularization is also needed to allow the arrival of osteoblast precursor cells [34].

Angiogenesis and migration of vascular endothelial cells are stimulated by pro-angiogenic factors such as VEGF, BMPs, TGF-β, FGF and angiopoietins (especially angiopoyectina I and II) [26].

Finally, over the course of months to years, the third stage, the **remodeling phase** of bone healing occurs, whose main objective is to reshape the bone in order to restore its original structure and strength. During this phase, osteoclasts reabsorb recently formed bone tissue, due to the stimulation of growth factors and cytokines that promote osteoclastogenesis as TNF-α, TGF and BMPs. Osteoblasts deposit more osteoid and calcium phosphate in the newly regenerated bone, increasing the density of mineralized matrix. Therefore, the transverse diameter of the bone decreases but the density of internal structure increases, closer and closer to the architecture of the intact bone. As this stage keeps going, cellularity is gradually reduced and bone density is enhanced [26].

3. Biological factors in bone regeneration

During the process of bone regeneration, the release of growth factors occurs as a series of highly time-space regulated biological events. These soluble molecules are able to regulate signaling cascades that specifically influence cellular responses such as differentiation and proliferation [28].

Biological signaling molecules function effectively by a limited window of time to get a proper result in the target cell. Therefore, it is necessary to have a precise understanding of the temporal pathways for natural bone regeneration. Biological signaling agents can be classified into the following categories: pro-inflammatory cytokines, growth and differentiation factors and angiogenic factors. Pro-inflammatory cytokines are activated immediately after bone injury and establish and maintain the acidic and hypoxic environment for the initial destruction phase. Growth and differentiation factors function during the constructive and destructive phases, while angiogenic factors are focal points for the revascularization of the wounded area [25, 26, 35] (**Table 1**).

Signaling Molecules	Expression Pattern	Source	Target cells	function
Cytokines (IL-1,IL-6, TNF-α)	Increased levels from days 1 to 3 and during bone remodeling	Macrophages Inflammatory cells Cells of mesenchymal origin	Mesenchymal and inflammatory cells	Chemotactic effect on other inflammatory cells Stimulation of extracellular matrix synthesis, angiogenesis, recruitment of endogenous fibrogenic cells to the injury site and at later stages bone resorption
TGF-β	Expressed from very early stages throughout fracture healing	Degranulating platelets Inflammatory cells endothelium, extracellular matrix, chondrocytes, osteoblasts	MSCs, osteoprogenitor cells, osteoblasts, chondrocytes	Potent mitogenic and chemotactic for bone-forming cells, chemotactic for macrophages
PDGF	Released at very early stages of fracture healing	Degranulating platelets, macrophages, monocytes (during the granulation stage) and endothelial cells, osteoblasts (at later stages)	Mesenchymal and inflammatory cells, osteoblasts	Mitogenic for mesenchymal cells and osteoblasts, chemotactic for inflammatory and mesenchymal cells
BMPs	Various temporal expression patterns	Osteoprogenitors and mesenchymal cells, osteoblasts, bone extracellular matrix and chondrocytes	Mesenchymal and osteoprogenitor cells, osteoblasts	Differentiation of undifferentiated mesenchymal cells into chondrocytes and osteoblasts and osteoprogenitors into osteoblasts
FGFs	Expressed from the early stages until osteoblasts formation	Monocytes, macrophages, mesenchymal cells, osteoblasts, chondrocytes	Mesenchymal and epithelial cells, osteoblasts and chondrocytes	Angiogenic and mitogenic for mesenchymal and epithelial cells, osteoblasts, chondrocytes α-FGF mainly effects chondrocyte proliferation β-FGF (more potent) involved in chondrocytes maturation and bone resorption
IGFs	Expressed throughout fracture healing and endochondral ossification	Bone matrix, endothelial and mesenchymal cells (in granulation stage) and osteoblasts and non-hypertrophic chondrocytes (in bone and cartilage formation)	MSCs, endothelial cells, osteoblasts, chondrocytes	IGF-I: mesenchymal and osteoprogenitor cells recruitment and proliferation IGF-II: cell proliferation and protein synthesis

http://dx.doi.org/10.5772/62523

Signaling Molecules	Expression Pattern	Source	Target cells	function
VEGFs	Expressed during endochondral and bone formation	Bone matrix, endothelial and mesenchymal cells	Endothelial progenitor cells	Potent stimulators of endothelial cell proliferation
Angiopoietin (I and II)	Expressed from the early stages throughout fracture healing	Extravascular tissue cells	Endothelial progenitor cells	Formation of larger vessel structures, development of co-lateral branches from existing vessels

Essential signaling molecules in bone regeneration: their time of expression, source, target cells and their major functions (Adapted with the permission from Dimitriou et al. [25]. Copyright© 2005).

Table 1. Biological factors in bone regeneration.

In the next section, we will list some of the common molecules associated with the bone regeneration process, and describe their biological significance.

3.1. Transforming growth factor-beta (TGF-β) superfamily

Members of the TGF- ▪ β are the most widely studied growth factors in recent years. This family includes, among others, five isoforms of TGF-β (1–5), bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs), which participate in a complex series of molecular events that lead to mesenchymal precursors during bone morphogenesis [25, 29, 33, 36]. They originate from high molecular weight precursors and are activated by proteolytic enzymes. They act on serine/threonine kinase membrane receptor on target cells. This ligand-receptor interaction activates intracellular signaling pathways which ultimately affects gene expression in the nucleus [25].

3.1.1. Bone morphogenetic proteins (BMPs)

The BMPs are a unique family of proteins within the TGF-β superfamily that play an essential role in regulating the formation, maintenance and bone repair [30]. To date, about 20 different proteins have been termed BMPs, but not all of them have osteogenic potential [37]. Among the BMPs with osteogenic potential we have, BMPs-2, -3 (osteogenin), -4, -6, -7 (also known as osteogenic protein-1 [OP-1]), -12 (also known as growth/differentiation factor 7 [GDF-7]) and -14 (also known as GDF-5, or cartilage-derived morphogenetic protein-1 [CDMP-1]). These proteins have been evaluated for healing and bone regeneration in clinical and preclinical models showing enhanced and accelerated bone formation [30]. In bone tissue, BMPs are produced by osteoprogenitor cells, osteoblasts, chondrocytes and platelets. Their regulatory effects depend on the target cell, stage of differentiation, local concentration, as well as interactions with other secreted proteins. BMPs induce a sequential cascade of events leading to chondrogenesis, osteogenesis, controlled angiogenesis and extracellular matrix synthesis [37]. Large number of preclinical studies has shown that BMPs are capable of inducing bone

formation at ectopic sites and induce critical size defects healing [29]. It has been shown that BMPs 2, 4 and 7 play an important role in determining, migration, condensation, proliferation and apoptosis of skeletal cells. It has also been reported that BMP-4 and BMP-7 are responsible for inducing the cells of the neural crest, while BMP-2 is involved in the condensation of mesenchymal cells appearing before formation of immature bone structures during both endochondral and intramembranous ossification [33]. BMP-4 is predominantly active from days 1–5 after injury, with a peak closer to day 5. The BMP-2 is active during the bone regeneration process, culminating the bone remodeling to lamellar and haversian bone tissue, while BMP-7 is active after 14 days [23]. Target cells of BMPs include MSC, bone marrow cells, osteoblasts, myoblasts, prefibroblast and neuronal cells. The general effects on osteoblasts and cells of the periosteum involve an increase in the activity of DNA synthesis and transcription of genes involved in the synthesis of bone matrix proteins [23].

Scientific evidence of the role of BMPs in bone regeneration is overwhelming. There are a number of publications confirming that the delivery of BMP at the site of injury promotes bone regeneration in animal and human models [38–40].

BMP-2 and BMP-7 have been extensively evaluated in clinical studies of nonunion, bone defects, open tibial fractures and spinal fusion, demonstrating their efficacy in the acceleration of bone regeneration and healing of fractures [29]. In order to be used in the clinical practice, a local and controlled delivery of BMPs is required; so, it is important to consider its short half-life time. Various delivery systems have been developed to overcome this limitation [37]. Currently, there are several forms of the human recombinant proteins commercially available. For example, for rh-BMP2: InductOs® (United Kingdom) and InFUSE (United States), (Medtronic Sofamor Danek, Inc., Minneapolis, MN), which are supplied in a bovine collagen sponge allowing slow release over time, and for rhBMP-7, Osigraft® (United Kingdom) and OP-1™ (United States) (Stryker Biotech, Hop-kinton, MA), in a bovine collagen granular form [34, 36, 37].

3.1.2. Transforming growth factor-beta (TGF-β)

The five isoforms of TGF-β regulate cellular functions such as proliferation, apoptosis, differentiation and cell migration. TGF-β is produced by osteoblasts and chondrocytes, and is stored in the bone matrix [25, 41]. TGF-β is also released by platelets and TGF-β1 indeed, was the first member of the family to be described in human platelets, as a 25 kDa protein with a possible role in the healing process [42]. During the initial phase of inflammation resulting from a bone injury, platelets release TGF-β and therefore this factor seems to be involved in the initial callus formation stage [25, 41].

TGF-β is a multifunctional, secreted protein, with different functions in the cell, such as control of cell growth and proliferation, differentiation and apoptosis. TGF-β induces the proliferation of MSCs, pre-osteoblasts, osteoblasts and chondrocytes and stimulates the extracellular production of proteins such as collagen, proteoglycans, osteopontin, osteonectin and alkaline phosphatase [25, 41]. It is also a potent chemotactic agent for MSCs. During chondrogenesis

http://dx.doi.org/10.5772/62523

and endochondral bone formation, it induces the synthesis of BMP by osteoprogenitor cells, and it inhibits the activation and promotes osteoclast apoptosis [41].

3.2. Platelet-derived growth factors (PDGF)

This polypeptide growth factor has potent chemotactic and mitogenic stimulatory effects on MSCs [30], plays an important role in the differentiation of pre-osteoblasts to osteoblasts [43] with the ability to promote angiogenesis during wound healing [30]. The PDGF family includes four isoforms: PDGF-A, PDGF-B, the more recently discovered PDGF-C and PDGF-D [44]. PDGF-A and B form homodimers (AA or BB) and a heterodimer (AB) [30]. PDGF-AB and PDGF-BB are variants circulating in alpha platelet granules and are released when platelets bind to the site of injury. The PDGF-BB variant has an active role in mitogenesis and chemotaxis of cells in the injured area [15] and plays a key role in bone regeneration [23]. After bone injury, PDGF is released by macrophages and platelets and acts as a potent chemo-attractant and mitogenic factor for cells of mesenchymal lineage, recruit fibroblasts, endothelial cells, osteoblasts and cells of the immune system. PDGF is active during the first 72 hours after injury, and as a promoter of angiogenesis plays a role in revascularization of bone defects [23].

3.3. Fibroblast growth factor (FGF)

They constitute a family of structurally related polypeptides with a potent mitogenic effect on osteoprogenitor cells [29]. They are humoral factors originally identified by their ability to stimulate cell proliferation [33]. During bone healing, they can be secreted by monocytes, macrophages, mesenchymal cells, osteoblasts and chondrocytes in the early stages of bone fractures healing [33]. Members of the FGF family are present at the site of the wound for up to three weeks and its main activity is to stimulate endothelial cell migration and subsequent angiogenesis and mesenchymal cell mitogenesis [25, 34]. α-FGF mainly affects chondrocyte proliferation and is probably important for chondrocyte maturation, while β-FGF is expressed by osteoblasts and is generally more potent than α-FGF [45].

3.4. Vascular endothelial growth factor (VEGF)

Two separate pathways are involved in the regulation of angiogenesis during bone healing: a VEGF dependent pathway and the angiopoietin-dependent pathway [31]. VEGF is a potent angiogenic [29, 43] and vasculogenic [23] factor that not only increases the differentiation and proliferation of endothelial cells but also increases the tubular formation and mobilization and recruitment of endothelial progenitor cells [34]. VEGF is increased in response to hypoxia, ischemia and during healing of bone tissue [15, 34]. It has been shown that VEGF works synergistically with BMPs. VEGF by itself does not promote bone regeneration, but rather acts in coordination with BMPs to increase the recruitment of MSCs to the defect site and induce active differentiation of osteoblasts [46]. VEGF is expressed predominantly 14 to 21 days after the injury; and therefore, it is a candidate for early in situ application to promote mineralization and bone regeneration remodeling [23].

3.5. Insulin-like growth factors (IGF)

IGF-1 and -2 play a critical role in stimulation of organogenesis and growth during the first stages of embryogenesis as well as in regulating the functions of specific tissues and organs in later stages of development [47]. The sources of IGF-1 and IGF-2 are the bone matrix, endothelial cells, osteoblasts and chondrocytes [25]. IGF-1 promotes bone matrix formation (type I collagen and non-collagenous matrix proteins) by fully differentiated osteoblasts and is more potent than IGF-2 [45]. IGF-2 acts at a later stage of endochondral bone formation and stimulates type I collagen production, cartilage matrix synthesis and cellular proliferation [25].

4. Regenerative therapies

Clinical failure of bone tissue is defined as a discontinuity of the integrity of bone resulting from trauma, congenital malformation or surgical recession. Particularly, bone deficiency "critical size" is the bone defect that cannot regenerate spontaneously during the lifespan of the patient and, therefore, requires a surgical intervention for recovery [23].

The processes that drive the biology and biomechanics of bone regeneration remain largely unknown. During regeneration of bone tissue, many highly complex interactions between multiple cell types are mediated by soluble and insoluble factors and they have not been sufficiently characterized. The challenge for tissue engineering and regenerative medicine is to rebuild the regenerative healing process of bone tissue and then join the components to produce osteoangiogenic and, therefore, osteoregenerative therapies that fulfill the biomechanical parameters for the healing of a bone defect that exceeds the critical size.

We must remember that the bone has an inherent capacity for regeneration, so it is important to not only design therapies that do not interfere with the natural regenerative processes but also complement them and work synergistically with the endogenous bone healing process.

Regenerative therapy of bone tissue should include the three essential elements of bone regeneration: osteogenesis, osteoinduction and osteoconduction. Osteogenesis refers to the ability to produce new bone by bone-forming cells. Osteoinduction is the process whereby the presence of biological mediators stimulates the recruitment of mesenchymal stem cells to the wound site and their subsequent differentiation into mature bone cells, and osteoconduction is the physical property of providing a matrix facilitating the invasion of blood vessels and the new bone formation [48, 49].

Based on these fundamental principles, the main goal of regenerative medicine in clinical treatment is to reduce surgical morbidity by applying biological signals or cellular components that allow the reconstruction and restoration of lost tissue without autologous tissue transfer.

4.1. Mesenchymal stem cells-based therapy

Bone cell-based therapies seek to create viable tissue equivalents, providing live and metabolically active cells to repair the site of injury by continuous synthesis of bone matrix [50].

Mesenchymal stem cells are the center of a multitude of clinical studies currently underway (http://clinicaltrials.gov) [51]. Scientific evidence shows that they are one of the best choices in cell therapy, because of their ease of access and isolation, great potential of expansion in culture, immunosuppressive properties, paracrine effect and ability to migrate to injured tissues [52]. Moreover, their great therapeutic potential has been documented in the repair and regeneration of injured tissues in nearly every organ of the body, including the heart [53], immune system [54], liver [55], kidneys [56] and bone and cartilage tissue [57].

Mesenchymal stem cells are defined as pluripotent cells capable of self-renewal and differentiation into various specialized types of mesenchymal cells, such as osteoblasts, chondrocytes, adipocytes, myocytes, fibroblasts [52, 58–61]. MSCs are a group cells that have been isolated from virtually every vascularized tissue [62]. MSCs are a group cells that have been isolated from virtually every vascularized tissue [52]; however, recent reports have documented that they can also be isolated from other sources as umbilical cord [62], peripheral blood [63], adipose tissue [64–67], hair follicle [68], periodontal ligament [69–72], gingival tissue [73] and dental pulp [74, 75], among others.

MSCs, for its ability to differentiate to multiple lineages, specifically, their osteogenic potential and their immunomodulatory, anti-inflammatory and anti-apoptotic properties, have become a major tool in cell therapy for the regenerative treatment of pathologies affecting functionally bone tissue [76–79]. In vitro analyzes show that MSCs induced by osteogenic differentiation medium increase the expression of osteogenic differentiation markers such as alkaline phosphatase, osteocalcin, osteopontin, bone sialoprotein and calcium deposits in the extracellular matrix. The progress in the study of the biology of bone tissue and the isolation and in vitro cultivation of MSCs opened the possibility of studying the molecular and biological mechanisms of bone regeneration, making significant progress, as evidenced by the more two thousand publications of experimental reports on the application of MSCs in bone defects in animal models promoting bone regeneration, and the more than five hundred clinical trials currently registered on the NIH clinical trials website (http://clinicaltrials.gov) [51].

4.1.1. MSCs mechanism of action

The mechanisms through which MSCs enhance the bone tissue repair process are complex, since they can participate in the three phases of bone healing: inflammation, proliferation and remodeling [80]. The in vivo identity and location of MSC have been difficult to establish. However, various reports, especially the work of Crisan et al., presented evidence of a relationship between MSCs and perivascular pericytes. Irrespective of their tissue origin, perivascular cells exhibit osteogenic, chondrogenic and adipogenic potentials and express MSC markers [81]. Based on these reports, Caplan suggests that all MSCs are pericytes, which would explain the presence of MSC in all vascularized tissues. When an injury disrupts the normal architecture of the blood vessels, pericytes are activated giving rise to MSCs that then contribute to tissue repair by secreting trophic factors that can control the endogenous inflammatory reaction, promote angiogenesis and stimulate the proliferation and differentiation of progenitor cells [82] (**Figure 2**).

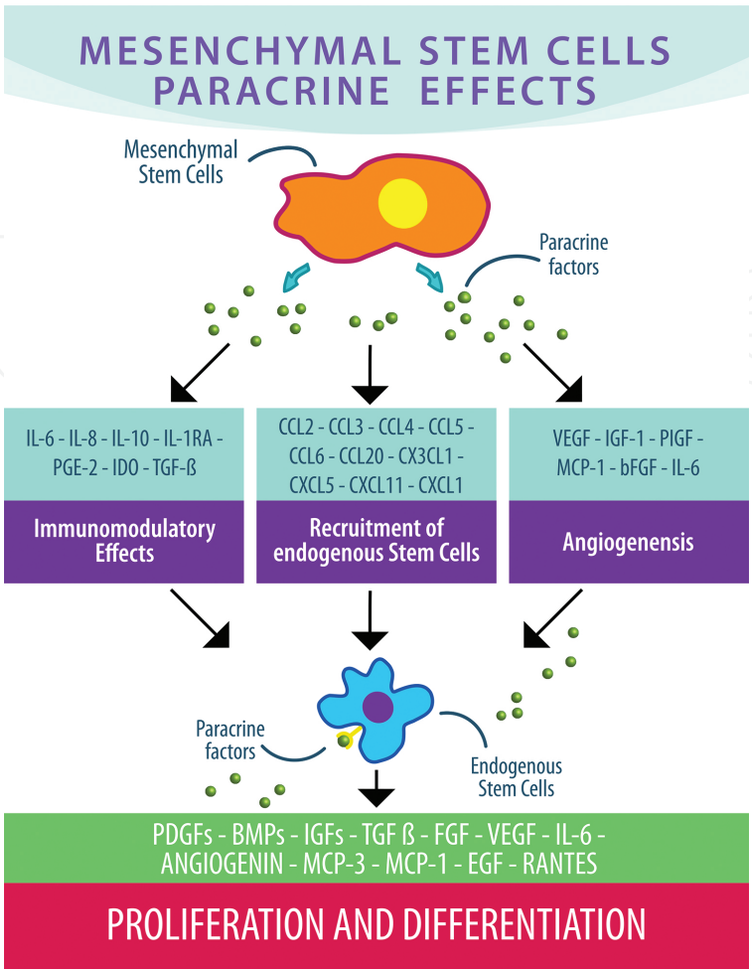

Figure 2. Schematic model of the MSCs' paracrine effect on tissue regeneration.

As mentioned before, a growing number of recent reports in the literature have revealed that even if a therapeutic effect can be observed, the implanted MSC cells do not differentiate and do not survive for a long time. For example, in an animal model of acute myocardial infarction, it was established that the MSCs implanted do not survive, and only 4.4% of grafted MSC could be found 1–2 weeks after transplantation [17], and MSC transplantation in a model of spinal cord injury in rats revealed that MSCs implanted disappeared from the host after 1–2 weeks [18]. It has been also reported that human adipose tissue-derived MSCs effectively induce bone regeneration in rabbit jaws, but they do not differentiate and do not survive more than 12 days in the site of implantation [21]. Recent reports have demonstrated that many of the therapeutic effects of MSCs can be mediated by the secretion of trophic factors, opening the possibility that direct administration of these mediators may replace the use of the cells in some instances [57]. This implies a shift from a paradigm centered on cell differentiation to a new vision where the MSCs can have a therapeutic effect even if they are not grafted or differentiated into specific tissue cells, which significantly increases the options of MSC therapeutic applications. According to this concept, Caplan has proposed that the most important feature of the MSC which determines its therapeutic potential is not their stemness

but the ability to secrete a large number of trophic factors, and he has proposed that their name to be changed to medicinal signaling cells, keeping the same MSC acronym [83].

Caplan also proposes a model whereby MSCs exert their therapeutic action at the site of the injury by two different activities: from the front of the cells, away from the area of injury, MSCs create a curtain, by the production of bioactive molecules that control local inflammation and prevent autoimmune reactions. From the back of the MSC, they produce molecules that: (1) stop scar formation, (2) inhibit cell apoptosis due to ischemia, (3) stimulate the formation and stabilization of blood vessels and (4) secrete trophic factors that induce the replication of endogenous tissue progenitors [84] (**Figure 3**).

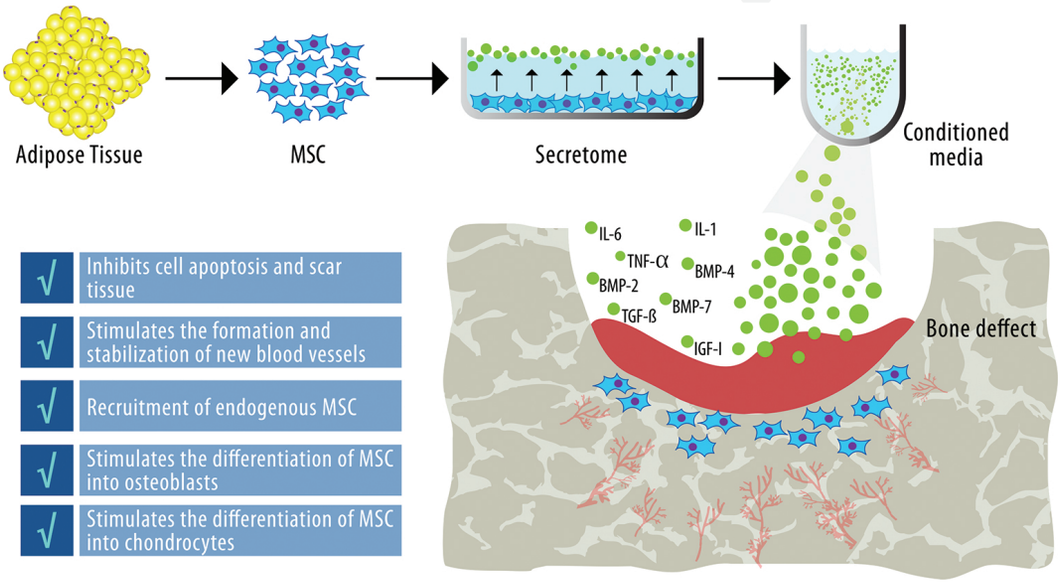

Figure 3. Schematic diagram illustrating the concept of application of MSC conditioned media in bone injuries. The MSC secretome, containing chemokines and growth factors, promote the recruitment of endogenous osteogenic cells and stimulate their migration to injured sites, inducing their differentiation and bone formation.

4.2. Secretome as a therapeutic strategy: conditioned media

The broad spectrum of factors secreted by the different types of MSCs is generally referred as MSC secretome. Recent data demonstrate that MSC secretome factors, collected as conditioned media (CM), are sufficient to exert the MSC therapeutic effects.

Previous studies have reported many growth factors and cytokines derived from the CM of various stem cells [19–21, 85–89], which could be responsible for the paracrine protective effects of stem cells against several diseases. Consequently, the use of stem cells CM instead of direct implantation of stem cells may be a feasible approach to overcome the limitations of current cell-based therapy. In addition, because CM is not a cell, but a conjugate of many growth factors, the administration of CM has no ethics concerns related with cell therapies.

However, secretomic signatures of the various types of MSC are not completely known, and the qualitative and quantitative characterization of MSC secretomes and their functions in

secretome-mediated repair will contribute to the development of new regenerative therapies that will not require cell transplants [90].

Recently, the great potential of tissue engineering and regenerative medicine strategies for bone augmentation has been demonstrated, and the feasibility of using CM from MSC as an osteoinductive agent for future clinical use is becoming more evident. CM from bone marrow-MSC increased the migration and proliferation of MSCs, vascularization and the early bone regeneration in rabbit sinus model, showing CM as a promising novel therapeutic agent to promote bone regeneration after maxillary sinus floor elevation [91]. It has been shown that CM can have stronger effects than MSCs, accelerating the mobilization of endogenous endothelial and MSC cells for bone regeneration in rat calvarial bone defect model [92]. Intravenous administration of MSC-CM provided the protection of osteoblasts and osteoclasts, induced angiogenesis, anti-apoptotic and anti-inflammatory effects in a rat bisphosphonate-related osteonecrosis of the jaw-like model [93]. It has also been reported that the use of MSC-CM may be an alternative therapy for periodontal tissue regeneration [94]. CM from human MSC accelerates the formation of new bone callus, shortening the time period required for distraction osteogenesis treatment in a mouse model by recruiting endogenous mouse bone marrow stem cells (mBMSCs) and EC/EPCs via MCP-1/-3 and IL-3/-6 signaling [95].

We have also reported that human Ad-MSCs and their CM induce bone regeneration in a jaw rabbit model, and that morphometric, radiographic and histological analysis demonstrate that the amount and quality of neoformed bone, repaired area, bone density, arrangement of collagen fibers, maturation and inorganic matrix calcification are very similar between Ad-MSC and CM-treated groups [21] (**Figure 3**).

5. Perspectives

All the scientific evidence on the paracrine effect of MSC provide the opportunity to exploit the therapeutic potential of MSC-CM and opens up scenarios for the identification of new candidate molecules for tissue repair via proteomic analysis of the MSC secretome. MSC-CM delivers osteoinductive growth factors and cytokines that modulate the behavior of endogenous cells contributing to the formation of new tissue. Furthermore, the use of MC allows us to avoid some of the limiting factors associated with the clinical application of stem cells, such as the risk of tumorigenesis and transmission of infectious diseases [80], immunological incompatibility, costs and waiting time for cell ex vivo expansion [80].

The use of MSC-CM as a novel therapeutic strategy has several practical advantages. CM storage and transportation procedures are not as complex as they are for MSC. CM production can be less expensive, enabling access to disadvantaged populations and reducing costs for health systems.

Despite the advantages of its use, CM application may not always supersede the use of MSC, and it is possible that for some type of disorders MSC could be a more effective alternative. The number of known molecules mediating the paracrine effect of MSC grows every day, and significantly increases the potential range of their therapeutic applications.

http://dx.doi.org/10.5772/62523

Author details

Orlando Chaparro* and Itali Linero

*Address all correspondence to: ochaparrog@unal.edu.co

Universidad Nacional de Colombia, Facultad de Medicina - Facultad de Odontología Biología de Células Madre, Ciudad Universitaria, Bogotá, Colombia

References

[1] Lane JM, Tomin E, Bostrom MP. Biosynthetic bone grafting. Clin Orthop Relat Res. 1999;(367 Suppl):S107-17.

[2] Meinel L, Hofmann S, Betz O, Fajardo R, Merkle HP, Langer R, et al. Osteogenesis by human mesenchymal stem cells cultured on silk biomaterials: comparison of adenovirus mediated gene transfer and protein delivery of BMP-2. Biomaterials. 2006;27(28): 4993-5002.

[3] Lewandrowski KU, Gresser JD, Wise DL, Trantol DJ. Bioresorbable bone graft substitutes of different osteoconductivities: a histologic evaluation of osteointegration of poly(propylene glycol-co-fumaric acid)-based cement implants in rats. Biomaterials. 2000;21(8):757-64.

[4] Pagni G, Kaigler D, Rasperini G, Avila-Ortiz G, Bartel R, Giannobile WV. Bone repair cells for craniofacial regeneration. Adv Drug Deliv Rev. 2012;64(12):1310-9.

[5] Pape HC, Evans A, Kobbe P. Autologous bone graft: properties and techniques. J Orthopaed Trauma. 2010;24(Suppl 1):S36-40.

[6] Roden RDJr. Principles of bone grafting. Oral Maxillofac Surg Clin North Am. 2010;22(3):295-300, v.

[7] Skovrlj B, Guzman JZ, Al Maaieh M, Cho SK, Iatridis JC, Qureshi SA. Cellular bone matrices: viable stem cell-containing bone graft substitutes. Spine J. 2014;14(11): 2763-72.

[8] Vo TN, Kasper FK, Mikos AG. Strategies for controlled delivery of growth factors and cells for bone regeneration. Adv Drug Deliv Rev. 2012;64(12):1292-309.

[9] Zimmermann G, Moghaddam A. Allograft bone matrix versus synthetic bone graft substitutes. Injury. 2011;42(Suppl 2):S16-21.

[10] Zouhary KJ. Bone graft harvesting from distant sites: concepts and techniques. Oral Maxillofac Surg Clin North Am. 2010;22(3):301-16, v.

[11] Cancedda R, Giannoni P, Mastrogiacomo M. A tissue engineering approach to bone repair in large animal models and in clinical practice. Biomaterials. 2007;28(29):4240-50.

[12] Shibuya N, Jupiter DC. Bone graft substitute: allograft and xenograft. Clin Podiatr Med Surg. 2015;32(1):21-34.

[13] Taba MJr., Jin Q, Sugai JV, Giannobile WV. Current concepts in periodontal bioengineering. Orthod Craniofac Res. 2005;8(4):292-302.

[14] Giannoudis PV, Einhorn TA, Schmidmaier G, Marsh D. The diamond concept--open questions. Injury. 2008;39(Suppl 2):S5-8.

[15] Khojasteh A, Behnia H, Naghdi N, Esmaeelinejad M, Alikhassy Z, Stevens M. Effects of different growth factors and carriers on bone regeneration: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(6):e405-23.

[16] Mesimaki K, Lindroos B, Tornwall J, Mauno J, Lindqvist C, Kontio R, et al. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg. 2009;38(3):201-9.

[17] Nakamura Y, Wang X, Xu C, Asakura A, Yoshiyama M, From AH, et al. Xenotransplantation of long-term-cultured swine bone marrow-derived mesenchymal stem cells. Stem Cells. 2007;25(3):612-20.

[18] Ide C, Nakai Y, Nakano N, Seo TB, Yamada Y, Endo K, et al. Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. Brain Res. 2010;1332:32-47.

[19] Perin EC, Silva GV. Autologous cell-based therapy for ischemic heart disease: clinical evidence, proposed mechanisms of action, and current limitations. Catheter Cardiovasc Interv 2009;73(3):281-8.

[20] Yoon SH, Kim SK, Kim JF. Secretory production of recombinant proteins in *Escherichia coli*. Recent Pat Biotechnol. 2010;4(1):23-9.

[21] Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PLo S One. 2014;9(9):e107001.

[22] Ratanavaraporn J, Furuya H, Tabata Y. Local suppression of pro-inflammatory cytokines and the effects in BMP-2-induced bone regeneration. Biomaterials. 2012;33(1):304-16.

[23] Shrivats AR, Mc Dermott MC, Hollinger JO. Bone tissue engineering: state of the union. Drug Discov Today. 2014;19(6):781-6.

[24] Shrivats AR, Alvarez P, Schutte L, Hollinger JO. Bone Regeneration. In: Lanza R, Vacanti J, Langer R, editors. Principles of Tissue Engineering. 4th ed. San Diego: Elseiver; 2014. p. 1201-1221. DOI: 10.1016/B978-0-12-398358-9.00055-0

[25] Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury. 2005;36(12):1392-404.

[26] Oryan A, Alidadi S, Moshiri A. Current concerns regarding healing of bone defects. Hard Tissue 2013;2(2):1-12.

[27] Lindahl A, Brittberg M, Gibbs D, Dawson JI, Kanczler J, Black C, Tare R, Oreffo ROC. Cartilage and bone regeneration. In: Van Blitterswijk CA, De Boer J, editors. Tissue Engineering. 2nd ed. San Diego: Elseiver; 2015. p. 529-582. DOI: 10.1016/B978-0-12-420145-3.00016-X

[28] Fernandez-Yague MA, Abbah SA, Mc Namara L, Zeugolis DI, Pandit A, Biggs MJ. Biomimetic approaches in bone tissue engineering: Integrating biological and physicomechanical strategies. Adv Drug Deliv Rev. 2014;84:1-29. DOI:10.1016/j.addr.2014.09.005

[29] Nauth A, Ristevski B, Li R, Schemitsch EH. Growth factors and bone regeneration: how much bone can we expect? Injury. 2011;42(6):574-9.

[30] Lee J, Stavropoulos A, Susin C, Wikesjo UM. Periodontal regeneration: focus on growth and differentiation factors. Dent Clin North Am. 2010;54(1):93-111.

[31] Keramaris NC, Calori GM, Nikolaou VS, Schemitsch EH, Giannoudis PV. Fracture vascularity and bone healing: a systematic review of the role of VEGF. Injury. 2008;39(Suppl 2):S45-57.

[32] Sun X, Kang Y, Bao J, Zhang Y, Yang Y, Zhou X. Modeling vascularized bone regeneration within a porous biodegradable CaP scaffold loaded with growth factors. Biomaterials. 2013;34(21):4971-81.

[33] Ji W, Wang H, van den Beucken JJ, Yang F, Walboomers XF, Leeuwenburgh S, et al. Local delivery of small and large biomolecules in craniomaxillofacial bone. Adv Drug Deliv Rev. 2012;64(12):1152-64.

[34] Hankenson KD, Dishowitz M, Gray C, Schenker M. Angiogenesis in bone regeneration. Injury. 2011;42(6):556-61.

[35] Gerstenfeld LC, Cullinane DM, Barnes GL, Graves DT, Einhorn TA. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem. 2003;88(5):873-84.

[36] Seo BB, Choi H, Koh JT, Song SC. Sustained BMP-2 delivery and injectable bone regeneration using thermosensitive polymeric nanoparticle hydrogel bearing dual interactions with BMP-2. J Control Release. 2015;209:67-76. DOI: 10.1016/j.jconrel.2015.04.023

[37] Lissenberg-Thunnissen SN, de Gorter DJ, Sier CF, Schipper IB. Use and efficacy of bone morphogenetic proteins in fracture healing. Int Orthopaed. 2011;35(9):1271-80.

[38] Jones AL, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, et al. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction

of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. J Bone Joint Surg Am. 2006;88(7):1431-41.

[39] Kanakaris NK, Calori GM, Verdonk R, Burssens P, De Biase P, Capanna R, et al. Application of BMP-7 to tibial non-unions: a 3-year multicenter experience. Injury. 2008;39(Suppl 2):S83-90.

[40] Zimmermann G, Wagner C, Schmeckenbecher K, Wentzensen A, Moghaddam A. Treatment of tibial shaft non-unions: bone morphogenetic proteins versus autologous bone graft. Injury. 2009;40(Suppl 3):S50-3.

[41] Tsiridis E, Upadhyay N, Giannoudis P. Molecular aspects of fracture healing: which are the important molecules? Injury. 2007;38(Suppl 1):S11-25.

[42] Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. J Biol Chem. 1983;258(11):7155-60.

[43] Khojasteh A, Behnia H, Dashti SG, Stevens M. Current trends in mesenchymal stem cell application in bone augmentation: a review of the literature. J Oral Maxillofac Surg. 2011;70(4):972-82.

[44] Bergsten E, Uutela M, Li X, Pietras K, Ostman A, Heldin CH, et al. PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. Nat Cell Biol. 2001;3(5):512-6.

[45] Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am. 2002;84-A(6):1032-44.

[46] Schilephake H. Bone growth factors in maxillofacial skeletal reconstruction. Int J Oral Maxillofac Surg. 2002;31(5):469-84.

[47] Butler AA, LeRoith D. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology. 2001;142(5):1685-8.

[48] Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury. 2005;36(Suppl 3):S20-7.

[49] Kalfas IH. Principles of bone healing. Neurosurg Focus. 2001;10(4):E1.

[50] Drosse I, Volkmer E, Capanna R, De Biase P, Mutschler W, Schieker M. Tissue engineering for bone defect healing: an update on a multi-component approach. Injury. 2008;39(Suppl 2):S9-20.

[51] clinicaltrials.gov. http://www.clinicaltrials.gov: http://www.clinicaltrials.gov/ct2/results?term=mesenchymal+stem+cells&Search=Search; 2016 [cited 2016 January].

[52] Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells. 2010;28(3):585-96.

[53] Badimon L, Onate B, Vilahur G. Adipose-derived mesenchymal stem cells and their reparative potential in ischemic heart disease. Revis Esp Cardiol. 2015;68(7):599-611.

[54] Coulson-Thomas VJ, Coulson-Thomas YM, Gesteira TF, Kao WW. Extrinsic and intrinsic mechanisms by which mesenchymal stem cells suppress the immune system. Ocul Surf. 2016;1-14. DOI:10.1016/j.jtos.2015.11.004

[55] Christ B, Bruckner S, Winkler S. The therapeutic promise of mesenchymal stem cells for liver restoration. Trends Mol Med. 2015;21(11):673-86.

[56] Hu J, Zhang L, Wang N, Ding R, Cui S, Zhu F, et al. Mesenchymal stem cells attenuate ischemic acute kidney injury by inducing regulatory T cells through splenocyte interactions. Kidney Int. 2013;84(3):521-31.

[57] Prockop DJ, Kota DJ, Bazhanov N, Reger RL. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med. 2010;14(9):2190-9.

[58] Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Trends Mol Med. 2001;7(6):259-64.

[59] Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006;98(5):1076-84.

[60] Asahara T, Kawamoto A. Endothelial progenitor cells for postnatal vasculogenesis. Am J Physiol Cell Physiol. 2004;287(3):C572-9.

[61] Clarke D, Frisen J. Differentiation potential of adult stem cells. Curr Opin Genet Dev. 2001;11(5):575-80.

[62] Hendijani F, Haghjooy Javanmard S, Sadeghi-Aliabadi H. Human Wharton's jelly mesenchymal stem cell secretome display antiproliferative effect on leukemia cell line and produce additive cytotoxic effect in combination with doxorubicin. Tissue Cell. 2015;47(3):229-34.

[63] Roufosse CA, Direkze NC, Otto WR, Wright NA. Circulating mesenchymal stem cells. Int J Biochem Cell Biol. 2004;36(4):585-97.

[64] Dicker A, Le Blanc K, Astrom G, van Harmelen V, Gotherstrom C, Blomqvist L, et al. Functional studies of mesenchymal stem cells derived from adult human adipose tissue. Exp Cell Res. 2005;308(2):283-90.

[65] Levi B, Longaker MT. Osteogenic differentiation of adipose-derived stromal cells in mouse and human: in vitro and in vivo methods. J Craniofac Surg. 2010;22(2):388-91.

[66] Levi B, Nelson ER, Brown K, James AW, Xu D, Dunlevie R, et al. Differences in osteogenic differentiation of adipose-derived stromal cells from murine, canine, and human sources in vitro and in vivo. Plast Reconstr Surg. 2010;128(2):373-86.

[67] Badimon L, Onate B, Vilahur G. Adipose-derived mesenchymal stem cells and their reparative potential in ischemic heart disease. Rev Esp Cardiol. 2015;68:599–611. DOI: 10.1016/j.rec.2015.02.025

[68] Shih DT, Lee DC, Chen SC, Tsai RY, Huang CT, Tsai CC, et al. Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells. 2005;23(7):1012-20.

[69] Lin NH, Gronthos S, Mark Bartold P. Stem cells and future periodontal regeneration. Periodontol 2000. 2009;51:239-51.

[70] Ishikawa I, Iwata T, Washio K, Okano T, Nagasawa T, Iwasaki K, et al. Cell sheet engineering and other novel cell-based approaches to periodontal regeneration. Periodontol 2000. 2009;51:220-38.

[71] Kawanabe N, Murata S, Murakami K, Ishihara Y, Hayano S, Kurosaka H, et al. Isolation of multipotent stem cells in human periodontal ligament using stage-specific embryonic antigen-4. Differentiation. 2010;79(2):74-83.

[72] Yang Y, Rossi FM, Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. Biomaterials. 2010;31(33):8574-82.

[73] Mitrano TI, Grob MS, Carrion F, Nova-Lamperti E, Luz PA, Fierro FS, et al. Culture and characterization of mesenchymal stem cells from human gingival tissue. J Periodontol. 2010;81(6):917-25.

[74] Yamada Y, Fujimoto A, Ito A, Yoshimi R, Ueda M. Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. Biomaterials. 2006;27(20):3766-81.

[75] Chen B, Sun HH, Wang HG, Kong H, Chen FM, Yu Q. The effects of human platelet lysate on dental pulp stem cells derived from impacted human third molars. Biomaterials. 2012;33(20):5023-35.

[76] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739-49.

[77] Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair: current views. Stem Cells. 2007;25(11):2896-902.

[78] Slater BJ, Kwan MD, Gupta DM, Panetta NJ, Longaker MT. Mesenchymal cells for skeletal tissue engineering. Expert Opin Biol Ther. 2008;8(7):885-93.

[79] Ankrum J, Karp JM. Mesenchymal stem cell therapy: two steps forward, one step back. Trends Mol Med. 2010;16(5):203-9.

[80] Wang Q, Jin Y, Deng X, Liu H, Pang H, Shi P, et al. Second-harmonic generation microscopy for assessment of mesenchymal stem cell-seeded acellular dermal matrix in wound-healing. Biomaterials. 2015;53:659-68.

[81] Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;3(3):301-13.

[82] Caplan AI. All MSCs are pericytes? Cell Stem Cell. 2008;3(3):229-30.

[83] Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell. 2011;9(1):11-5.

[84] Caplan AI, Sorrell JM. The MSC curtain that stops the immune system. Immunol Lett. 2015;168:136-139. DOI: 10.1016/j.imlet.2015.06.005

[85] Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, et al. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. Circulation. 2004;109(12):1543-9.

[86] Succar P, Breen EJ, Kuah D, Herbert BR. Alterations in the secretome of clinically relevant preparations of adipose-derived mesenchymal stem cells cocultured with hyaluronan. Stem Cells Int. 2015;2015:421253.

[87] Kwon HM, Hur SM, Park KY, Kim CK, Kim YM, Kim HS, et al. Multiple paracrine factors secreted by mesenchymal stem cells contribute to angiogenesis. Vascul Pharmacol. 2014;63(1):19-28.

[88] Tran C, Damaser MS. Stem cells as drug delivery methods: application of stem cell secretome for regeneration. Adv Drug Deliv Rev. 2015;82-83:1-11.

[89] Blaber SP, Webster RA, Hill CJ, Breen EJ, Kuah D, Vesey G, et al. Analysis of in vitro secretion profiles from adipose-derived cell populations. J Transl Med. 2012;10:172.

[90] Kim JM, Kim J, Kim YH, Kim KT, Ryu SH, Lee TG, et al. Comparative secretome analysis of human bone marrow-derived mesenchymal stem cells during osteogenesis. J Cell Physiol. 2013;228(1):216-24.

[91] Katagiri W, Osugi M, Kinoshita K, Hibi H. Conditioned medium from mesenchymal stem cells enhances early bone regeneration after maxillary sinus floor elevation in rabbits. Implant Dent. 2015;24(6):657-63.

[92] Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. Tissue Eng Part A. 2012;18(13-14):1479-89.

[93] Ogata K, Katagiri W, Osugi M, Kawai T, Sugimura Y, Hibi H, et al. Evaluation of the therapeutic effects of conditioned media from mesenchymal stem cells in a rat bisphosphonate-related osteonecrosis of the jaw-like model. Bone. 2015;74:95-105.

[94] Kawai T, Katagiri W, Osugi M, Sugimura Y, Hibi H, Ueda M. Secretomes from bone marrow-derived mesenchymal stromal cells enhance periodontal tissue regeneration. Cytotherapy. 2015;17(4):369-81.

[95] Ando Y, Matsubara K, Ishikawa J, Fujio M, Shohara R, Hibi H, et al. Stem cell-conditioned medium accelerates distraction osteogenesis through multiple regenerative mechanisms. Bone. 2014;61:82-90.

IntechOpen