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Technologies Applied to Stimulate Bone Regeneration

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1. Introduction

Regenerative medicine constantly encounters situations where specific tissues do not have sufficient regenerative capacity to cope with lesions. Routine techniques involve varied surgical procedures, and often involve mechanical, functional and/or aesthetic discomfort. The need to develop alternative techniques for reducing these inconveniences is therefore necessary.

Techniques that stimulate normal tissue repair represent a major advance in biology and regenerative medicine. Frequently applied to the repair of bone lesions and reconstructive surgery, these new biomedical technologies and procedures have afforded technical simplification, elimination of some surgical processes, ease in handling, availability, good levels of predictability and effectiveness, and cost reduction. All this results in an improvement in the quality of life of the patients involved.

All the components of the skeletal system, bones and cartilage as well as the connective tissue present in tendons and ligaments, are capable of repair after an injury. During morphogenesis, bone is formed in a particular sequence of events. First, mesenchymal cells proliferate and differentiate into chondroblasts. This process leads to the production of cartilaginous skeleton. In the following stages, cartilage hypertrophy is observed with mineralization of the cartilaginous matrix. The cartilage cells then replaced by osteoblasts. Vascular invasion is necessary for this stage. The bone is then remodeled. Such events are seen during bone morphogenesis and, in the adult, during fracture consolidation (Reddi, 2001; Tsonis, 2002).

A bone fracture results in the loss of mechanical stability, discontinuity of the bone tissue and partial destruction of its blood supply. Repair is a complex process of tissue regeneration, resulting in stabilization of the fragments, consolidation through bone union,

reconstruction of the tips of the avascular and partially necrotic fragments and, finally, internal and external remodeling of the newly formed tissue.

Hemorrhage caused by the blood vessel lesion, destruction of matrix and death of bone cells occurs at the site of a bone fracture. For the repair to begin, the blood clot and the cellular remnants from the matrix must be removed by the macrophages. The *periosteum* and the *endosteum* that are close to the fractured area respond with intense proliferation, forming tissue that is very rich in osteoprogenitor cells which constitute a collar around the fracture that will penetrate between the ruptured bone extremities, making a ring or collar that is located between the fractured bone extremities. This leads to the appearance of immature bone tissue, both through endochondral ossification of small pieces of cartilage that form there and through intramembranous ossification (Kierszenbaum, 2004; Junqueira & Carneiro, 2008). Areas of cartilage, areas of intramembranous ossification and areas of endochondral ossification can be found at the repair site. This process evolves with the appearance after a while of a bone callus covering the extremity of the fractured bones. The callus is formed of immature bone tissue that will temporarily join together the extremities of the fractured bone (Kierszenbaum, 2004; Junqueira & Carneiro, 2008).

Bone repair is also mediated by Mesenchymal stem cells (MSCs) (Bruder et al., 1994). These cells can be stimulated to differentiate into osteoblasts, cultivating them in the presence of serum, dexamethasone, beta-glycerophosphate and ascorbic acid. Moreover, MSCs can differentiate into osteoblasts due to the influence of vitamin D and BMP-2 (Pittenger et al, 1999). Human adipose tissue also contains stroma cells that are able to differentiate into chondrocytes and osteoblasts (Halvorsen et al, 2001).

The traction and pressure applied to the bone during fracture repair, and soon after the patient resumes normal activities, cause the remodeling of the bone callus and its complete substitution with lamellar bone tissue. When these tractions and pressures are identical to those applied to the bone before the fracture, the bone structure returns to its previous state; unlike other connective tissues, bone tissue, despite its rigidity, heals without scar formation (Kessel, 2001; Junqueira & Carneiro, 2008). Bone formation depends on the existence of an extensive vascular network and on the stability of the fracture focus that facilitates local vascularization, giving rise to the differentiation of the osteoprogenitor cells into osteoblasts; moreover, it is well established that the osteoblasts only synthesize the bone matrix in the presence of high oxygen tension.

The mechanical instability of the fracture hinders local vascularization and under these conditions the osteoprogenitor cells differentiate preferentially into chondroblasts. Accordingly, the fracture focus will initially be filled with cartilage (avascular tissue), which will provide a certain degree of stability to this focus, subsequently favoring vascularization of the site (Kierszenbaum, 2004). The quantity of cartilage formed during embryogenesis and at the site of a fracture (bone granulation tissue) is inversely proportional to the quantity of osteoprogenitor cells and of blood capillaries present at the site. If the bone callus tissue and the capillaries develop at the same time, the osteoprogenitor cells will differentiate into a vascularized environment and will consequently form bone. If there are proportionally few vessels or movement of the fracture segments, there will be cartilage formation followed by its substitution with bone tissue (endochondral ossification). If,

however, the movement is excessive and the vascular supply limited, the establishment of local fibrous connective tissue (fibrosis) is probable (Kierszenbaum, 2004).

In the *regeneration of a fracture without loss of bone mass*, the repair process occurs in a biologically determined order. The first priority is stabilization and consolidation through callus formation on the edges and between the fragments, followed by its remodeling, besides revascularization and substitution of the necrotic areas. External factors can deeply affect the regeneration process, but the tissues act according to biological rules that control proliferation and cell differentiation as well as the production of matrix, which may occur regardless of, yet influenced by, external interferences.

However, *fractures with loss of bone mass* call for the use of grafts or implants. The latter serve as a support to bone regeneration, interacting with the interface of the receptor fragments and stimulating the tissue restoration process. These devices developed to be implanted are currently known as biomaterials (Hench, 1998), and will be addressed subsequently over the course of this text, constituting the basis of procedures such as guided tissue regeneration and tissue engineering. We will also address other technologies applied to bone regeneration, seeking optimization and acceleration of the process.

2. Regeneration biology without loss of bone mass

The bone repair process can be characterized by 6 physiological stages: impact, induction, inflammation, formation of cartilaginous callus, formation of bone callus and remodeling (Heppenstall, 1980).

Impact consists of the period of energy absorption until the fracture. The quantity of energy absorbed depends on the bone volume and is related to the loading rate. The impact stage of the fracture occurs until energy dissipation.

The *induction* stage involves modulation and differentiation of cellular elements required during the regeneration process. In fractures there is always local hemorrhage caused by injury to the blood vessels of the bone and of the periosteum, besides destruction of the matrix and death of the bone cells adjacent to the fractured site (Fawcett, 1986). This process triggers the inflammatory stage that will persist until the remodeling stage, with phagocytic activity of macrophages that will remove tissue and clot remnants. Cells from the periosteum and from the endosteum, close to the fractured area, will be activated (induction stage) and will respond with intense proliferation of their fibroblasts. Mesenchymal tissues, undifferentiated osteogenic and chondrogenic cells will differentiate into functional osteoblasts and chondrocytes, respectively. The stimulus for this induction can be electrical, low oxygen tension, low pH, release of lysosomal enzymes, release of cytosine and the presence of a series of inductor proteins, including bone morphogenetic proteins (BMP) and cartilage growth factors (Reddi, 1981; Canalis, 1983; Zellin *et al*., 1996; Lieberman *et al*., 2002; Oringer, 2002). The induction stage actually occurs through a series of sub-stages in which the inducing phenomena for each subsequent repair stage can be quite distinct, with unique characteristics toward specific target cells (for example: chondrocytes in the cartilaginous callus and osteoblasts in the bone callus).

During the *cartilaginous callus formation* stage, there is a considerable increase of vascularity and cellularity, and of the production of collagen, proteoglycans and lipids.

The callus is electronegative and the osteoclasts continue to remove the necrotic bone. The cartilage formed undergoes modifications, with hypertrophy of the chondrocytes that generate compression on the preexisting cartilaginous matrix, and consequent enlargement of its gaps, being gradually reduced to fenestrated thin septa and spicules of irregular shapes (Probst & Spiegel, 1997). The hyaline matrix from this hypertrophic region becomes calcified, and small granular aggregates and crystals of calcium phosphate are deposited on it.

The *bone callus formation* stage is marked by the substitution of calcified cartilage in primary bone tissue (Heppenstall, 1980; Probst & Spiegel, 1997; Mandracchia *et al*., 2001). Cells with osteogenic potential, originating from the endosteum and particularly from the periosteum, are activated and a thin layer of bone (a periosteal ring or collar) is deposited around the central portion of the calcified cartilage. At the same time, periosteal blood vessels grow, invading the irregular cavities of the cartilaginous matrix created by the enlargement of the chondrocytes and by the confluence of its gaps. Vessels with thin walls branch out and grow into the cavities of the cartilaginous matrix with blind bottoms. Pluripotent cells are carried to the perivascular tissue of these blood vessels and, some differentiate into hematopoietic elements of the bone marrow. Other cells differentiate into osteoblasts, which deposit an aligned layer similar to an epithelium on the irregular walls of the spicules of the calcified cartilaginous matrix, and start the production of bone matrix. The osteoblasts covered by matrix become osteocytes and start to maintain contact with one another through cytoplasmic processes in a system of canaliculi. The callus remains electronegative, while the osteoclasts finish removing the necrotic bone. The cartilaginous matrix is gradually replaced by primary bone tissue.

During *remodeling*, the conversion of the primary bone tissue into secondary or lamellar bone tissue is completed. The collagen fibers are thicker and present preferential orientation alternating between layers or lamellae. These lamellae can be compacted if deposited on a flattened or concentric surface covering a blood vessel. The collagen fibers extend between the lamellae, thus increasing the bone strength. The blood vessels are contained in central canals (the Haversian canals), which intercommunicate through Volkmann's canals. Moreover, there are several canaliculi that extend to nourish the osteocytes. This assembly is known as the *osteon* or classically as *Haversian system*. Secondary osteons are formed when part of the concentric lamellar bone is converted into Haversian systems (Fawcett, 1986). The medullary canal is reestablished and the diameter and electronegativity of the callus decrease until they disappear.

3. Regeneration biology with loss of bone mass

As mentioned previously, the natural phenomenon of bone regeneration is insufficient, on its own, to reestablish the integrity of fractures with substantial loss of bone mass. For bone regeneration process to take place, it is necessary to have four components: a) a morphogenetic signal, b) host cells that respond to the signal, c) an appropriate carrier of this signal that can deliver it to the specific sites and thus serve as support to the growth of responsive cells of the host and d) a viable and well-vascularized bed (Burg *et al*., 2000). Consequently, the need to use materials that would serve as support for the regeneration process is present and currently appears as an alternative to the use of grafts, where there are difficulties involved in obtaining bone tissue and in molding for the fracture in question.

These devices produced to serve as implants are known as biomaterials (Hench, 1998) and should exhibit characteristics that stimulate (osteoinduction) and/or guide (osteoconduction) the bone regeneration process.

Osteoinduction consists of a set of chemical, humoral or physical signals that initiate and sustain the various stages of the bone regeneration process and several factors may be involved (Nakagawa & Tagawa, 2000). The concept of osteoinduction was explored in 1965 by Urist, who showed ectopic bone formation when the demineralized bone matrix was implanted in muscles of rabbits, rats, mice and guinea pigs (Urist, 1965). Afterwards, it was concluded that a protein, called *bone morphogenetic protein* (BMP), was involved in the sequence of events involving chemotaxis, mitosis, bone differentiation and formation (Urist *et al*., 1979). BMP is a glycoprotein of 17,500 daltons, and one of the factors currently identified in bone formation (Adriano *et al*., 2000; Nakagawa & Tagawa, 2000; Reddi, 2000). Nowadays, several groups have shown that BMPs have the capacity to induce new formation of bone tissue by the endochondral route when implanted in ectopic sites in animals used for experimentation (Habibovic & de Groot, 2007). Besides BMP, other factors such as electromagnetic fields and direct currents also manifest inductive properties. The common result of osteoinduction is the modulation and differentiation of cells for bone production.

O*steoconduction,* in turn, is related to the establishment of an appropriate environment model on which osteoprogenitor cells, when adequately stimulated, can produce bone (Heppenstall, 1980). Osteoconduction also facilitates production and bone deposition in the appropriate three-dimensional arrangement and increases the ability of the regeneration process in large segmental defects. Collagen, a natural organic component of bone and of bone surfaces, is the prototype of an osteoconductive substance (Kimura *et al*., 2000; Lee *et al*., 2001). A large number of natural and manufactured substances can also stimulate a favorable environment for bone formation (Mandracchia *et al*., 2001; Pineda *et al*., 1996), as discussed below. Osteoconduction is the phenomenon in which a single vehicle physically conducts the proliferation of osteogenic cells.

Many studies have investigated the inductive signals for bone morphogenesis, but the greatest emphasis has been placed on BMPs (Reddi, 2000). These proteins were found to have highly specialized patterns of expression during bone repair (Bostrom, 1998; Groeneveld & Burger, 2000). During the initial phases of consolidation, some primordial cells express BMPs in the bone callus. Expression is greater in MSCs and chondrocytes when endochondral ossification occurs. Expression decreases as the cartilaginous component of the callus matures. BMPs are expressed by osteoblasts, but decrease as the primary bone is replaced by lamellar bone. BMP-2, -3, -4, -5, -7, and -8 are responsible for the induction of bone and cartilage formation. BMP-12, -13 and -14, are cartilage derived. BMPs have also been used in clinical trials for the treatment fractures and pseudarthrosis, for example (Reddi, 2001).

A large number of natural substances can be extracted and/or manufactured to stimulate a favorable environment for bone formation (Mandracchi *et al*., 2001; Pineda *et al*., 1996). Among all the biomaterials of natural origin that aim to assist in bone regeneration, special emphasis is placed on those of bovine origin, where different protocols of chemical treatment of the bovine bone are evaluated with the purpose of preserving the organic

components and inorganic components, such as collagen and hydroxyapatite repectively, which results in a mixed bovine bone (MBB) with an increase of the material's mechanical resistance. The MBB scaffold used in tissue regeneration has the appearance of a porous sponge that will occupy the space of the bone defect, preventing the migration of epithelial and connective cells, so that the osteoblastic cells have access to the regenerating tissue and start to populate the scaffold. Another role of the scaffold is its use as a vehicle for drugs that induce tissue regeneration and inhibit the progression of the disease.

Autogenous bone grafts are considered very advantageous, since they avoid complications of immunological rejection and supply cells that can immediately start the regenerative process (Cunha et al., 2005, 2006). The use of bone grafts is becoming frequent in orthopedics, as a method for resolution of comminuted fractures, (fractures in which the bone is splintered or crushed) thus significantly reducing the need to amputate an affected limb (Cavassini *et al*., 2001).

One option in the treatment of partial bone defects is to perform the transportation of small bone fragments - called parietal transportation. In this technique (seldom reported in medical literature), the viable bone segment contiguous to the bone cavity is preserved. A bone fragment is created in the healthy region adjacent to the cavity and transported, according to the Llizarov method, filling a cavity of approximately 50% of the bone diameter (Rodrigues & Mercadante, 2005). The option of performing resection of viable bone occupying the complete cortical, transforming the partial defect into segmental for application of the conventional bone transportation technique, appears to us to be absolute nonsense: removing healthy bone when this is what is missing. The main advantage of parietal transportation is bone formation, even during infection.

The exact mechanism of osteoinduction by biomaterials is still largely unknown. Neither is it known whether the mechanisms of osteoinduction by BMPs and biomaterials are the same. In a recent review (Habibovic & de Groot, 2007) striking differences were shown in osteoinduction by BMPs and biomaterials, namely: (1) bone induced by biomaterials is always intramembranous, while bone induced by BMP is formed mainly by the endochondral route; (2) in small animals, just as in rodents, the bone is very rarely induced by biomaterials, but easily by BMPs; (3) bone is never observed on the edge of biomaterials but instead is always formed inside their pores, while bone formation by BMPs is regularly seen on the outside of the carrier and the soft tissue distant from the surface of this carrier.

4. Bone regeneration scaffolds

4.1 Autogenous grafts and allografts

The advantages cited for autogenous grafts in the bone regeneration process allow us to classify it as "gold standard". The presence of osteoprogenitor cells and osteoblasts confer the property of *osteogenesis*; the proteins that are contained in the bone matrix (for example, transforming growth factor β (TGF–β) , and the BMPs) confer the aspect of *osteoinduction;* and the actual mineralized bone matrix provides a structural base for the growth of newly formed tissue, favoring the lodging of cells, the growth of blood vessels, and the deposition of bone matrix, characterizing *osteoconduction* (Bauer & Muschler, 2000).

The autogenous graft of *spongy* or *cortical* origin, whether vascularized or not, presents good integration with the adjacent tissue (Khan et al., 2005). In general autogenous grafts are obtained from the iliac crest, due to ease of access and as the obtainment of spongy bone of good quality, which is considered more osteoconductive, osteogenic and osteoinductive than the cortical bone graft, once it favors the diffusion of nutrients and revascularization of the treated area, and presents in its structure osteoprogenitor cells and osteoinductor proteins. Cortical bone graft acts mainly as a support for bone regeneration, changing the direction of the tissue regeneration process (Cypher & Grossman, 1996).

As regards *mechanical resistance*, the spongy graft does not offer immediate resistance at the grafting site. But the osseointegration process favors the acquisition of resistance during bone neoformation, and in an interval of 6 to 12 months it acquires resistance similar to that offered by the cortical graft (Dell et al., 1985; Stevenson, 1999). The opposite occurs with the cortical bone graft, which initially presents good mechanical resistance; however, over the first 6 months of grafting, this resistance is decreased by the presence of a mixture of newly formed bone and necrotic bone at the grafting site (Goldberg & Stevenson, 1992).

The different properties of spongy and cortical autogenous grafts establish the direction of their clinical use. The spongy autogenous graft can be used in cases involving difficulty in the consolidation of long bone fractures, and in the reconstruction of depressed lateral tibial plateau fractures (Marsh, 2006; Marino & Ziran, 2010; Nandi et al., 2010). A case report on autograft use in osteotomy for ulnar lengthening demonstrates the use of the trabecular autograft in functional recovery of the humeroulnar joint, resulting from difficulty in bone union. The patient presented recovery of movements in the two years of follow-up, achieving 105˚of humeroulnar movement (Doornberg & Marti, 2010).

The cortical bone graft can be used in cases that require greater initial mechanical resistance at the graft site, even with the need for stabilization of the fracture with implants. Bone defects larger than 5 or 6cm are indications for the use of cortical grafts, as they require immediate mechanical support and a longer period of graft use (Nandi et al., 2010).

In spite of clinical results demonstrating the efficacy and safety of use of autogenous grafts in bone regeneration, some *disadvantages* of their clinical application limit their use (Arrington et al., 1996). We can mention high morbidity of the graft obtainment procedure, the aesthetic discomfort of this route, complications related to the surgical technique that mainly include infection and hemorrhage, and the actual limitations of indication, such as young or elderly patients and cases of recurring surgeries (Seiler & Johnson, 2000; Giannoudis et al., 2005).

As possible alternatives to autograft surgeons have allografts, demineralized bone matrix and natural or synthetic bone graft substitutes at their disposal.

Allografts have a growing clinical use, favored by the upgrading of techniques for obtaining, preparing and storing these materials. Fresh, frozen or freeze-dried grafts can be obtained, but fresh materials are used less often due to their technical difficulty and associated risks (Boyce et al., 1999; Keating & McQueen, 2001). Frozen and freeze-dried allografts are kept in specific tissue banks, properly processed and sterilized prior to storage. Their processing ends up eliminating cells naturally present in the graft, so there is no osteogenic activity here. It is considered that the allograft retains osteoinductive activity, and in spite of its

processing some proteins are maintained. But its main activity is conduction of bone formation. However, even the osteoconductive activity can be affected by the material processing. The freezing, drying and sterilization stages, normally using Gamma rays, end up weakening the graft structure, reducing its mechanical properties (Pelker & Friedlaender, 1987; Henman & Finlayson, 2000).

The advantages of allograft use include immediate availability in a sufficient quantity for any treatment and in varied forms, facilitating clinical handling of this graft (Nandi et al., 2010). Li and collaborators described allograft use in the treatment of malignant humeral resection in patient treated between 2005 and 2008, with bone regeneration occurring at 26.3 weeks on average (Li et al., 2011). In another study, Virolainen and collaborators performed a survey of 10 years of allograft use for the treatment of periprosthetic fractures. This type of fracture can entail some surgical complication, and in this case the fractures occur soon after the prosthesis implant surgery, while fractures occurring at a later stage usually result from osteolytic lesions or osteoporosis. In both cases there is bone impairment at the implant site, hindering corrective surgical treatment. There were 71 patients treated between 1999 and 2008 with the use of cortical allograft and stabilization of the site with metal implants, and the patients presented a bone union rate of 91%. Allograft use was considered adequate, allowing biomechanical stability of the site (Virolainen et al., 2010).

4.2 Demineralized bone matrix

An alternative to bone tissue regeneration induction is the use of demineralized bone matrix (DBM) (Pietrzak et al., 2005). This type of biomaterial, obtained by acid hydrolysis of the bone matrix, through the action of hydrochloric acid, basically presents osteoinductive properties (Tuli & Singh, 1978; Katz et al., 2009). Its principle of action is based on preservation of the trabeculated collagen structure of the matrix and of bone formation inductor proteins, even with the processing of the tissue, obtained preferentially from human or bovine bones. The use of DBM to replace grafts should be observed with restrictions, as it does not present osteoconductive properties, due to the absence of the calcified bone matrix, and osteogenic properties, since processing for demineralization ends up killing the cells initially present in the tissue.

Nowadays there is a wide variety of available forms of DBM, either rigid or malleable. One of their main applications is the treatment of unconsolidated fractures (Pietrzak et al., 2005), in addition to the filling of bone cysts and cavities (Docquier & Delloye, 2005) and long bone fractures (Tiedeman et al., 1995; Keating & McQueen, 2001). Pieske and collaborators presented data on 20 patients with unconsolidated diaphyseal long bone fractures, treated between the years 2000 and 2006. The patients received autogenous grafts (n=10) or demineralized bone matrix (n=10), with bone formation having been observed in all the patients treated with DBM, while 20% of the patients treated with autogenous graft did not obtain the expected result (Pieske et al., 2009). The use of demineralized bone matrix has also been indicated for treatments of arthrodesis of the spinal column on account of its bone formation inducing action, and there may be an association with osteoconductive graft substitutes (Morone and Boden, 1998; Park et al., 2009). However, one of the disadvantages of demineralized bone matrix is related to the significant variability of donor sources, and corresponding variability of results obtained (Pietrzak et al., 2005).

Therefore in spite of the *availability* of natural materials as autogenous grafts, allografts and demineralized bone matrix, some limitations of use or clinical disadvantages of these materials drive the development of new technologies for bone tissue regeneration. *Natural and synthetic bone graft substitutes* are available to perform this role. The synthetic bone graft substitutes include ceramic and polymeric biomaterials, while biopolymers represent natural bone graft substitutes, including collagen and chitosan.

4.3 Bioceramics

Bioceramics are biocompatible biomaterials with a long history of clinical applications for bone regeneration. Among the advantages of these biomaterials we can cite their synthetic origin, eliminating the risk of autograft morbidity, or the risk of immunorejection and transmission of diseases of allografts or even biomaterials of human and animal origin. The structural similarity between some bioceramics, such as hydroxyapatite and beta-tricalcium phosphate, and spongy bone, allows us to classify them as biomimetic in relation to physical structure and chemical composition (Giannoudis et al., 2005). This mimicry favors the differentiation of osteoprogenitor cells and the deposition of bone matrix, characterizing bioceramics as essentially *osteoconductive*. The porous structure of bioceramics, or even the crystalline structure of calcium sulfate, also allows neoangiogenesis, which is essential in the osteoconduction process. Bioceramics of interest in the bone tissue regeneration process are those classified as temporary, since they are gradually replaced by newly formed bone (Tormala et al., 1998). Calcium sulfate and beta-tricalcium phosphate do this. Resorption time varies depending on the bioceramic in question, but is generally consistent with the bone callus formation time, sustaining tissue regeneration as osteoconductive agents. Hydroxyapatite is not considered resorbable by many authors, since the resorption process of this bioceramic averages 5 years, which corresponds to the period of natural bone remodeling of the body. Therefore it is considered that this bioceramic is integrated to the newly formed bone tissue and its resorption occurs during the intrinsic remodeling of the tissue.

Calcium Sulfate is one of the synthetic biomaterials with a long history of clinical use as a graft substitute for bone regeneration (Peltier et al., 1957; Tay et al., 1999). The dihydrated form of calcium sulfate, also called "gypsum", presents a crystalline structure that is not very uniform, and is currently used as a raw material in a calcination process that results in hemi-hydrated calcium sulfate (CaSO4.½ H2O), also called "Plaster of Paris" (Peltier et al., 1957). Calcium sulfate presents optimal biocompatibility, with reports of sporadic cases of inflammatory reaction after its use, with good evolution and spontaneous resolution in most cases. The length of stay in the organism is 8 weeks on average, a relatively short time, yet sufficient for bone callus formation to begin (Coetzee , 1980; Kelly et al., 2001). Calcium sulfate has ample clinical application potential, including bone defects resulting from trauma or created surgically, such as osteotomies and resection of tumors (Finkemeier, 2002; Kelly et al., 2001), as well as spinal surgery, for filling or bone fusion (Hadjipavlou et al., 2001).

More recently there was a proposal for the expansion of the clinical use of calcium sulfate as an *antibiotic release* agent, since it ensures high local concentration of the drug, avoiding its systemic circulation (Gogia et al., 2009). Reports demonstrate the control of osteomyelitis through the application of calcium sulfate pellets with antibiotics such as tobramycin,

vancomycin and gentamicin (Bibbo & Patel, 2006; Chang et al., 2007). A randomized, prospective clinical study, published in 2010, presents data on local control of chronic osteomyelitis of long bones and cases of infection at non-bone consolidation sites. Thirty patients were treated, with half receiving calcium sulfate associated with tobramycin and the other half bone cement (polymethyl methacrylate) impregnated with antibiotic. The results demonstrated the mean follow-up of the patients for 38 months (ranging between 24 and 38 months) with the resolution of 86% of the cases in both experimental groups, concluding on the efficacy of calcium sulfate application in the local control of osteomyelitis (McKee et al., 2010).

In turn, *calcium phosphates* constitute bioceramics with a nanoparticulated physical structure, porous with pores of 100µm to favor the osteoconductive aspect of the biomaterial. The pore density can range between 40% and 60%, with Ca:P stequiometric ratio similar to spongy bone, imitating it (Gautier et al., 1998; Tanaka et al., 2008; Porter et al., 2009). Osteoconduction with calcium phosphate, often used in beta conformation, as *beta-tricalcium phosphate* (β-TCP, CA3(PO4)2), results in resorption of the biomaterial and osseointegration of the treated region in approximately 12 weeks. The bioresorption process occurs through a combination of dissolution and osteoclastic resorption at the implant site (Dong et al., 2002).

The persistence of the biomaterial favors the treatment of cavities resulting from bone resection, filling of osteotomy regions, defects of critical size of the bone (Gaasbeek et al., 2005; Tanaka et al., 2008) or even spinal fusion. Le Huec and collaborators reported the use of β-TCP for spinal fusion in 30 patients in association with bone graft, in comparison to another 24 patients treated with cortical allograft. The authors did not report pseudarthrosis and demonstrated the formation of bone callus 6 months after the β-TCP implant, with full resorption in 2 years (Le Huec et al., 1997).

The physical properties of β-TCP favor its association with liquids such as blood and bone marrow aspirate. In an experimental study with dogs, Bruder and collaborators demonstrated bone formation and the refinement of the bioceramic in association with mesenchymal cells obtained from bone marrow aspirate (Bruder et al., 1998). In 2007 the same author published, together with collaborators, the result of an experimental application of β-TCP grafts in sheep for posterolateral fusion (Gupta et al., 2007). In this experiment the authors compared the results of the fusion process with the use of autograft, biomaterial enriched with mesenchymal cells, biomaterial associated with total bone marrow aspirate and pure biomaterial. The radiological findings, in line with histological data, demonstrated a high rate of bone formation after 6 months in the presence of autograft (25%) and in the presence of the biomaterial enriched with cells (33%), whereas the biomaterial associated with the total bone marrow aspirate presented a low rate of bone formation (8%) and no bone formation was observed with the use of pure biomaterial, reinforcing the need for association of characteristics such as osteogenesis for guided tissue regeneration.

Unlike beta-tricalcium phosphate, *hydroxyapatite* ($Ca_{10}(PO_4)_6(OH)2$) a bioceramic with a low resorption rate and greater mechanical resistance, is commonly used in association with beta-tricalcium phosphate, in the proportion of 60/40 to improve osseointegration of the graft substitute (Balcik et al., 2007). The porosity of the biomaterial is essential for its action, requiring pores of 100-200µm, at a density of 60 to 65% for cellular lodging and

vascularization of the treated area, confirming the osteoconductive action of hydroxyapatite (Giannoudis et al., 2005). The regeneration of defects of critical size and defects in long bones, created surgically or resulting from trauma, are general indications for its use, either pure or in association with β-TCP. Hydroxyapatite can also be used in spinal fusion procedures. The report on the use of hydroxyapatite in orthopedic lesions, including the resection of bone tumors, and the treatment of cystic lesions in rheumatoid arthritis, without the occurrence of adverse reactions and with good clinical evolution of the patients, was published by the group of Yoshikawa and collaborators (2009).

Bioactive glass, or *bioglass*, is a biocompatible bioceramic that allows good integration with newly formed tissue (Hench et al., 1971). It is basically composed of silica, sodium oxide, calcium oxide and phosphates. Some factors influence the integration of bioglass with the surrounding environment, such as composition of the biomaterial, pH of the environment, temperature, and porosity, directing its osteoconductive function (Nandi et al., 2010). Bioglass is indicated for filling bone cavities in general, in reconstructive surgery, including craniofacial defects, besides spinal column fusion procedures (Asano et al., 1994; Suominen &, Kinnunen, 1996).

4.4 Polymeric biomaterials

Among the polymeric, biocompatible and bioresorbable biomaterials used for bone tissue regeneration, the poly (L-lactic acid) (PLLA), poly (glycolic acid) (PGA), polycaprolactone (PCL) polyesters, and their copolymers, such as poli (D,L-lactic-co-glycolic acid) (PLGA) (Santos & Wada, 2007; Santos, 2010) deserve special emphasis. These polymers are often associated with bone formation induction proteins, such as BMPs or even with osteoconductive bioceramics, such as hydroxyapatite. One of the advantages of the use of polymers for tissue regeneration resides in the wide variety of possible applications, not just as graft substitutes, but also as fastening elements, including screws and plates. Bone tissue regeneration is guided by the polymer structure used, whereas proliferation induction and cellular differentiation are observed in these specific scaffolds (Ishaug-Riley et al., 1998; Santos et al., 2001; Santos et al., 2004). The PLGA copolymers implanted in bones induce bone tissue neoformation at the implant site, over a variable period of time, depending on the ratio of polyesters present in the copolymers (Reed & Gilding, 1981).

Polymer/bioceramic composites have the advantage of conferring on polymers the intrinsic biomechanical property of calcium phosphates, such as hydroxyapatite, favoring osteoconductive characteristic of the biomaterial (Hutmacher et al., 2007). Osteoblast cell cultures in porous PLLA/hydroxyapatite composites (PLLA-HA) enable cell proliferation, the lodging of cells throughout the scaffold of the biomaterial and the differentiation of these cells with synthesis of mineralized matrix (Ma et al., 2001). These results are corroborated by the study of Rizzi and collaborators with the biomaterial of PLA-HA and PCL-HA (Rizzi et al. 2001). HA induces the activity of the bone cells preferentially adhered to these particles, exposed on the surface of the composite.

The application of HA-PLLA to two cases of mandibular reconstruction after tumor resection was published recently (Matsuo et al., 2010). The plates were designed with the use of computed tomography. In one of the cases there was association of the composite

biomaterial with growth factors obtained from platelets harvested from the patient and in the second case there was a dental graft. Both cases presented good clinical evolution, without the observation of bone resorption in two years of follow-up, and with the formation of good quality bone.

4.5 Other biomaterials

Besides the synthetic bioceramics and polymeric biomaterials, some biomaterials obtained in nature present considerable potential for application in bone regeneration: coralline hydroxyapatites and chitosan.

Similar to hydroxyapatite, *coralline hydroxyapatites* have been explored recently for their osteoconductive potential. They derive from marine corals, with a calcium carbonate base, a porous structure, and pore size ranging between 100 and 500μm, suitable for the proposed function. They can be obtained directly in nature (and processed mainly for sterilization), or obtained from hydroxyapatite (Keating & McQueen, 2001). Indications for use include long bone fractures and tibial plateau fractures, presenting a behavior similar to the autogenous graft (Bucholz et al., 1989).

Chitosan is another biocompatible biomaterial with potential for clinical application under analysis, and is considered very promising for the area of tissue regeneration. It is a natural biopolymer, obtained from the polysaccharide chitin, common in the exoskeleton of crustaceans (such as shrimps and lobsters). It presents encouraging results demonstrating its performance as an osteoconductive biomaterial guiding osseointegration. A study published in 2003 uses chitosan glutamate associated with hydroxyapatite for the treatment of defects of critical size in rat calvaria. The results were obtained after 9 and 18 weeks. The association with osteoprogenitor cells obtained from bone marrow proved ideal for tissue regeneration according to the protocol under investigation, including with mineralization of the treated areas (Mukherjee et al., 2003).

A recently published study (Jayasuriya & Kibbe, 2010) demonstrates the preparation of chitosan microparticles on a wide scale, and the incubation of these particles in concentrated physiological fluid for the stimulation of in vitro biomineralization and subsequent incorporation of insulin-like growth factor (IGF-1). The study evidenced the release of IGF-1 over a 30-day period, characterizing the possibility of the biomaterial's use as a drug release agent.

Collagen, in turn, exhibits a series of possible clinical applications, such as a scaffold for the regeneration of various tissues, including skin, cartilage and bone. It is a natural biopolymer, obtained from animal tissue, generally bovine, with low toxicity and immunogenicity. It can be made available in the form of gels, films and sponges, favoring cell adhesion and resorption, driving the regenerative process. In the case of bone regeneration, collagen is often associated with osteoconductive materials such as beta-tricalcium phosphate or hydroxyapatite (Wahl & Czernuszka, 2006). These composites aim to reproduce the natural conditions of bone and thus to drive cell behavior, with the differentiation of osteoblasts and the synthesis of mineralized bone matrix (Zhang et al., 2010). A randomized, prospective clinical study brings data on the clinical application of collagen biomaterial associated with calcium phosphate bioceramic in the treatment of long bone fractures, having the use of autogenous grafts as a form of control. The

fractures were stabilized with metal implants suitable for each case. There was a follow-up on 213 patients, and a total of 249 fractures. According to the authors the collagen-based composite had the same performance observed for the autograft as regards fracture union rate and functional measurements, and is a possible treatment alternative (Chapman et al., 1997).

Associations of biomaterials, initially used as *scaffolds* for the conduction of bone formation, with tissue regeneration *inductor proteins,* are not just a promise for regenerative medicine, but are already taking shape as potential and usual clinical applications. At the same time associations with *osteoprogenitor cells* or bone marrow aspirate are also consolidating for the refinement of the functions of these scaffolds.

5. Stem cells and bone regeneration

Cells are the essential elements during repair and regeneration, with *stem cells* playing an important role in this process, as already mentioned previously. Nowadays there are a growing number of studies seeking therapeutic strategies and applications using stem cells to minimize clinical problems caused by injury or diseases in the bone tissue (Meyer, et al., 2006; Charbord, 2010), which present increasing demand, considering the demographic growth of the population and the rise in the number of elderly citizens, where the frequency of diseases in the musculoskeletal system is higher (De Peppo et al., 2010).

6. Stem cells and their application to regeneration and to bioengineering of bone tissue

Stem cells correspond to a group of *undifferentiated cells* with the capacity for unlimited self-renewal, as they are capable of successive divisions throughout the entire lifetime of the organism. Moreover, these cells, once stimulated by specific signals and under ideal conditions, will be able to differentiate into cell types with specialized forms and functions and that will maintain the homeostasis of the body. Therefore the proliferative capacity associated with the potential to differentiate into different specific cell types, confer immense potential for application to different areas of biomedicine including gene therapy and tissue engineering on stem cells (Kirschstein & Skirboll, 2001).

Thus, the success of tissue engineering depends on the use of the appropriate cells, on the ability to predict the cell response and on culture techniques for proliferation and differentiation into specific cell types. Nowadays tissue engineering applications are allowing, among others, the use of cells from the actual patient (autologous cells), from donors (allogenic), from different species (xenogeneic), from immortalized lineages (both allogeneic and xenogeneic) and fetal and adult stem cells (Parenteau, 2002); which can be cultivated on molds of biocompatible materials, and subsequently implanted to the injured tissue or inoculated directly or onto the biomaterials at the implant sites. This methodology opens vast perspectives for application in the medical area, allowing the performance of graft implants in injured tissues leading to a greater benefit to the patient, with the initial use of a small number of cells, which will be expanded *in vitro* by means of culture techniques, and also due to the fact that it will be possible to either minimize or avoid immunological problems such as rejection of non-autogenous transplants (Calvert et al.,

2000; Temenoff & Mikos, 2000a,b). To this effect, several strategies are being applied to improve the efficiency of tissue engineering such as growth factors and recombinant differentiation factors, use of autologous cells, gene therapy through the incorporation of vectors and genetic engineering of cells (Satija et al., 2007).

7. Embryonic stem cells

The self-renewal capacity of *human embryonic stem cells (ESCs)* over prolonged periods and their ability to differentiate into different tissues from the three embryonic layers, were characterized by Thomson and collaborators (Thomson et al., 1998). These oocyte-derived cells fertilized in the morule phase or derived from the inner cell mass of embryos in the blastula phase, are able to divide in an unlimited manner, keeping their original characteristics and genetic information, besides being pluripotent, that is, they can differentiate into practically all cell types, derived from the three embryonic germ layers, mesoderm, ectoderm and endoderm (Figure 1) (Doetschman et al., 1985; Smith, 2001).

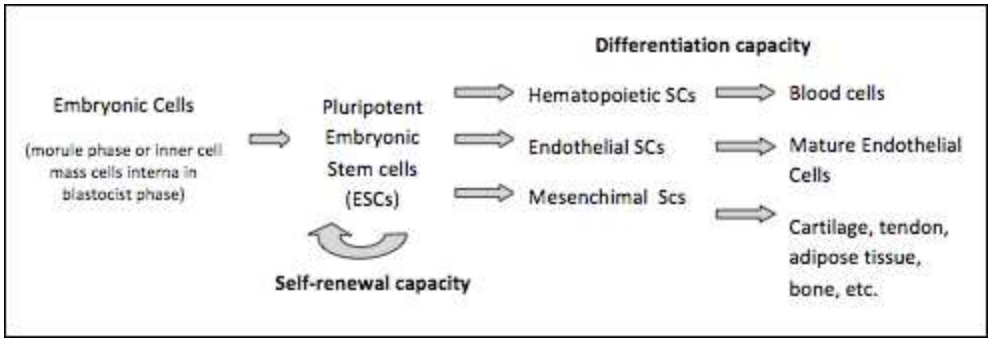

Fig. 1. Diagram showing pluripotent property of embryonic stem cells and their capacity to originate cell types from the three embryonic germ layers.

Although there is immense potential for the use of embryonic stem cells, due to their pluripotency, in practical terms their use is still very limited due to problems including cell regulation, immunological incompatibility, and possible development of neoplasias upon their administration (Passier & Mummery, 2003). These complications are also accentuated by ethical and religious issues and government regulations vis-à-vis the use of human embryonic cells in research (Zuk et al., 2001; Lee et al., 2003, Undale et al. 2009). Such factors led scientists to seek options with greater application potential, such as adult stem cells.

8. Adult stem cells

Although they decrease with age, *adult stem cells* are present in a wide variety of tissues throughout the lifetime of an individual. These cells, like the ESCs, also have the capacity for unlimited self-renewal and the potential to differentiate into cell types with specific morphologic and functional characteristics. This differentiation process generally involves intermediate cell types called precursor or progenitor cells that, although with a reduced self-renewal capacity, can split up to produce specific cell types (Robey, 2000; Gamradt & Lieberman, 2004). Accordingly, adult stem cells are being identified by different methods

and there is a growing number of tissues and organs identified as carriers of the so-called mesenchymal stem cells (MSCs), including the bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelium of the skin and of the digestive system, cornea, retina, liver, pancreas and others, whereas the umbilical cord and the placenta are also carriers of cells similar to the mesenchymal stem cells. In spite of the fact that they have similar characteristics, MSCs of different origins present varied differentiation and gene expression potentials. Bone marrow is known to present considerable potential for obtaining stem cells and they have been studied with clinical and therapeutic objectives for fractures with substantial loss of bone mass and metabolic diseases involving the bone tissue.

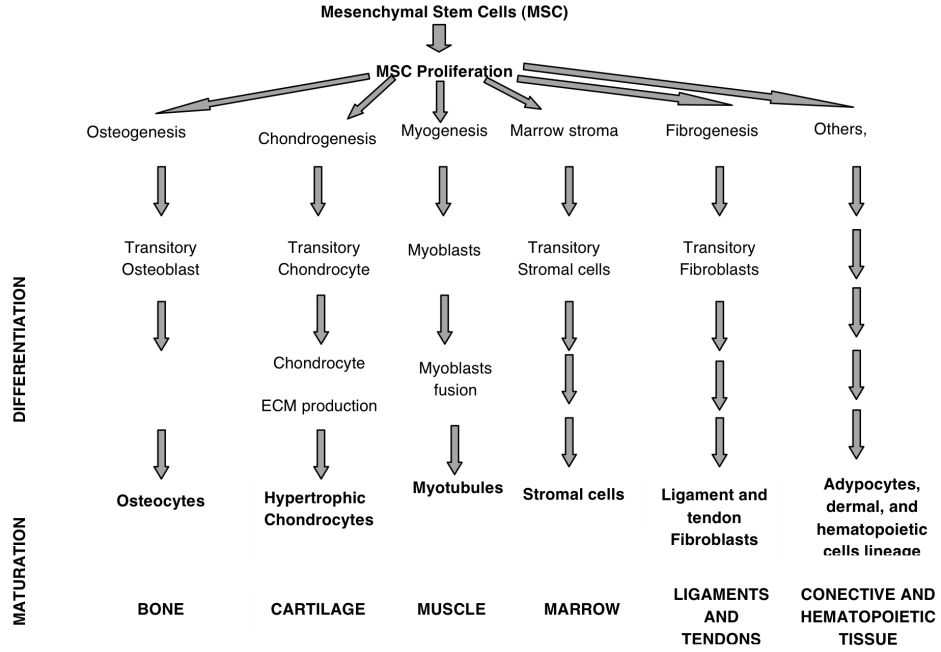

Fig. 2. Summary diagram showing the capacity of mesenchymal stem cells (MSCs) to differentiate into bone, cartilage, muscle, tendons/ligaments and other tissues. Each stage of this differentiation and maturation process involves the control and the interaction of growth factors and cytokines (Caplan, 2010).

9. Bone marrow stem cells

The pioneer studies that evidenced the separation of a population of cells with the capacity to differentiate into a variety of cell types including osteoblasts, chondrocytes, adipocytes and hematopoietic cells, were carried out by Friedenstein *et a*l, in the sixties (Friedenstein et. al., 1968). Their studies demonstrated the existence of precursor *mesenchymal cells*, with the potential to differentiate into osteoblasts and fibrous tissue (Figure 2) (Charbord, 2010; Hidalgo-Bastida et al., 2010).

These stem cells in the muridae are frequently obtained from femoral or tibial flushing, obtaining the bone marrow mononuclear cells (BMMNCs) that are isolated by density gradient centrifugation then cultivated *in vitro*. On the other hand, human cells are aspirated from the iliac crest and cultivated directly, since gradient centrifugation techniques have not been seen to increase the separation efficiency and frequently present contamination with hematopoietic cells. This methodology is used to obtain populations that undergo a cloning process, and are characterized by the presence of both positive markers (Stro-1, CD29, CD73, CD90, CD105, CD166 and CD44) and negative markers (CD43, CD45, CD14, CD11b, CD19, CD79a and HLA-DR). The positive expression for Stro-1, identifies cells with osteogenic potential and expression of the three markers for osteoblast differentiation: alkaline phosphatase, 1,25-dihydroxy-vitamin D that has induction dependent on the specific bone protein: osteocalcin and hydroxyapatite production (mineralized matrix). Recent studies have pointed to other markers present in mesenchymal stem cells of the bone marrow that present osteogenic potential (Undale et al., 2009).

The molecular regulatory mechanism involved in the MSC differentiation control process has been extensively studied *in vitro*, whereas *in vivo* control is little known due to the difficulties inherent in the study process. However, the properties of the MSCs *in vivo* and *in vitro* vary according to the method of removal of these cells from their natural environment and the use of chemical and physical factors to keep them in culture, which can lead to alterations in their characteristics. Heterogeneity and diversity of types of MSCs and their ability to undergo phenotypic rearrangements in culture, modifies the expression of markers and hinders the comparison of data or renders it unfeasible in some situations (Augello & De Bari, 2010).

In vitro, the classical methodology to induce osteogenic differentiation in human MSCs consists of incubation with bovine fetal serum, in a medium supplemented with ascorbic acid, β-glycerophosphate and dexamethasone, which leads to the increase of alkaline phosphatase and calcium deposition (Jaiswal et al, 1997; Pittenger et al. 1999).

On the other hand, *in vivo*, the information obtained from studies of embryonic development indicates that different signaling routes and transcription factors may play a critical role in the differentiation of MSCs. Different molecules have been described in the regulation of MSC differentiation, including Wnt and the TGF-β superfamily.

Wnt proteins, coded by a family of 19 genes in humans and in mice, are involved in cell proliferation, differentiation and apoptosis. They act directly on MSCs, and are crucial for embryonic development and regeneration of different tissues in adults, including bone. Etheridge and collaborators (2004) demonstrated that MSCs express a series of ligands including Wnt2, Wnt4, Wnt5a, Wnt11, and Wnt16, and different Wnt receptors, FZD2, 3, 4, 5, and 6, as well as several co-receptors and inhibitors.

Several studies have shown that osteogenic differentiation *in vitro* is upregulated by some molecules related to the Wnt family and downregulated by others. For example, the administration of exogenous Wnt3 leads to osteogenesis repression. The TGF–β superfamily represents a set of growth factors and morphogens that play a role in skeletogenesis and in postnatal skeletal homeostasis. The TGF–β superfamily of ligands includes BMPs, growth and differentiation factors (GDFs), anti-mullerian hormone (AMH), activin, nodal, and TGF–β (Piek et al., 1999; Derynck & Miyazono, 2008).

The expression of growth factors from the TGF–β family is crucial for bone repair in adults and has been described during the embryonic phase as essential for the development of cartilage and bones. TGF–β1 promotes the specific gene expression, initializing chain events with the participation of the SMAD proteins, which lead to the process of chondrogenesis and differentiation of the MSCs (Tuli et al., 2003).

BMPs are important morphogens involved in the regulation of chondrogenesis and osteogenesis during normal embryonic development (Hogan, 1996). The effects of BMPs on MSCs has been investigated, demonstrating that the culture of MSCs in the presence of BMP2 increases alkaline phosphatase activity and osteocalcin expression, both indicators of osteoblast differentiation, whereas this effect is intensified in the presence of dexamethasone. Other factors are also known to influence the differentiation of MSCs, interacting at different levels with the metabolism of the Wnts and/or TGF–β/BMP. One of these factors is FGF-2 (fibroblast growth factor - 2), which promotes cell proliferation and maintains the populations of MSCs undifferentiated for prolonged periods of time (Martin et al., 1997).

10. Applications and clinical potential

Different characteristics of MSCs, including their availability, potential for autologous use and absence of immunological rejection, make them very promising for clinical and therapeutic applications, especially in fractures with significant bone loss and metabolic diseases (Caplan, 2010). In spite of the major advances that have occurred in orthopedic surgery, fractures that involve considerable bone loss and non-union still represent a very important clinical problem. During the normal regeneration of a fracture, as seen previously, undifferentiated MSCs, with the assistance of BMPs and regulatory cytokines, proliferate and differentiate into chondrocytes and osteoblasts, which will form bone tissue reconstituting the lesion. Although related to the site where they occur, around 5 to 20% or more of fractures present failure in regeneration and consolidation (Kimelman et al., 2007; Undale, et al, 2009). Experiments on animal models using autologous MSCs and different scaffolds have resulted in bone regeneration (Arinzeh et al, 2003; Bruder et al. 1998a, b; Kon et al, 2000; Petite et al, 2000). Clinical studies on humans with the use of MSCs aspirated from the iliac crest and subsequently expanded in cultures on different biomaterials (Quarto et al., 2001; Marcacci et al, 2007), or percutaneously injected (Hernigou et al., 2005), have also been conducted, indicating clinical success positively correlated with greater capacity for *in vitro* formation of colonies and concentration of injected MSCs. Clinical applications in humans have also been described in patients with metabolic diseases of the bone tissue such as osteogenesis imperfecta and hypophosphatasia. Cultures of allogeneic MSCs and intravenous administration have mainly been used in these diseases, demonstrating the ability of these stem cells to stimulate bone mineralization and regeneration (Undale et al., 2009)

11. Mesenchymal stem cells of the adipose tissue

Stem cells play a crucial role for the body's homeostasis, as they maintain the functional state of the tissues and also replace cells killed by injury or disease. These cells are very rare in the adult (Kirschstein & Skirboll, 2001). For example, it is estimated that in the bone

marrow only one among ten to fifteen thousand cells is a hematopoietic source cell (Weissman, 2000).

Although the bone marrow is the place where the presence and the differentiation process of MSCs is currently best known and characterized, they are also found in other places (Gamradt & Lieberman, 2004).

Studies have indicated that MSCs are also found in animal (Lee et al., 2002) and human (Zuk et al., 2002) *adipose tissue*, and can be obtained by the lipoaspiration process. They are frequently referred to in literature as PLA (processed lipoaspirative) or ADAS (adipose-derived adult stem cells). Different studies have evidenced that mesenchymal stem cells obtained from the adipose tissue, when stimulated by different factors, can also differentiate into adipose cells (Halbleib et al., 2003), osteoblasts (Hicok et al., 2004), chondroblasts, myocytes and neural cells, which means that they draw great interest for applications in regenerative medicine and in tissue engineering (Barry & Murphy, 2004; Ogawa et al., 2004a,b). Since they are easily and abundantly obtained by lipoaspirative process, which is therefore less invasive, using local anesthesia, mesenchymal cells from the adipose tissue offer advantages over the bone marrow (Mizuno & Hyakusoku, 2003; Macleod et al, 2010). In the latter, the obtainment of mesenchymal cells is generally performed using aspiration and flushing of the upper part of the iliac crest, involving a process that is extremely painful for the patient, with the risk of a general or spinal anesthesia, usually implying morbidity of the donor site, resulting in a small number of functional cells. Now the obtainment of mesenchymal cells from the adipose tissue has presented more homogeneous populations with normal karyotype, and can be kept *in vitro* for long periods, with constancy in the cell doubling time and low levels of senescence (Zuk et al., 2002; Aust et al., 2004). Comparative studies between the mesenchymal cells of the bone marrow and of adipose tissue have shown that they both exhibit similarity in their ability to differentiate into adipose cells, from the bone, cartilaginous and muscle tissues; share similarities in the kinetics of growth and senescence, with the capacity for gene transduction and also among the cell surface markers (Mizuno & Hyakusoku, 2003; De Urgate et al., 2003a,b; Mosna et al., 2010)

Thus the autologous mesenchymal stem cells of the adipose tissue are also being used in the construction of three-dimensional scaffolds and applied to patients with severe problems of bone mass loss (Gamradt & Lieberman, 2004).

12. Stem cells and gene therapy: prospects of future applications

Genetic engineering of adult stem cells with genes presenting osteogenic potential has gained considerable emphasis in the repair of fractures and bone tissue formation. Studies have indicated that these genetically modified cells can produce autocrine and paracrine effects on the stem cells present in the actual patient, leading to a greater response in the osteogenic effect. These strategies involve both the use of viral and non-viral vectors, presenting genes that code different BMPs, as well as genetically modified cells containing these implanted transgenes. The advance of these studies may be essential for the future prospects of clinical use of stem cells for bone regeneration (Kimelman et al, 2007), bringing more efficient solutions in the field of orthopedics.

The proliferation capacity of MCSs is a measure of the number of cell divisions that can occur *in vitro* after the culture has been started. Many studies suggest that MCSs have doubling capacity of up to 50 times; after this period the culture is characterized by alteration of a series of cellular characteristics and properties, followed by senescence or even cell transformation. The senescence process is characterized by modifications to morphology and increase of cell volume, reduction in surface marker expression and decrease in differentiation potential. Several molecular mechanisms have already been identified in the senescence process, including DNA injury, accumulation of the cyclin-dependent kinase inhibitor, oxidative stress, telomeric modifications, action of epigenetic factors, and others (Wagner et al, 2010).

Accordingly, the safe and efficient clinical application of stem cells to bone tissue regeneration depends on the elucidation of mechanisms associated with senescence. Moreover, it is essential to understand the mechanisms of action and interaction with other cell types, with different biomaterials, soluble factors, extracellular matrix components (Hidalgo-Bastida et al, 2010) and biochemical and mechanical agents present in the micro-environment *in vitro* and *in vivo*, as well as to keep the proliferation of stem cells restricted to the implanted site and to know the gene control mechanism for safe induction of the desired functions (Gronthos et al., 2000; Discher et al., 2009). The identification of growth factors and the signaling mechanisms involved in the actual control of stem cell renewal and differentiation will allow the design of strategies to block senescence and to safely drive cellular differentiation (Satija et al, 2007).

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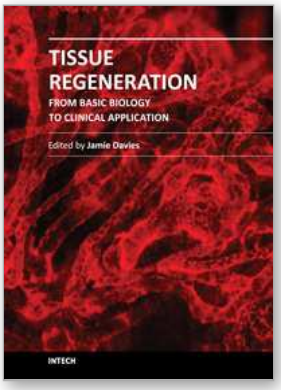

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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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