5

Skeletal Regeneration by Mesenchymal Stem Cells: What Else?

José A. Andrades[1,2] et al.*
[1]*Laboratory of Bioengineering and Tissue Regeneration (LABRET–UMA), Department of Cell Biology, Genetics and Physiology, Faculty of Sciences, University of Málaga, Málaga,*
[2]*Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER–BBN)*
Spain

1. Introduction

1.1 The definition of MSCs

Bone marrow (BM) was the first tissue described as a source of plastic-adherent, fibroblast-like cells that develops colony-forming unit fibroblastic (CFU-F) when seeded in tissue culture plates (Friedenstein et al., 1982; 1987). These cells, originally designated stromal cells, elicited much attention, and the main goal of thousands of studies conducted using these cells was to find an ultimate pure cell population that could be further utilized for regenerative purposes. In these studies, cells were isolated using several methods and were given names such as mesenchymal stem cells (MSCs), mesenchymal progenitors, stromal stem cells, among others. Lately, a committee of the International Society for Cytotherapy suggested the name "multipotent mesenchymal stromal cells" (Dominici et al., 2006). However, most scientists have been referring to them simply as "MSCs".

The precise definition of these cells remains a matter of debate. Nevertheless, to date MSCs are widely defined as a plastic-adherent cell population that, under closely controlled conditions, can be directed to differentiate *in vitro* into cells of osteogenic, chondrogenic, adipogenic, myogenic, tenogenic, or hematopoietic-supportive stromal lineages (Pittenger et al., 1999; Javazon et al., 2004; Alonso et al., 2008; Prockop, 2009) (Fig. 1).

As part of their stem cell nature, MSCs proliferate and give rise to daughter cells that have the same pattern of gene expression and phenotype and, therefore, maintain the "stemness"

* Silvia Claros[1,2], Pedro Jiménez-Palomo[1,2], José Mª López–Puerta[3,2], Plácido Zamora–Navas[4,2], Enrique Guerado[5,2], Manuel Monleón[6,2], María C. Araque[6,2] and José Becerra[1,2]
[1]*Laboratory of Bioengineering and Tissue Regeneration (LABRET–UMA), Department of Cell Biology, Genetics and Physiology. Faculty of Sciences, University of Málaga, Málaga,*
[2]*Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER–BBN),*
[3]*Department of Orthopaedic Surgery and Traumatology, Universitary Hospital Virgen del Rocío, Sevilla,*
[4]*Department of Orthopaedic Surgery and Traumatology, Universitary Hospital Virgen de la Victoria, Málaga,*
[5]*Department of Orthopaedic Surgery and Traumatology, Hospital Costa del Sol, Marbella,*
[6]*Center of Biomaterials and Tissue Engineering, Universidad Politécnica de Valencia,*
Center of Research Príncipe Felipe, Valencia
Spain

of the original cells. Self-renewal and differentiation potential are two criteria that define MSCs as real stem cells; however, these characteristics have only been proved after *in vitro* manipulation, in bulk and at single-cell level, and there is no clear description of the characteristics displayed by unmanipulated MSCs *in vivo* (Lee et al., 2010).

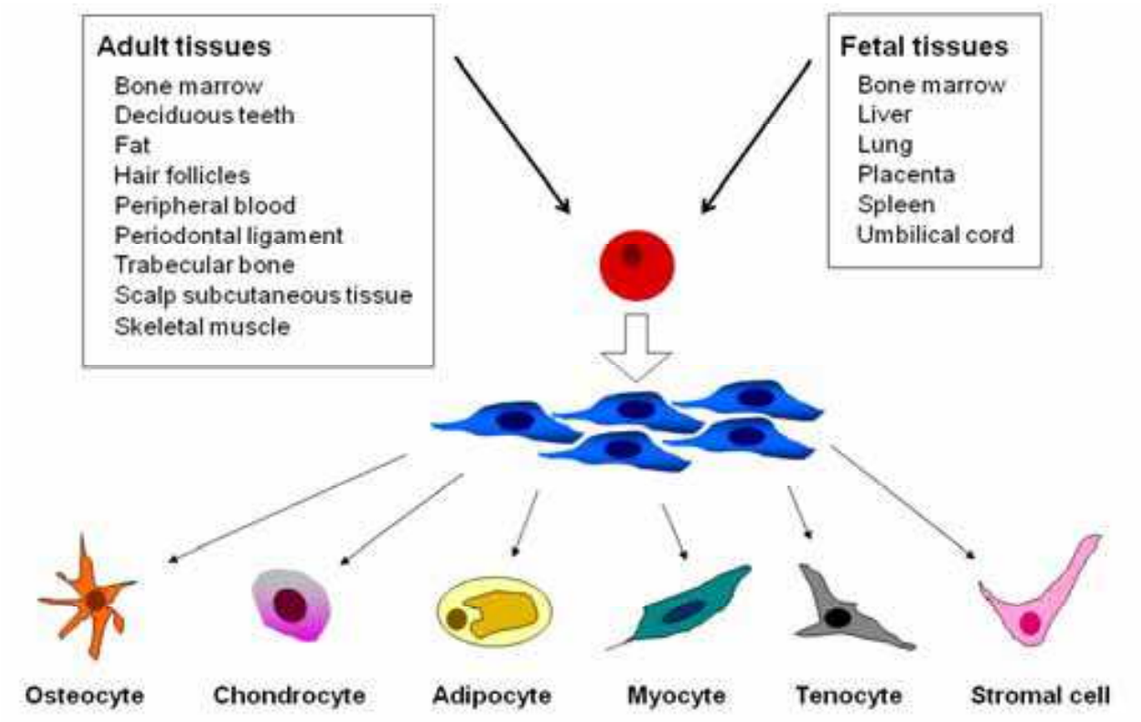

Fig. 1. Source of MSCs and multipotent differentiation capacity. MSCs can be isolated from bone marrow, deciduous teeth, fat, hair follicles, peripheral blood, periodontal ligament, trabecular bone, scalp subcutaneous tissue, and skeletal muscle in adult tissues, and from bone marrow, liver, lung, placenta, spleen and umbilical cord in fetal tissues. MSCs can generate multiple mesoderm-type cell lineages, such as osteocytes, chondrocytes, adipocytes, myocytes, tenocytes, and stromal cells

In contrast to other stem cells such as hematopoietic stem cells (HCSs), which are identified by the expression of the CD34 surface marker, MSCs lack a unique marker. The CD105 surface antigen (endoglin) has been recently used to isolate human MSCs (hMSCs) from BM and such an approach enabled the characterization of freshly isolated hMSCs before culture. A distinct expression of certain surface antigens such as CD31 and CD45 was demonstrated in freshly isolated hMSCs and the expression of these molecules was lower in culture-expanded hMSCs (Aslan et al., 2006). These data suggest, again, the alterations that hMSCs may undergo during culture (Boquest et al., 2005).

In several studies, cultured MSCs have been characterized either by using cell surface antigens and/or by examining the cells' differentiation potential. Lately, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to define human MSCs: (1) MSCs must be plastic-adherent when maintained in standard culture conditions and form CFU-Fs in primary cultures, (2) MSCs must express CD73, CD90, CD105, and lack expression of CD11b, CD14, CD19, CD34, CD45,

CD79alpha, and HLA-DR surface molecules, and (3) MSCs must differentiate to osteoblasts, adipocytes, and chondroblasts *in vitro* (Dominici et al., 2006; Claros et al., 2008).

1.2 The stem cell nature of MSCs

Stem cells are defined by their ability to self-renew and by their potential to undergo differentiation into functional cells under the right conditions. The ongoing public discussion regarding whether MSCs are strictly stem cells requires a revision of the definition of stem cells, as MSCs apply to a wide cluster of non-hematopoietic stem-like cells isolated from mesenchymal tissues such as BM, adipose, amniotic fluid, and blood vessels. The central question would be whether they might be differentiated into cells of other than a mesenchymal nature. Researchers have reported that MSCs from BM and other tissues can be differentiated into epithelial, endothelial, and neural cells (Spees et al., 2003; Greco & Rameshwar, 2007; Yue et al., 2008). As stated above, there is a consensus on specific MSC markers, but a unique marker of "stemness" and multipotentiality has not yet been defined, since culture-expanded MSCs may lose some of these markers and acquire others, which are non-specific, but cells retain their multipotentiality (Jones & McGonable, 2008). The molecular signature and *in vivo* distribution status of MSCs remain unknown and, as such, subject to investigation, even though *ex vivo*-expanded MSCs have been widely used in numerous studies (Prockop, 2007; Kubo et al., 2009; Pricola et al., 2009).

In local models, direct injection of hMSCs into the brain tissue of rats resulted in the cells' long-term engraftment and subsequent migration along pathways similar to those used by neural stem cells (Azizi et al., 1998). The results of these studies demonstrate the multilineage differentitation potential of BM-derived adult MSCs and aid in defining them as suitable candidates for the regeneration of several mesenchymal tissues. These data suggest that stem cells require a specific tissue environment to develop their intrinsic potency (Weissman, 2000). A stem cell niche is defined as a complex, multifactorial local microenvironment required for the maintenance of the stem cell biology. The stem cell niche consists of stem cells, non-stem cells, an extracellular matrix (ECM) and molecular signals. Inside the niche, the stem cell can divide asymmetrically giving rise to both new stem cells and proliferating progenitor cells. These proliferating cells give rise to a cell population that undergoes differentiation (Becerra et al., 2011). Recent scientific advances have lead to a substantial increase in the amount of information regarding stem cell niche data. Some of the best-characterized stem cell niche models are *Drosophila* germarium or testis, vertebrate hair follicle, intestinal crypts, BM, and brain subventricular/subgranular zones (Fuchs et al., 2004; Mitsiadis et al., 2007). *In vitro* culture conditions of single stem cells form intestinal crypts can give rise to organoids which may behave as self-organizing structures in the absence of other non-epithelial cellular niche components (Sato et al., 2009).

1.3 MSCs and tissue engineering

The chronic shortage of donor organs and tissues for donor organs and tissues for transplantation has provided the impetus for intense research in the field of tissue engineering (TE). Unlike pharmacology and physiotherapies they are mainly palliative, TE and cellular therapy seek to augment, replace, or reconstruct damage of diseased tissues (Chai & Leong, 2007). Tissue engineering is an emerging field that offers outstanding opportunities for regenerative medicine. The most common concept underlying TE is to combine a scaffold or matrix, living cells and/or biologically active molecules to form a "TE

construct" to promote the repair and regeneration of tissues. The scaffold supports cell colonization, migration, growth and differentiation, and often guides the development of the required tissue or acts as a drug delivering vehicle. Hence, TE can be defined as a discipline that seeks to create or to induce the formation of a specific tissue in a specific location through the selection and manipulation of cells, scaffolds, biologic stimuli (Muschler & Midura, 2002), and vascular support (angiogenesis and/or vasculogenesis), on which the TE paradigm is based on (Fig. 2).

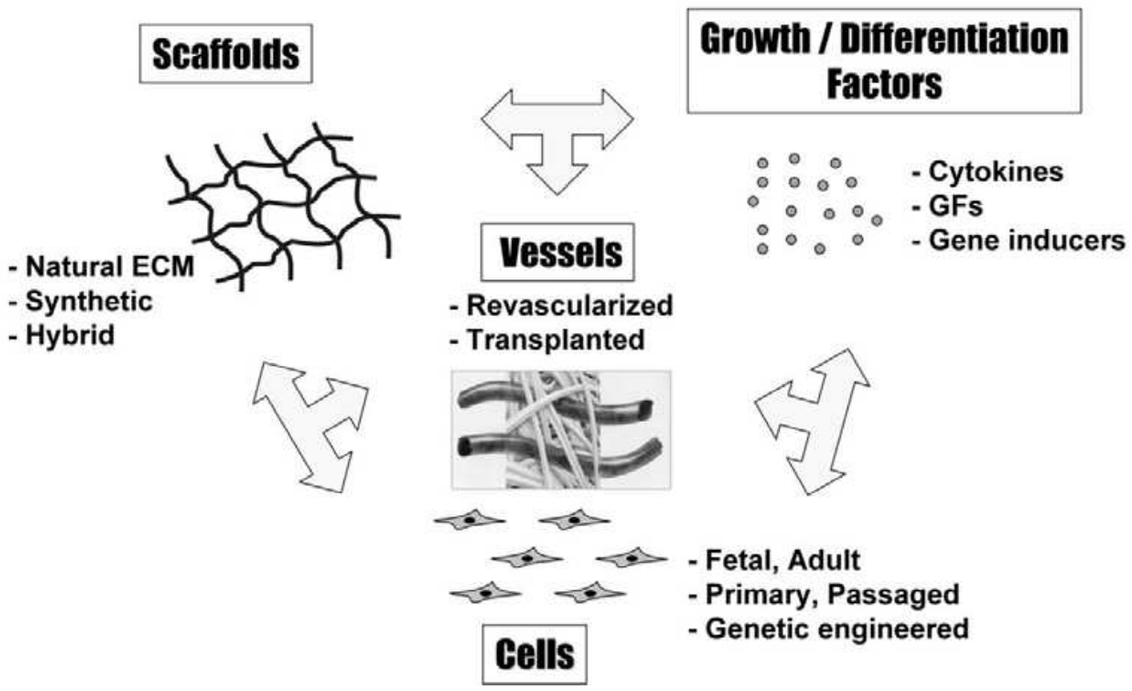

Fig. 2. Graphical illustrations showing the principles underlying tissue engineering. *From the same authors, published by Cellular and Molecular Biology, vol. 54 (1), 40-51. Copyright 2008 C.M.B. Edition*

The right knowledge of these interactions will create exciting new opportunities that might be useful in a broad array of clinical applications. As a logic consequence, today, a great number of multi-disciplinary groups with different backgrounds (Fig. 3) focus on various problems associated with TE, including cell isolation, characterization, and manipulation of cell proliferation/differentiation for stem cell therapy, design and elaboration of appropriate biomaterials as well as for development of bioreactors to enlarge tissue/organ engineering as a strategy to be applied in regenerative medicine.

MSCs have recently received much attention for their therapeutic potential in regenerative medicine, due to their capacity to secrete soluble factors that have beneficial effects (Caplan & Dennis, 2006; Cuenca-López et al., 2008; Caplan, 2009). Several studies have demonstrated that these MSC trophic factors may enhance regeneration ability of injured tissues, inhibit apoptosis, limit pathologic fibrotic remodelling, stimulate proliferation and differentiation of endogenous stem-like progenitors, decrease inflammatory oxidative stress and modulate immune reactions (Meirelles et al., 2009; Lee, 2010; Rodrigues et al., 2010).

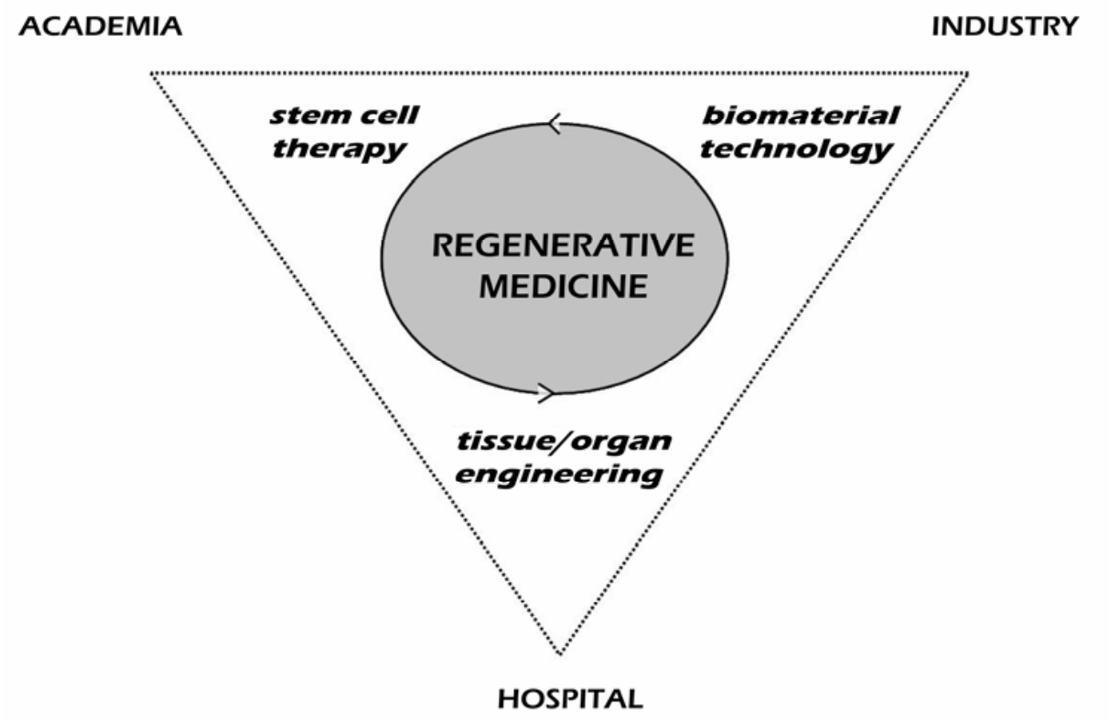

Fig. 3. Graphical illustration showing the multidisciplinary and complexity of interactions within the context of regenerative medicine. *From the same authors, published by Cellular and Molecular Biology, vol. 54 (1), 40-51. Copyright 2008 C.M.B. Edition*

Moreover, it has been described that growth factors (GFs) can act by an autocrine, paracrine, endocrine, juxtacrine, ECM mediated or intracrine process (Fig. 4) (Nimni, 1997). Autocrine action is the secretion of GFs for which the cell possesses receptors (1). Some experiments have suggested that this interaction may even occur within the cell, a process called intracrine interaction (6). Paracrine action is defined as the release of soluble GFs which diffuse into the extracellular space and act upon adjacent or closely located cells (2). In the case of endocrine action, GFs are carried in the bloodstream and may act on distant sites much like a hormone (3). Juxtacrine stimulation is when one cell has surface bound GFs which interact with an adjacent cell containing receptors for the GF (4). Furthermore, some ECM molecules can bind GFs, modulating their activities and regulating how they interact with cells (5).

Protein chain reaction, flow cytometry, enzyme-linked immunosorbent assay and Western blotting have shown that the secretion of MSCs include many major GF families and multitude of chemokine, including transforming growth factor beta-1 (TGF-β1), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), nerve growth factor beta (NGF-β), Wnts, interleukin-1 (IL-1), IL-1β, IL-3, IL-6, IL-7, IL-11, among others (Gnecchi et al., 2008; Caplan, 2009; Zisa et al., 2009).

TGF-β1 is a multifunctional GF with a broad range of biological activities in various cell types in many different tissues. In general, TGF-β1 is known to influence cells from the chondro- and osteogenic lineage, promoting initial stages of mesenchymal condensation, regulating cell proliferation and cell differentiation, and stimulating production of ECM

(Andrades et al., 2003; van der Kraan et al., 2009; Janssens et al., 2005). Other of the trophic benefits produced by TGF-β1 is the immunomodulatory effect. Several authors have reported that MSCs suppress natural killer (NK) cell proliferation, alloreactive T-lymphocyte proliferation and activation, cytokine production, and cytotoxicity against HLA-class 1 expressing targets via TGF-β1 and prostaglandin E2 secretion (Groh et al., 2005; Sotiropoulou et al., 2006).

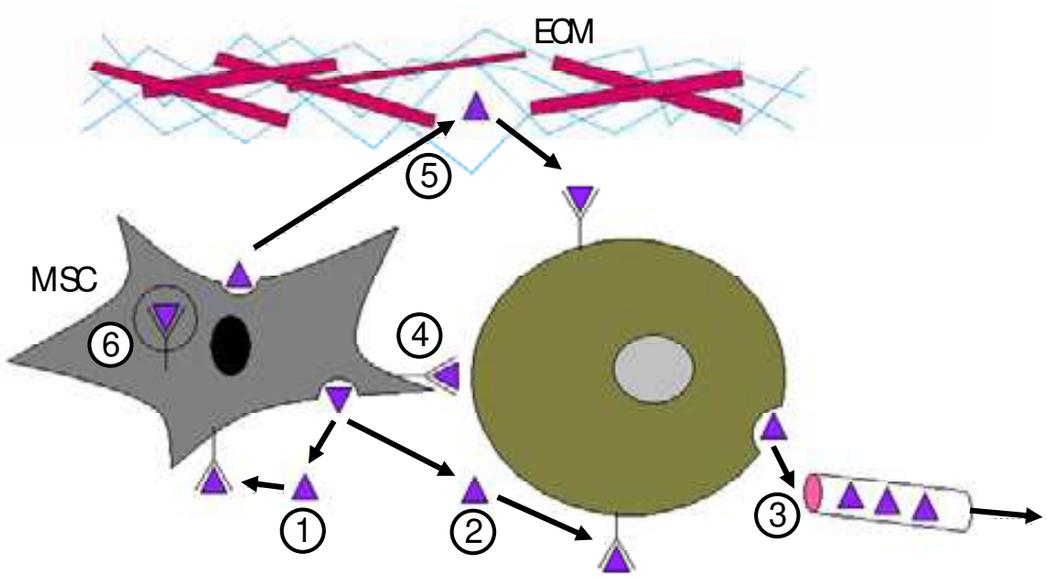

Fig. 4. Chemical signaling mechanisms of growth factors. GFs (triangles) produced by the cells can act within itself or in adjacent or remote cells to modulate their activities by reacting with specific receptors. (1) Autocrine; (2) paracrine; (3) endocrine; (4) juxtacrine; (5) extracellular matrix mediated; (6) intracrine. *Modified from Nimni ME. Polypeptide growth factors: targeted delivery systems. Biomaterials. 1997; 18(18):1201-1225*

FGFs are a family of GFs that act in an autocrine and paracrine way as a mitogen on many cell types (Chiou et al., 2006; Makino et al., 2010). They also regulate events in normal embryonic development, angiogenesis, wound repair, and cell differentiation. FGFs are involved in endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures. They thus promote angiogenesis, the growth of new blood vessels from the pre-existing vasculature (Cao et al., 2003; Behr et al., 2010). Moreover, FGFs are important players in wound repair, stimulating the proliferation of fibroblasts that give rise to granulation tissue, which fills up a wound cavity early in the wound healing process (Schmid et al., 2009). Respect to cell differentiation, it is known that FGFs not only maintain MSC proliferation, they also retain osteogenic, adipogenic and chondrogenic differentiation potentials throughout many mitotic divisions (Tsutsumi et al., 2001; Kawazoe et al., 2008; Rodrigues et al., 2010).

The anti-fibrotic effects of MSCs have been demonstrated in different animal models, but the molecular mechanisms are not yet fully understood. Recently, HGF has been shown to be involved in the prevention of fibrosis (Li et al., 2009). The results reveal that, in a situation of tissue injury, MSCs become proliferative and secrete HGF, which in turn mediates anti-

fibrotic and immunomodulatory effects. Additionally, the secretion of HGF and IGF-I is essential for activation of cardiac stem cells, which may contribute to endogenous repair mechanisms (Linke et al., 2005). On the other hand, IGFs play an important role in stem cell maintenance within the niche (Bendall et al., 2007), and bone metabolism. During osteogenesis, bone cells secrete amounts of IGFs that are stored at the highest concentration of all GFs in the bone matrix (Wang et al., 2011). Principally, IGFs have an anti-apoptotic effect on (pre)osteoblast and enhance bone matrix synthesis (Meinel et al., 2003; Niu & Rosen, 2005).

PDGF is considered one of the key regulators of general tissue repair. The main functions of PDGF are to stimulate cell replication of healing capable stem cells, as well as proliferation of endothelial cells. This will cause budding of new capillaries into the wound (angiogenesis), a fundamental part of all wound healing. In addition, PDGF also seems to promote the migration of perivascular healing capable cells into a wound and to modulate the effects of other GFs (Hollinger et al., 2008).

VEGF is best known for inducing angiogenesis (Gerstenfeld et al., 2003). Further, VEGF induce vascular changes: increased vascular support network and permeabilization of capillaries (Neufeld et al., 1999; Hansen-Algenstaedt et al., 2006), which could be responsible for the stimulation of the recruitment of MSCs (Fiedler et al., 2005). In addition, VEGF secretion is also increased during MSC osteogenesis (Wang et al., 2010), thus accounting for the infiltration of blood vessels during bone development (Shum et al., 2003).While VEGF has been reported to decrease the synthesis of osteocalcin (a marker of osteoblast maturation) in osteogenic MSCs (Villars et al., 2000), it has also been observed to increase mineralization (Mayer et al., 2005) and to be regulated by proteins involved in osteoblast differentiation (Deckers et al., 2000), suggesting that it usually acts as and enhancer of both angiogenesis and osteogenesis.

The neuroprotective effects of MSCs are principally attributed to production of neurotrophic factors, such as BDNF and NGF-β, that support neuronal cell survival, induce endogenous cell proliferation and promote nerve fiber regeneration at sites of injury (Li et al., 2002; Mahmood et al., 2004; Alexanian et al., 2010). Additionally, MSCs also express various neuro-regulatory proteins, adhesion molecules and receptors that likely contribute to the MSC-induced effects on neuronal cell survival and neurite formation (Crigler et al., 2006).

Another family of proteins critical in MSC biology is the Wnt family of signalling molecules (Ling et al., 2009). Wnts are highly conserved proteins that are essential to limb development and musculoskeletal morphogenesis in vertebrates (Yang, 2003). At least Wnts 2, 4, 5a, 11 and 16 are expressed by MSCs along with several Wnt receptors (Frizzleds 2-6) (Etheridge et al., 2004). Wnts are very active in determining MSC patterns of proliferation and differentiation (Boland et al., 2004; Baksh & Tuan, 2007), and they also play a role in cell adhesion and migration through their indirect interactions with the cadherin pathway.

Therefore, MSCs themselves secrete a large number of regulatory proteins. *In vivo*, these signals work together to regulate the regenerative abilities of MSCs. By learning more about the mechanisms of action and interactions of each member of this microenvironment, it will be possible to develop the full potential of MSCs for future therapeutic applications.

2. Skeletal tissue regeneration by MSCs

2.1 Therapy for bone fractures and arthrodesis

The treatment aim of bone fractures and of many orthopaedic deformities is the fusion between two or more bone ends or bone structures. Therapy for diaphyseal, metaphyseal or

paraarticular fractures of long or short bones, either simple or complex, is always addressed to achieve permanent solid bone continuity between fractured bone fragments. The same comes true for orthopaedic corrections such as bone osteotomies, scoliosis or whatever situation where a bone discontinuity, either traumatic or therapeutic mediated, exists (Guerado, 2005; Guerado et al., 2005). Nowadays surgical treatment of these conditions is the choice for both young and old patients, as conservative treatment requires prolonged neighbor joints rest which results in articular stiffness and unacceptable deformities. Further, in many instances, non-surgical treatment will be unsuccessful in dealing with many low or high energy fractures, and also with orthopaedic corrections as non-union or malunion will occur.

Current surgical treatment of fractures or orthopaedic deformities aimed to promote osteogenesis for bony fusion is still far away from being undoubtedly successful. Surgical site infection, implant failure, and many other complications will facilitate failure of achieving the main aim of surgical treatment. In many instances, even carrying out appropriately surgical technique, failure of what had been planned will be the final result.

Although many techniques have been developed for non union treatment, complexity of them is, as far as biomechanics and biology are concerned, very difficult for accomplishing a successful outcome (Guerado, 2005; Guerado et al., 2005). Nonetheless, diaphyseal non-unions and even paraarticular ones, although in some cases present difficult challenges to bony union, can be managed, at least, under favorable biomechanical situations; further, its biology can be managed with autograft. Conversely, since in spinal fusion the size of any vertebra and the distance to each other make the biomechanical and biological situation very difficult for addressing a successful treatment of fusion or non-union, current and forthcoming new "biological" therapies have important perspectives. In relation to bone therapy, spinal fusion is currently the most significant surgical technique expecting for progress in biological treatments.

Surgical technique of spinal arthrodesis combines a hardware system for mechanical stabilization and a biological substance for bone formation enhancement. Both surgical steps have the aim of creating an optimal biomechanical situation together with a biological environment which promotes definite spinal bony fusion (Guerado, 2005; Guerado et al., 2005).

2.1.1 Biomechanics

Biomechanics is indeed a very important issue in spinal surgery. As a general concept, spinal arthrodesis is just the treatment of multiple foci of fractures once neat decortication of host bone has been carried-out; consequently, correct application of any of the four main principles of osteosynthesis (compression, neutralization, buttressing, and tension band, together with accessory bridging) play a major role depending on the sort and position of graft used; further, not only biology but also biomechanical features of host and donor bones are decisive. Using the same graft, either cortical or cancellous, different placements may need different nature of osteosynthesis, and may make the own graft behaves in different ways. Therefore, only when correct stabilization has been performed, as in any fracture treatment, biology behaves in the wished way. Likewise provided biomechanics is correct, the same graft behaves in different ways depending on the biomechanical forces acting on it (Guerado et al., 2005). So far, there has been a long list of bone graft substitutes, and strategies for bone promotion (Hecht et al., 1999; Bostrom & Seigerman, 2005; Huang et al., 2006; Ma et al., 2007; Thalgott et al., 2009). However only cells synthesize osteoid, and

none of the nowadays known bone substitutes, apart from cells transplantation, are real "bone substitutes", since they do not have osteoprogenitor cells.

2.1.2 Biology

Grafting enhances bone fusion, and that results in permanent stability. Otherwise hardware fails with time. The three main properties a therapy for optimum fusion enhacement must have are osteogenesis, osteoconduction, and osteoinduction, together with avoidance of histoincompatibility, and, in the clinical setting, as said above, a favorable biomechanical situation (Boatright & Boden, 2002). Osteogenesis consists basically on cells differentiation into osteogenic linage, being bone cells the only agent with osteogenetic power. Osteoinduction is also very important; as mentioned before, molecules commonly called GFs induce MSCs to amplification (increase of cell population in number) and subsequently to differentiation into osteoprogenitor cells, specially the TGFs superfamily which also includes the well-known BMPs (Li et al., 2002). Osteoconduction is also the complement to osteogenesis and osteoinduction; a tridimensional scaffold structure of bone or bone substitute is an indispensable scaffolding for cell population growth. An osteoconductive microstructure of 200-400 microns is nowadays very easy to be manufactured. However osteoinductive GFs doses to be added to it are unknown; further osteogenetic properties of "bone substitutes" are lacking for osteoconductive materials, as osteogenesis is an exclusive property of mature osteoprogenitor cells.

Since bone synthesis is exclusively made by bone cells, a large population of osteoprogenitor cells is necessary for successful fracture or spinal fusion. The larger the area to be fused the more number of osteoprogenitor cells are needed. Because MSCs population is very scarce in elderly people, this age group has a higher risk for non-union (Caplan, 1991). Nowadays it is known that differentiated cells do not reproduce themselves, being undifferentiated cells, particularly MSCs, responsible for cell amplification and subsequent differentiation into osteogenic linage; moreover mature osteoblasts can recapitulate back to former less differentiated MSCs in order to achieve amplification for increasing the final population of differentiated osteoblasts (McCulloch & Till, 2005; Daley & Scadden, 2008; Jones & Wagers, 2008; Kuang & Rudnicki, 2008; Morrison & Sprading, 2008). During the entire process, the new-forming blood vessels that grow inside the callus act as a source of new MSCs; in fact, recent publications have given strong evidences of a perivascular origin for the MSCs (Crisan et al., 2008; Da Silva Meirelles et al., 2008; Koob et al., 2010); therefore neat decortication of host bone until bleeding, becomes of overwhelming importance. On the other hand fracture focus stability prevents new vessels and incipient callus from breakage.

Currently, the combination of biomechanical stabilization ensuring appropriate environment for bone growth, osteoprogenitor bone cells for osteoid synthesis, vascularization of fusion site for cells and metabolite transport, signaling molecules (TGFs, particularly BMPs) to encourage cells to amplification and differentiation, and scaffold for tridimensional cells growth has been denominated the "diamond concept" (Giannoudis et al., 2007; 2008), as to the ideal situation for fusion to take place. Hence clinical situations lacking of any of these variables are in less than optimal condition for successful spinal fusion. This concept is indeed extended to any fracture of the skeleton or joint arthrodesis (Giannoudis et al., 2007; 2008).

MSCs are, therefore, the centre of the osteogenesis concept, as to its amplification and commitment to osteoprogenitor line signifies that biomechanics is already achieved into its optimum, and that the appropriate environment has been created. Finding a natural source of viable MSCs for therapy is the star point of the diamond concept.

2.1.3 Autograft as a source of osteoprogenitor cells. The relation between biomechanics and biology in spinal surgery

Autograft is the standard biological agent to be added in fracture or arthrodesis focus for bone repair enhancement (Figs. 5-8). Autograft is the appropriate biological source of cells whenever bone healing is required in any part of the skeleton, as its biological properties are considered to be optimum: autograft has a good volume effect, and also potent osteogenic, osteoinductive, and osteoconductive properties (Boatright & Boden, 2002; Niu et al., 2009). Autograft is also the standard for spinal arthrodesis as the natural source of osteoprogenitor cells (Fig. 6). It can be used in spinal surgery either as cancellous chips or as a tricortical alive implant in order to improve the immediate strength of the construct. So far, no allogenic or synthetic bone substitutes have achieved its osteogenicity, although careful autograft implantation for spinal surgery must include shortening harvesting-to-implant interim, because these properties diminish with time (Sandhu et al., 1999).

Nevertheless, morbidity in donor site after autograft collection is very high, and fractures of iliac bone, particularly after tricortical graft has been harvested, with ensuing non-union are not rare, making the need for new bone substitutes (Arrington et al., 1999; Banwart et al., 1995; Delawi et al., 2010; Epstein, 2008; Glassman et al., 2010; Slosar et al., 2007). Limited availability together with longer operative time are also the most important burdens in conventional autograft harvesting, and new less invasive aspiration techniques of cancellous bone from femoral and tibial metaphysis by a "Reamer Irrigator Aspirator" (RIA) apparently provide larger graft amounts than conventional iliac crest harvesting (Belthur et al., 2008; Kobbe et al., 2008a; 2008b) and with higher concentration of GFs (Porter et al., 2008; Schmidmaier et al., 2006); further, RIA would provoke less postoperative pain and shorter length of stay (Belthur et al., 2008). However studies on RIA for spinal surgery are lacking.

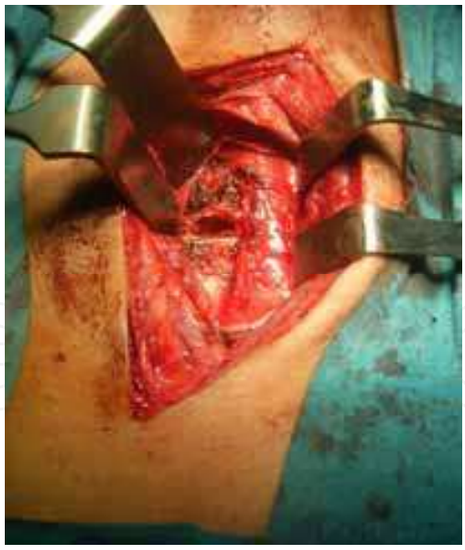

Fig. 5. Cervical spine. C_6-C_7 interbody disc has been removed for interbody fusion by a tricortical autograft harvested from iliac crest

This basic knowledge on autograft has been achieved after animal experiments, making clinical inference to human very uncertain. Cancellous bone graft has greater cellular activity than cortical, whereas cortical graft is much more dense and stronger (Day et al., 2000). Spongy structure of cancellous bone allows osteoprogenitor cells to have better

vascularization, developing superior osteoconduction and osteogenesis. Anyhow although histological incorporation with prompt vascularization and MSCs invasion stars at the second day of implantation, mechanical properties of cancellous bone are compromised during remodelation phase, taking a few months to become structurally stronger (Pape et al., 2010). Cortical autograft having less biological properties than cancellous bone also suffers biomechanical weakening up to the 75% (19) at 6 to 24 weeks postimplantation, returning to normal strength some 48 weeks thereafter (Enneking et al., 1975).

Fig. 6. Harvested tricortical autograft is prepared for implantation. Cortical layers provide strength for interbody compression. Cancellous bone provides biological support for osteogenesis enhacement and faster fusion

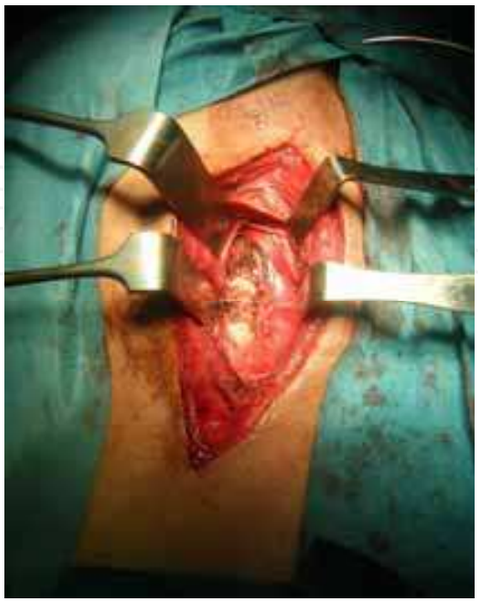

Fig. 7. Tricortical graft in place

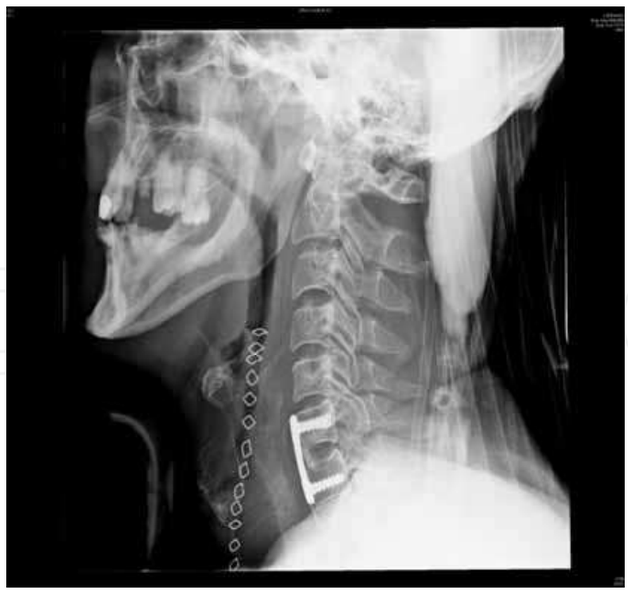

Fig. 8. X-ray control shows good position of graft and osteosynthesis stabilization by a plate. The cortical layers of the graft are positioned anteriorly facing the plate, superiorly facing the above vertebral body, and inferiorly facing the below vertebral body. The posterior face has not cortical bone

Consequently, according to biological and biomechanical properties of autograft, spinal posterior artrodesis is better enhanced by cancellous autograft whereas tricortical bone under interbody compression is more suitable for buttressing in anterior interbody fusion, together with a posterior transpedicular screw system in order to provide a tension band principle; alternatively an interbody plating together with the interbody tricortical autograft provides buttressing leaving the tricortical graft healing without supporting compression; this last possibility may be used in any anterior surgical approach (Figs. 5-8); however in clinical setting it is usually preferred, by some surgeons, a 360º fusion, as surgeons feel more confident in case of implant failure. Eventually, during the course of thoracotomy, removed ribs for anterolateral thoracic or thoracolumbar approaches can substitute the use of tricortical grafts, suppressing the morbidity provoked by iliac crest graft harvesting. In this case the graft can provide limited buttressing, as ribs are not as strong as tricortical iliac crest grafts; full buttressing and neutralization must be supported by the hardware; anterior interbody plating for this purpose appears to be ideal, and ultimately posterior pedicular instrumentation either alone or, ideally, within a 360º fusion concept. In conclusion, failure in the correct combination of graft type with proper instrumentation principles will result in poorer outcome, although the right graft had been used or the appropriate osteosynthesis applied.

2.1.4 Therapy with laboratory treated MSCs

Many studies have been made during the last years in experimental MSCs application for therapy in animals. Nevertheless biomechanics and biology of animal used are quite different from humans (Heineck et al., 2010); moreover clinical situations are different from one patient to another. Stem cells are currently being studied for use in numerous clinical applications, ranging from neurodegenerative diseases to cardiac insufficiency. The use of MSCs in spinal surgery is also compelling, especially with the increasing age of the general

population. In spinal surgery, the use of MSCs is focused in intervertebral disc repair and regeneration and in spinal arthrodesis procedures. Although the routine use of cellular therapies by spine surgeons to improve outcome after a variety of surgical procedures is rapidly approaching with uncontrolled enthusiasm (Helm & Gazit, 2005), knowledge on its real effectiveness is far from being identified.

Experimental studies on the effect of low intensity pulsed ultrasound on rabbit posterolateral intertransverse processes spinal fusion with MSC-derived osteogenic cells and bioceramic composite have shown that this combination promote clinical fusion. The mechanism was likely to be mediated through better osteointegration between the host bone and implanted materials and enhanced endochondral ossification at the fusion site (Hui et al., 2011). However it is known that osteogenesis in spinal rabbit is achieved just by stripping the periostium. Also MSCs that had been cultured with osteogenic differentiation medium may induce the formation of new bone in experimental posterolateral intertransverse process spinal fusion in rabbits. Nonetheless suitability level of osteogenic differentiation of MSCs as well as the most appropriate carrier for MSCs is unkown (Nakajima et al., 2007).

New approaches and carrier have been introduced in the armamentarium for MSCs transplantation. It has been shown that MSCs and platelet lysate seeded in a fibrin or collagen scaffold can improve the new bone formation around an uncemented hip prosthesis stem in a sheep model. In vitro expanded MSCs suspended in platelet lysate and either mixed with collagen or fibrin gel as delivery vehicle inserted inside the femoral canal, in a press-fit femoral stem model provides higher bone-prosthesis contact (Dozza et al., 2011). But again biology is subjected to biomechanics and human and animal hip biomechanics are not similar.

MSCs transplantation will be a very important therapeutic principle, however deep knowledge not only on cells in cultures but also in biomechanical situations resembling clinical setting is needed. We know now that MSCs behave in different ways depending on the biomechanical situation. In fact this principle is worldwide known since more than 100 years by any orthopaedic surgeon as Wolf's law (Wolf, 2010).

2.2 Cartilage regeneration

Degenerative disease of articular cartilage (AC), generically known as osteoarthritis (OA), is an irreversible evolution process towards terminal articular failure. Due to its high prevalence on population and its socioeconomic impact, this condition is of great concern, and this way more resources and effort are dedicated to the research on its development.

OA is the result of several mechanical changes and biological events that destabilize the balance between normal degradation and synthesis of AC, ECM and subchondral bone. Knowledge on OA physiopathology has improved considerably, evolving from a purely mechanic approach to a molecular and inflammatory view, taking into consideration that cartilage, synovium and subchondral bone contribute to the joint restructuring.

Mature AC is a tissue free of blood vessels and nerves, located at the diarthrodial joints of the skeleton, making easier its gliding and the lubrication between the articular surfaces; it absorbs traumatisms and distributes load on the adjacent bone. It is made up of one ECM with proteoglycan, rich in type II collagen, and about only 5% of the tissue volume occupied by cells. These cells, the chondrocytes, are spherical, and can be found embedded inside lacunae filled with pericellular matrix and have no contact with the distant neighbour cells. Although human cartilage can reach up to 7-8 mm thickness, its supply with nutrients and oxygen is limited by diffusion, which is, however, facilitated by a cyclic compressive

loading; this provides a pumping mechanism during joint movements. There are several reasons that contribute to a lessened capacity of response from chondrocytes before an injury regarding other parenchymal cells. As it has no vascularisation, the typical provisional matrices made from fibrin deposits do not exist, nor the inflammatory cells afflux occurs, there is no delivery to the environment of cell mediators through degranulation, whereas in other tissues, arrival to the focus of such mediators with mitogenic, fibrogenic and phagocytosis-inducing capacities are responsible for the repair. Although most of the actions promoted by the mediators of inflammation can enhance the injury magnitude (such as the proteolythic capacity, free radicals production, cell death or proliferation), the best guarantee for the *restitutio ad integrum* is its early delivery at the injured focus.

Chondrocytes are well differentiated cells with a limited proliferative and migratory capacity. Before physiological stimuli, it synthesizes its own ECM at a very constant and slow rate. A macroscopic structural injury involves higher demand of matrix synthesis, resulting in an insufficient cell response to achieve the tissue regeneration. However, the regenerative capacity of the organs lies on the persistence of cell progenitors in some areas of the adult tissues. Mesenchymal non-differentiated cells found in the AC prove that cartilage, as other tissues, has the capacity to regenerate its own structure. This suggests the existence of a lapse of time in which the capacity or repair is complete; this period would possibly cover the early stages of the disease when defects are microscopic. The most adequate resolution of a chondral defect should involve the regeneration into a tissue identical to hyaline cartilage. A simple repair means the filling with a non-identical tissue, which should be able to seal the defective area with good adhesiveness to subchondral bone and total integration within the surrounding cartilage, as well as to resist the mechanic wear and incorporate into the natural turnover of the normal tissue.

All this makes the AC a tissue with almost no healing capacity for intrinsic healing in most *in vivo* situations, although tissue culture demonstrates that *in vitro* conditions may reactivate a significant regenerative potential for juvenile tissue and articular chondrocytes. In partial cartilage defects, the subchondral plate remains intact, without access to the vascular system and they are not usually repaired spontaneously. On the other hand, defects that penetrate the full chondral thickness are generally associated to the violation of the subchondral plate, thus exposing the defect to the vascular system though the marrow area. This leads to the migration of MSCs to the damaged area, where they undergo the chondrogenic differentiation. Nonetheless, in many instances the repair tissue is made up of fibrocartilage that contains mainly collagen type I fibres, and does not meet the criteria precise for a functional tissue. Small defects can be repaired spontaneously with hyaline cartilage production, whereas larger defects will only be able to be repaired with the production of fibrous or fibrocartilaginous tissue, which are biomechanically and biochemically different from the normal hyaline cartilage. As a result, degeneration subsequently appears which can evolve into osteoarthritic change in some cases.

Focal cartilage defects have been detected in up to 63% of patients undergoing arthroscopy of the knee and fortunately most of them remain symptomless for a long time. However, symptomatic lesions can result in significant pain and morbidity and a prospective clinical study has demonstrated that the risk of patients with a cartilage lesion to progress to osteoarthritis is enhanced more than fivefold. Thus, such patients require a treatment filling the gap between palliation and resurfacing via arthroplastia. This need as well as the detailed knowledge we have about the functional elements of cartilage tissue, have turned cartilage repair into a pioneering and very successful area of regenerative medicine.

2.2.1 Therapeutic interventions without active biologics

2.2.1.1 Bone marrow stimulation

First approaches to heal cartilage by *in situ* regeneration date back to 1959. Pridie technique was directed to BM cells recruitment to be used in cartilage defects by drilling small holes into the subchondral BM space underlying the damaged cartilage regions. It was improved later on by reducing the size of the perforations and being then called microfracture technique which is now a frequently performed and well studied procedure (Steadman et al., 1999). This technique is based on the mechanism of mesengenesis or capacity of the non-differentiated mesenchymal cells in choosing a determined phenotype as a response to inducing or GFs. A non-differentiated cell from the BM can be promoted to different cell types such as osteoblasts, with a later maturing to osteocytes, chondroblasts and chondrocytes, but also to endothelial cells, mesothelial cells, fibroblasts or adipocytes. It is a cell signalling process of local cytokines on local cells. In order to achieve all this, the surgical technique is based on drilling the subchondral plate to get bleeding and a superclot that will become a scaffold and supply cells and proteins, starting this way the physiological cascade of the chondrogenic cell differentiation. Other alternative techniques of BM stimulation to regenerate cartilage would be abrasion chondroplasty and in case the articular surface remained untouched, the retrograde stimulation technique. Cartilage defects are repaired only with fibrous tissue or fibrocartilage when using these methods, probably because the number of chondroprogenitors recruited from the BM is too small to promote the hyaline cartilage repair and results are often followed by degeneration of the repair tissue. This was used as an explanation for the observations of other studies that good short term results may be followed by deterioration starting about 18 months after surgery.

Clinical observations and theoretical considerations pointed towards several possible limitations of marrow stimulation techniques. The non-adhesive properties of the cartilage surface and the softness and shrinking of the superclot can lead to only partial defect filling and facilitate an early loss of repair tissue from the cartilage lesion. To avoid this, the treatment has been recently advanced into a matrix-supported technique in which the performed defect was stabilized in an additional way with a biomaterial. The microfractured lesion is covered with a collagen type I/III scaffold and it is called autologous matrix induced chondrogenesis (AMIC) (Kramer et al., 2006; Steinwachs et al., 2008). This technique has been developed to allow the treatment of larger defects by microfracturing and it is used as alternative treatment to autologous chondrocytes transplantation (ACT).

2.2.1.2 Autologous osteochondral transplantation: mosaicplasty

Autologous osteochondral mosaicplasty, sometimes known as osteoarticular transfer system, OATS, is an effective method for the resurfacing of osteochondral defects of the knee. The technique consists in transplantation of many osteocondral autologous plugs obtained from the periphery of the femoral condyle articular surface, which supports less weight and transferring them to create a durable resurfaced area in the defect (Fig. 9). The procedure shows some advantages regarding other repair techniques, such as the viable hyaline cartilage transplantation, a relatively short rehabilitation period and the possibility of carrying out the procedure in one only operation.

However, the OATS limitations are the donor-site morbidity and a limited availability of grafts that can be obtained from the femoropatellar joint or the area adjacent to the intercondylar fossa. Other possible limitations are differences in bearing, thickness and mechanical properties between the donor's and the receiver's cartilages, as well as the graft

sinking into the surface due to the support of weight after surgery. Besides, the lack of filling and the possible dead space between cylindrical grafts can limit the repair quality and integrity. Lane et al. transplanted autologous osteochondral grafts into sheep knee joints and reported the lack of integration of the cartilage, which determined the persistence of gaps through the full thickness in all the specimens (Mishima et al., 2008).

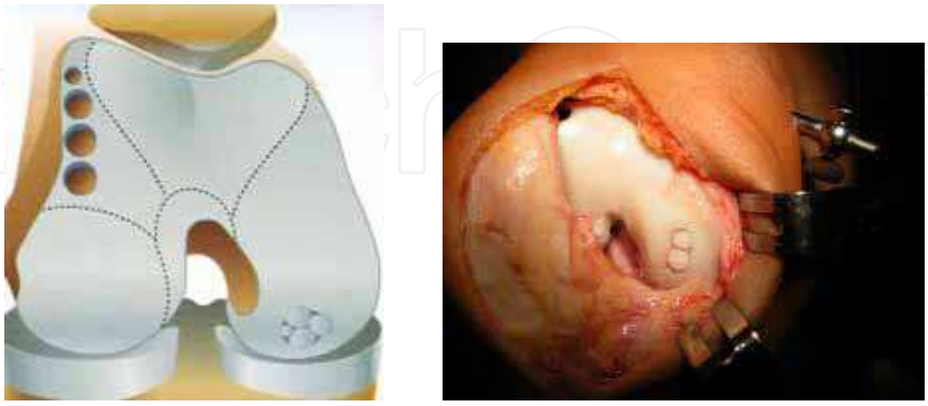

Fig. 9. In mosaicplasty cylindrical osteochondral plugs are harvested from nonload-bearing sites in the affected joint and pressed into place within the osteochondral defect, creating an autograft "mosaic" to fill the lesion

2.2.1.3 Alogenic osteochondral transplantation

Osteochondral allograft transplantation is a procedure for cartilage resurfacing which involves the transplantation into the defect a cadaveric graft composed of viable, intact AC and its underlying subchondral bone. It is a well known resource, especially for tumour surgery. The defect size, its location and its depth are crucial factors for the suitability of the donor graft. Advantages of using osteochondral allografts are the possibility if achieving a precise architecture of the surface, the immediate transplantation of viable hyaline cartilage in a one-time procedure, the possibility to repair large defects, even half-condyles and the donor-site lack of morbidity. Gross et al. have reported results from fresh allografts in 123 patients with good clinical results in 95% of the patients after five years (Gross et al., 2002). There are different possible allografts. Fresh osteochondral allografts are generally used because both freezing and cryopreservation have proved to reduce the chondrocytes viability. Traditionally grafts have been obtained, kept in lactated Ringer's solution at 4 °C and then transplanted in a week. Another alternative for allografts conservation and implantation is cryopreservation, which involves freezing at a controlled speed of specimens within a nutrients rich medium, a cryoprotector agent (glycerol or dymethil sulfoxide), to minimize the cells freezing and keep their viability; finally, there is the possibility of fresh-frozen allografts, with the advantages of lower immunogenic capacity and less transmission of diseases but with lower chondrocyte viability.

2.2.1.4 Soft tissues transplantation

Two main theories support the practicing of covering the cartilage defects with soft tissues, such as perichondrium or periosteum. On one hand, the defect has to be covered mechanically and on the other, we know about the presence of pluripotential stem cells in the perichondrium and the periosteum cambium layer. The different factors able to promote these cells differentiation into active chondrocytes still remain unknown.

2.2.2 Therapeutic interventions with active biologics

2.2.2.1 Autologous chondrocytes implant

The clinical use of the autologous chondrocytes implant (ACI) technique was first reported by Brittberg et al. in 1994, following animal studies which had shown its effectiveness (Grande et al., 1989). In this method, chondrocytes are obtained from a biopsy taken from a non-weight bearing part of the patients cartilage, and are expanded *in vitro*, followed by the injection of a suspension of chondrocytes into cartilage defects, covered with autologous periosteal flap (Fig. 10). This technique premises are based on the capacity of adhesiveness of the cells to certain surfaces, they spread on them and proliferate producing their specific ECM. Although clinical results of the original ACI looked promising (Minas, 2001; Peterson et al., 2000), this procedure has some potential disadvantages, such as leakage of transplanted cells, invasive surgical method, hypertrophy of periosteum (Haddo et al., 2005; Kreuz et al., 2009) and loss of chondrogenic phenotype of expanded chondrocytes in monolayer culture (Benya & Shaffer, 1982). Second generation ACI, named membrane autologous chondrocyte implantation (MACI), has a similar procedure, but a collagen type I and III membrane instead of periosteum. This technique was introduced to improve the ACI problems, and biomaterials such as collagen type I gel (Ochi et al., 2002), hyaluronan-based scaffold (Manfredini et al., 2007) and collagen type I/III membrane (Bartlett et al., 2005) were applied to secure cells in the defect area, to restore chondrogenic phenotype by way of three dimensional cultures (Gigante et al., 2007]) and to replace the periosteum as defect coverage. This is the way MACI technique is created *a posteriori*, by implanting autologous chondrocytes in three dimensional matrices of collagen types I and III, or hyaluronic acid.

Fig. 10. In autologous chondrocytes implantation (ACI) a chondral biopsy is taken from a donor site at the time of clinical examination to be treated with enzymes in the laboratory to obtain chondrocytes cultures that are re-injected under the periosteal flap

At present, only two prospective studies comparing the original and second generation ACI are available (Bartlett et al., 2005; Manfredini et al., 2007) and both studies show no significant differences in the short term clinical results. As for the first generation ACI, the newly regenerated cartilage often consists of fibrous tissue (Horas et al., 2003; Tins et al., 2005), possibly due to the limited number of chondrocytes and their low proliferation potential. Bone overgrowth that causes thinning of the regenerated cartilage and the violation of the tidemark are also of concern. Moreover, this method still sacrifices healthy

cartilage. Thus, these aspects limit ACI in the treatment of large defects and may increase the long-term risk of osteoarthritis development.

2.2.2.2 Mesenchymal stem cells therapy

TE based on cell and genetic therapy offers some of the most promising strategies of tissue repair, including AC repair. It is the science able to create alive tissue to replace, repair or strengthen ill tissue. Thus, the term tissue engineering refers to a wide variety of techniques. Regardless of the used technique, it needs four components (Fig. 2): a growth inducing stimulus (GF), cells that may respond to such influences, a scaffold that might provide a support for the tissue generation, and vascular support (angiogenesis and/or vasculogenesis). This approach involves the use of different cell types acting as chondroprogenitor cells and/or vehicles for supply of genes that synthesize therapeutic proteins. MSCs provide a new potential for cartilage regeneration, as their differentiation to several lineages can be induced, in this case into chondrocytes. Destiny of these cells within tissues is determined by specific cell–to-cell and cell–to-matrix interactions and is controlled by extracellular signalling molecules and their respective receptors and by events that control genetic transcription in a cell-specific way. It has been shown that several differentiation factors are required, such as the BMPs, the FGF and Wnt molecules, although they are not specific for chondrogenesis (Jorgensen et al., 2004). These factors promote the formation of cartilage as well as of bone *in vivo* (Noel et al., 2004), and the precise molecular pathways that rule each specific lineage are being researched.

Beside the characteristics of MSCs exposed before, these cells have self-renewal potential as well as multilineage differentiation potential, including chondrogenesis (Johnstone et al., 1998; Pittenger et al., 1999; Prockop 1997; Sacchetti et al., 2007). MSCs chondrogenesis was first reported by Ashton et al. (1980) and the first ones to describe a defined medium for *in vitro* chondrogenesis of MSCs were Johnstone et al. (1998), who used micromass culture with TGF-β and dexamethasone. Sekiya et al. (2001, 2005) reported that addition to bone BMPs enhanced chondrogenesis under the conditions employed by Johnstone et al. (1998). Nowadays, the micromass culture is widely used to evaluate chondrogenic potential of MSCs *in vitro*. However, this *in vitro* chondrogenesis does not mimic cartilage formation during development. During micromass culture, MSCs increase expressions of both collagen type II (chondrocytes marker) and X (hypertrophic chondrocytes marker) (Barry et al., 2001; Ichinose et al., 2005). Other cytokines such as IGF (Pei et al., 2008) and parathyroid hormone-related peptide (PTHrP) had been tried for better differentiation cocktails, but it is still difficult to obtain *in vitro* MSC-based cartilage formation comparative to native cartilage tissue.

Although BM is considered an acceptable source of MSCs some comparative studies show that MSCs from BM have more chondrogenic potential *in vitro* than those from the adipose tissue (Colter et al. 2001; Huang et al. 2005; Liu et al. 2007; 53, 54). Sakaguchi et al. (47) harvested human BM, synovium, periosteum, muscle and adipose tissue and isolated and expanded MSCs in a similar condition. They demonstrated that MSCs derived from synovium had higher chondrogenic potential than those from other mesenchymal tissues. Yoshimura et al. (71) also demonstrated in a similar way higher chondrogenic differentiation potential of MSCs from synovium in rats. Park et al. (72) showed that MSCs from BM and periosteum are superior to cells isolated from fat with respect to forming hyaline cartilaginous tissue when transplanted into cartilage defects in rats.

To start any regeneration based on MSC activity, first the cells have to be recruited to the damage site. Second step is adhesion to local matrix, followed by activation and extensive

proliferation to provide the necessary number of chondroprogenitor cells to build up new tissue. Finally, it is required to switch from expansion to chondrogenic matrix production via chondrogenesis induction, to build up the shock absorbance and gliding characteristics for a proper tissue function. Seamless integration with neighbouring cartilage and bone tissues depends on successful crosstalk between new and old tissues. For durable cartilage repair, the tissue eventually needs to regenerate a tidemark, get adapted to mechanical loading and build up a balanced tissue homeostasis.

Cell migration is a requisite for development from conception to adulthood and plays a major role in regeneration of all tissues. A number of studies demonstrated that chondrocytes migrate under the action of different stimuli, on or within planar and 3D matrices. Attracting factors include BMPs (Frenkel et al., 1996), hepatocyte growth factor (HGF) (Takebayashi et al., 1995), IGF-1 (Chang et al., 2003), TGF-β (Chang et al., 2003), PDGF (Fujita et al., 2004), FGF (Hidaka et al., 2006), fibronectin, fibrin and collagen type I (Maniwa et al., 2001). However, results remain contradictory for some of these factors, as testing of human chondrocytes revealed no effects for BMP-2, BMP-4, BMP-7, IGF and TGF-β in other studies (Mishima &, Lotz, 2008), being IGF-1, PDGF (Fiedler et al., 2004; Ozaki et al., 2007; Ponte et al., 2007) and VEGF the ones that induced higher response (Mishima & Lotz, 2008). Effects of GFs vary *in vitro* and *in vivo*, regarding animal species and specimens' age, among other aspects. But it is generally accepted that GFs stimulate the synthesis of the cartilage ECM components, they inhibit proteases and activate their inhibition systems. Thus, TGF-β illustrates perfectly the complex as well as paradoxical nature of the action of the GFs, which on one hand can increase or decrease the expression and activity of some metalloproteinases (MMP) produced by articular chondrocytes, increasing MMP-9 and reducing MMP-2. Another anabolic factor for chondrocytes is IGF-I, which can keep chondrocytes phenotype *in vitro*, stimulate intensely the synthesis of proteoglycans and type II collagen, as well as block the harmful effects induced by IL1 on the proteoglycans degradation. On the other hand, both IGF-1 and TGF-β stimulate the expression on the cell surface of the subunits of integrins a3- and a5-, as well as adhesion of chondrocytes to fibronectin and collagen type II, being integrins, accepted as the main receptors, the molecules used by the cells (chondrocytes, in cartilaginous tissue case) to adhere to ECM. A new GFs family has been reported, Wnt, with a major role in chondrocyte differentiation. Wnt family members are important regulators of several development processes, including skeletogenesis. After the binding of Wnt to the Frizzled receptors family and the LRP5/6 co-receptors family, the canonical Wnt pathway will stabilize the β-catenin, which translocates to the nucleus and interacts with members of the TCF/LEF (β-catenin-T-cell factor/lymphoid enhancer factor) families to activate target genes. Whereas inactivation of β-catenin causes ectopic formation of chondrocytes at the expense of osteoblasts formation, the canonical Wnt pathway leads to enhanced ossification and suppression of chondrocytes due to the transcriptional downregulation of Sox9 (Boyden et al., 2002; Guo et al., 2004). In fact, Church and colleagues have shown that Wnt4 blocks the chondrogenesis start and accelerates the terminal chondrocyte differentiation *in vitro*, while Wnt5a and Wnt5b promote early chondrogenesis and inhibit terminal differentiation *in vivo* (Church et al., 2002). On the other hand, Wnt7 blocks chondrogenesis (Tufan et al., 2002). These studies illustrate that Wnt/β-catenin signalling play an essential role on MSCs by controlling osteoblastic and chondrocytic differentiations.

Once the process of activation and attraction for the tissue repair has been started, we have just to review MSCs proliferation or expansion. Requisites for human MSCs growth are

different than those for other species and several factors have been identified as potent mitogens, taking PDGF-BB, EGF and TGF-β as the most important ones, leading jointly cell migration and proliferation steps. MSCs have been expanded with foetal bovine serum (FBS), supplemented with GF, for research as well as for clinical use; this supplementation with FBS has several risks such as diseases transmission or immune reactions, which promoted the development of research lines with autologous human serum as substitute for FBS. Human platelet lysate (hPL) has been also proposed as a substitute for culture and expansion of MSCs, and some reports showed that there is a higher proliferation in hPL regarding FBS, keeping their differentiation potential including chondrogenesis.

Finally, as one of the basic columns of the tissue repair we have to highlight the importance of scaffolds, because treatment with MSCs requires cells and scaffold transplantation. Importance of such structures is based on their ability for a fast filling of defects, their raised persistence to carry out repair, they also achieve an uniform distribution of cells in an enhance volume and also because they provide an active environment that may allow the local delivery of local molecules to stimulate repair (Andrades et al., 2010). Many studies have reported that using several scaffolds improve the new tissue quality. Such scaffolds are based mainly on hyaluronic acid, polylactic acid and/or polyglicolic acid, which can help to keep the cells inside the defect and provide a chondroinductive matrix. Natural materials, such as agarose, alginate, gelatine and collagen derivatives are inferior than synthetic and hybrid materials, due to a poor resistance to mechanical stress, so their clinical usefulness is severely limited. We have to take on account that MSCs chondrogenic potential is favoured by hypoxia and that it does not only depend on hydrostatic pressure, but also on cell density within the matrix, as well as on the presence of GFs. It is suggested by the obtained information that scaffolds or matrices allow the cell differentiation and the maintenance of a mature phenotype (Fig. 11); this combined with the use of stem cells, provides us promising perspectives for the regeneration into a functional tissue (Becerra et al., 2010; Reddi et al., 2011).

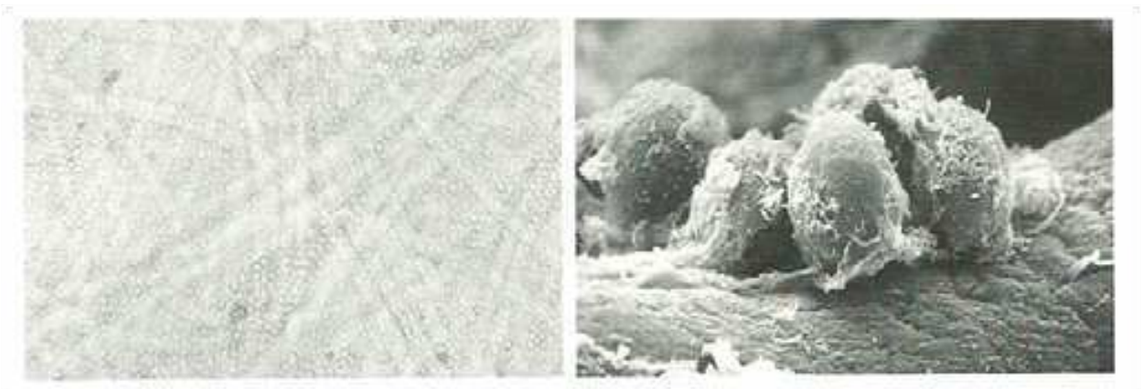

Fig. 11. Scanning electron microscopy of natural alginate wire mesh containing chondrocytes

From the knowledge of these biological phenomena of in situ repair, adequate conditions for cartilage repair based on MSCs are considered. Although some studies demonstrate that non-differentiated MSCs transplantation in cartilage defects, without scaffolds and not having been chondroinduced provided good, it has been reported that the before mentioned cytokines and particularly the association of TGF-β and BMPs improve the cartilage repair when combined with MSCs. Such stimuli could be obtained by direct administration of recombining GFs in the culture media or via transfer of the respective genes. Thus, the

possibility of considering genetic therapy as an applicable measure for the treatment of cartilaginous lesions arises.

2.3 Approaches for tendon regeneration

Tendons make part of a biomechanical chain formed by muscle, bones and tendon itself. Muscle contraction is converted by tendons into bone motion or viceversa; thus, tendon's main function is the transmission of force. Far from being a passive element, thanks to its composition and structure tendon has an adaptive, dynamic behaviour to face special functional requirements.

2.3.1 Tendon composition, structure and mechanical properties

Tendon is a dense connective tissue. Having to withstand basically tensile stresses, tendons are bundles of fibrillar proteins aligned parallelly to the tendon's longitudinal axis, with small amounts of other ECM components, specialized cells (tenocytes, fibroblasts), vessels and neuroreceptors. Between a 75% and a 96% of a tendon's dry weight is constituted by collagen, around a 2% by elastin. These proteins are imbibed in a mucopolysaccharide gel, the ground substance, formed mainly by hyaluronic acid and chondroitin sulfate, proteoglycans (decorin) and glycoproteins (fibronectin), which may account for a 1% of the tendon's dry weight (Crisp, 1972; Hooley, 1977). Collagen comes mainly as type-I collagen, with its characteristic hierarchical organization (Fig. 12) so important to understand the tendon's overall mechanical behaviour (Kastelic et al., 1978; Kastelic & Baer, 1980; Baer et al., 1975; Silver et al., 2003; Wang 2006): tropocollagen molecules assemble into microfibrils, these microfibrils into subfibrils, and several subfibrils give rise to a collagen fibril, with a characteristic 65 nm periodicity visible in scanning electron microscopy. Assemblies of

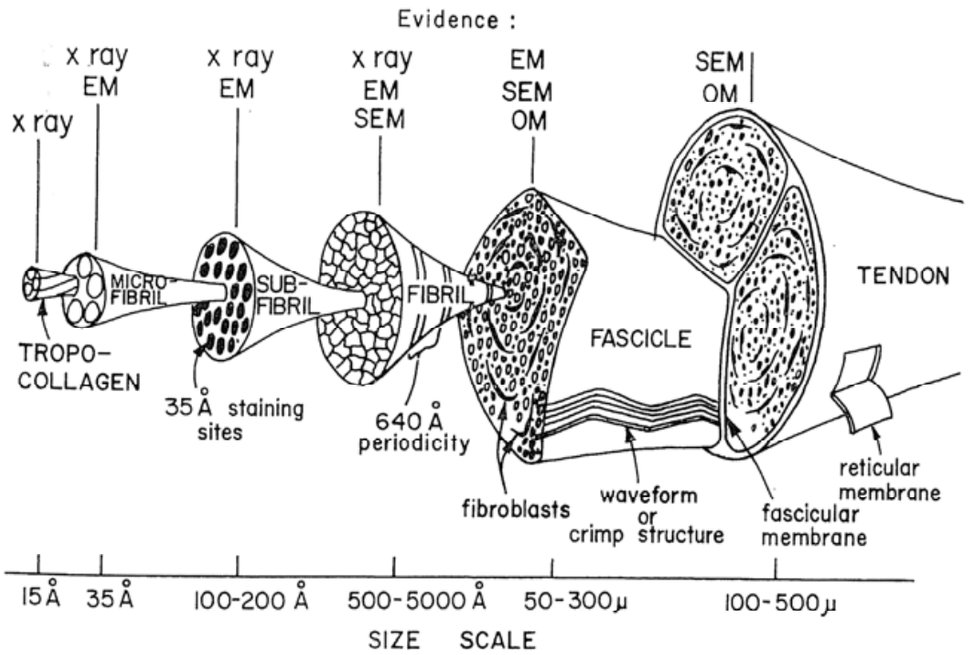

Fig. 12. Type I collagen hierarchical organization in tendon. *Modified from Kastelic J et al. The multicomposite structure of tendon. Connective Tissue Research 1978; 6:11-23*

fibrils bound by a thin layer of connective tissue (the endotenon) form typically crimped planar ribbons, with a periodicity of the order of a hundred microns, visible for the optical microscope. Packages of these ribbons surrounded by the epitenon (a sheath of loose connective tissue) constitute one tendinous bundle or fascicle; several fascicles may constitute larger units, and a tendon may consist of several of these units surrounded, again, by a layer of connective tissue (paratenon). Blood and lymphatic vessels, nerves and cells reside in the endo-, epi- and paratenon. Elastin, as said, is much less abundant in tendon than collagen, and is believed to help recover the original configuration of collagen fibres after cessation of applied stresses thanks to its rubberlike elasticity. The ground substance is a highly hydrated gel that allows for the diffusion of nutrients, signals and metabolytes to and from the cellular component of the tendon, contributing also to its mechanical behaviour as it is sheared during fascicle extension, (Minns et al., 1973; Kastelic & Baer, 1980).

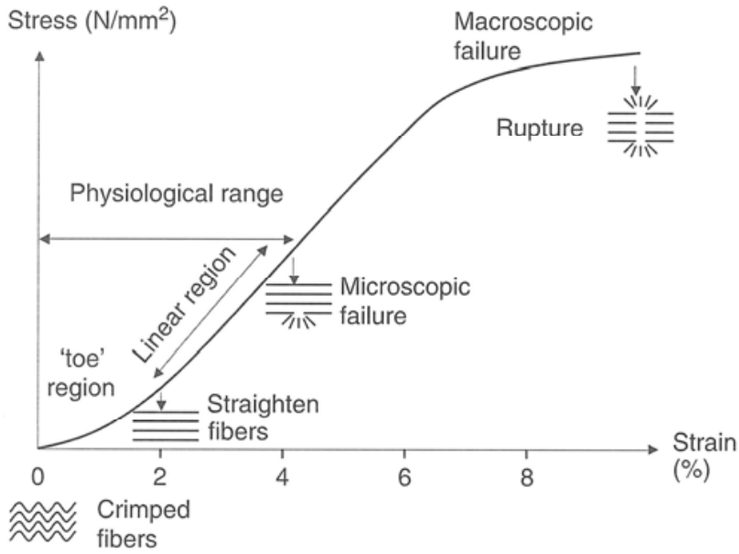

Fig. 13. Mechanism of internal deformation of tendon. *Modified from Wang JH. Mechanobiology of tendon. Journal of Biomechanics 2006; 39:1563-1582*

The crimped geometry of the parallelly aligned collagen-I fibre bundles and the properties of the highly crystalline collagen fibrils are the main responsibles for the tendon's overall mechanical behaviour. The main feature of this behaviour is its nonlinear viscoelasticity, 'nonlinear' meaning that the tendon's stiffness increases as it is progressively stretched, and 'viscoelastic' meaning that the mechanical response of the tissue to an external stimulus does not adjust instantaneously but lags in time, giving rise to the phenomena of stress relaxation and creep (Hooley et al., 1978; Monleón & Díaz, 1990). Thanks to both characteristics tendon is able to damp sudden stresses transmitted to muscles and to achieve a progressive adaptation of the whole biomechanical chain to varying mechanical stimuli. The origin of nonlinearity lies in the mechanism of internal deformation of tendon: the undulation ('crimping') of the collagen ribbons is not uniform, but its angles with the longitudinal axis of the tendon are distributed; thus, as the tendon is progressively deformed a varying number of ribbons first straightens out, and then become stretched (Kastelic & Baer, 1980; Monleón & Díaz, 1990). This gives rise to a typical nonlinear, convex stress-strain relationship, with an initial ('toe') region of low modulus extending up to some

2-3 % of strain, and a subsequent ('linear') high-modulus part, which corresponds to a state in which most of the tendon fascicles have lost their rest wavy crimp and are bearing load (Fig. 13). Physiological regime of tendons lies in the strain interval up to 5-7 %, but this figure may vary considerably depending on age, weight, tendon type and other factors.

2.3.2 Cell response to mechanical stimuli

Even though tendon is a very poorly cellularized tissue, its cells are very important during development and healing, and thus a consideration of their role is critical for cell therapy purposes. Endothelial cells and synovial cells are present in the peritendon, but specialized fibroblasts (tenoblasts and tenocytes) are the dominant cell type. Tendon fibroblasts align in rows between collagen fibre bundles, and are responsible for synthesizing the ECM proteins, organizing them into their characteristic structures, and remodelling the ECM during tendon healing. These processes occur as a consequence of the biochemical and mechanical stimuli transmitted to the cells by their ECM. Tendon cells respond to mechanical forces by altering gene expression, protein synthesis, and cell phenotype. Mechanotransduction (the conversion of mechanical stimuli into biochemical signals leading to cell response) is a not entirely understood complex process which involves the interaction of proteins from the ECM, transmembrane integrins and G proteins, and cytoskeleton proteins such as actin. This interaction triggers signalling pathways that lead to differential gene expression and protein synthesis by the cells (Wang, 2006). Increased ECM protein production occurs as a consequence of GF release stimulated by mechanical loading (Skutek et al., 2001; Kim et al., 2002); matrix remodelling is also influenced by mechanical stimuli, which enhance the secretion of matrix metalloproteinases (Archambault et al., 2002; Tsuzaki et al. 2003). Different mechanical loading conditions lead to varying proportions of ECM proteins produced.

2.3.3 Lesions, standard approaches and challenges posed to cell therapy

Weakly injured tendons tend to heal spontaneously, but the remodelled tissue usually has poorer mechanical properties than the original one. More extensive trauma and degenerative processes, tumors or congenital malformations may require different degrees of surgery, including transplantation. Autologous grafts are in these cases the golden standard, but they may result in donor site morbidity and there may be lack of sufficient supply; artificial prostheses have not until today stood up to the requirements to represent an alternative. Cell therapy and engineered tendon constructs would be of utility in these cases (Thorfinn et al., 2010). The pluripotency discovered in several cell types, embryonic and adult, opens the way to their differentiation into tenocytes and their transplantation into injured or defective sites. A pure cell supply strategy, however, may be appropriate to small defects and injuries, but does not seem to be sufficient in cases of extensive tissue loss or rupture; here cell supply will presumably have to be combined into an engineered tendon construct with biomaterials acting as supporting or guiding structures and maybe GFs enhancing matrix production, remodelling and neovascularization. It is a challenge for this strategy to identify suitable candidates for a scaffold material and architecture, for the cell type to be supplied, for the GFs, their concentration and their release kinetics, and, maybe, for a cell training strategy through convenient *ex vivo* stimuli in bioreactors.

2.3.4 Tissue engineering and cell therapy of tendons

The paradigm of the TE approach is a biodegradable scaffold seeded with cells and bioactive factors, such that the scaffold is progressively replaced by neotissue formed *in situ*,

hystologically and functionally normal. The scaffold must initially bear the full mechanical load, but this must soon be gradually transferred to the ingrowing tissue to ensure the mechanical stimuli on the cells necessary for them to differentiate and produce and organize the ECM. A part of this process may take place *ex vivo*, in bioreactors. *In vitro* and *in vivo* animal studies have been undertaken along lines aiming to understand the relative influence and cross-talk of all these factors: *(i)* scaffolds, *(ii)* cell types supplied, *(iii)* biochemical signals, and *(iv)* mechanical stimulation.

i. Three dimensional environments for cells have proved to be necessary to achieve *in vitro* significant tendon-like matrix production (Garvin et al., 2003), and such 3D scaffolds are also likely to make part of any successful *in vivo* strategy. Synthetic materials available for scaffold fabrication belong to the standard families of polyesthers (polylactic and polyglycolic acid and their copolymers, polycaprolactone), and they have been assayed with different morphologies, including knitted and electrospun versions (Ouyang et al., 2003; Guo & Spector, 2006; Thorfinn et al., 2010; Ladd et al., 2011; Jiang et al., 2011). None of these synthetic materials, however, possesses mechanical properties approaching those of natural tendon; the less so when they have a porous or microfibrilar structure. Moreover, the effects of their degradation process have to be taken into account, since they imply local acidification. This is why many researchers show preference for different collagen-based gels, where cells are seeded and reside as in a 3D matrix, and can be tested *in vitro* (Garvin et al., 2003; Kuo & Tuan, 2008); these gels could also serve as vector (transport, localization) for the supply of cells *in vivo*. However, these collagen gels are even less resistant than the synthetic polymers, and thus their utility *in vivo* has to be limited to those cases involving only limited injuries and defects, and where more complex regeneration and extensive reconstruction is not required. Nonetheless, systems based on these gels could be the solution also for these latter cases if they could be engineered *in vitro* to develop a high modulus construct which would be then transplanted, and this is the hope of much current work. All in all, scaffold choice, its composition and structure, remains an open problem for tendon TE.
ii. The cells to be employed in tendon regeneration should be capable of producing and organizing into its functional structure the ECM of tendons; they may be adult or embryonic, differentiated or pluripotent (stem) cells, autologous or not. For obvious reasons, adult autologous cells seem those more probable to enter a clinical solution; and, since BM MSCs and adipose tissue-derived stem cells (ADSC) have been successfully differentiated to functional tenocyte-like cells (Altman et al., 2001; Hoffmann et al., 2006; Juncosa-Melvin et al., 2007; Lee et al., 2007), it is they who are receiving most attention. Bone MSCs, in particular, are cells likely to enter wounded areas, and they have demonstrated a paracrine effect on tendon fibroblasts, secreting factors which enhance cell migration and proliferation (Shimode et al., 2007). Significant results have been obtained with ADSC *in vivo* (de Matos Carvalho et al., 2011). However, the sole transplantation of MSC into tendon defects does not improve significantly the tendon's microstructure, and the cells should be induced into the tenocytic linage by specific molecules (Araque et al., 2011).
iii. Since a natural regeneration accompanies healing processes, an understanding of natural tendon healing and the cascade of signals involved in it is crucial for the design of cell therapy strategies. A number of GFs that play an important role during the inflammatory, repairing and remodelling stages of the healing process has been identified, the most important ones being IGF-1, PDGF, VEGF, bFGF and some of the

TGF-β family (Molloy et al., 2003). Specific molecules which selectively induce the tenocytic differentiation of MSC while supressing their osteogenic and chondrogenic differentiation have been identified (Hoffmann et al., 2006); they may provide a powerful platform for successful tendon regeneration when combined with mechanical stimulation within scaffolds.

iv. Though MSC have been made to express tendon-like phenotype and produce aligned collagen-I, mechanical properties of the neotissue produced could not match those of native tendon unless the cells had been subjected to mechanical stimulation (Juncosa-Melvin et al., 2006; Benhardt & Cosgriff-Hernández, 2009). Several types of bioreactors have been developed which allow to apply load uniaxially, in a static or dynamic (cyclical) mode, to seeded constructs (Guo & Spector, 2006; Garvin et al., 2003; Abousleiman et al., 2009). With their help it has been possible to check hypotheses as to the importance of force magnitude, strain amplitude, time frequency of loading, loading duration, and synergisms with GFs on the proliferation, differentiation and ECM production of cells. An excessive mechanical conditioning has been shown to lead to tendon disorders and matrix degeneration, however; thus, optimal values of those mechanical parameters must be determined (Wang, 2006). Generally, cyclic stress application has resulted in better results than static stresses as regards matrix formation and fibre alignment (Kuo & Tuan, 2008; Benhardt & Cosgriff-Hernández, 2009; Nguyen et al., 2009).

Taking into account those concepts, we are currently investigating the potential of human MSCs to differentiate into functional tenocytes. This cell application is directed to heal tendon and ligament injuries using either biosutures (Fig. 14) or poly-l-lactic acid (PLLA) scaffolds microparticles (Fig. 15) that could represent adequate surfaces for MSCs adhesion, proliferation and differentiation to tenocytes.

3. Conclusion

In the last ten years we have published reports suggesting that MSCs have a promising potential to be utilized for regenerative medicine. For example, our laboratory has accumulated experience working *in vitro* with blood-, fat-, skeletal muscle-, BM-, and now synovial-derived MSCs, performing ectopic implants (in mouse and rat), preclinical trial with small animals (rabbit, using an osteochondral defect model), with big animals (sheep, using a spinal fusion model), and with a pilot clinical trial in a patient affected by osteomyelitis. Cells to be used for an efficient regenerative medicine should be chosen. Undifferentiated versus differentiated or predifferentiated cells will be the choice. A permanent solution will come when the new tissue built in the defect is of the same nature and is perfectly integrated in the whole structure in any pathology and in any age. Only in such as way structure and function will be fully recovered.

When regenerative medicine started several years ago, the main goal was for the implanted cells to directly participate in the reconstruction of the damaged tissue. But now, after the reported paracrine effects in several MSCs therapies we know that MSCs, far from building those tissues, they exert immunomodulatory functions, secreting, in addition, several bioactive molecules that inhibit apoptosis and scarring at sites of injury, and stimulate angiogenesis and mitosis of tissue-specific progenitors (Caplan, 2009; Ito et al., 2010; Park et al., 2010). These actions have been found either when the implanted MSCs coming from BM or adipose derived in adherent cell cultures (García-Olmo et al., 2010) or when the

mononuclear fraction of BM was infused. We cannot predict the extent of the paracrine effect, immunomodulatory, or if the effective replenishment of differentiated cells can be assigned, in each case, to the MSCs, or whether these effects have some degree of integration between them. We cannot even know if all those effects can influence the surrounding tissue positively during regeneration, but perhaps negatively towards the pathogenesis of cancer and metastasis (Kuhn & Tuan, 2010). For now, we can only say that in many cases, these actions have a certain synergy to the purpose they claim. In the future, in might be useful to know the responsibility of each action in the regenerative process in order to control it appropriately. All this indicates the necessity to highlight again the importance of a tight control over the stem cell culture method in order to define the cell products for transplantation properly, according to the specific functional outcome sought.

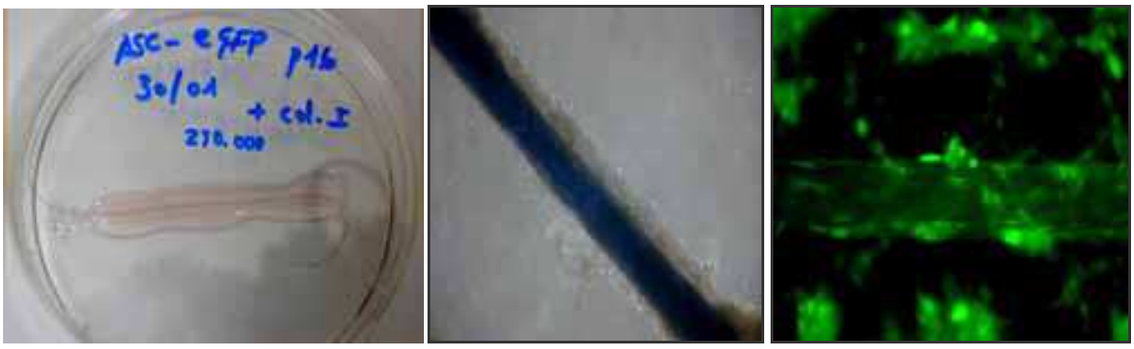

Fig. 14. Fresh collagen type I containing human MSCs embedding a resorbable polyglactic suture in vitro. After 30 min. of incubation at 37 ^{o}C, the collagen gelify and cells oriented around and along the suture line, as we can see under a fluorescence microscope (grean: hMSCs transfected with the green fluorescent protein)

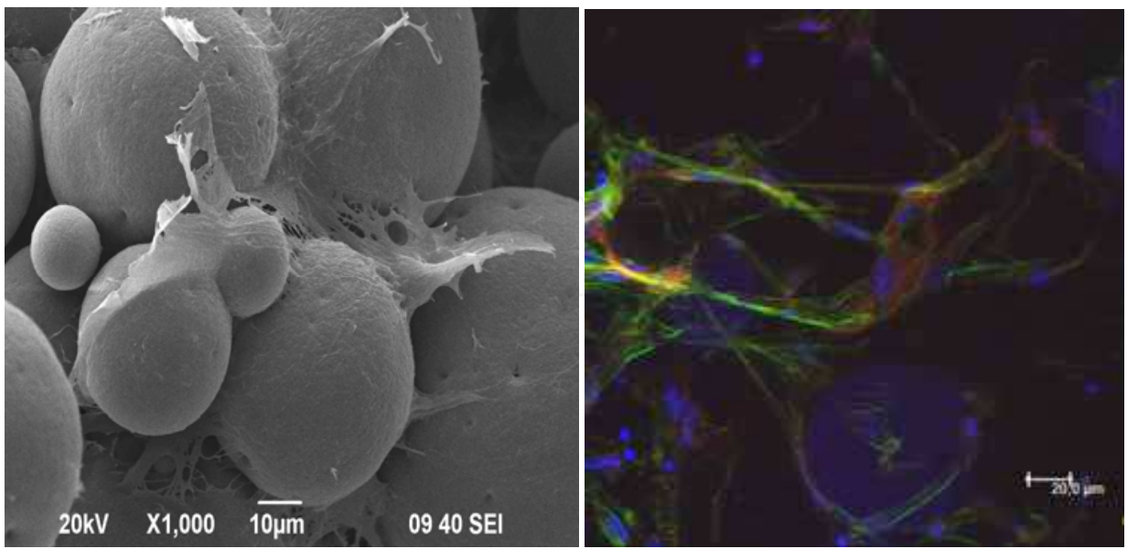

Fig. 15. Scanning electron microscopy of PLLA microparticles containing human MSCs attached to their surfaces after 14 days in culture, and their phenotypic features by confocal microscopy (blue: cell nuclei, grean: actin cytoskeleton, red: collagen type I)

4. Acknowledgments

Laboratory of Bioengineering and Tissue Regeneration-University of Málaga (LABRET-UMA) is supported by grants from the Spanish (BIO2009-13903-C02-01, PLE2009-0163, PI10/02529, and Red de Terapia Celular, RD06/00100014), and the Andalusian Governments (P07-CVI-2781, PI-0729-2010, and PAID BIO217). CIBER-BBN is an initiative funded by the VI National R&D&I Plan 2008-2011, *Iniciativa Ingenio 2010*, *Consolider Program*, *CIBER Actions* and financed by the Instituto de Salud Carlos III with assistance from the *European Regional Development Fund*.

5. References

Abousleiman, R.I.; Reyes, Y.; McFetridge, P. & Sikavitsas, V. (2009). Tendon tissue engineering using cell-seeded umbilical veins cultured in a mechanical stimulator. *Tissue Engineering*, 15, 787-795.

Alexanian, A.R.; Kwok, W.M.; Pravdic, D.; Maiman, D.J. & Fehlings, M.G. (2010). Survival of neurally induced mesenchymal cells may determine degree of motor recovery in injured spinal cord rats. Restoration Neurology and Neuroscience, 28(6):761-767.

Alonso, M.; Claros, S.; Becerra, J. & Andrades, J.A. (2008). The effect of type I collagen on osteochondrogenic differentiation in adipose-derived stromal cells in vivo. *Cytotherapy*, 10(6), 597-610.

Altman, G.H.; Horan, R.L.; Martin, I.; Farhadi, J.; Stark, P.R.H.; Volloch, V.; Richmond, J.C.; Vunjak-Novakovic, G. & Kaplan, D.L. (2001). Cell differentiation by mechanical stress. *FASEB J.* 16, 270-272.

Andrades, J.A.; Han, B.; Nimni, M.E.; Ertl, D.C.; Simpkins, R.J.; Arrabal, M.P. & Becerra, J. (2003). A modified rhTGF-beta1 and rhBMP-2 are effective in initiating a chondro-osseous differentiation pathway in bone marrow cells cultured in vitro. *Connective Tissue Research*, 44(3-4), 188-197.

Andrades, J.A.; López-Puertas, J.M.; Cuenca-López, M.D.; Jiménez-Palomo, P. & Becerra, J. (2010). Mesenchymal stem cells and a composed membrane for osteocondral wound treatment. *Patent application* nº 201031016, 29 pages.

Araque, M.C.; Claros, S.; Jiménez-Palomo, P.; Becerra, J.; Monleón, M. & Andrades, J.A. (2011). Differentiation of human mesenchymal stem cells into tenogenic lineage on different designs of microparticles. *Journal of Orthopaedic Research*, in press.

Arrington, E.D.; Smith, W.J.; Chambers, H.G., et al. (1996). Complications of iliac crest bone harvesting. *Clinical Orthopaedic Related Research*, 329, 300-9.

Archambault, J.; Tsuzaki, M.; Herzog, W. & Banes, A.J. (2002). Stretch and interleukin-1beta induce matrix metalloproteinases in rabbit tendon cells in vitro. *Journal of Orthopaedic Research*, 20, 36–39.

Aslan, H.; Zilberman, Y.; Arbeli, V.; Sheyn, D.; Matan, Y.; Liebergall, M. et al. (2006). Nucleofection-based ex vivo nonviral gene delivery to human stem cells as a plataform for tissue regeneration. *Tissue Engineering Part A*, 12(4), 877-889.

Azizi, S.A.; Stokes, D.; Augelli, B.J.; DiGirolamo, C. & Prockop, D.J. (1998). Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats-similarities to astrocyte grafts. *Proceedings of National Academic of Sciences USA*, 95(7), 3908-3913.

Baer, E.; Gathercole, L. & Keller, A. (1975). Structure hierarchies in tendon collagen: an interim summary. In: Atkins, E.D.T. & Keller, A. (eds.). Structure of fibrous biopolymers. Butterworth, London.

Baksh, D. & Tuan, R.S. (2007). Canonical and non-canonical Wnts differentially affect the development potential of primary isolate of human bone marrow mesenchymal stem cells. *Journal of Cell Physiology*, 212(3), 817-826.

Banwart, J.C.; Asher, M.A. & Hassanein, R.S. (1995). Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. *Spine*, 20, 1055-60.

Barry, F.; Boynton, R.E.; Liu, B. & Murphy, J.M. (2001). Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Experimental Cell Research*, 268, 189–200.

Bartlett, W.; Skinner, J.A.; Gooding, C.R.; Carrington, R.W.; Flanagan, A.M.; Briggs, T.W. & Bentley, G. (2005). Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. *Journal of Bone and Joint Surgery Brithis*, 87, 640–645.

Becerra J.; Andrades J.A.; Guerado, E.; Zamora-Navas, P.; López-Puertas, J.M. & Reddi, A.H. (2010). Articular cartilage: structure and regeneration. *Tissue Engineering Part B Review*, 16(6), 617-627.

Becerra J.; Santos-Ruiz, L.; Andrades, J.A. & Marí-Beffa, M. (2011). The stem cell niche should be a key issue for cell therapy in regenerative medicine. *Stem Cell Review*, 7, 248-255

Behr, B.; Leucht, P.; Longaker, M.T. & Quarto, N. (2010). Fgf-9 is required for angiogenesis and osteogenesis in long bone repair. *Proceedings of the National Academic of Sciences USA*, 107(26):11853-11858.

Belthur, M.V.; Conway, J.D.; Jindal, G., et al. (2008). Bone graft harvest using a new intramedullary system. *Clinical Orthopaedic and Related Research*, 466, 2973-80.

Bendall, S.C.; Stewart, M.H.; Menendez, P.; George, D.; Vijayaragavan, K.; Werbowetski-Ogilvie, T.; Ramos-Mejia, V.; Rouleau, A.; Yang, J.; Bossé, M.; Lajoie, G. & Bhatia, M. (2007). IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. *Nature*, 448(7157), 1015-1021.

Benhardt, H.A. & Cosgriff-Hernández, E.M. (2009). The role of mechanical loading in ligament tissue engineering. *Tissue Engineering*, 15, 467-475.

Benya, P.D. & Shaffer, J.D. (1982). Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell*, 30, 215–224.

Boatright, K.C. & Boden, S.D. (2002). Biologic enhacement of spinal arthrodesis: past, present, and future. In: Fardon, D.F.; Grafin, S.R.; Abitbol, J.J.; Boden, S.D.; Herkowitz, H.N. & Mayer, T.G. Orthopaedic Knowledge Update, *Spine*, 2, 459-68.

Boyden, L.M.; Mao, J.; Belsky, J. *et al.* (2002). High bone density due to a mutation in LDL-receptor-related protein 5. *New England Journal of Medicine*, 346, 1513–1521.

Boland, G.M.; Perkins, G.; Hall, D.J. & Tuan, R.S. (2004). Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. *Journal of Cell Biochemistry*, 93(6), 1210-1230.

Boquest, A.C.; Shahdadfar, A.; Fronsdal, K.; Sigurjonsson, O.; Tunheim, S.H.; Collas, P. et al. (2005). Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro culture. *Molecular Biology and Cell*, 16(3), 1131-1141.

Bostrom, M.P. & Seigerman, D.A. (2005). The clinical use of allografts, demineralized bone matrices, synthetic bone graft substitutes and osteoinductive growth factors: a survey study. *Hospital for Surgery Survery Journal*, 1, 9-18.

Brittberg, M.; Lindahl, A.; Nilsson, A.; Ohlsson, C.; Isaksson, O. & Peterson, L. (1994). Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *New England Journal of Medicine*, 331, 889–895.

Cao, R.; Bråkenhielm, E.; Pawliuk, R.; Wariaro, D.; Post, M.J.; Wahlberg, E.; Leboulch, P. & Cao, Y. (2003). Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nature Medicine*, 9(5), 604-613.

Caplan, A.I. (1991). Mesenchymal stem cells. *Journal Orthopaedic Research*, 9, 641-50.

Caplan, A.I. (2009). Why are MSCs therapeutic? New data: new insight. *Journal of Pathology*, 217, 318-324.

Caplan, A.I. & Dennis, J.E. (2006). Mesenchymal stem cells as trophic mediators. *Journal of Cell Biochemistry*, 98(5), 1076-1084.

Chai, C. & Leong, KW. (2007). Biomaterials approach to expand and direct differentiation of stem cells. *Molecular Therapy*, 15, 467-480.

Chang, C.; Lauffenburger, D.A. & Morales, T.I. (2003). Motile chondrocytes from newborn calf: migration properties and synthesis of collagen II. *Osteoarthritis and Cartilage*, 11, 603–12.

Chiou, M.; Xu, Y. & Longaker, M.T. (2006). Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells. *Biochemical and Biophysic Research Communication*, 343(2), 644-652.

Church, V.; Nohno, T.; Linker, C. *et al.* (2002). Wnt regulation of chondrocyte differentiation. *Journal of Cell Sciences*, 115, 4809–4818.

Claros, S.; Alonso, M.; Becerra, J. & Andrades, J.A. (2008). Selection and induction of rat skeletal muscle-derived cells to the chondro-osteogenic lineage. *Cell and Molecular Biology*, 54(1), 1-10.

Colter, D.C.; Sekiya, I. & Prockop, D.J. (2001). Identification of a subpopulation of rapidly self-renewing and multypotential adult stem cells in colonies of human marrow stromal cells. *Proceedings of National Academic of Sciences USA*, 98, 7841–7845.

Crigler, L.; Robey, R.C.; Asawachaicharn, A.; Gaupp, D. & Phinney, D.G. (2006). Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. *Experimental of Neurology*, 198(1), 54-64.

Crisan, M.; Yap, S.; Casteilla, L., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*, 3, 301-13.

Crisp, J. (1972). Properties of tendon and skin. In: Fung, Y.C.; Perrone, N. & Anliker, M. (eds.). Biomechanics: its foundations and objectives, pp. 141-171. Prentice-Hall, Englewood Cliffs, New Jersey.

Cuenca-López, M.D.; Zamora-Navas, P.; García-Herrera, J.M.; Godino, M.; López-Puertas, J.M.; Guerado, E.; Becerra, J. & Andrades, J.A. (2008). Adult stem cells applied to tissue engineering and regenerative medicine. *Cell and Molecular Biology*, 54(1), 40-51.

Da Silva Meirelles, L.; Caplan, A.I. & Nardi, N.B. (2008). In search of the in vivo identity of mesenchymal stem cells. *Stem Cells*, 26, 2287-99.

Daley, G.Q. & Scadden, D.T. (2008). Prospect for stem cell-based therapy. *Cell*, 132, 544-8.

Day, S.; Ostrum, R.; Clinton, R.; et al. (2000). Bone injury, regeneration, and repair. In: *Biology and Biomechanics of Musculoskeletal System*. Buckwalter, J.; Einhorn, T. & Simon, S.; (Eds.). Chicago, Rosemont IL: American Academy of Orthopaedic Surgeons 388-75.

Deckers, M.M.; Karperien, M.; van der Bent, C.; Yamashita, T.; Papapoulos, S.E. & Löwik, C.W. (2000). Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. *Endocrinology*, 141(5), 1667-1674.

Delawi, D., Dhert, W.J.; Rillardon, L. et al. (2010). A prospective, randomized, controlled, multicenter study of osteogenic protein-1 in instrumented posterolateral fusions: report on safety and feasibility. *Spine*, 35, 1185-91.

De Mattos Carvalho, A.; Garcia-Alves, A.L.; Galvão Gomes de Oliveira, P.; Cisneros Álvarez, L.E.; Laufer-Amorim, R.; Hussni, C.A. & Deffune, E. (2011). Use of adipose tissue-derived mesenchymal stem cells for experimental tendinitis therapy in equines. *Journal of Equine Veterinary Science*, 31, 26-34.

Dominici, M.; le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D. et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, 8(4), 315-317.

Enneking, W.F.; Burchardt, H.; Puhl, J.J., et al. (1975). Physical and biological aspects of repairs in dog cortical bone transplants. *Journal of Bone and Joint Surgery American*, 57, 237-52.

Epstein, N.E. (2008). Efficacy of different bone volume expanders for augmenting lumbar fusions. *Surgical Neurology*, 69, 16-9.

Etheridge, S.L.; Spencer, G.J.; Heath, D.J. & Genever, P.G. (2004). Expression profiling and functional analysis of wnt signaling mechanisms in mesenchymal stem cells. *Stem Cells*, 22(5), 849-860.

Fiedler, J.; Etzel, N. & Brenner, R.E. (2004). To go or not to go: migration of human mesenchymal progenitor cells stimulated by isoforms of PDGF. *Journal of Cell and Biochemistry*, 93, 990–8.

Fiedler, J.; Leucht, F.; Waltenberger, J.; Dehio, C. & Brenner, R.E. (2005). VEGF-A and PlGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. *Biochemical and Biophysic Research Communication*, 334(2), 561-568.

Frenkel, S.R.; Clancy, R.M.; Ricci, J.L.; Di Cesare, P.E.; Rediske, J.J. & Abramson, S.B. (1996). Effects of nitric oxide on chondrocyte migration, adhesion, and cytoskeletal assembly. *Arthritis and Rheumatism*, 39, 1905–12.

Friedenstein, A.J.; Chailakhyan, R.K. & Gerasimov, U.V. (1987). Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Tissue Kinetic*, 20(3), 263-272.

Friedenstein, A.J.; Latzinik, N.W.; Grosheva, A.G. & Gorskaya, U.F. (1982). Marrow microenvironment transfer by heterotopic transplantation of freshly isolated and cultured cells in porous sponges. *Experimental Hematology*, 10(2), 217-227.

Fuchs, E.; Tumbar, T. & Guasch, G. (2004). Socializing with the neighbors: stem cell and their niche. *Cell*, 116, 769-778.

Fujita, T.; Azuma, Y.; Fukuyama, R. et al. (2004). Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-Akt signaling. *Journal of Cell Biology*, 166, 85–95.

García-Olmo, D.; Herreros, D.; De-La-Quintana, P. et al. (2010). Adipose-derived stem cells in Crohn's rectovaginal fistula. *Case Reports in Medicine*, 961758.

Garvin, J.; Qi, J.; Maloney, M. & Banes, A.J. (2003). Novel system for engineering bioartificial tendons and application of mechanical load. *Tissue Engineering*, 9, 967-979.

Gerstenfeld, L.C.; Cullinane, D.M.; Barnes, G.L.; Graves, D.T. & Einhorn, T.A. (2003). Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *Journal of Cell Biochemistry*, 88(5), 873-884.

Giannoudis, P.V.; Einhorn, T.A. & Marsh, D. (2007). Fracture healing: the diamond concept. *Injury*, 38, Suppl 4 S3-6.

Giannoudis, P.V.; Einhorn, T.A.; Schmidmaier, G. & Marsh, D. (2008). The diamond concept: open questions. *Injury*, 39, Suppl 2 S5-8.

Gigante, A.; Bevilacqua, C.; Ricevuto, A.; Mattioli-Belmonte M. & Greco, F. (2007). Membrane-seeded autologous chondrocytes: cell viability and characterization at surgery. *Knee Surgery of Sports and Traumatology Arthroscopyc*, 15, 88–92.

Glassman, S.D.; Howard, J.; Dimar, J. 2nd., et al. (2010). Complications With rhBMP-2 in Posterolateral Spine Fusion: A Consecutive Series of One Thousand Thirty-Seven Cases. *Spine*, 10 doi: 10.1097/BRS.0b013e3181d133d0.

Gnecchi, M.; Zhang, Z.; Ni, A. & Dzau, V.J. (2008). Paracrine mechanisms in adult stem cell signaling and therapy. *Circulation Research*, 103(11), 1204-1219.

Grande, D.A.; Pitman, M.I.; Peterson, L.; Menche, D. & Klein, M. (1989). The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. *Journal of Orthopaedic Research*, 7, 208–218.

Greco, S.J. & Rameshwar, P. (2007). Enhancing effect of IL-1alpha on neurogenesis from adult human mesenchymal stem cells: implication for inflammatory mediators in regenerative medicine. *Journal of Immunology*, 179(5), 3342-3350.

Groh, M.E.; Maitra, B.; Szekely, E. & Koç, O.N. (2005). Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. *Experimental of Hematology*, 33(8), 928-934.

Gross, A.E.; Aubin, P.; Cheah, H.K.; Davis, A.M. & Ghazavi, M.T. (2002). A fresh osteochondral allograft alternative. *The Journal of Arthroplasty*, 17, (4 suppl 1).

Guerado, E. (2005). Osteogénesis terapéutica en cirugía del raquis. Bases científicas de la artrodesis vertebral. I: Fundamentos biomecánicos. *Revista Ortopédica de Traumatología*, 49, 29-45.

Guerado, E.; Godino, M.; Andrades, J.A. & Becerra, J.(2005). Osteogénesis terapéutica en cirugía del raquis. Bases científicas de la artrodesis vertebral. II. Fundamentos biológicos. *Revista Ortopédica de Traumatología*, 49, 46-58.

Guo, X.; Day, T.F.; Jiang, X. *et al.* (2004). Wnt/β- catenin signaling is sufficient and necessary for synovial joint formation. *Genes Development*, 18, 2404–2417.

Guo, Ch. & Spector, M. (2006). Tissue engineering of tendons and ligaments. In: Ma, P.X., & Elisseeff, J. (eds.), Scaffolding in tissue engineering. CRC Press, Boca Raton.

Haddo, O.; Mahroof, S.; Higgs, D.; David, L.; Pringle, J.; Bayliss, M.; Cannon, S.R. & Briggs, T.W. (2004). The use of chondroguide membrane in autologous chondrocyte implantation. *Knee*, 11, 51–55.

Hansen-Algenstaedt, N.; Joscheck, C.; Wolfram, L.; Schaefer, C.; Müller, I.; Böttcher, A.; Deuretzbacher, G.; Wiesner, L.; Leunig, M.; Algenstaedt, P. & Rüther W. (2006). Sequential changes in vessel formation and micro-vascular function during bone repair. *Acta Orthopaedic*, 77(3), 429-439.

Hecht, B.P.; Fischgrund, J.S.; Herkowitz, H.N. et al. (1999). The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. *Spine*, 24, 629-636.

Heineck, J.; Haupt, C.; Werner, K. et al. (2010). Fracture models in the lumbar sheep spine: a biomechanical investigation. *Journal of Orthopaedic Research*, 28, 773-7.

Helm, G.A. & Gazit, Z. (2005). Future uses of mesenchymal stem cells in spine surgery. *Neurosurgery Focus*, 19(6), E13.

Hidaka, C.; Cheng, C.; Alexandre, D.; Bhargava, M. & Torzilli, P.A. (2006). Maturational differences in superficial and deep zone articular chondrocytes. *Cell and Tissue Research*, 323, 127–35.

Hollinger, J.O.; Hart, C.E.; Hirsch, S.N.; Lynch, S. & Friedlaender GE. Recombinant human platelet-derived growth factor: biology and clinical applications. *Journal of Bone and Joint Surgery American*, 90 Suppl 1, 48-54.

Hoffmann, A.; Pelled, G.; Turgeman, G.; Eberle, P.; Zilberman, Y.; Shinar, H.; Keinan-Adamsky, K.; Winkel, A.; Shahab, S.; Navon, G.; Gross, G. & Gazit, D. (2006). Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *The Journal of Clinical Investigation*, 116, 940-952.

Hooley, C. (1978). The viscoelastic properties of tendon. PhD Thesis, Oxford University (1977).

Horas, U.; Pelinkovic, D.; Herr, G.; Aigner, T. & Schnettler, R. (2003). Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. *Journal of Bone and Joint Surgery American*, 85-A, 185–192.

Huang, J.I.; Kazmi, N.; Durbhakula, M.M.; Hering, T.M.; Yoo, J.U. & Johnstone, B. (2005). Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient matched comparison. *Journal of Orthopaedic Research*, 23, 1383–1389.

Huang, P.; Gupta, M.C.; Sarigul-Klijn, N. & Hazelwood, S. (2006). Two in vivo surgical approaches for lumbar corpectomy using allograft and a metallic implant: a controlled clinical and biomechanical study. *Spine Journal*, 6, 648-58.

Ichinose, S.; Yamagata, K.; Sekiya, I.; Muneta, T. & Tagami, M. (2005). Detailed examination of cartilage formation and endochondral ossification using human mesenchymal stem cells. *Clinical Experimental Pharmacology and Physiology*, 32, 561–570.

Ito, T.; Itakura, S.; Todorov, I. et al. (2010). Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation*, 89, 1438-1445.

Janssens, K.; ten Dijke, P.; Janssens, S. & Van Hul W. (2005). Transforming growth factor-beta1 to the bone. *Endocrinology Review*, 26(6), 743-774.

Javazon, E.H.; Beggs, K.J. & Flake, A.W. (2004). Mesenchymal stem cells: paradoxes of passaging. *Experimental Hematology*, 32(5), 414-425.

Jiang, Y.; Liu, H.; Li, H.; Wang, F.; Cheng, K.; Zhou, G.; Zhang, W.; Ye, M.; Cao, Y.; Liu, W. & Zou, H.A. (2011). Proteomic analysis of engineered tendon formation under dynamic mechanical loading in vitro. *Biomaterials*, 32, 4085-4095.

Johnstone, B.; Hering, T.M.; Caplan, A.I.; Goldberg, V.M. & Yoo, J.U. (1998). In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Experimental Cell Research*, 238, 265–272.

Jones, D.L., & Wagers, A.J. (2008). No place like home: anatomy and function of the stem cell niche. *Nature Review Molecular Cell Biology*, 9, 11-21.

Jorgensen, C.; Gordeladze, J. & Noel, D. (2004). Tissue engineering through autologous mesenchymal stem cells. *Current Opinion in Biotechnology*, 15, 406–410.

Juncosa-Melvin, N.; Shearn, J.T.; Boivin, G.P.; Gooch, C.; Galloway, M.T.; West, J.R.; Nirmalanandhan, V.S.; Bradica, G. & Butler, D.L. (2006). Effects of mechanical stimulation on the biomechanics and histology of stem cell-collagen sponge constructs for rabbit patellar tendon repair. *Tissue Engineering*, 12, 2291-2300.

Juncosa-Melvin, N.; Matlin, K.S.; Holdcraft, R.W.; Nirmalanandhan, V.S. & Butler, D.L. (2007). Mechanical stimulation increases collagen type I and collagen type III gene expression of stem cell-collagen sponge constructs for patellar tendon repair. *Tissue Engineering*, 13, 1219-1226.

Kastelic, J. & Baer, E. (1980). Deformation in tendon collagen. In: Vincent, J. & Currey, J. (eds.), The mechanical properties of biological materials. Cambridge University Press.

Kastelic, J.; Galeski, A. & Baer, E. (1978). The multicomposite structure of tendon. *Connective Tissue Research*, 6, 11-23.

Kawazoe, Y.; Katoh, S.; Onodera, Y.; Kohgo, T.; Shindoh, M. & Shiba T. (2008). Activation of the FGF signaling pathway and subsequent induction of mesenchymal stem cell differentiation by inorganic polyphosphate. *International Journal of Biology Sciences*, 4(1), 37-47.

Kim, S.G.; Akaike, T.; Sasagaw, T.; Atomi, Y. & Kurosawa, H. (2002). Gene expression of type I and type III collagen by mechanical stretch in anterior cruciate ligament cells. *Cell Structure and Function*, 27, 139–144.

Kobbe, P.; Tarkin, I.S.; Frink., et al. (2008). Voluminous bone graft harvesting of the femoral marrow cavity for autologous transplantation. An indication for the "Reamer-Irrigator-Aspirator" (RIA) technique. *Unfallchirurg*, 111, 469-72.

Koob, S.; Torio-Padron, N.; Stark, G.B.; Hannig, C.; Stankovic, Z. & Finkenzeller, G. (2011). Bone formation and neovascularization mediated by mesenchymal stem cells and endothelial cells in critical-sized calvarial defects. *Tissue Engineering Part A*, 17(3-4), 311-321.

Kobbe, P.; Tarkin, I.S. & Pape, H.C. (2008). Use of the "reamer irrigator aspirator" system for non-infected tibial non-union after failed iliac crest grafting. *Injury*, 39, 796-800.

Kreuz, P.C.; Steinwachs, M.; Erggelet, C.; Krause, S.J.; Ossendorf, C.; Maier, D.; Ghanem, N.; Uhl, M. & Haag, M. (2009). Classification of graft hypertrophy after autologous chondrocyte implantation. *Knee Surgery of Sports Traumatology Arthroscopy*, 17, 1289–1297.

Kubo, H.; Shimizu, M.; Taya, Y.; Kawamoto, T.; Michida, M.; Kaneko, E.; et al. (2009). Identification of mesenchymal stem cell (MSC)-transcription factors by microarray and knockdown analyses, and signature molecule-marked MSC in bone marrow by immunohistochemistry. *Genes Cells*, 14(3), 407-424.

Kuhn, N.Z. & Tuan, R.S. (2010). Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metástasis. *Journal of Cellular Physiology*, 222, 268-277.

Kuo, C.K. & Tuan, R.S. (2008). Mechanoactive tenogenic differentiation of human mesenchymal stem cells. *Tissue Engineering Part A*, 14(10), 1615-1627.

Ladd, M.R.; Lee, S.Y.; Stitzel, J.D.; Atala, A. & Yoo, J.J. (2011). Co-electrospun dual scaffolding system with potential for muscle-tendon junction tissue engineering. *Biomaterials*, 32, 1549-1559.

Lee, I.C.; Wang, J.H.; Lee, Y.T. & Young, T.H. (2007). The differentiation of mesenchymal stem cells by mechanical stress or/and co-culture system. *Biochemical and Biophysical Research Communications*, 352, 147-152.

Lee, T. (2010). Host tissue response in stem cell therapy. *World Journal of Stem Cells*, 2(4), 61-66.

Li, H.; Pujic, Z.; Xiao, Y. et al. (2002). Identification of bone morphogenetic proteins 2 and 4 in commercial demineralized freeze-dried allograft preparations: pilot study. *Clinical Implant Dentistry and Related Research*, 2, 110-7.

Li, L.; Zhang, S.; Zhang, Y.; Yu, B.; Xu, Y. & Guan, Z. (2009). Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. *Molecular Biology Reports*, 36(4), 725-731.

Li, Y.; Chen, J.; Chen, X.G.; Wang, L.; Gautam, S.C.; Xu, Y.X.; Katakowski, M.; Zhang, L.J.; Lu, M.; Janakiraman, N. & Chopp, M. (2002). Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology*, 59(4), 514-523.

Ling, L.; Nurcombe, V. & Cool, S.M. (2009). Wnt signaling controls the fate of mesenchymal stem cells. *Gene*, 433(1-2), 1-7.

Linke, A.; Müller, P.; Nurzynska, D.; Casarsa, C.; Torella, D.; Nascimbene, A.; Castaldo, C.; Cascapera, S.; Böhm, M.; Quaini, F.; Urbanek, K.; Leri, A.; Hintze, T.H.; Kajstura, J. & Anversa P. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proceedings of National Academic of Sciences USA*, 102(25), 8966-8971.

Liu, T.M.; Martina, M.; Hutmacher, D.W.; Hui, J.H.; Lee, E.H. & Lim, B. (2007). Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. *Stem Cells*, 25, 750–760.

López-Puertas, J.M.; Cuenca-López, M.D.; Jiménez-Palomo, P.; Becerra, J. & Andrades, J.A. (2011). Bone marrow mesenchymal stem cells and a biphasic collagen membrane are effective for articular cartilage regeneration. *Journal of Biomedicine and Biotechnology*, in press.

Ma, T.; Gutnick, J.; Salazar, B.; Larsen, M.D., et al. (2007). Modulation of allograft incorporation by continuous infusion of growth factors over a prolonged duration in vivo. *Bone*, 41, 386-92.

McCulloch, E.A. & Till, J.E. (2005). Perspectives on the properties of stem cells. *Nature Medicine*, 11, 1026-1028.

Mahmood, A.; Lu, D. & Chopp, M. (2004). Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. *Neurosurgery*, 55(5), 1185-1193.

Makino, T.; Jinnin, M.; Muchemwa, F.C.; Fukushima, S.; Kogushi-Nishi, H.; Moriya, C.; Igata, T.; Fujisawa, A.; Johno, T. & Ihn H. Basic fibroblast growth factor stimulates the proliferation of human dermal fibroblasts via the ERK1/2 and JNK pathways. *Brithish of Journal Dermatology*, 162(4), 717-723.

Manfredini, M.; Zerbinati, F.; Gildone, A. & Faccini, R. (2007). Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique. *Acta of Orthopædic Belgium*, 73, 207–218.

Maniwa, S.; Ochi, M.; Motomura, T.; Nishikori, T.; Chen, J. & Naora, H. (2001). Effects of hyaluronic acid and basic fibroblast growth factor on motility of chondrocytes and synovial cells in culture. *Acta Orthopaedic Scandinavia*, 72, 299–303.

Mayer, H.; Bertram, H.; Lindenmaier, W.; Korff, T.; Weber, H. & Weich, H. (2005). Vascular endothelial growth factor (VEGF-A) expression in human mesenchymal stem cells: autocrine and paracrine role on osteoblastic and endothelial differentiation. *Journal of Cell Biochemistry*, 95(4), 827-839.

Meinel, L.; Zoidis, E.; Zapf, J.; Hassa, P.; Hottiger, M.O.; Auer, J.A.; Schneider, R.; Gander, B.; Luginbuehl, V.; Bettschart-Wolfisberger, R.; Illi, O.E.; Merkle, H.P. & von Rechenberg, B. (2003). Localized insulin-like growth factor I delivery to enhance new bone formation. *Bone;* 33(4), 660-672.

Meirelles, Lda.S.; Fontes, A.M.; Covas, D.T. & Caplan AI. (2009). Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Review*, 20(5-6), 419-427.

Minas, T. (2001). Autologous chondrocyte implantation for focal chondral defects of the knee. *Clinical Orthopaedic and Related Research*, S349–S361.

Minns, R.J.; Soden, P.D. & Jackson, D.S. (1973). The role of the fibrous components and ground substance in the mechanical properties of biological tissues: a preliminary investigation. *Journal of Biomechanics*, 6, 153-165.

Mishima, Y. & Lotz, M. (2008). Chemotaxis of human articular chondrocytes and mesenchymal stem cells. *Journal of Orthopaedic Research*, 26, 1407–12.

Mitsiadis, T.A.; Barrandon, O.; Rochat, A. et al. (2007). Stem cell niches in mammals. *Experimental Cell Research*, 313, 3377-3385.

Molloy, T.; Wang, Y. & Murrell, G. (2003). The roles of growth factors in tendon and ligament healing. *Sports Medicine*, 33, 381–394.

Monleón, M. & Díaz-Calleja, R. (1990). Nonlinear viscoelastic behaviour of the fllexor tendon of the human hand. *Journal of Biomechanics*, 23, 773-781.

Morrison, S.J. & Sprading, A.C. (2008). Stem cells and niches: mechanics that promote stem cell maintenance throughout life. *Cell*, 132, 598-611.

Muschler, G.F. & Midura, R.J. (2002). Connetive tissue progenitors: practical concepts for clinical applications. *Clinical Orthopaedic*, 395, 66-80.

Neufeld, G.; Cohen, T.; Gengrinovitch, S. & Poltorak, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *FASEB Journal*, 13(1), 9-22.

Nimni, M.E. (1997). Polypeptide growth factors: targeted delivery systems. *Biomaterials*, 18(18), 1201-1225.

Nguyen, T.D.; Liang, R.; Woo, S.L.Y.; Burton, S.D.; Wu, Ch.; Almarza, A.; Sacks, M.S. & Abramowitch, S. (2009). Effects of cell seeding and cyclic stretch on the fiber remodeling in an extracellular matrix–derived bioscaffold. *Tissue Engineering Part A*, 15, 957-963.

Niu, T. & Rosen, C.J. (2005). The insulin-like growth factor-I gene and osteoporosis: a critical appraisal. *Gene*, 361, 38-56.

Niu, C.C.; Tsai, T.T.; Fu, T.S.; Lai, P.L.; Chen, L.H. & Chen, W.J. (2009). A comparison of posterolateral lumbar fusion comparing autograft, autogenous laminectomy bone with bone marrow aspirate, and calcium sulphate with bone marrow aspirate: a prospective randomized study. *Spine*, 34, 2715-9.

Noel, D.; Gazit, D. & Bouquet, C. (2004). Short-term BMP-2 expression is sufficient for *in vivo* osteochondral differentiation of mesenchymal stem cells. *Stem Cells*, 22, 74–85.

Ochi, M.; Uchio, Y.; Kawasaki, K.; Wakitani, S. & Iwasa, J. (2002). Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. *Journal of Bone and Joint Surgery Brithish*, 84, 571–578.

Ouyang, H.W.; Goh, J.C.H.; Thambyah, A.; Teoh, S.H. & Lee, E.H.L. (2003). Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. *Tissue Engineering Part A*, 9, 431-439.

Ozaki, Y.; Nishimura, M.; Sekiya, K. et al. (2007). Comprehensive analysis of chemotactic factors for bone marrow mesenchymal stem cells. *Stem Cells Development*, 16, 119–29.

Pape, H.C.; Evans, A. & Kobbe, P. (2010). Autologous Bone Graft: Properties and Techniques. *Journal of Orthophaedic and Trauma*, 24, S36-S40.

Park, K.S.; Kim, Y.S.; Kim, J.H. et al. (2010). Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. *Transplantation*, 89, 509-517.

Pei, M.; He, F. & Vunjak-Novakovic, G. (2008). Synovium-derived stem cell-based chondrogenesis. *Differentiation*, 76(10), 1044-1056.

Peterson, L.; Minas, T.; Brittberg, M.; Nilsson, A.; Sjogren-Jansson, E. & Lindahl, A. (2000). Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clinical Orthopaedic and Related Research*, 212–234.

Pittenger, M.F.; Mackay, A.M.; Beck, S.C.; Jaiswal, R.K.; Douglas, R.; Mosca, J.D.; Morman, M.A.; Ponte, A.L.; Marais, E.; Gallay, N. et al. (2007). The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells*, 25, 1737–45.

Porter, R.M.; Liu, F.; Pilapil, C. et al. (2008). Osteogenic potential of reamer irrigator aspirator (RIA) aspirate collected from patients undergoing hip arthroplasty. *Journal of Orthopaedic Research*, 27, 42-9.

Pricola, K.L.; Kuhn, N.Z.; Haleem-Smith, H.; Song, Y. & Tuan, R.S. (2009). Interleukin-6 maintains bone marrow-derived mesenchymal stem cells stemness by an ERK1/2-dependent mechanism. *Journal of Cell Biochemistry*, 108(3), 577-588.

Prockop, D.J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*, 276, 71–74.

Prockop, D.J. (2007). "Stemness" does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). *Clinical Pharmacology Therapy*, 82(3), 241-243.

Prockop, D.J. (2009). Repair of tissue by adult stem/progenitor cells (MSCs), controversies, myths, and changing paradigms. *Molecular Therapy*, 17(6), 939-946.

Reddi, A.H.; Becerra, J. & Andrades J.A. (2011). Nanomaterials and hydrogel scaffolds for articular cartilage regeneration. *Tissue Engineering Part B Review*, 20 (pending).

Rodrigues, M.; Griffith, L.G. & Wells A. Growth factor regulation of proliferation and survival of multipotential stromal cells. *Stem Cell Research Therapy*, 1(4), 32.

Sacchetti, B.; Funari, A.; Michienzi, S.; Di Cesare, S.; Piersanti, S.; Saggio, I.; Tagliafico, E.; Ferrari, S.; Robey, P.G.; Riminucci, M. & Bianco, P. (2007). Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell*, 131, 324–336.

Sandhu, H.S.; Grewal, H.S. & Parvataneni, H. (1999). Bone grafting for spinal fusion. *Orthopaedic Clinical North America*, 30, 685-98.

Sato, T.; Vries, R.G.; Snippert, H.J., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal Niche. *Nature*, 459, 262-266.

Schmid, G.J., Kobayashi, C.; Sandell, L.J. & Ornitz, D.M. (2009). Fibroblast growth factor expression during skeletal fracture healing in mice. *Developmental Dynamic*, 238(3), 766-774.

Schmidmaier, G.; Hermann, S.; Gree, J. et al. (2006). Quantitative assessment of growth factors in reaming aspirate, iliac crest, and platelet preparation. *Bone*, 39, 1156-63.

Shimode, K.; Iwasaki, N.; Majima, T.; Funakoshi, T.; Sawaguchi, N.; Onodera, T. & Minami, A. (2007). Bone marrow stromal cells act as feeder cells for tendon fibroblasts through soluble factors. *Tissue Engineering Part A*, 13, 333-341.

Shum, L.; Coleman, C.M.; Hatakeyama, Y. & Tuan, R.S. (2003). Morphogenesis and dysmorphogenesis of the appendicular skeleton. *Birth Defects Research C Embryo Today*, 69(2), 102-122.

Silver, F.H.; Freeman, J.W. & Seehra, G.P. (2003). Collagen selfassembly and the development of tendon mechanical properties. *Journal of Biomechanics*, 36, 1529–1553.

Skutek, M.; van Griensven, M.; Zeichen, J.; Brauer, N. & Bosch, U. (2001). Cyclic mechanical stretching modulates secretion pattern of growth factors in human tendon fibroblasts. *European Journal of Applied Physiology*, 86, 48–52.

Slosar, P.J.; Josey, R. & Reynolds, J. (2007). Accelerating lumbar fusions by combining rhBMP-2 with allograft bone: a prospective analysis of interbody fusion rates and clinical outcomes. *Spine Journal*, 7, 301-7.

Simonetti, D.W.; Craig, S. & Marshak, D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, 284, 143–147.

Sotiropoulou, P.A.; Perez, S.A.; Gritzapis, A.D.; Baxevanis, C.N. & Papamichail, M. (2006). Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells*, 24(1), 74-85.

Spees, J.L.; Olson, S.D.; Ylostalo, J.; Lynch, P.J.; Smith, J.; Perry, A. et al. (2003). Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. *Proceedings of National Academic of Sciences USA*, 100(5), 2397-2402.

Steadman, J.R.; Rodkey, W.G.; Briggs, K.K. & Rodrigo JJ (1999). The microfracture technic in the management of complete cartilage defects in the knee joint. *Orthopade*, 28, 26-32.

Takebayashi, T.; Iwato, M.; Jikko, A. et al. (1995). Hepatocyte growth factor / scatter factor BMdulates cell BMtility, proliferation, and proteoglycan synthesis of chondrocytes. *Journal of Cell Biology*, 129, 1411–9.

Thalgott, J.S.; Fogarty, M.E.; Giuffre, J.M.; Christenson, S.D.; Epstein, A.K. & Aprill, C. (2009). A prospective, randomized, blinded, single-site study to evaluate the clinical and radiographic differences between frozen and freeze-dried allograft when used as part of a circumferential anterior lumbar interbody fusion procedure. *Spine*, 34, 1251-6.

Tins, B.J.; McCall, I.W.; Takahashi, T.; Cassar-Pullicino, V.; Roberts, S.; Ashton, B. & Richardson, J. (2005). Autologous chondrocyte implantation in knee joint: MR imaging and histologic features at 1-year follow-up. *Radiology*, 234, 501–508.

Thorfinn, J.; Angelidis, I.; Pridgen, B. & Chang, J. (2010). Tendon engineering. In: Chang, J. & Gupta, G. (eds.). Tissue engineering for the hand. World Scientific Publishers, Singapore.

Tsutsumi, S.; Shimazu, A.; Miyazaki, K.; Pan, H.; Koike, C.; Yoshida, E.; Takagishi, K. & Kato, Y. (2001). Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. *Biochemical and Biophysic Research Communication*, 288(2), 413-9.

Tsuzaki, M.; Bynum, D.; Almekinders, L.; Yang, X.; Faber, J. & Banes, A.J. (2003). ATP modulates load-inducible IL-1beta, COX 2, and MMP-3 gene expression in human tendon cells. *Journal of Cellular Biochemistry*, 89, 556–562.

Tufan, A.C.; Daumer, K.M.; DeLise, A.M. *et al.* (2002). AP-1 transcription factor complex is a target of signals from both WnT-7a and N-cadherin-dependent cell-cell adhesión complex during the regulation of limb mesenchymal chondrogenesis. *Experimental Cell Research*, 273, 197–203.

Van der Kraan, P.M.; Blaney-Davidson, E.N.; Blom, A. & van den Berg, W.B. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. *Osteoarthritis and Cartilage*, 17(12), 1539-1545.

Villars, F.; Bordenave, L.; Bareille, R. & Amédée. J. (2000). Effect of human endothelial cells on human bone marrow stromal cell phenotype: role of VEGF? *Journal of Cell Biochemistry*, 79(4), 672-685.

Wang, J.H.C. (2006). Mechanobiology of tendon. *Journal of Biomechanics*, 39, 1563-1582.

Wang, L.; Fan, H.; Zhang, Z.Y.; Lou, A.J.; Pei, G.X.; Jiang, S.; Mu, T.W.; Qin, J.J.; Chen, S.Y. & Jin. D. (2010). Osteogenesis and angiogenesis of tissue-engineered bone constructed by prevascularized β-tricalcium phosphate scaffold and mesenchymal stem cells. *Biomaterials*, 31(36), 9452-9461.

Wang, Y.; Cheng, Z.; Elalieh, H.Z.; Nakamura, E.; Nguyen, M.T.; Mackem, S.; Clemens, T.L.; Bikle, D.D. & Chang, W. (2011). IGF-1R Signaling in chondrocytes modulates growth plate development by interacting with the PTHrP/Ihh pathway. *Journal of Bone and Mineral Research*, Feb 10.

Weissman, IL. (2000). Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science*, 287, 1442-1446.

Yang, Y. (2003). Wnts and wing: Wnt signaling in vertebrate limb development and musculoskeletal morphogenesis. *Birth Defects Research C Embryo Today*, 69(4), 305-317.

Yue, W.M.; Liu, W.; Bi, Y.W.; He, X.P.; Sun W.Y., Pang, X.Y. et al. (2008). Mesenchymal stem cells differentiate into an endothelial phenotype, reduce neointimal formation, and enhance endothelial function in a rat vein grafting model. *Stem Cells Development*, 17(4), 785-793.

Zisa, D.; Shabbir, A.; Suzuki, G., Lee T. Vascular endothelial growth factor (VEGF) as a key therapeutic trophic factor in bone marrow mesenchymal stem cell-mediated cardiac repair. *Biochemical and Biophysical Research Communication*, 390(3), 834-838.

Regenerative Medicine and Tissue Engineering - Cells and Biomaterials

Edited by Prof. Daniel Eberli

ISBN 978-953-307-663-8
Hard cover, 588 pages
Publisher InTech
Published online 29, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen