JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
*J Tissue Eng Regen Med* 2009; **3**: 327–337.
Published online 5 May 2009 in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/term.173

**REVIEW ARTICLE**

# Progenitor and stem cells for bone and cartilage regeneration

M. K. El Tamer[1,2]* and R. L. Reis[1,2]

[1]*3Bs Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal*
[2]*Institute for Biotechnology and Bioengineering (IBB), PT Government Associated Laboratory, Guimarães, Portugal*

## Abstract

**Research in regenerative medicine is developing at a significantly quick pace. Cell-based bone and cartilage replacement is an evolving therapy aiming at the treatment of patients who suffer from limb amputation, damaged tissues and various bone and cartilage-related disorders. Stem cells are undifferentiated cells with the capability to regenerate into one or more committed cell lineages. Stem cells isolated from multiple sources have been finding widespread use to advance the field of tissue repair. The present review gives a comprehensive overview of the developments in stem cells originating from different tissues and suggests future prospects for functional bone and cartilage tissue regeneration. Copyright © 2009 John Wiley & Sons, Ltd.**

**Received 7 January 2009; Accepted 17 March 2009**

**Keywords stem cells; stem cell sources; progenitor cells; regeneration; bone; osteogenesis; cartilage; chondrogenesis; tissue engineering; compromised tissue replacement; functional tissue repair; regenerative medicine**

## 1. Introduction to bone and regenerative capacity

Bones are characterized by patterns of microstructural organization which govern the mechanical interaction of the elementary components of bone (hydroxyapatite, collagen, water) and provide effective elastic properties. At a scale of 10 nm, long cylindrical collagen molecules, attached to each other at their ends by ~1.5 nm long crosslinks and hosting intermolecular water in between, form a contiguous matrix called wet collagen. At a scale of several hundred nanometers, wet collagen and mineral crystal agglomerations interpenetrate each other, forming the mineralized fibril. At a scale of 5–10 m, the extracellular solid bone matrix is represented as collagen fibril inclusions embedded in a foam of largely disordered (extrafibrillar) mineral crystals. At a scale above the ultrastructure, where lacunae are embedded in extracellular bone matrix, the extravascular bone material is observed (Fritsch and Hellmich, 2007). Human femoral trabecular bone has an apparent density and an apparent ash density of 0.43 g/cm$^3$ and 0.26 g/cm$^3$, respectively. The human vertebral body has an apparent density of $0.14 \pm 0.06$ g/cm$^3$ (Liebschner *et al*., 2004). Adult human bone has a secondary osteonal structure, i.e. osteons >100 m containing blood vessels and with cement lines forming a boundary between adjacent lamellae (Wang *et al*., 1998). Bone has the unique capacity to regenerate without the development of a fibrous scar, which is symptomatic of soft tissue healing of wounds. This is achieved through the complex interdependent stages of the healing process, which mimics the tightly regulated development of the skeleton (Kanczler and Oreffo, 2008).

## 2. Bone cells and ossification

Bone tissue consists of specialized cells and the extracellular matrix that these cells secrete and remodel (Huang *et al*., 2007). Osteoblasts, which mature into osteocytes, are responsible for depositing the proteinaceous and calcified matrix and secreting the growth factors necessary for osteogenesis. Osteoclasts, derived from the

*Correspondence to: M. K. El Tamer, American University of Beirut, PO Box 11-0236, Abuhaidar Neuroscience Institute, Faculty of Medicine and Medical Center, Riad El-Solh, Beirut 1107 2020, Lebanon. E-mail: mazen.eltamer@aub.edu.lb

monocyte–macrophage lineage, participate in the critical function of bone remodelling. The extracellular matrix is composed of collagenous proteins (predominantly collagen type I), non-collagenous proteins (osteocalcin, matrix gla protein, osteopontin and bone sialoprotein) and mineralized matrix (hydroxyapatite) (Kwan *et al*., 2008). The cell responsible for bone formation, the osteoblast, is derived from a marrow stromal fibroblastic stem cell. These marrow stromal fibroblastic stem cells exist postnatally, are multipotent and have the ability to generate myelosupportive stroma, osteoblasts, adipocytes, chondrocytes, smooth muscle cells and astrocytes. This population of cells are also referred to as osteogenic stem cells, marrow stromal fibroblastic cells, bone marrow stromal stem cells, mesenchymal stem cells, stromal precursor cells and skeletal stem cells (Caplan, 1991; Bianco and Robey, 2001; Barry and Murphy, 2004).

Development and formation of the skeleton (ossification) occurs by two distinct processes: intramembraneous and endochondral ossification. Both intramembraneous and endochondral bone ossification occur in close proximity to vascular ingrowth. Intramembraneous ossification is characterized by invasion of capillaries into the mesenchymal zone and the emergence and differentiation of mesenchymal cells into mature osteoblasts. These osteoblasts constitutively deposit bone matrix, leading to the formation of bone spicules. These spicules grow and develop, eventually fusing with other spicules to form trabeculae. As the trabeculae increase in size and number they become interconnected, forming woven bone (a disorganized weak structure with a high proportion of osteocytes), which eventually is replaced by more organized, stronger lamellar bone. This type of ossification occurs during embryonic development and is involved in the development of flat bones in the cranium, various facial bones, parts of the mandible and clavicle and the addition of new bone to the shafts of most other bones. In contrast, bones of load-bearing joints form by endochondral formation (Marks and Hermey, 1996).

# 3. Regenerative medicine and stem cells

Regenerative medicine offers novel therapeutic approaches, not only to control the progression of diseases but also, for the first time, to promote repair through tissue regeneration, a complex process of events encompassing stem cell differentiation and tissue patterning with architectonic organization and functional restoration.

Cell-based bone tissue engineering is a rapidly evolving therapy option in bone reconstruction strategies. The discovery of stem and progenitor cells opened a new frontier in regenerative medicine, which aims to replace cells and tissues in a broad range of conditions associated with damaged cartilage, bone, muscle, tendon and ligament.

Stem cells are undifferentiated cells with the capability to regenerate tissues (Blau *et al*., 2001). Adult stem cells are sparsely distributed in the body and perform functions such as, first, to produce identical copies of themselves for long periods of time, which is also referred to in the stem cell literature as the capacity for long-term self-renewal, and second, to engender transitional cell types before they reach the end of the differentiation cascade. The intermediate cell is defined as a progenitor or precursor cell, a cell that is regarded as committed to differentiate along a particular cellular pathway (Bruder and Fox *et al*., 1999; Caplan and Bruder, 2001).

Among the different stem cells types, one can find different populations proposed for regenerative medicine applications. Research on skeletal tissue engineering has remained focused on identifying an ideal cellular source. When considering potential cells for bone and cartilage regenerative medicine, options include osteoblasts, bone marrow mesenchymal stem cells, adipose-derived stem cells, embryonic stem cells, genetically modified cells, mesenchymal cambial layer cells, skeletal muscle-derived stem cells, muscle satellite cells, muscle-derived stem cells, umbilical cord stem/progenitor cells, cells from cord blood, amniotic fluid stem cells, veins and Wharton jelly cells. The present review presents an overview of these different sources for bone and cartilage tissue regeneration.

# 4. Cell sources for bone regeneration

Cell sources for bone tissue engineering applications can be categorized with respect to their state of differentiation. With this idea, four different cell-based tissue engineering approaches have been described for the regeneration of bone. These strategies are based on the implantation of: (a) unfractionated fresh bone marrow; (b) purified, culture-expanded stem cells; (c) differentiated osteoblasts; or (d) cells that have been modified genetically to express rhBMP (Bruder and Fox *et al*., 1999). In general, the less differentiated cells will be more easily expanded *in vitro* due to their high proliferation rate, while the differentiated cells will be more effective *in vivo* due to their higher and rapid production of mineralized extracellular matrix. For each type of cells use, advantages and disadvantages can be found.

## 4.1. Osteoblasts

Despite their lineage commitment to bone formation, osteoblasts derived from autologous bone represent a relatively limited source. In the early phase, the non-stem-cell approach was usually used to prove the concept of bone regeneration. For example, Vacanti *et al*. reported that bone tissue could be generated in the subcutaneous tissue of nude mice after implantation of degradable polymer seeded with osteoblasts isolated from periosteum (Vacanti *et al*., 1993; Vacanti and Upton, 1994). Studies of animal model and human osteoblasts have described



DOI: 10.1002/term

an attenuation of osteogenic differentiation and the proliferative response to mitogenic stimuli with aging (Zuk *et al*., 2001; Simonsen *et al*., 2002).

## 4.2. Mesenchymal stem cells

Friedenstein *et al*. (1968) found there were osteogenic precursor cells located in the bone marrow in a population of fibroblastic cells that could form a cell colony, named fibroblastic colony-forming units (CFU-Fs). The mammalian bone marrow (BM) is composed of different types of stem cells, among which are cells termed mesenchymal stromal cells or mesenchymal stem cells (MSCs) (Prockop, 1997; Blau *et al*., 2001). Mesenchymal cells are originally defined as primordial cells of mesodermal origin, giving rise to cells such as adipocytes (Young *et al*., 1998; Pittenger *et al*., 1999; Endres *et al*., 2003), osteoblasts (Pittenger *et al*., 1999; Donald *et al*., 1996; Jaiswal *et al*., 1997; Kadiyala *et al*., 1997; Nilsson *et al*., 1999) chondrocytes (Kadiyala *et al*., 1997; Pittenger *et al*., 1999; Johnstone *et al*., 1998; Mackay *et al*., 1998), tenocytes (Awad *et al*., 1999), skeletal myocytes (Pereira *et al*., 1995; Horwitz *et al*., 1999; Jiang *et al*., 2002; Bhabavati *et al*., 2004; Smith *et al*., 2004; Beyer and da Silva, 2006; Sethe *et al*., 2006). MSCs can also differentiate into cells of ectodermal origin, such as neurons (Woodbury *et al*., 2000), and of endodermal origin, such as hepatocytes (Petersen *et al*., 1999).

Adult MSCs have also been isolated from muscles (Deasy *et al*., 2001), peripheral blood (Kuznetsov *et al*., 2001; Roufosse *et al*., 2004), fat (Lee RH *et al*., 2004), hair follicles and scalp subcutaneous tissue (Shih *et al*., 2005), periodontal ligament (Trubiani *et al*., 2005), fetal bone marrow, blood, lung, liver and spleen (In't Anker *et al*., 2003), as well as pre-natal tissues such as cord blood (Erices *et al*., 2000) and placenta (Fukuchi *et al*., 2004; In't Anker *et al*., 2004). As a result, significant efforts have been directed at identifying postnatal sources for multipotent cells. Multipotent cells have been identified in bone marrow, adipose tissue, placenta, umbilical cord, human amniotic fluid, dental pulp and skeletal muscle among others (Freeman, 1997; Clarkson, 2001; Mitka, 2001; Kadner *et al*., 2002; Kaviani *et al*., 2002, 2003; Rosser and Dunnett, 2003; Savitz *et al*., 2004).

Currently, MSCs are isolated through a methodology based on gradient centrifugation and adherence to plastic culture surfaces, as described by Haynesworth and co-workers in the early 1990s (Haynesworth *et al*., 1992). Compared to unfractionated bone marrow, mesenchymal stem cells generate greater bone formation in preclinical studies (Kahn *et al*., 1995; Inoue *et al*., 1997). However, gradual loss of both their proliferative and differentiation potential has been observed during *in vitro* expansion (Mauney *et al*., 2005).

The cells from unfractionated fresh bone marrow are relatively easy to collect but it will not be possible to use these cells in allotransplantation, as bone marrow contains T lymphocytes that encounter and respond to host antigens in virtually all tissues in the body, leading to multi-system graft-versus-host syndrome (Weissman *et al*., 2000). Mesenchymal stem cells isolated from bone marrow aspirate, adult peripheral blood, neonatal cord blood or liver, for example, could present advantages from an immunological point of view (Javazon *et al*., 2004). However, as one of every 100 000 nucleated cells derived from bone marrow is a stem cell, a procedure of isolation is required in order to decrease the volume of material injected (Connolly *et al*., 1989).

MSCs express a complex pattern of molecules, including CD105 (SH2), CD73 (SH3 and SH4), CD106 (VCAM-1), CD54 (ICAM-1), CD44, CD90, CD29, STRO-1, as well as immune molecules such as HLA class I and II [the latter only upon the effect of interferon-$\gamma$ (IFN-$\gamma$)] and CD119 (IFN-$\gamma$ receptor). Haematopoietic markers, such as CD45 and CD34, are normally not expressed (Krampera *et al*., 2005, 2006a). MSCs also express cytokines, growth factors, extracellular matrix and adhesion-related receptors (Ringe *et al*., 2002).

Among all adult stem cells, bone marrow stem cells remain the most commonly used cell source for bone regeneration and repair in the studies using different animal models. On the basis of *in vitro* observation that MSCs can differentiate into osteocytes and chondrocytes, many attempts have been made to use expanded MSCs for *in vivo* tissue repair (Barry, 2001; Long, 2001; Fibbe, 2002). Osteogenic induction is conducted by culturing human BMSCs in an induction medium containing the synthetic glucocorticoid dexamethasone, L-ascorbic acid, 1,25-dihydroxyvitamin $D_3$ and the organic phosphate $\beta$-glycerophosphate playing a role in the mineralization and modulation of osteoblast activities (Bellows *et al*., 1990; Chung *et al*., 1992; Tenenbaum *et al*., 1992; Liu *et al*., 1999). After 16 days of culture, induced cells exhibited an osteogenic phenotype by alkaline phosphatase expression, reactivity with anti-osteogenic cell surface monoclonal antibodies, modulation of osteocalcin mRNA production and the formation of a mineralized extracellular matrix containing hydroxyapatite. The differentiation of MSCs into the osteogenic lineage is also stimulated by the addition of vitamin $D_3$ (Rickard *et al*., 1994; Jorgensen *et al*., 2004). A similar inducing effect can also be achieved by using growth factors such as the bone morphogenetic protein (BMP) family (Hanada *et al*., 1997; Yeh *et al*., 2002; Gregory *et al*., 2005), and if the cells are cultured on collagen (Yang *et al*., 2004; Salasznyk *et al*., 2004a; Salasznyk *et al*., 2004b) and calcium phosphates (Murphy *et al*., 2005; Salgado *et al*., 2005). Mesenchymal progenitors derived from juvenile bone marrow have the potential to undergo multiple differentiation pathways. Although it has been suggested that osteoblasts and adipocytes share common precursors within the adult stromal system (Bennett *et al*., 1991), human bone marrow-derived precursors showed no obvious differentiation into adipocytic cells, when stimulated with osteogenic medium supplemented with dexamethasone (Dex) in monolayers. In other studies,



DOI: 10.1002/term

depending on the presence of Dex in primary or secondary cultures of marrow stromal cells, an inverse relationship between the differentiation of adipocytic and osteogenic cells in marrow stromal cells has been reported (Beresford *et al*., 1992).

Bone marrow-derived MSCs have been seeded on extracellular matrices such as hydroxyapatite and then implanted *in vivo* into NOD/SCID mice, subsequently observing bone formation (Krebsbach *et al*., 1997). In combination with scaffolds/matrices, it was possible for seeded MSCs to repair segmental defects of critical size in various animal models (Bruder *et al*., 1998; Kon *et al*., 2000; Petite *et al*., 2000; Arinzeh *et al*., 2003; Holy *et al*., 2003). Similarly, bone marrow cells infused in children with osteogenesis imperfecta also increased, 3 months later, the mean number of osteoblasts, the formation of new lamellar bone and the total body mineral content. In addition, they eventually lowered the frequency of fractures and enhanced the body growth rate (Beyer and da Silva, 2006). Other studies in animals showed that the best route of MSC administration to induce local repair or regeneration of bone, cartilage or tendon is the *in situ* injection or implant (Richards *et al*., 1999). Particularly promising for orthopaedic applications, especially for bone formation, is the use of natural or synthetic biomaterials as carriers for MSCs delivery (Cancedda *et al*., 2003). A number of clinical studies have shown the efficacy of this approach in humans. Porous ceramic scaffolds loaded with *in vitro* expanded autologous bone marrow-derived MSCs were successfully implanted in three patients with large bone defects (Quarto *et al*., 2001). MSCs can also potentially be used to engineer cartilage–bone composites for the repair of defects extending from the articular surface into the underlying bone (Martin *et al*., 1998).

Stem cells have recently evoked interest as a promising alternative cell source for treating articular cartilage defects, helped by the development of MSC-based strategies of tissue engineering to induce *in situ* differentiation of mesenchymal progenitors into cartilage (Schultz *et al*., 2000; Jorgensen *et al*., 2001).

Autologous chondrocytes have a limited capacity to proliferate, on the other hand, MSCs are quickly amplified in monolayers. The easy availability of MSCs from various tissues, such as bone marrow, adipose tissue, synovial membrane and other tissues, together with their high proliferation capacity, make them attractive as a distinguished cell substitute for chondrocytes in cartilage regeneration (Friedenstein *et al*., 1970; Castro-Malaspina *et al*., 1980; De Bari *et al*., 2001; Noth *et al*., 2002; Baksh *et al*., 2004).

MSCs have been used *in vivo* to repair full-thickness joint cartilage defects in animal models, using various carrier matrices (Wakitani *et al*., 1994; Caplan *et al*., 1997; Murphy *et al*., 2000; Adachi *et al*., 2002; Wakitani *et al*., 2002; Wakitani and Yamamoto, 2002). Indrawattana and co-workers (2004) described the use of three different growth factors, TGF$\beta$3, BMP-6 and IGF-1, in combination with pellet cultures of human bone marrow cells, for cell induction. Cells exhibited features of chondrocytes in their morphology and extracellular matrix, in both inducing patterns of combination and cycling induction. Expression of gene markers of chondrogenesis, collagen type II and aggrecan was noticeable. In rabbits, full repair of full-thickness defects of joint cartilage was observed after transplantation of autologous MSCs dispersed in a type I collagen gel, which was then transplanted into a large and full-thickness defect in the weight-bearing surface of the medial femoral condyle (Wakitani *et al*., 1994). Twenty-four weeks after transplantation, the reparative tissue was stiffer and less compliant than the tissue derived from empty defects, but less stiff and more compliant than the normal cartilage. MSCs have been successfully used for intervertebral disc regeneration in a rat model, using local injection of fluorescently labelled MSCs (Crevensten *et al*., 2004). After an initial decrease at 7 and 14 days after injection, fluorescent MSCs inside the disc returned to the initial number of injected cells at 28 days, with 100% cell viability. Autologous bone-marrow-derived MSCs have been applied to patients with osteoarthritis (Wakitani *et al*., 2002).

For *in vitro* chondrocyte differentiation, the most commonly used method in this field established over many years has involved culturing MSCs in chondrogenic medium as cell aggregates, often referred to as pellet culture, which was originally developed using rabbit MSCs and later with human bone marrow-derived stem cells (Yoo *et al*., 1998). In 2007, this method was modified with a different format for the culture, employing a porous membrane support for the cells that initially creates a shallow multilayer of stem cells, which then differentiate and grow into a disc of cartilage-like tissue. This resulted in a more uniform differentiation of the MSCs and more efficient production of matrix by the cells (Murdoch *et al*., 2007). Attempts to use these cells in the clinical setting have been restricted to implantation in human knees in a carrier gel (Wakitani *et al*., 2002; Kuroda *et al*., 2007). Human mesenchymal stem cells were cultured *in vitro* in a poly(DL-lactic-co-glycolic acid)–collagen biodegradable polymer scaffold in serum-free DMEM containing TGF$\beta$3 (Chen *et al*., 2004). After 4 weeks, the matrices were positively stained by safranin O and toluidine blue, as well collagen type ll and proteoglycan were detected around the cells. Three-dimensional PLGA scaffolds seeded with cultured rabbit MSCs were also transplanted into large defects in rabbit knees and analysed histologically after the operation. A hyaline-like cartilage structure was shown at 12 weeks after the transplantation (Uematsu *et al*., 2005). MSCs and chondrocytes embedded in a polylactid acid matrix placed into a full-thickness cartilage defect in rabbits showed a hyaline cartilage-like histology (Yan and Yu, 2007). The histology scores in these groups were significantly higher than in groups where the defect was filled with fibroblasts or without cells. Studies demonstrated that mechanical stress strongly improves cartilage regeneration through the maintenance of hyaline cartilage, and that cyclic mechanical compression enhances the expression of



DOI: 10.1002/term

chondrogenic markers in mesenchymal progenitor cells differentiated *in vitro*, resulting in increased cartilaginous matrix formation (Guilak *et al*., 2004; Schumann *et al*., 2006; Mauck *et al*., 2007).

## 4.3. Adipose-derived stem cells

Isolated adipose stem cells (ASCs) acquire a fibroblast-like morphology, similar to that observed for MSCs, and have been shown to have potential for osteogenic, adipogenic, chondrogenic and other lineage differentiation (Huang *et al*., 2000; Zuk *et al*., 2002; Gimble, 2003; Gimble and Guilak, 2003a; Awad *et al*., 2004; Dragoo *et al*., 2004; Hicok *et al*., 2004; Rodriguez *et al*., 2005; Betre *et al*., 2006). Osteogenic differentiation can be assessed by the identification of osteoblast phenotype markers, such as ALP activity, extracellular matrix production by the presence of bone matrix proteins such as osteopontin, osteonectin, bone sialoprotein-2, osteocalcin and collagen type I, among others, and by the calcification and formation of bone nodules (Gimble and Guilak, 2003b).

Chondrogenic differentiation can be verified by the presence of chondrocyte phenotypic markers, such as aggrecan, collagen type lI, Sox-9, collagen type 6, collagen type 10 and collagen type 9. A study has shown that ASCs have the same ability as bone marrow MSCs to regenerate bone and repair bone defects *in vivo* (Cowan *et al*., 2004). Significant osteogenic formation and defect bridging was evident after 2–12 weeks, respectively, following a critical-sized mouse calvarial bone defect. Clonal populations within ASCs are multipotent and possess the potential for differentiation along adipose, chondrogenic and osteogenic lineages. The ability of mouse ASCs to regenerate bone in a critical-sized calvarial defect model was demonstrated. ASCs were seeded on to apatite-coated poly(DL-lactic-co-glycolic acid) (PLGA) scaffolds and implanted into 4 mm parietal bone defects of adult mice (Kwan *et al*., 2008). Radiographical analysis of calvarial healing revealed that mice treated with ASCs had greater bone regeneration than mice treated with osteoblasts at the 4 week time point.

The potential of these cells to be used for bone tissue engineering was shown by Hicok *et al*. (2004), who demonstrated the ability of ASCs to form bone *in vivo*. When seeded on hydroxyapatite/tricalcium phosphate subcutaneously implanted into SCID mice for 6 weeks, ASCs were shown to be capable of causing the formation of human osteoids in 80% of the implant. Hennig *et al*. (2007) demonstrated that adipose tissue-derived MSCs reveal an altered bone morphogenic protein (BMP) profile compared to MSCs from bone marrow and required exogenous application of BMP, in addition to TGF$\beta$, to compensate for the reduced endogenous expression of BMP2, −4 and −6. Application of BMP6 in combination with TGF$\beta$ completely eliminated the reduced chondrogenic differentiation potential of MSCs derived from adipose tissue. This demonstrated that MSCs isolated from different tissues do not represent identical cell populations, but vary in the expression profile of some growth factors relevant for chondrogenesis.

## 4.4. Embryonic stem cells

Embryonic stem cells (ESCs) are harvested from the inner cell mass of the blastocyst and are acclaimed for their unlimited capacity for self-renewal (Allison *et al*., 2002; Preston *et al*., 2003). They were primarily isolated during early 1980s from mouse embryos (Evans and Kaufman, 1981; Martin, 1981), then later from human embryos (Thomson *et al*., 1998; Reubinoff *et al*., 2000). Human ESCs are pluripotent, as they can give rise to essentially all cell types in the body (Buckwalter *et al*., 1997; Hentthorn, 2002). *In vitro* and *in vivo* experiments have demonstrated the ability of ESCs for osteogenic differentiation (Whang and Lieberman, 2003). In spite of this broad differentiation capability and potential to be used in regenerative medicine, the predisposition of these cells for teratoma formation and the political and ethical debate currently surrounding their use pose substantial challenges for forward progress on these cells (Lauffenburger and Schaffer, 1999; Montjovent *et al*., 2004).

## 4.5. Multipotent periosteum cells

The periosteum is a bilayered tissue membrane that is attached to bone cortex (Taylor, 1992). A number of studies (Nakahara *et al*., 1990; Nakata *et al*., 1992; Fang and Hall, 1996; O'Driscoll *et al*., 2001) have also shown that the periosteum cambium layer also possesses chondroprogenitor cells that can also promote new cartilage. Perka *et al*. (2000) reported that multipotent cells isolated from the periosteum were seeded on PLGA scaffolds and placed in critical-sized defects in the metadiaphyseal ulnas of New Zealand white rabbits. After 28 days, the constructs had bone formation and adequate transplant integration at the margins to the surrounding bone tissue. Vacanti *et al*. (2001) reported the replacements of an avulsed phalanx with lamellar bone, coral, blood vessels and soft tissue. Shantz *et al*. (2002) reported on the *in vivo* endochondral bone formation with osteoid production detectable through Von Kossa and osteocalcin staininq after 6 and 17 weeks. Schmelzeisen *et al*. (2003) showed that MPCs give rise to viable osteocytes in trabecular bone.

MPCs have also been used in cartilage tissue engineering. Stevens *et al*. (2004a) used a rapid-curing alginate gel system to support periosteum-derived chondrogenesis. After 6 weeks of culture, significant quantities (>50%) of the total area of the periosteal explants were composed of cartilage that was hyaline-like in appearance and contained cartilage-specific proteoglycans and type-ll collagen. In another study, the combined use of two growth factors, FGF-2 and TGF$\beta$1, with periosteal explants cultured *in vitro* within



DOI: 10.1002/term

alginate or agarose-based gels, significantly enhanced cell proliferation, resulting in increased neocartilage formation at later stages (Stevens *et al*., 2004b).

## 4.6. Skeletal muscle-derived stem cells

Two muscle stem cell populations with a possible mesenchymal character have been described: satellite cells (SCs) and muscle-derived stem cells (MDSCs).

Muscle satellite cells are found adjacent to skeletal muscle myofibres and lie underneath the basal lamina. When initiating division, satellite cells express either myf-5 or Myo-D (O'Brien *et al*., 2002). Furthermore, this cell population has also shown to express a number of other proteins, including desmin, c-met, M-cadherin, PaxT and Bcl-2. Satellite cells have for long been considered as precursor rather than stem cells. Studies reported that from SCs and upon stimulation with BMPs, it was possible for these cells to differentiate into osteogenic lineage expressing both alkaline phosphatase (ALP) and osteocalcin (Asakura *et al*., 2001; Wada *et al*., 2002).

Muscle-derived stem cells (MDSCs) are believed to be located either in the connective tissue regions of the skeletal muscle or in the capillaries surrounding the myofibres (Lee *et al*., 2000; Qu-Petersen *et al*., 2002; Peng and Huard, 2004). In culture, MDSCs express desmin and Myo D and stem cell markers such as CD34, sca-1 and Bcl-2 (Torrente *et al*., 2001; Cao and Huard, 2004). McKirmey-Freeman *et al*. (2002) and Adachi *et al*. (2002) showed that CD45-MDSCs have both chondrogenic and osteogenic potential. Other studies described positive results regarding the regeneration of critical-sized bone defects (Qu *et al*., 1998; Peng *et al*., 2001; Lee JY *et al*., 2002; Wright *et al*., 2002).

## 4.7. Prenatal stem cells

Several sources for adult stem cells with putative mesenchymal character have been described from prenatal tissues and fluid, and these include cord blood, amniotic fluid, umbilical vein and Wharton's jelly (Erices *et al*., 2000; Rosada *et al*., 2003; Gang *et al*., 2004; Kogler *et al*., 2004; Lee OK *et al*., 2004; Tondreau *et al*., 2005).

In cord blood, Erices *et al*. (2000) reported the presence of a population of mesenchymal progenitors that expressed several MSC-related antigens, such as SH2, SH3, SH4, ASMA, MAB 1470, CD13, CD29 and CD49e. These cells could be directed toward the osteogenic lineage, showing bone nodule formation and ALP activity. Lee *et al*. (2004) clonally expanded adult stem cells derived from umbilical cord blood with a mesenchymal character, which were able to differentiate into several lineages, including osteogenic and chondrogenic lineages. Rosada *et al*. (2003) subcutaneously implanted immunocompromised mouse cord blood MSCs that had previously been mixed with hydroxyapatite/tricalcium phosphate powder. After 8 weeks, It was possible to observe the presence of bone at the interface of the HA/TCP powder and the surrounding tissues. Kogler *et al*. (2004) implanted in mice, either subcutaneously or in femoral defects, a three-dimensional (3D) scaffold seeded with cord blood MSCs. After 3 weeks, a cartilage tissue-like formation was observed in the subcutaneous model and after 12 weeks bony reconstitution was observed.

Amniotic fluid is known to contain multiple cell types derived from the developing fetus (Priest *et al*., 1978; Polgar *et al*., 1989). De Coppi *et al*. (2007) showed that cKit-expressing cells within this heterogeneous population can give rise to differentiated cells of bone lineage. Human amniotic fluid stem cells (AFSCs) of the same clone can be induced to express markers characteristic of osteocytes, such as Runx2, osteocalcin and alkaline phosphatase. Calcium deposition was shown in human AFS cells maintained in osteogenic differentiation medium *in vitro* by measuring calcium–cresolophthalein complex levels. Von Kossa staining of AFS cells in alginate/collagen scaffold recovered 8 weeks after implantation indicated strong mineralization. The formation of tissue-engineered bone from printed constructs of osteogenically differentiated human AFS cells in immune-deficient mice was measured using micro-CT scan of mouse 18 weeks after implantation of printed constructs. At sites of implantation of the scaffolds containing AFS cells, blocks of bone-like material were observed with a density somewhat greater than that of mouse femoral bone. Control scaffolds lacking AFSCs did not promote the formation of bony tissue.

The umbilical cord vein was shown by Romanov *et al*. (2003) to possess mesenchymal progenitors that showed alkaline phosphatase activity. According to Kim *et al*. (2004), it was possible to obtain these MSC-like cells from only 6% of the cords. Fibroblastic cells were negative for endothelial markers such as Von Willenbranf factor (vWF) and PECAM-1. As in other cases, when exposed to osteogenic conditions these cells revealed typical signs of osteogenic differentiation through the deposition of a mineralized extracellular matrix and cells expression of Runx2 and osteopontin.

In Wharton's jelly, the primitive connective tissue of the umbilical cord, Sarugaser *et al*. (2004) described a cell population designated human umbilical cord perivascular cells (HUCPVCs). Upon culture, these cells displayed a fibroblast-like morphology, expressing at the same time $\alpha$-actin, desmin, vimentin, 3G5 and typical MSC markers such as SH2, SH3 and CDl44, and a colony-forming unit fibroblast frequency (CFU-F) of 1 : 333. These HUCPVCs had a subpopulation that exhibited osteogenic phenotype and elaborated bone nodules. Wang *et al*. (2004) showed that, upon stimulation of cells positive for SH2 and SH3 with osteogenic supplements, it was possible to observe a cell population with high indices of ALP activity as well as the expression of osteopontin. This cell population were also shown to possess chondrogenic potential, expressing collagen ll.



DOI: 10.1002/term

### 4.8. Genetically modified cells

The transfection of the cells can be done either in two steps with an *in vitro* transfection followed by injection of the modified cells (a procedure called '*ex vivo* gene therapy') or in one step by transfecting the cells directly in the body (a procedure called '*in vivo* gene therapy'). Cells genetically modified to express bone formation cytokines could be used to take advantage of genetic therapy (Lauffenburger and Schaffer, 1999). BMPs have promising potential for clinical bone and cartilage repair, working as bone-inducing components in diverse tissue-engineering products. Current clinical uses include spinal fusion, healing of long bone defects and craniofacial and periodontal applications, among others (Bessa *et al*., 2008). Combining gene therapy and tissue-engineering methodologies to enhance tissue regeneration, cells overexpressing BMP have been developed and used in animal studies (Gazit *et al*., 1999). Gene transfection of osteogenesis-related transcription factors such as Osterix (Tu *et al*., 2006) and Runx2 (Byers *et al*., 2004) has been shown to induce an osteogenic phenotype of BMSCs.

### 4.9. Human skin fibroblasts

The Yamanaka and Thompson groups demonstrated that the ectopic expression of a select group of genes can enable postnatal, human fibroblasts and other somatic cells to exhibit many of the hallmarks of human embryonic stem cells (Park *et al*., 2003; Hohlfeld *et al*., 2005, Takahashi *et al*., 2007). These findings are especially important, as they demonstrate the potential for reprogramming postnatal somatic cells to a pluripotent state (Wernig *et al*., 2007). These studies provide a promising direction for generating patient-, tissue- and disease-specific stem cells, presumably without immunological rejection concerns. Given the abundance and ease with which skin fibroblasts can be harvested autogenously, such an approach may provide patients with specific cell types needed for tissue/organ regeneration, including bone and cartilage.

## 5. Future directions

The presented stem cell populations have shown promising results, key characteristics and differentiation potential to be used for bone and cartilage tissue regeneration. The amazement starts when reviewers (Krampera *et al*., 2006b; Pioletti *et al*., 2006; Salgado *et al*., 2006; Mano and Reis, 2007) start to gaze at the number of these populations, how their diversity might have arisen and when one considers the evolutionary processes behind this difference. This inextricably drove researchers to closely examine processes responsible for cell differentiation into bone and cartilage, scrutinizing matrix sources nature has readily provided for millions of years (Cruz *et al*., 2008; Gomes *et al*., 2008; Oliveira *et al*., 2008). Most experiments with bone and cartilage tissue engineering have been carried out with small animals or small-sized defects. However, human defects are normally larger and more complicated, thus requiring larger repair tissues and structural and mechanical properties similar to human normal tissues. In the coming years, further clinical trials involving stem and progenitor cells have the potential to deepen our knowledge of stem cell biology and dramatically improve their application for tissue regeneration. It specifically remains necessary to investigate further the different signalling pathways involved in the proliferation and differentiation of stem cells and the further identification of related markers for these cells, so that functional stem cells can be obtained. It is our belief that the isolation and use of SCs with proper electrical, physical and chemical maturity for tissue engineering is an essential factor. If donor SCs are engrafted in order to support the strength and tensile capacity of compromised bone or skin, functional electrical cell coupling should first be satisfied. In contrast to host osteoblasts, stem cell-differentiated osteoblasts might be lacking a proper functional coupling, thereby creating a heterogeneous focus upon implantation. To optimally support a compromised tissue upon engraftment, donor cells should preferably be similar to, and integrate mechanically and electrically with, the host tissue (van Veen *et al*., 2006). If not, implantation of cells will not only chronically fail to increase the strength/tensile capacity of that tissue but, even more deleterious, disturb the repartition of forces applied to regenerated tissue. The *ex vivo* formation of complex 3D hybrid tissues (i.e. joint cartilage with subchondral bone and integrated vascular access for implantation) may also revolutionize the treatment of damaged skeletal tissue. Further developments in these areas of stem cell research will have a significant impact on functional bone and cartilage regeneration and open a novel avenue for regenerative medicine.

### Acknowledgement

The European Network of Excellence EXPERTISSUES (Project No. NMP3-CT-2004-500283), under which this work was carried out, is acknowledged.

## References

Adachi N, Sato K, Usas A, *et al*. 2002; Muscle-derived, cell-based *ex vivo* gene therapy for treatment of full-thickness articular cartilage defects. *J Rheumatol* **29**: 1920–1930.

Allison MR, Poulsom R, Forbes S, *et al*. 2002; An introduction to stem cells. *J Pathol* **197**: 419–423.

Arinzeh TL, Peter SJ, Archambault MP, *et al*. 2003; Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. *J Bone Joint Surg Am* **85A**: 1927–1935.

Asakura A, Komaki M, Rudnicki MA. 2001; Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic and adipogenic differentiation. *Differention* **68**: 245–253.

Awad HA, Butler DL, Boivin GP, *et al*. 1999; Autologous mesenchymal stem cell-mediated repair of tendon. *Tissue Eng* **5**: 267–277.

Awad HA, Wickham MQ, Leddy HA, *et al*. 2004; Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate and gelatin scaffolds. *Biomaterials* **25**: 3211–3222.
Baksh D, Song L, Tuan RS. 2004; Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med* **8**: 301–316.
Barry FP, Boynton RE, Liu B, *et al*. 2001; Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res* **268**: 189–200.
Barry FP, Murphy JM. 2004; Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol* **36**: 568–584.
Bellows CG, Heersche JN, Aubin JE. 1990; Determination of the capacity for proliferation and differentation of osteoprogenitor cell in the presence of dexamethasone. *Dev Biol* **140**: 132–138.
Bennett JH, Joyner CJ, Triffitt JT, *et al*. 1991; Adipocytic cells cultured from marrow have osteogenic potential. *J Cell Sci* **99**: 131–139.
Beresford JN, Bennett JH, Devlin C, *et al*. 1992; Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. *J Cell Sci* **102**: 341–351.
Bessa PC, Casal M, Reis RL. 2008; Bone morphogenetic proteins in tissue engineering: The road from the laboratory to clinic. *J Tissue Engineering Regen Med* **2**: 81–96.
Betre H, Ong SR, Guilak F, *et al*. 2006; Chondrocytic differentiation of human adipose-derived adult stem cells in elastin-like polypeptide. *Biomaterials* **27**: 91–99.
Beyer NN, da Silva ML. 2006; Mesenchymal stem cells: isolation, *in vitro* expansion and characterization. *Handb Exp Pharmacol* **174**: 249–282.
Bhabavati S, Xu W. 2004; Isolation and enrichment of skeletal muscle progenitor cells from mouse bone marrow. *Biochem Biophys Res Commun* **21**: 119–124.
Bianco P, Robey PG. 2001; Stem cells in tissue engineering. *Nature* **414**: 118–121.
Blau HM, Brazelton TR, Weimann JM. 2001; The evolving concept of a stem cell: entity or function? *Cell* **105**: 829–841.
Bresolin N. 2001; Intra-arterial injection of muscle-derived $CD34^+$ $Sca\text{-}l^+$ stem cells restores dystrophin in mdx mice. *J Cell Biol* **152**: 335.
Bruder SP, Fox BS. 1999; Tissue engineering of bone; cell-based strategies. *Clin Orthop*, **S**: S68–83.
Bruder SP, Kraus KH, Goldberg VM, *et al*. 1998; The effects of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg* **80A**: 985–995.
Buckwalter JA, Mankin HJ. 1998; Articular cartilage: degeneration and osteoarthrosis, repair, regeneration, and transplantation. *Instr Course Lect* **47**: 487–504.
Byers BA, Guldberg RE, Garcia AJ. 2004; Synergy between genetic and tissue engineering: Runx2 overexpression and *in vitro* construct development enhance *in vivo* mineralization. *Tissue Eng* **10**: 1757–1766.
Cao B, Huard J. 2004; Muscle-derived stem cells. *Cell Cycle* **3**: 104–107.
Cancedda R, Dozin B, Giannoni P, *et al*. 2003; Tissue engineering and cell therapy of cartilage and bone. *Matrix Biol* **22**: 81–91.
Caplan AI. 1991; Mesenchymal stem cells. *J Orthop Res* **9**: 641–650.
Caplan AI, Bruder SP. 2001; Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* **7**: 259–264.
Caplan AI, Elyaderani M, Mochizuki Y, *et al*. 1997; Principles of cartilage repair and regeneration. *Clin Orthop* **342**: 254–269.
Castro-Malaspina H, Gay RE, Resnick G, *et al*. 1980; Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood* **56**: 289–301.
Chen G, Liu D, Tadokoro M, *et al*. 2004; Chondrogenic differentiation of human mesenchymal stem cells cultured in a cobweb-like biodegradable scaffold. *Biochem Biophys Res Commun* **322**: 50.
Chung CH, Golub EE, Forbes E, *et al*. 1992; Mechanisms of action of $\beta$-glycerophosphate on bone cell mineralization. *Cell Tissue Int* **51**: 212–219.
Clarkson ED. 2001; Fetal tissue transplantation for patients with Parkinson's disease: a database of published clinical results. *Drugs Aging* **18**: 773–785.
Connolly J, Guse R, Lippiello L, *et al*. 1989; Development of an osteogenic bone-marrow preparation. *J Bone Joint Surg Am* **71**: 684–691.
Cowan CM, Shi YY, Aalami OO, *et al*. 2004; Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat Biotechnol* **22**: 560–567.
Crevensten G, Walsh AJ, Ananthakrishnan D, *et al*. 2004; Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. *Ann Biomed Eng* **32**: 430–434.
Cruz DM, Ivirico JL, Gomes MM, *et al*. 2008; Chitosan microparticles as injectable scaffolds for tissue engineering. *J Tissue Eng Regen Med* **2**: 378–380.
Deasy BM, Jankowski RJ, Huard J. 2001; Muscle-derived stem cells: characterization and potential for cell-mediated therapy. *Blood Cells Mol Dis* **27**: 924–933.
De Bari C, Dell'Accio F, Tylzanowski P, *et al*. 2001; Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* **44**: 1928–1942.
De Coppi P, Bartsch G Jr, Minhaj Siddiqui M, *et al*. 2007; Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* **25**: 100–106.
Donald PL, Haynesworth SE, Buder SP, *et al*. 1996; Human and animal mesenchymal progenitor cells from bone marrow: identification of serum for optimal selection and proliferation. *In Vitro Cell Dev Biol Anim* **32**: 602.
Dragoo JL, Choi JY, Lieberman JR, *et al*. 2003; Bone induction by BMP2-transduced stem cells derived from human fat. *J Orthop Res* **21**: 622–629.
Endres M, Hutmacher DW, Salgado AJ, *et al*. 2003; Osteogenic induction of human bone marrow-derived mesenchymal progenitor cells in novel synthetic polymer–hydrogel matrices. *Tissue Eng* **9**: 689–702.
Erices A, Conget P, Minguel JJ. 2000; Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* **109**: 235–242.
Evans MJ, Kaufman MH. 1981; Establishment in culture of pluripotent cells from mouse embryos. *Nature* **292**: 154–156.
Fang J, Hall BK. 1996; *In vitro* differentiation potential of the periosteal cells from a membrane bone. the quadratojugal of the embryonic chick. *Dev Biol* **180**: 701–712.
Fibbe WE. 2002; Mesenchymal stem cells. A potential source for skeletal repair. *Ann Rheum Dis* **61**: 29–31.
Freeman TB. 1997; From transplants to gene therapy for Parkinson's disease. *Exp Neurol* **144**: 47–50.
Friedenstein AJ, Chailakhjan RK, Lalykina KS. 1970; The development of fibroblast colonies in monolayer cultures of guinea pig bone marrow and spleen cells. *Cell Tissue Kinet* **3**: 393–403.
Friedenstein AJ, Petrakova KV, Kurolesova AI, *et al*. 1968; Heterotopic transplants of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* **6**: 230–247.
Fritsch A, Hellmich C. 2007; Universal microstructural patterns in cortical and trabecular, extracellular and extravascular bone materials: micromechanics-based prediction of anisotropic elasticity. *J Theoret Biol* **244**: 597–620.
Fukuchi Y, Nakajima H, Sugiyama D, *et al*. 2004; Human placenta-derived cells have mesenchymal stem/progenitor cell potential. *Stem Cells* **22**: 649–658.
Gang EJ, Hong SH, Jeong JA, *et al*. 2004; *In vitro* mesengenic potential of human umbilical cord blood-derived mesenchymal stem cells. *Biochem Biophy Res Commun* **321**: 102–108.
Gazit D, Turgeman G, Kelley P, *et al*. 1999; Engineered pluripotent mesenchymal cells integrate and differentiate in regenerating bone: a novel cell-mediated gene therapy. *J Gene Med* **1**: 121–133.
Gregory CA, Gunn WG, Reyes E, *et al*. 2005; How Wnt signaling affects bone repair by mesenchymal stem cells from the bone marrow. *Ann NY Acad Sci* **1049**: 97–106.
Gimble JM. 2003; Adipose tissue-derived therapeutics. *Expert Opin Biol Ther* **3**: 1–9.
Gimble JM, Guilak F. 2003a; Differentiation potential of adipose-derived adult stem (ADAS) cells. In *Current Topics in Developmental Biology*. Academic Press: New York.
Gimble J, Guilak F. 2003b; Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy* **5**: 362–369.
Gomes ME, Azevedo HS, Moreira AR, *et al*. 2008; Starch-poly($\varepsilon$-caprolactone) and starch-poly(lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: structure, mechanical

properties and degradation behaviour. *J Tissue Eng Regen Med* **2**: 243–252.
Guilak F, Fermor B, Keefe FJ, *et al*. 2004; The role of biomechanics and inflammation in cartilage injury and repair. *Clin Orthop Relat Res* **423**: 17–26.
Hanada K, Dennis JE, Caplan AI. 1997; Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein 2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. *J Bone Min Res* **12**: 1606–1614.
Haynesworth SE, Goshima J, Goldberg VM, *et al*. 1992; Characterization of cells with osteogenic potential from human marrow. *Bone* **13**: 81–88.
Hennig T, Lorenz H, Thiel A, *et al*. 2007; Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGF$\beta$ receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol* **211**: 682–691.
Hentthorn PS. 2002; Alkaline phosphatase. In *Principles of Bone Biology*. Academic Press: New York.
Hicok KC, Du Laney TV, Zhou YS, *et al*. 2004; Human adipose-derived adult stem cells produce osteoid *in vivo*. *Tissue Eng* **10**: 371–380.
Hohlfeld J, de Buys Roessingh A, Hirt-Burri N, *et al*. 2005; Tissue engineered fetal skin constructs for paediatric burns. *Lancet* **366**: 840–842.
Holy CE, Fialkov JA, Davies JE, *et al*. 2003; Use of a biomimetic strategy to engineer bone. *J Biomed Mat Res A* **65**: 447–453.
Horwitz EM, Prockop DJ, Fitzpatrick LA, *et al*. 1999; Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Medicine* **5**: 262–264.
Huang JI, Hedrick MH, Lorenz HP, *et al*. 2000; Chondrogenesis of human adipo-derived mesodermal stem cells. *J Am Coll Surg* **191**: S47.
Huang W, Yang S, Shao J, *et al*. 2007; Signaling and transcriptional regulation in osteoblast commitment and differentiation. *Front Biosci* **12**: 3068–3092.
Indrawattana N, Chen G, Tadokoro M, *et al*. 2004; Growth factor combination for chondrogenic induction from human mesenchymal stem cells. *Biochem Biophys Res Comun* **320**: 914–919.
Inoue K, Ohgushi H, Yoshikawa T, *et al*. 1997; The effect of aging on bone formation in porous hydroxyapatite: biochemical and histological analysis. *J Bone Miner Res* **12**: 989–994.
In't Anker PS, Noort WA, Scherjon SA, *et al*. 2003; Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica* **88**: 845–852.
In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, *et al*. 2004; Isolation of mesenchymal stem cells of fetal and maternal origin from human placenta. *Stem Cells* **22**: 1338–1345.
Jaiswal N, Haynessworth SE, Caplan AI, *et al*. 1997; Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. *J Cell Biochem* **64**: 295–312.
Javazon EH, Beggs KJ, Flake AW. 2004; Mesenchymal stem cells: paradoxes of passaging. *Exp Hematol* **32**: 414–425.
Jiang Y, Jahagirdar BN, Reinhardt RL, *et al*. 2002; Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**: 41–49.
Johnstone B, Hering TM, Caplan AI, *et al*. 1998; *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* **238**: 265–272.
Jorgensen NR, Henriksen Z, Sorensen OH, *et al*. 2004; Dexamethasone, BMP-2, and 1,25-dihydroxyvitamin D enhance a more differentiated osteoblast phenotype: validation of an *in vitro* model for human bone marrow-derived primary osteoblasts. *Steroids* **69**: 219–226.
Jorgensen C, Noel D, Apparailly F, *et al*. 2001; Stem cells for repair of cartilage and bone: the next challenge in osteoarthritis and rheumatoid arthritis. *Ann Rheum Dis* **60**: 305–309.
Kadiyala S, Young RG, Thiede MA, *et al*. 1997; Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential *in vivo* and *in vitro*. *Cell Transpl* **6**: 125–134.
Kadner A, Hoerstrup SP, Tracy J, *et al*. 2002; Human umbilical cord cells: a new cell source for cardiovascular tissue engineering. *Ann Thorac Surg* **74**: S1422–1428.
Kahn A, Gibbons R, Perkins S, *et al*. 1995; Age-related bone loss: a hypothesis and initial assessment in mice. *Clin Orthop Relat Res* **313**: 69–75.
Kanczler JM, Oreffo ROC. 2008; Osteogenesis and angiogenesis: the potential for engineering bone. *Eur Cells Mater* **15**: 100–114.
Kaviani A, Guleserian K, Perry TE, *et al*. 2003; Fetal tissue engineering from amniotic fluid. *J Am Coll Surg* **196**: 592–597.
Kaviani A, Perry TE, Barnes CM, *et al*. 2002; The placenta as a cell source in fetal tissue engineering. *J Pediatr Surg* **37**: 995–999.
Kim JW, Kim SY, Park SY, *et al*. 2004; Mesenchymal progenitor cells in the human umbilical cord. *Ann Hematol* **83**: 733–738.
Kogler C, Sensken S, Airey JA, *et al*. 2004; A new human somatic stem cells from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* **200**: 123–135.
Kon E, Muraglia A, Corsi A, *et al*. 2000; Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. *J Biomed Mater Res* **49**: 328–337.
Krampera M, Cosmi L, Angeli R, *et al*. 2006a; Role of the interferon-$\gamma$ in the immunomodulatory activity of human mesenchymal stem cells. *Stem Cells* **24**: 386–398.
Krampera M, Cosmi L, Angeli R, *et al*. 2002; Stem cells for regenerative medicine advances in the engineering of tissues and organs. *Naturwissenschaften* **89**: 338–351.
Krampera M, Pasini A, Rigo A, *et al*. 2005; HB-EGF/HER-1 signalling in bone marrow mesenchymal stem cells: inducing cell expansion and reversibly preventing multi-lineage differentiation. *Blood* **106**: 59–66.
Krampera M, Pizzolo G, Aprili G, *et al*. 2006b; Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair. *Bone* **39**: 678–683.
Krebsbach PH, Kuznetsov SA, Satomura K, *et al*. 1997; Bone formation *in vivo*: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation* **63**: 1059–1069.
Kuroda R, Ishida K, Matsumoto T, *et al*. 2007; Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthr Cartil* **15**: 226–231.
Kuznetsov SA, Mankani MH, Gronthos S, *et al*. 2001; Circulating skeletal stem cells. *J Cell Biol* **153**: 1133–1140.
Kwan MD, Slater BJ, Wan DC, *et al*. 2008; Cell-based therapies for skeletal regenerative medicine. *Hum Mol Genet* **17**: 93–98.
Lauffenburger DA, Schaffer DV. 1999; The matrix delivers. *Nat Med* **5**: 733–734.
Lee JY, Peng H, Usas A. 2002; Enhancement of bone healing based *ex vivo* therapy using human muscle-derived cells expressing bone morphogenetic protein 2. *Human Gene Ther* **13**: 1201–1211.
Lee JY, Qu-Peterson Z, Cao B, *et al*. 2000; Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. *J Cell Biol* **150**: 1085–1099.
Lee OK, Kuo TK, Chen WM, *et al*. 2004; Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* **103**: 1669–1675.
Lee RH, Kim B, Choi I, *et al*. 2004; Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem* **14**: 311–324.
Liebschner MA. 2004; Biomechanical considerations of animal models used in tissue engineering of bone. *Biomaterials* **25**: 1697–1714.
Liu P, Oyajobi BO, Russel RG, *et al*. 1999; Regulation of osteogenic differentiation of human bone marrow stromal cells: interaction between transforming growth factor $\beta$ and 1,25$(OH)_2$ vitamin $D_3$ *in vitro*. *Calcif Tissue Int* **65**: 173–180.
Long MW. 2001; Osteogenesis and bone marrow-derived cells. *Blood Cells Mol Dis* **27**: 677–690.
Mackay AM, Beck SC, Murphy JM, *et al*. 1998; Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* **4**: 415–428.
Mano JF, Reis RL. 2007; Osteochondral defects: present situation and tissue engineering approaches. *J Tissue Eng Regen Med* **1**: 261–273.
Marks SC, Hermey DC. 1996; The structure and development of bone. In *Principles of Bone Biology*. Academic Press: San Diego, CA.
Martin GR. 1981; Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* **78**: 7634–7638.
Martin I, Padera RF, Vunjak-Novakovic G, *et al*. 1998; *In vitro* differentiation of chick embryo bone marrow stromal cells into cartilaginous and bone-like tissues. *J Orthop Res* **16**: 181–189.

Mauck RL, Byers BA, Yuan X, *et al*. 2007; Regulation of cartilaginous ECM gene transcription by chondrocytes and MSCs in 3D culture in response to dynamic loading. *Biomech Model Mechanobiol* **6**: 113–125.
Mauney JR, Volloch V, Kaplan DL. 2005; Role of adult mesenchymal stem cells in bone tissue engineering applications: current status and future prospects. *Tissue Eng* **11**: 787–802.
Mitka M. 2001; Amniotic cells show promise for fetal tissue engineering. *J Am Med Assoc* **286**: 2083.
Montjovent MO, Burri N, Mark S, *et al*. 2004; Fetal bone cells for tissue engineering. *Bone* **35**: 1323–1333.
Murdoch AD, Grady LM, Ablett MP, *et al*. 2007; Chondrogenic differentiation of human bone marrow stem cells in transwell cultures: generation of scaffold-free cartilage. *Stem Cells* **25**: 2786–2796.
Murphy JM, Kavalkovitch KW, Fink D, *et al*. 2000; Regeneration of meniscal tissue and protection of articular cartilage by injection of mesenchymal stem cells. *Osteoarthr Cartilage* **8B**: S25.
Murphy WI, Hsiong S, Richardson TP, *et al*. 2005; Effects of bone-like mineral film on phenotype of adult mesenchymal stem cells *in vitro*. *Biomaterials* **26**: 303–310.
Nakahara H, Bruder SP, Haynesworth SE, *et al*. 1990; Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. *Bone* **11**: 181–188.
Nakata K, Nakahara H, Kimura T, *et al*. 1992; Collagen gene expression during chondrogenesis from chick periosteum-derived cells. *FEBS Lett* **299**: 278.
Nilsson SK, Dooner MS, Weier HU, *et al*. 1999; Cells capable of bone production engraft from whole bone marrow transplant in nonablated mice. *J Exp Med* **189**: 729–734.
Noth U, Osyczka AM, Tuli R, *et al*. 2002; Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. *J Orthop Res* **20**: 1060–1069.
O'Brien K, Muskiewicz K, Gussoni E. 2002; Recent advances in and therapeutic potential of muscle-derived stem cells. *J Cell Biochem* **S38**: 80–87.
O'Driscoll SW, Saris DB, Ito Y, *et al*. 2001; The chondrogenic potential of periosteum decrease with age. *J Orthop Res* **19**: 95–103.
Oliveira JT, Correlo VM, Sol PC, *et al*. 2008; Assessment of the suitability of chitosan/polybutylene succinate scaffolds seeded with mouse mesenchymal progenitor cells for a cartilage tissue engineering approach. *Tissue Eng A* **14**: 1651–1661.
Park J, Ries J, Gelse K, *et al*. 2003; Bone regeneration in critical size defects by cell-mediated *bmp-2* gene transfer: a comparison of adenoviral vectors and liposomes. *Gene Ther* **10**: 1089–1098.
Peng H, Chen ST, Wergedal JE, *et al*. 2001; Development of a MGF-based retroviral vector system for secretion of high levels of functional active human BMP-4. *Mol Ther* **4**: 95–104.
Peng H, Huard J. 2004; Muscle-derived stem cells for musculoskelelal tissue regeneration and repair. *Transpl Immunol* **12**: 311–319.
Pereira RF, Halford KW, O'Hara MD, *et al*. 1995; Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Nat Acad Sci USA* **92**: 4857–4861.
Perka C, Schultz O, Spitzer RS, *et al*. 2000; Segmental bone repair by tissue-engineered periosteal cell transplants with biosorbable fleece and fibrin scaffolds in rabbits. *Biomaterials* **11**: 1145–1153.
Petersen BE, Bowen WC, Patrene KD, *et al*. 1999; Bone marrow as a potential source of hepatic oval cells. *Science* **284**: 1168–1170.
Petite H, Viateau V, Bensaid W, *et al*. 2000; Tissue-engineered bone regeneration. *Nat Biotechnol* **18**: 959–963.
Pioletti DP, Montjovent MO, Zambelli PY, *et al*. 2006; Bone tissue engineering using foetal cell therapy. *Swiss Med Wkly* **136**: 557–560.
Pittenger MF, Mackay AM, Beck SC, *et al*. 1999; Multilineage potential of adult mesenchymal stem cells. *Science* **284**: 143–147.
Polgar K, Adany R, Abel G, *et al*. 1989; Characterization of rapidly adhering amniotic fluid cells by combined immunofluorescence and phagocytosis assays. *Am J Hum Genet* **45**: 786–792.
Preston SL, Alison MR, Forbes SJ, *et al*. 2003; The new stem cell biology: something for everyone. *Mol Pathol* **56**: 86–96.
Priest RE, Marimuthu KM, Priest JH. 1978; Origin of cells in human amniotic fluid cultures: ultrastructural features. *Lab Invest* **39**: 106–109.
Prockop DJ. 1997; Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* **276**: 71–74.
Qu Z, Balkir L, van Deutekom JC, *et al*. 1998; Development of approaches to improve cell survival in myoblast transfer therapy. *J Cell Biol* **142**: 1257–1267.
Qu-Petersen Z, Deasy B, Jankowsky R. 2002; Identification of a novel cell population of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol* **157**: 851–864.
Quarto R, Mastrogiacomo M, Cancedda R, *et al*. 2001; Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* **344**: 385–386.
Reubinoff BE, Pera MF, Fong CY, *et al*. 2000; Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat Biotechnol* **18**: 399–404.
Richards M, Huibregtse BA, Caplan AI, *et al*. 1999; Marrow-derived progenitor cell injections enhance new bone formation during distraction. *J Orthop Res*, **17**: 900–908.
Rickard DJ, Sullivan TA, Shenker BJ, *et al*. 1994; Induction of rapid osteoblast differentiation in rat bone marrow stromal cell cultures by dexamethasone and BMP-2. *Dev Biol* **161**: 218–228.
Rodriguez AM, Elabd C, Amri E-Z, *et al*. 2005; The human adipose tissue is a source of multipotent stem cells. *Biochimie* **87**: 125–128.
Romanov YA, Svintsitskaya A, Smirnov VN. 2003; Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* **21**: 105–110.
Rosada C, Justesen J, Melsvik D, *et al*. 2003; The human umbilical cord blood: a potential source of osteoblast progenitor cells. *Calcif Tissue Int* **72**: 135–142.
Rosser AE, Dunnett SB. 2003; Neural transplantation in patients with huntington's disease. *CNS Drugs* **17**: 853–867.
Roufosse CA, Direkze NC, Otto WR, *et al*. 2004; Circulating mesenchymal stem cells. *Int J Biochem Cell Biol* **36**: 585–597.
Salasznyk RM, Klee RF, Hughlock MK, *et al*. 2004a; ERK signalling pathways regulate the osteogenic differentiation of human mesenchymal stem cells on collagen I and vitronectin. *Cell Commun Adhes* **11**: 137–153.
Salasznyk RM, Williams WA, Boskey A, *et al*. 2004b; Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. *J Biomed Biotechnol* **1**: 24–34.
Salgado AJ, Figueiredo JE, Coutinho OP, *et al*. 2005; Biological response to pre-mineralized starch based scaffolds for bone tissue engineering. *J Mater Sci Mater Med* **16**: 267–275.
Salgado AJ, Oliveira JT, Pedro AJ, *et al*. 2006; Adult stem cells in bone and cartilage tissue engineering. *Curr Stem Cell Res Ther* **1**: 345–364.
Savitz SI, Dinsmore JH, Wechsler LR, *et al*. 2004; Cell therapy for stroke. *Neuro Res* **1**: 406–414.
Sarugaser R, Lickorish D, Baksh D, *et al*. 2004; Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. *Stem Cells* **23**: 220–229.
Schantz JT, Hutmacher DW, Chim H, *et al*. 2002; Induction of ectopic bone formation using human periosteal cells in combination with a novel scaffold technology. *Cell Transpl* **11**: 125–138.
Schmelzeisen R, Schimming R, Sittinger M. 2003; Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation – a preliminary report. *J Craniomaxillfac Surg* **31**: 34–39.
Schultz O, Sittinger M, Haeupl T, *et al*. 2000; Emerging strategies of bone and joint repair. *Arthritis Res* **2**: 433–436.
Schumann D, Kujat R, Nerlich M, *et al*. 2006; Mechanobiological conditioning of stem cells for cartilage tissue engineering. *Biomed Mater Eng* **16**: S37–52.
Sethe S, Scutt A, Stolzing A. 2006; Aging of mesenchymal stem cells. *Ageing Res Rev* **5**: 91–116.
Shih DT, Lee DC, Chen SC, *et al*. 2005; Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. *Stem Cells* **23**: 1012–1020.
Simonsen JL, Rosada C, Serakinci N, *et al*. 2002; Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat Biotechnol* **20**: 592–596.
Smith JR, Pochampally R, Perry A, *et al*. 2004; Isolation of a highly clonogenic and multipotential subfraction of adult stem cells from bone marrow stroma. *Stem Cells* **22**: 823–831.
Stevens MM, Qanadilo HF, Langer R, *et al*. 2004a; A rapid curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. *Biomaterials* **25**: 887–894.

Stevens MM, Marini RP, Martin I, *et al*. 2004b; FGF-2 enhances TGF-$\beta$1-induced periosteal chondrogenesis. *J Orthopaed Res* **22**: 1114–1119.
Takahashi K, Okita K, Nakagawa M, *et al*. 2007; Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* **2**: 3081–3089.
Taylor IF. 1992; The periosteum and bone growth. *Bone* **6**: 21.
Tenenbaum HC, Limeback H, McCulloch CA, *et al*. 1992; Osteogenic phase-specific co-regulation of collagen synthesis and mineralization by $\beta$-glycerophosphate in chick periosteal cultures. *Bone* **13**: 129–138.
Thomson JA, Itskovitz J, Shapiro SS, *et al*. 1998; Embryonic stem cell lines derived from human blastocysts. *Science* **282**: 1145–1147.
Tondreau T, Meuleman N, Delforge A, *et al*. 2005; Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, oct4 expression and plasticity. *Stem Cells* **23**: 1105–1112.
Torrente Y, Tremblay JP, Pisati F, *et al*. 2002; Muscle derived hematopoietic stem cells are hematopoietic in origin. *Proc Natl Acad Sci USA* **99**: 1341–1346.
Trubiani O, Di Primio R, Traini T, *et al*. 2005; Morphological and cytofluorimetric analysis of adult mesenchymal stemcells expanded *ex vivo* from periodontal ligament. *Int J Immunopathol Pharmacol* **18**: 213–221.
Tu Q, Valverde P, Chen J. 2006; Osterix enhances proliferation and osteogenic potential of bone marrow stromal cells. *Biochem Biophys Res Commun* **341**: 1257–1265.
Uematsu K, Hattori K, Ishimoto Y, *et al*. 2005; Cartilage regeneration using mesenchymal stem cells and a three-dímensional polylactic-glycolic acid (PLGA) scaffold. *Biomaterials* **26**: 4273–4279.
Vacanti CA, Bonassar LJ, Vacanti MP, *et al*. 2001; Replacement of an avulsed phalanx with tissue-engineered bone. *N Engl J Med* **30**: 1511–1514.
Vacanti CA, Kim W, Upton J, *et al*. 1993; Tissue-engineered growth of bone and cartilage. *Transpl Proc* **25**: 1019–1021.
Vacanti CA, Upton J. 1994; Tissue-engineered morphogenesis of cartilage and bone by means of cell transplantation using synthetic biodegradable polymer matrices. *Clin Plast Surg* **21**: 445–462.
van Veen TA, de Bakker JM, van der heyden MA. 2006; Mesenchymal stem cells repair conduction block. *J Am Coll Cardiol* **48**: 219–220.
Wada RW, Inagawa-Ogasgiwa M, Shimizu S, *et al*. 2002; Generation of different fates from multipotent muscle stem cells. *Development* **129**: 2987–2995.
Wakitani S, Goto T, Pineda SJ, *et al*. 1994; Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* **76**: 579–592.
Wakitani S, Imoto K, Yamamoto T, *et al*. 2002; Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthr Cartilage* **10**: 199–206.
Wakitani S, Yamamoto T. 2002; Response of the donor and recipient cells in mesenchymal cell transplantation to cartilage defect. *Microsc Res Technol* **58**: 14–18.
Wang HS, Hung SC, Peng ST, *et al*. 2004; Mesenchymsal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* **22**: 130–137.
Wang X, Mabrey JD, Agrawal CM. 1998; An interspecies comparison of bone fracture properties. *Biomed Mater Eng* **8**: 1–9.
Weissman IL. 2000; Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science* **287**: 1442–1446.
Wernig M, Meissner A, Foreman R, *et al*. 2007; *In vitro* reprogramming of fibroblasts into a pluripotent ES cell-like state. *Nature* **448**: 318–324.
Whang PG, Lieberman JR. 2003; Clinical issues in the development of cellular systems for use as bone graft substitutes. In *Bone Graft Substitutes*. ASTM International: USA.
Woodbury D, Schwarz EJ, Prockop DJ, *et al*. 2000; Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* **61**: 364–370.
Wright V, Peng H, Usas A, *et al*. 2002; BMP-4 expressing muscle-derived stem cells differentiation to osteogenic lineage and improve bone healing in immunocompetent mice. *Mol Ther* **6**: 169–178.
Yan H, Yu C. 2007; Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy* **23**: 178–187.
Yang XB, Bhatnagar RS, Li S, *et al*. 2004; Biomimetic collagen scaffolds for human bone cell growth and differentiation. *Tissue Eng* **10**: 1148–1159.
Yeh LC, Tsai AD, Lee JC. 2002; Osteogenic protein-1 (OP-1, BMP-7) induces osteoblastic cell differentiation of the pluripotent mesenchymal cell line C2C12. *J Cell Biochem* **87**: 292–304.
Yoo JU, Barthel TS, Nishimura K, *et al*. 1998; The chondrogenic potential of human bone marrow-derived mesenchymal progenitor cells. *Bone Joint Surg Am* **80**: 1745–1757.
Young RG, Butler DL, Weber W, *et al*. 1998; Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res* **16**: 406–413.
Zuk PA, Zhu M, Ashjian P, *et al*. 2002; Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* **13**: 4279–4295.
Zuk PA, Zhu M, Mizuno H, *et al*. 2001; Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* **7**: 211–228.