**Request # 21291624**

**NOV 17, 2006**

**Email (PDF) To: ill@nshs.edu**

North Shore University Hospital
Daniel Carroll Payson Medical Library ILL
300 Community Drive
Manhasset, NY 11030

**DOCLINE: Journal Copy EFTS Participant**

| | |
|---|---|
| Title: | Cells, tissues, organs |
| Title Abbrev: | Cells Tissues Organs |
| Citation: | 2006;183(3):112-122 |
| Article: | Mesenchymal Stem Cells in Tissue Engineering. |
| Author: | Leo AJ;Grande DA |
| NLM Unique ID: | 100883360 Verify: PubMed |
| PubMed UI: | 17108682 |
| ISSN: | 1422-6405 (Print) 1422-6421 (Electronic) |
| Publisher: | Karger,, Basel ; |
| Copyright: | Copyright Compliance Guidelines |
| Authorization: | er |
| Need By: | N/A |
| Maximum Cost: | **$11.00** |
| Patron Name: | Pantazopoulos, Christina RES |
| Referral Reason: | Not owned (title) |
| Library Groups: | LILRC,NSLIJS |
| Phone: | 1.516.562-4324 |
| Fax: | 1.516.562-2865 |
| Email: | ill@nshs.edu |
| Alternate Delivery: | Email(PDF),Email(TIFF),Fax,Mail,Web(PDF),Web(TIFF) |
| Comments: | *****WE PREFER TO RECEIVE ILLS AS PDF FILES VIA E-MAIL***** |
| Routing Reason: | Routed to NYUNUM in Serial Routing - cell 2 |
| Received: | Nov 17, 2006 ( 08:10 AM EST ) |
| Lender: | New York University School of Medicine/ New York/ NY USA (NYUNUM) |



**Bill to: NYUNSU**

North Shore University Hospital
Daniel Carroll Payson Medical Library ILL
300 Community Drive
Manhasset, NY 11030

Cells Tissues Organs

Cells Tissues Organs 2006;183:112–122
DOI: 10.1159/000095985

Accepted after revision: July 25, 2006

# Mesenchymal Stem Cells in Tissue Engineering

Andrew J. Leo Daniel A. Grande

Department of Orthopedic Surgery, Feinstein Institute for Medical Research, North Shore – Long Island Jewish Health System, Manhasset, N.Y., USA

**Key Words**
Mesenchymal stem cells · Engineering, tissue · Repair, tissue

**Abstract**
The repair of diseased or damaged cartilage remains one of the most challenging problems of musculoskeletal medicine. Tissue engineering advances in cartilage repair have utilized autologous and allogenic chondrocyte and cartilage grafts, biomaterial scaffolds, growth factors, stem cells, and genetic engineering. The mesenchymal stem cell has specifically attracted much attention because of its accessibility, potential for differentiation, and manipulability to modern molecular, tissue and genetic engineering techniques. Mesenchymal stem cells provide invaluable tools for the study of tissue repair when combined with a carrier vehicle/matrix scaffold, and/or bioactive growth factors. However, an underappreciated source of knowledge lies in the relationship between fetal development and adult tissue repair. The multitude of events that take place during fetal development which lead from stem cell to functional tissue are poorly understood. A more thorough understanding of the events of development as they pertain to cartilage organogenesis may help elucidate some of the unknowns of adult tissue repair.



**Abbreviations used in this paper**

| | |
|---|---|
| BMP | bone morphogenic protein |
| FGF | fibroblast growth factor |
| IGF | insulin-like growth factor |
| MSC | mesenchymal stem cell |
| PDGF | platelet-derived growth factor |
| TGF | transforming growth factor |

## Introduction

With the increasing proportion of elderly individuals in the US, degenerative joint diseases such as osteoarthritis are rapidly becoming a significant healthcare burden, both to the patients that must live with these conditions and to the society that has little to definitively offer them short of major surgery [Centers for Disease Control and Prevention, 2003]. In the US alone 43 million people suffer from such arthritic conditions [Centers for Disease Control and Prevention, 1999] and osteoarthritis is currently the most common musculoskeletal disorder of industrial nations [Jackson et al., 2001]. Traumatic injuries to cartilage pose a similar problem both to patients and the healthcare community. Despite the immense natural healing and regenerative capabilities the human body possesses, the repair of diseased or damaged cartilage remains one of the most daunting problems of musculoskeletal medicine. Adult articular cartilage retains a poor capacity for growth and regeneration, and in the limited circumstances of partial repair, it is replaced with suboptimal fibrocartilage. Recent tissue engineering advances in the augmentation of cartilage repair have included the use of autologous and allogenic chondrocyte and cartilage grafts [Lane et al., 1977; Grande et al., 1989; Wakitani

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com


1422–6405/06/1833–0112$23.50/0

Accessible online at:
www.karger.com/cto

Dr. Daniel A. Grande
Department of Orthopedic Surgery, Feinstein Institute for Medical Research
North Shore – Long Island Jewish Health System
350 Community Drive, Manhasset, NY 11030 (USA)
Tel. +1 516 562 1138, Fax +1 516 562 2866, E-Mail dgrande@nshs.edu

et al., 1989; Minas and Peterson, 1999; Peterson et al., 2002], biomaterials [Vacanti et al., 1991; Grande et al., 1997; Sherwood et al., 2002; Cao et al., 2003], growth factors [Redini et al., 1988; Sellers et al., 1997; Nixon et al., 1999; O'Connor et al., 2000; Fukumoto et al., 2003; Tanaka et al., 2004], mesenchymal stem cells (MSCs) [Wakitani et al., 1994; Grande et al., 1995; Caplan et al., 1997; Im et al., 2001], and genetic engineering [Mason et al., 2000; Madry et al., 2002; Gelse et al., 2003; Grande et al., 2003; Noel et al., 2004]. However, the progress made with such tissue engineering techniques is still limited by our current fund of knowledge. A largely untapped source of knowledge lies in the relationship between fetal development and adult tissue repair. Despite the lack of attention given to this relationship, the events that take place during organogenesis may bear great significance to the tissue repair process that occurs later in life, and to the scientific community studying that process. Where our knowledge is limited in the biology and specific molecular interactions between stem cells and their environment, a deeper understanding of the events of fetal development as they pertain to cartilage organogenesis may help elucidate some of the unknowns of adult tissue repair.

## Mesenchymal Stem Cells

The utilization and manipulation of stem cells has offered some of the most promising technological advances in cartilage repair. These cells have attracted great attention because of their accessibility, relative ease of expansion by culture, and their manipulability to modern molecular and genetic engineering techniques. Stem cells are by definition undifferentiated cells that have a high capacity for proliferation and self-renewal, and enable the production of differentiated daughter cell lines for the purpose of tissue maintenance and repair. With our recent appreciation of their heterogeneity and plasticity, a revised definition has been proposed to reflect these functional attributes. The proposed definition regards stem cells as a potentially heterogeneous population of functionally undifferentiated cells that maintain flexibility and reversibility in their capabilities of: self-renewal, homing to an appropriate growth environment, proliferation, production of differentiated progeny, and regeneration of functional tissue after injury [Loeffler and Roeder, 2002]. Stem cells vary in their potential for differentiation depending on their subtype. Totipotent stem cells have ability to produce daughter cells of every cell lineage of an organism, including the necessary tissues for implantation within the uterus. Pluripotent stem cells have the same ability minus the production of trophoblasts, and so are unable to give rise to an entire organism. Multipotent stem cells produce progeny of a limited number of tissue types. MSCs are an example of multipotent cells, originally named for their differentiation potential.

MSCs are multipotent for the mesoderm-derived cell lines, such as chondrocytes, osteocytes, myocytes, adipocytes, and fibroblasts [Caplan, 1991]. MSCs have thus far demonstrated the ability to give rise to cartilage [Wakitani et al., 1994; Rogers et al., 1995; MacKay et al., 1998; Johnstone et al., 1998; Pittenger et al., 1999; Barry et al., 2001], bone [Aubin et al., 1995; Rogers et al., 1995; Jaiswal et al., 1997; Karsenty, 2000; Long, 2001; Aslan et al., 2006], fat [Beresford et al., 1992; Rogers et al., 1995; Pittenger et al., 1999], muscle [Rogers et al., 1995; Saito et al., 1995; Wakitani and Saito, 1995; Reyes and Verfaille, 1999a], tendon [Young et al., 1998; Awad et al., 1999], skin [Deng et al., 2005], hematopoietic-supporting stroma [Cheng et al., 2000; Koc and Lazarus, 2001] and neural tissue [Kopen et al., 1999; Reyes and Verfaille, 1999b; Deng et al., 2006]. Although MSCs are well known for their abundance in bone marrow, similar adult-derived stem cells have also been isolated from a variety of tissue types, such as periosteum [Nakahara et al., 1991; Mason et al., 1998; De Bari et al., 2001; O'Driscoll and Fitzsimmons, 2001; Grande et al., 2003], trabecular bone [Noth et al., 2002; Osyczka et al., 2002], muscle [Bosch et al., 2000; Asakura et al., 2001; Young et al., 2001; Huard et al., 2003], fat [Zuk et al., 2001; Mizuno, 2003; Wickham et al., 2003; Dicker et al., 2005; Kern et al., 2006], synovial tissue [De Bari et al., 2001], dermis [Young et al., 2001], adult peripheral blood [Zvaifler et al., 2000], and cord blood [Erices et al., 2000; Romanov et al., 2003; Kern et al., 2006]. Of particular interest, a progenitor cell population has also been recently isolated from articular cartilage [Dowthwaite et al., 2004; Alsalameh et al., 2004]. Whether these adult-derived stem cells are in fact MSCs, are similar progenitors that are further differentiated, or are cells of a different line altogether is still debated. Also questioned is the actual source of stem cells from these adult tissues, with vascular endothelium being an example of a plausible contributor of progenitor cells in some tissues. With such a broad spectrum of tissues from which MSCs and similar stem cells have been isolated and shown to give progeny, it is not surprising that there is still no agreement in the literature as to a specific definition for the MSC.

With the current broad range of cells thought to be MSCs or progenitors of similar origin, efforts have been made to differentiate and classify them. To aid in the isolation and characterization of these cells, different cell surface markers have been identified using monoclonal antibodies. Although certain typical surface antigens are recognized, no unique combination is universally agreed upon for the MSC or any of its subtypes [Ringe et al., 2002]. Differential up-regulation and down-regulation of many of those antigens further frustrates attempts to uniquely identify these cells. The most commonly studied MSCs are those derived from bone marrow, and also those thought to be derived from muscle and fat. Some of the surface markers that have been identified specifically on marrow-derived MSCs are: collagen types I, II, III, IV, V, VI; proteoglycan; fibronectin; hyaluronan; laminin; IL-6, -7, -8, -11, -12, -13, -14, -15, 1α, 1R, 3R, 4R, 6R, 7R; M-CSF; SCF; F/t-3 ligand; LIF; GM-CSF; G-CSF; integrin-α1, α2, α3, β1, β3, β4; ALCAM; endoglin; hyaluronate receptor; ICAM-1, -2; VCAM-1; LFA-3; L-selectin; PDGFR; TNFIR; TNFIIR; TGFβ1R; TGFβIIR; IFNγR; bFGFR; EGFR; LIFR; G-CSFR, and SCFR [Haynesworth et al., 1996; Majumdar et al., 1998; Pittenger et al., 1999; Conget and Minguell, 1999; Minguell et al., 2001; Ringe et al., 2002]. Some of the known surface markers of muscle-derived MSCs are Sca-1, Bcl-2, CALLA, aminopeptidase N, CD34, and NCAM [Miller et al., 1999; Young et al., 1999; Seale and Rudnicki, 2000]. Adipose-derived MSCs have been associated with tetraspan, CALLA, aminopeptidase N, integrin α5, β1, hyaluronate, complement protectin, endoglin, VCAM, and ALCAM [Wickham et al., 2003]. Marrow-derived MSCs have demonstrated the potential to give rise to bone, cartilage, muscle, and hematopoietic-supporting stroma [Pereira et al., 1995; Wakitani and Saito 1995; Prockop, 1997; Pittenger et al., 1999; Barry et al., 2001] but are themselves non-hematopoietic [Prockop, 1997]. Muscle-derived stem cells have been shown to differentiate into myogenic, osteogenic, chondrogenic, adipogenic, and hematopoietic cells [Pate et al., 1993; Young et al., 1993; Williams et al., 1999; Herzog et al., 2003]. Adipose-derived stem cells have been induced to differentiate into adipogenic, myogenic, osteogenic, and chondrogenic lineages [Halvorsen et al., 2001; Erickson et al., 2002; Dicker et al., 2005]. Each of these stem cell types can be isolated using similar methods, such as the preplate technique [Qu et al., 1998; Pittenger et al., 1999; Caterson et al., 2002; Erickson et al., 2002; Winter et al., 2003]. However, in spite of their differences in the tissue of origin, surface markers, and tissue progeny, there still remains a lack of agreement as to whether each of these cell types are all the same MSC, are different stages of a common MSC, or are truly distinct cells.

The differentiation capabilities of MSCs are highly plastic, influenced and determined by cell–cell and cell–environment interactions [Bennett et al., 1991; Beresford et al., 1992; Pittenger et al., 1999; Liang and Bickenbach, 2002; Loeffler and Roeder, 2002; Ball et al., 2004]. The extent of the potential and plasticity of differentiation of these cells is a subject of intense investigation. Conflicting reports debating the level of plasticity of specific stem cell lines have often used different cell markers with varying sensitivity and selectivity [Prockop et al., 2003], preventing a valid comparison between studies. Other reports pose the question of whether some of the plasticity attributed to such cells is due to cell fusion with local tissue rather than cell differentiation [Terada et al., 2002; Ying et al., 2002; Prockop et al., 2003]. The presence of other multipotential stem cells in the same compartments that MSCs are found further complicates our efforts. Multipotent adult progenitor cells, found in bone marrow and other tissue types [Jiang et al., 2002; Bedada et al., 2006], also co-purify with MSCs and grow as adherent cells. They have been shown to produce progeny of endodermal, mesodermal, and ectodermal cell lines, including hematopoietic cells [Herzog et al., 2003]. It is plausible that muscle-derived and adipose-derived stem cells are imprinted in some fashion so that they are more likely to produce progeny specific to their tissue of origin. It is also possible that MSCs commonly originating in bone marrow can become imprinted to favor myogenic or adipogenic differentiation based alone on the local environmental milieu. An incomplete understanding of the cell markers that can be reliably used to identify and distinguish these cells and their individual subtypes is a current limitation to the study of the MSC and other multipotent stem cells. In addition to varying methods of identification, the multitude of techniques used to obtain, isolate, and expand such cells have prevented a consensus on a universally agreed upon definition of the MSC. Despite these limitations, the potentially vast differentiating capabilities of the MSC, along with its accessibility, ease of culture and expansion, phenotypic stability, and overall manipulability make it an attractive target of investigation in tissue repair.

## MSCs in Tissue Repair

Following injury, there is a local influx of inflammatory cells from the circulation that help remove damaged cellular and extracellular debris. During and/or subse-

quent to this immediate inflammatory response, there is a local regeneration and proliferation of most tissue types. Poorly vascularized tissues, such as articular cartilage, benefit little from these reparative mechanisms. Tissues with little regenerative capacity attempt to overcome injury largely with scar formation. Repair and regeneration is in part accomplished by locally resident, early precursor cells that provide tissue-specific progeny. To provide a few examples, periosteum and endosteum contain fibroblast-like osteoprogenitor cells that have the potential to differentiate into osteoblasts. Except for fibrocartilage and articular hyaline cartilage, the perichondrium surrounding other types of cartilage contains fibroblast-like chondroblasts that differentiate into chondrocytes. Satellite cells, quiescent mesenchymal cells found just outside of the sarcolema, differentiate into myoblasts that fuse to form new myofibers. Pericytes are perivascular mesenchymal cells found along capillaries and small venules that differentiate into endothelial cells. The basal layer of progenitor cells of the epidermis replenishes the turnover of epithelial cells. Each of these progenitor cells are themselves thought to be replenished by stem cells that originate in bone marrow. Marrow-derived stem cells may become imprinted once they reach local tissue to be more selective in producing progeny of that tissue type during repair. Some types of repair may even require importation of marrow-derived progenitor cells for complete repair to be achieved at all. As an example, while periosteal progenitors may provide subperiosteal bone formation following fracture, vascular invasion of long bone fractures imports marrow-derived cells for the bone formation at the fracture ends. The properties of MSCs discussed earlier makes them some of the most likely candidates as the source for many or even all of these progenitors. There is debate as to exactly which level of cellular differentiation is responsible for the source of marrow-derived cells in tissue-specific repair, and whether these cells leave the marrow at a stage of even greater multipotentiality than the MSC [Prockop et al., 2003]. Our understanding of tissue repair is further complicated when taking into consideration the number of tissue types other than bone marrow from which MSCs are thought to possibly be derived. It remains unclear to what extent the locally resident MSC/adult progenitor participates in repair versus the abundant marrow-derived MSCs. Given the great potential of these cells and our lack of a complete understanding of that potential, they make excellent subjects of investigation for the current state of tissue repair science.

## Modern Techniques in Tissue Engineering

Earlier efforts towards cartilage repair focused on autologous and allogenic chondrocyte grafts. However, such grafting techniques are hampered by morbidity to donor sites, limited quantity of tissue, immunogenic reactions, transmission of infections, biological inferiority, inconsistent local incorporation into host tissue, morbidity of lengthy, multiple, technique-dependent procedures, and ultimately limited clinical applicability. Additionally, native chondrocytes isolated for in vitro culture and expansion demonstrate a low proliferative capability with a limited number of replication cycles [Saadeh et al., 1999] and poor phenotypic stability [Benya and Shaffer, 1982]. These limitations have spurred the scientific community towards the investigation of cellular and biosynthetic alternatives to autologous and allogenic chondrocyte grafting.

Current tissue engineering techniques utilize any combination of three critical components: a cellular component, a carrier vehicle/matrix scaffold, and a bioactive component. The cellular component should consist of healthy, viable cells that are accessible, manipulable, and nonimmunogenic. These cells should also be responsive to environmental cues while maintaining a certain degree of phenotypic stability. The carrier component has a dual function, acting as both a delivery vehicle and a matrix scaffold. The carrier function may pertain to a bioactive growth factor and/or to a cellular component. The carrier should be biologically compatible to both the growth factor/cellular component and the recipient host tissue. The scaffold should enable in vitro/in vivo cellular seeding and adhesion without adversely altering cellular phenotype, and enable integration into local host tissue without causing a detrimental inflammatory reaction. It should be biodegradable, acting as a temporary structural framework until its role is replaced by naturally synthesized matrix. In addition, the scaffold would also need to be biomechanically sound, strong enough to withstand at least the compressive and shear forces of the growth and repair process, and preferably resemble the properties of the local host tissue. The bioactive component should act as an inductive factor to tissue repair. These growth factors should augment chemotaxis, cell differentiation, proliferation, and/or matrix production.

### *MSCs in Tissue Engineering*

An excellent candidate for the cellular component is the MSC. Fibroblast-like precursor cells isolated from bone marrow that manifested the potential to produce cell

lines resembling osteocytes and chondrocytes were first identified by Friedenstein et al. [1976] three decades ago, and were later confirmed to be multipotential mesenchymal precursors [Friedenstein et al., 1987]. If these cells are required for the replenishment of local progenitors in adult tissue repair, then a logical extrapolation is that their regional availability would act as a limiting factor to the extent of repair possible. Therefore, tissues like articular cartilage that have a limited or non-existent blood supply, and thus a limited natural source of these cells, may exhibit the greatest benefit to tissue engineering techniques that utilize and locally provide MSCs as the cellular component. These cells are readily accessible from bone marrow sources such as the iliac crest [Haynesworth et al., 1992; DiGirolamo et al., 1999; Pittenger et al., 1999], tibial and femoral shafts [Oreffo et al., 1998; Murphy et al., 2002], and vertebra [D'Ippolito et al., 1999], among a long list of other potential sources discussed earlier, and are easily expanded in vitro while maintaining phenotypic stability [Haynesworth et al., 1992; Martin et al., 1997; DiGirolamo et al., 1999; Pittenger et al., 1999]. When such cultured MSCs are implanted alone into surgically created full-thickness cartilage defects in rabbit and rat animal models, they demonstrate increased cartilage repair over controls [Wakitani et al., 1994; Grande et al., 1995; Caplan et al., 1997; Im et al., 2001; Oshima et al., 2005]. Similar repair has been reported in femoral bony defects in rats [Kadiyala et al., 1997] and Achilles tendon defects in rabbits [Young et al., 1998].

*MSCs and Biomaterial Scaffolds*

Various biomaterials have been utilized as vehicles of delivery for cells and growth factors, and as scaffolds for cellular propagation and matrix production. Some of the more commonly studied synthetic scaffolds in cartilage repair are made of polyglycolic acid, poly-L-lactic acid and the copolymer poly-DL-lactic-co-glycolic acid [Sherwood et al., 2002; Giurea et al., 2003; Moran et al., 2003]. The more common natural scaffold materials include collagen, hyaluronic acid, chitosan and alginate [Cao et al., 1998; Cherubino et al., 2003; Abe et al., 2004]. Biosynthetic scaffolds offer the advantage of manipulability in design (fiber diameter, pore size, degradation time) and reproducibility in production. Natural scaffolds offer the advantage of using materials that are native to the local environment. Matrix scaffolds have demonstrated increased cartilage growth and/or repair over controls when used alone [Grande et al., 1999], when seeded with a growth factor [Kim and Valentini, 2002], and when seeded with chondrocytes [Vacanti et al., 1991, 1994; Freed et al., 1994; Chu et al., 1995; Frenkel et al., 1997; Grande et al., 1997; Saldanha and Grande, 2001; Weng et al., 2001] in both in vitro and in vivo model systems. More recently, these scaffolds are being used in combination with MSCs [Martin et al., 1998; Radice et al., 2000; Caterson et al., 2001; Williams et al., 2003; Huang et al., 2006; Shao et al., 2006]. Seeding MSCs onto a scaffold offers the advantage of providing an accessible, manipulable, self-renewing source of otherwise locally limited progenitor cells, on a biodegradable template for proliferation and matrix production.

*MSCs, Bioactives, and Genetic Engineering*

Many bioactive growth factors have been investigated for their chondroprogenitive potential. The most common of these factors are the members of the transforming growth factor (TGF) superfamily; specifically TGF-β and the bone morphogenic proteins (BMP-2 and 7). Other growth factors that likely play a role in neochondrogenesis include acidic and basic fibroblast growth factor (FGF-1 and 2), insulin-like growth factor (IGF-1), and platelet-derived growth factor (PDGF). An additional growth factor currently under investigation is growth and differentiation factor-5 (a.k.a. BMP-13). Bioactives other than growth factors have also been studied, such as synthetic thrombin peptide-508. Much of the existing evidence of the chondroprogenitive role of these growth factors was obtained using them either alone or with cultured chondrocytes [Redini et al., 1988; Nixon et al., 1999; Hunziker et al., 2001; Hickey at al., 2003; Tanaka et al., 2004]. However, stromal cell populations have also been found to expand efficiently and/or undergo chondrogenic differentiation under the influence of many of the above-mentioned bioactive factors, such as TGF-β, IGF, PDGF, and FGF [Kuznetsov et al., 1997; Worster et al., 2000, 2001].

Newer technologies under investigation are combining the use of growth factors and MSCs. But most interesting are the techniques that utilize genetic engineering to transfect MSCs with genes of specific growth factors. These cells have recently demonstrated the ability to function as efficient vehicles of transduced genes [Dao and Nolta 1998; Mason et al., 1998; Mosca et al., 2000; Guo et al., 2001, 2002; McMahon et al., 2006]. Various vector systems are constructed with the bioactive gene of interest and allowed to transduce a MSC population in vitro before the cells are transplanted into the host tissue. This indirect method of gene therapy enables the controlled transfer of a specific growth factor gene into a select cell population. The vectors currently utilized are viral or less commonly nonviral. Viral vectors are manipulated to

render them nonpathogenic, loaded with the DNA or RNA of the desired growth factor, and allowed to infect a specific cell line in order to transfer the specific gene of choice. The vectors most commonly used are adenoviral, adeno-associated, retroviral, and herpes simplex. When considering the most appropriate vector, the characteristics of importance are transgene insertion size capacity, genome state after transduction, efficiency of infection, ability to infect nondividing cells, degree and duration of transgene expression, native viral gene expression, and cost of vector production [Moynihan and Grande, 2002]. As this technology develops, the number of reports illustrating the chondroprogenitive potential of MSCs transduced with the genes of bioactive growth factors continues to grow in the literature [Carlberg et al., 2001; Madry et al., 2002; Steinert et al., 2003; Noel et al., 2004; Palmer et al., 2005]. However, the real potential of transduced MSCs goes beyond the induction of these cells into chondrocytes. Cells transduced using these techniques function as bioactive factories, providing a sustained local delivery of specific growth factors to the site of diseased or damaged tissue. This combined approach, termed 'gene-enhanced tissue engineering' by one of the authors (D.A.G.), delivers a local supply of otherwise limited cells that not only participate directly in the repair process, but also act indirectly to augment the local host tissue response by inducing cell recruitment, propagation, and matrix production. Investigators have gone beyond in vitro cell culture and have utilized this technology to demonstrate in vivo cartilage repair. Periosteal-derived MSCs transduced with BMP-7 and sonic hedgehog have been recently reported to significantly increase the quality of repair over controls in surgically created osteochondral defects in a rabbit model [Mason et al., 2000; Grande et al., 2003]. Similarly, periosteal-derived MSCs expressing BMP-2 and IGF-1 improved cartilage repair in the surgical defects of rat femurs [Gelse et al., 2003]. With such a powerful combination of tools, gene-enhanced tissue engineering will likely provide a highly useful methodology in the near future of tissue repair.

## The Role of Understanding Fetal Development in Future Directions of Tissue Repair

*MSCs in Development*

Our current understanding of the role of MSCs in development is extremely limited. The multitude of events that take place during fetal development which lead from stem cell to functional tissue have not been adequately studied, are still poorly understood, and few reports are available. A lineage scheme has been proposed for the embryonic development of bone, starting with the MSC and progressing through several stages before ending with the osteocyte [Bruder and Caplan, 1989, 1990a, 1990b, 1990c]. However, with our still incomplete knowledge of the various surface markers present on these cells during their different stages, it is difficult to determine the completeness of such a scheme. Interpreting such information is made more complicated with our recent appreciation of the heterogeneity and plasticity of MSCs.

*Cell Signals in Development*

Even more elusive are the cellular and environmental signals that regulate the progression from one cellular stage to the next. The specifics of the molecular interactions of MSCs between each other and their environment are largely unknown. Efforts towards elucidating musculoskeletal organogenesis have made some progress, such as determining the role of FGF in embryonic osteogenesis [Frenkel et al., 1990] and limb-bud formation [Niswander and Martin, 1992], the influence of the inhibitory protein I-mfa on embryonic mesenchymal precursors and skeletal development [Kraut et al., 1998], and the suspected role of cartilage homeoprotein-1 [Zhao et al., 1993] and Notch1 [Watanabe et al., 2003] in embryonic chondrocyte differentiation. But these reports are sparse and explain relatively little of the complete mechanism of progression from stem cell to functional tissue. Are the cellular signaling events, intracellular cascades, and steps of cell differentiation observed in experimental models and in tissue repair the same as those taking place during development? Previous investigations of the events of long bone formation during development demonstrated that osteogenesis occurs in a manner that is directed along invading vasculature, suggesting that marrow-derived elements are essential for this process [Pechak et al., 1986a, 1986b]. Does the invading vasculature following an adult long bone fracture bring in the same marrow-derived elements that are responsible for developmental osteogenesis, and are they subject to a local microenvironment that is similar or drastically different in the two scenarios? Although the current opinion is that these processes are similar, the existing evidence is thin and sometimes conflicting. For example, although the growth factor Tenascin-C has been shown to participate in chondrogenesis in the embryonic limb-bud [Mackie et al., 1987; Gluhak et al., 1996], one report demonstrated that it fails to improve cartilage repair in partial thickness defects over controls in the knees of adult pigs [Hunziker et al., 2001].

*Cell Signals in Fetal Tissue Repair*

Older reports that describe scarless healing in the early fetal lamb [Burrington, 1971] might suggest that there is a certain significant difference between adult and fetal tissue repair. Why should tissue injury at one stage of life result in perfect repair and at a later stage lead to scar formation? One plausible explanation is that the same growth factor may play different roles at different stages of life. In comparing fetal and adult tissue concentrations of different growth factors known to participate in repair in adult models, various investigators have reported lower concentrations of TGF-β1, TGF-β2 [Whitby and Ferguson, 1991a, 1991b] and hyaluronidase [Whitby and Ferguson, 1991a; West et al., 1997], and higher concentrations of hyaluronan [DePalma et al., 1989; Alaish et al., 1994; Iocono et al., 1998] and tenacin [Shah et al., 1995] in fetal tissue. It is difficult to interpret such data with the large body of evidence supporting the chondroprogenitive and osteoinductive role of factors such as TGF-β in adult models. The available literature is still too sparse and inconclusive to lead to any definitive conclusions about the relation of these growth factors between fetal and adult tissue repair [Akeson et al., 2001], and even less understood is their role in relation to the MSC.

## Discussion

A more aggressive approach is required in order to increase our understanding of the possible link between fetal development and adult tissue repair. Some of the questions left to be answered are whether there are additional cytokines and growth factors released by the local environment during organogenesis that can be used to augment adult tissue repair, what those bioactive agents are, with what concentration are they present, and in what temporal relationship. Other unknowns center around the stem cell, such as the extent of any differences between the embryonic and adult MSC.

Despite the progress made in the use of MSCs in tissue, molecular, and genetic engineering, the role of the MSC in development is an understudied area, one which may bear great significance to the study of adult tissue repair. Perhaps a better working knowledge of the molecular mechanisms of organogenesis, and specifically chondrogenesis, as they occur in development can be used to better understand the role of MSCs in tissue repair. Can the cellular signals and interactions that take place during development provide new insights to potential manipulations to improve repair of damaged or diseased tissue in adult life? If the events of development and adult tissue repair share a high similarity, then a deeper understanding of one can be utilized towards advancing the technology implemented to augment the other. If they are in fact very different, then the study of one may lead to entirely new approaches towards improving the other. In either circumstance, it is clear that MSCs have enormous potential, and the next step towards harnessing that full potential likely lies in the many remaining unknowns of the role of MSCs in fetal development.

## References


Abe M., M. Takahashi, S. Tokura, H. Tamura, A. Nagano (2004) Cartilage-scaffold composites produced by bioresorbable beta-chitin sponge with cultured rabbit chondrocytes. Tissue Eng *10:* 585–594.

Adzick N.S., H.P. Lorenz (1994) Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg *220:* 10–18.

Akeson W.H., W. Bugbee, C. Chu, A. Giurea (2001) Differences in mesenchymal tissue repair. Clin Orthop *391(suppl):* S124–S141.

Alaish S.M., D. Yager, R.F. Diegelmann, I.K. Cohen (1994) Biology of fetal wound healing: hyaluronate receptor expression in fetal fibroblasts. J Pediatr Surg *29:* 1040–1043.

Alsalameh S., R. Amin, T. Gemba, M. Lotz (2004) Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis Rheum *50:* 1522–1532.

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

Aslan H., Y. Zilberman, A. Kendel, M. Liebergall, R.J. Oskouian, D. Gazit, Z. Gazit (2006) Osteogenic differentiation of noncultured immunoisolated bone marrow-derived CD105+ cells. Stem Cells *24:* 1728–1738.

Aubin J.E., F. Liu, L. Malaval, A.K. Gupta (1995) Osteoblast and chondroblast differentiation. Bone *17(suppl):* 77S–83S.

Awad H.A., D.L. Butler, G.P. Boivin, F.N. Smith, P. Malaviya, B. Huibregtse, A.I. Caplan (1999) Autologous mesenchymal stem cell-mediated repair of tendon. Tissue Eng *5:* 267–277.

Ball S.G., A.C. Shuttleworth, C.M. Kielty (2004) Direct cell contact influences bone marrow mesenchymal stem cell fate. Int J Biochem Cell Biol *36:* 714–727.

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

Bedada F.B., S. Gunther, T. Kubin, T. Braun (2006) Differentiation versus plasticity: fixing the fate of undetermined adult stem cells. Cell Cycle *5:* 223–226.

Bennett J.H., C.J. Joyner, J.T. Triffitt, M.E. Owen (1991) Adipocytic cells cultured from marrow have osteogenic potential. J Cell Sci *99:* 131–139.

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

Beresford J.N., J.H. Bennett, C. Devlin, P.S. Leboy, M.E. Owen (1992) Evidence for an inverse relationship between the differentiation of adipocytes and osteogenic cells in rat marrow stromal cell cultures. J Cell Sci *102:* 341–351.

Bosch P., D.S. Musgrave, J.Y. Lee, J. Cummins, T. Shuler, T.C. Ghivizzani, T. Evans, T.D. Robbins, J. Huard (2000) Osteoprogenitor cells within skeletal muscle. J Orthop Res *18:* 933–944.

Bruder S.P., A.I. Caplan (1989) First bone formation and the dissection of an osteogenic lineage in the embryonic chick tibia is revealed by monoclonal antibodies against osteoblasts. Bone *10:* 359–375.

Bruder S.P., A.I. Caplan (1990a) A monoclonal antibody against the surface of osteoblasts recognizes alkaline phosphatase isoenzymes in bone, liver, kidney, and intestine. Bone *11:* 133–139.

Bruder S.P., A.I. Caplan (1990b) Terminal differentiation of osteogenic cells in the embryonic chick tibia is revealed by a monoclonal antibody against osteocytes. Bone *11:* 189–198.

Bruder S.P., A.I. Caplan (1990c) Osteogenic cell lineage analysis is facilitated by organ cultures of embryonic chick periosteum. Dev Biol *141:* 319–329.

Burrington J. (1971) Wound healing in the fetal lamb. J Pediatr Surg *6:* 523–528.

Cabrera R.C., J.W. Siebert, Y. Eidelman, L.I. Gold, M.T. Longaker, H.G. Garg (1995) The in vivo effect of hyaluronan associated protein-collagen complex on wound repair. Biochem Mol Biol Int *37:* 151–158.

Cao T., K.H. Ho, S.H. Teoh (2003) Scaffold design and in vitro study of osteochondral coculture in a three-dimensional porous polycaprolactone scaffold fabricated by fused deposition modeling. Tissue Eng *9(suppl):* S103–S112.

Cao Y., A. Rodriguez, M. Vacanti, C. Ibarra, C. Arevalo, C.A. Vacanti (1998) Comparative study of the use of poly(glycolic acid), calcium alginate and pluronics in the engineering of autologous porcine cartilage. J Biomater Sci Polym Ed *9:* 475–487.

Caplan A.I. (1991) Mesenchymal stem cells. J Orthop Res *9:* 641–650.

Caplan A.I., M. Elyaderani, Y. Mochizuki, S. Wakitani, V.M. Goldberg (1997) Principles of cartilage repair and regeneration. Clin Orthop *342:* 254–269.

Carlberg A.L., B. Pucci, R. Rallapalli, R.S. Tuan, D.J. Hall (2001) Efficient chondrogenic differentiation of mesenchymal cells in micromass culture by retroviral gene transfer of BMP-2. Differentiation *67:* 128–138.

Caterson E.J., L.J. Nesti, K.G. Danielson, T.J. Albert, A.R. Vaccaro, R.S. Tuan (2001) Three-dimensional cartilage formation by bone marrow-derived cells seeded in polylactide/alginate amalgam. J Biomed Mater Res *57:* 394–403.

Caterson E.J., L.J. Nesti, K.G. Danielson, R.S. Tuan (2002) Human marrow-derived mesenchymal progenitor cells: isolation, culture expansion, and analysis of differentiation. Mol Biotechnol *20:* 245–256.

Centers for Disease Control and Prevention (1999) CDC targets arthritis, a leading cause of disability. Public Health Rep *114:* 102.

Centers for Disease Control and Prevention (2003) Direct and indirect costs of arthritis and other rheumatic conditions – United States, 1997. MMWR Morb Mortal Wkly Rep *52:* 1124–1127.

Cheng L., P. Qasba, P. Vanguri, M.A. Thiede (2000) Human mesenchymal stem cells support megakaryocyte and pro-platelet formation from CD34(+) hematopoietic progenitor cells. J Cell Physiol *184:* 58–69.

Cherubino P., F.A. Grassi, P. Bulgheroni, M. Ronga (2003) Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. J Orthop Surg *11:* 10–15.

Chu C.R., R.D. Coutts, M. Yoshioka, F.L. Harwood, A.Z. Monosov, D. Amiel (1995) Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study. J Biomed Mater Res *29:* 1147–1154.

Conget P.A., J.J. Minguell (1999) Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol *181:* 67–73.

Dao M.A., J.A. Nolta (1998) Use of the bnx/hu xenograft model of human hematopoiesis to optimize methods for retroviral-mediated stem cell transduction. Int J Mol Med *1:* 257–264.

De Bari C., F. Dell'Accio, P. Tylzanowski, F.P. Luyten (2001) Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum *44:* 1928–1942.

Deng W., Q. Han, L. Liao, C. Li, W. Ge, Z. Zhao, S. You, H. Deng, F. Murad, R.C. Zhao (2005) Engrafted bone marrow-derived flk-(1+) mesenchymal stem cells regenerate skin tissue. Tissue Eng *11:* 110–119.

Deng Y.B., X.G. Liu, X.L. Liu, X.L. Liu, Y. Liu, G.Q. Zhou (2006) Implantation of BM mesenchymal stem cells into injured spinal cord elicits de novo neurogenesis and functional recovery: evidence from a study in rhesus monkeys. Cytotherapy *8:* 210–214.

DePalma R.L., T.M. Krummel, L.A. Durham 3rd, B.A. Michna, B.L. Thomas, J.M. Nelson, R.F. Diegelmann (1989) Characterization and quantitation of wound matrix in the fetal rabbit. Matrix *9:* 224–231.

Dicker A., K. Le Blanc, G. Astrom, V. van Harmelen, C. Gotherstrom, L. Blomqvist, P. Arner, M. Ryden (2005) Functional studies of mesenchymal stem cells derived from adult human adipose tissue. Exp Cell Res *308:* 283–290.

DiGirolamo C.M., D. Stokes, D. Colter, D.G. Phinney, R. Class, D.J. Prockop (1999) Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. Br J Haematol *107:* 275–281.

D'Ippolito G., P.C. Schiller, C. Ricordi, B.A. Roos, G.A. Howard (1999) Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res *14:* 1115–1122.

Dowthwaite G.P., J.C. Bishop, S.N. Redman, I.M. Khan, P. Rooney, D.J. Evans, L. Haughton, Z. Bayram, S. Boyer, B. Thomson, M.S. Wolfe, C.W. Archer (2004) The surface of articular cartilage contains a progenitor cell population. J Cell Sci *117:* 889–897.

Erices A., P. Conget, J.J. Minguell (2000) Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol *109:* 235–242.

Erickson G.R., J.M. Gimble, D.M. Franklin, H.E. Rice, H. Awad, F. Guilak (2002) Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. Biochem Biophys Res Commun *290:* 763–769.

Freed L.E., G. Vunjak-Novakovic, R.J. Biron, D.B. Eagles, D.C. Lesnoy, S.K. Barlow, R. Langer (1994) Biodegradable polymer scaffolds for tissue engineering. Biotechnology *12:* 689–693.

Frenkel S.R., B. Toolan, D. Menche, M.I. Pitman, J.M. Pachence (1997) Chondrocyte transplantation using a collagen bilayer matrix for cartilage repair. J Bone Joint Surg Br *79:* 831–836.

Frenkel S.R., D.A. Grande, M. Collins, I.J. Singh (1990) Fibroblast growth factor in chick osteogenesis. Biomaterials *11:* 38–40.

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

Friedenstein A.J., J.F. Gorskaja, N.N. Kulagina (1976) Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol *4:* 267–274.

Fukumoto T., J.W. Sperling, A. Sanyal, J.S. Fitzsimmons, G.C. Reinholz, C.A. Conover, S.W. O'Driscoll (2003). Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro. Osteoarthritis Cartilage *11:* 55–64.

Gelse K., K. von der Mark, T. Aigner, J. Park, H. Schneider (2003) Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum *48:* 430–441.

Giurea A., T.J. Klein, A.C. Chen, R.S. Goomer, R.D. Coutts, W.H. Akeson, D. Amiel, R.L. Sah (2003) Adhesion of perichondrial cells to a polylactic acid scaffold. J Orthop Res *21:* 584–589.

Gluhak J., A. Mais, M. Mina (1996) Tenascin-C is associated with early stages of chondrogenesis by chick mandibular ectomesenchymal cells in vivo and in vitro. Develop Dynam *205:* 24–40.

Grande D.A., A.S. Breitbart, J. Mason, C. Paulino, J. Laser, R.E. Schwartz (1999) Cartilage tissue engineering: current limitations and solutions. Clin Orthop *367:* 176–185.

Grande D.A., C. Halberstadt, G. Naughton, R. Schwartz, R. Manji (1997) Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts. J Biomed Mater Res *34:* 211–220.

Grande D.A., J. Mason, E. Light, D. Dines (2003) Stem cells as platforms for delivery of genes to enhance cartilage repair. J Bone Joint Surg *85A(suppl 2):* 111–116.

Grande D.A., M.I. Pitman, L. Peterson, D. Menche, M. Klein (1989) The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res *7:* 208–218.

Grande D.A., B.S. Southerland, R. Manji (1995) Repair of articular cartilage defects using mesenchymal stem cells. Tissue Eng *1:* 345–352.

Guo X., J. Du, Q. Zheng, Y. Liu, D. Duan, Y. Wu (2001) Molecular tissue engineering: applications for modulation of mesenchymal stem cells proliferation by transforming growth factor beta 1 gene transfer. J Tongji Med Univ *21:* 314–317.

Guo X., J. Du, Q. Zheng, S. Yang, Y. Liu, D. Duan, C. Yi (2002) Expression of transforming growth factor beta 1 in mesenchymal stem cells: potential utility in molecular tissue engineering for osteochondral repair. J Huazhong Univ Sci Technolog Med Sci *22:* 112–115.

Halvorsen Y.D., D. Franklin, A.L. Bond, D.C. Hitt, C. Auchter, A.L. Boskey, E.P. Paschalis, W.O. Wilkison, J.M. Gimble (2001) Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived stromal cells. Tissue Eng *7:* 729–41.

Haynesworth S.E., J. Goshima, V.M. Goldberg, A.I. Caplan (1992) Characterization of cells with osteogenic potential from human marrow. Bone *13:* 81–88.

Haynesworth S.E., M.A. Baber, A.I. Caplan (1996) Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol *166:* 585–592.

Herzog E.L., L. Chai, D.S. Krause (2003) Plasticity of marrow-derived stem cells. Blood *102:* 3483–3493.

Hickey D.G., S.R. Frenkel, P.E. Di Cesare (2003) Clinical applications of growth factors for articular cartilage repair. Am J Orthop *32:* 70–76.

Huang J.I., M.M Durbhakula, P. Angele, B. Johnstone, J.U. Yoo (2006) Lunate arthroplasty with autologous mesenchymal stem cells in a rabbit model. J Bone Joint Surg Am *88:* 744–752.

Huard J., B. Cao, Z. Qu-Petersen (2003) Muscle-derived stem cells: potential for muscle regeneration. Birth Defects Res Part C Embryo Today *69:* 230–237.

Hunziker E.B., I.M.K. Driesang, E.A. Morris (2001) Chondrogenesis in cartilage repair is induced by members of the transforming growth factor-beta superfamily. Clin Orthop *391(suppl):* 171–181.

Im G.I., D.Y. Kim, J.H. Shin, C.W. Hyun, W.H. Cho (2001) Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow. J Bone Joint Surg *83B:* 289–294.

Iocono J.A., H.P. Ehrlich, K.A. Keefer, T.M. Krummel (1998) Hyaluronan induces scarless repair in mouse limb organ culture. J Pediatr Surg *33:* 564–567.

Jackson D.W., T.M. Simon, H.M. Aberman (2001) Symptomatic articular cartilage degeneration: the impact in the new millennium. Clin Orthop *391(suppl):* S14–S25.

Jaiswal N., S.E. Haynesworth, A.I. Caplan, S.P. Bruder (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem *164:* 295–312.

Jiang Y., B. Vaessen, T. Lenvik, M. Blackstad, M. Reyes, C.M. Verfaillie (2002) Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol *30:* 896–904.

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

Jorgensen C., D. Noel, F. Apparailly, J. Sany (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., N. Jaiswal, S.P. Bruder (1997) Culture expanded bone marrow-derived mesenchymal stem cells can regenerate a critical-sized segmental bone defect. Tissue Eng *3:* 173–185.

Karsenty G. (2000) Bone formation and factors affecting this process. Matrix Biol *19:* 85–89.

Kern S., H. Eichler, J. Stoeve, H. Kluter, K. Bieback (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells *24:* 1294–1301.

Kim H.D., R.F. Valentini (2002) Retention and activity of BMP-2 in hyaluronic acid-based scaffolds in vitro. J Biomed Mater Res *59:* 573–584.

Koc O.N., H.M. Lazarus (2001) Mesenchymal stem cells: heading into clinic. Bone Marrow Transplant *27:* 235–239.

Kopen G.C., D.J. Prockop, D.J. Phinney (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA *96:* 10711–10716.

Kraut N., L. Snider, C.M. Chen, S.J. Tapscott, M. Groudine (1998) Requirement of the mouse I-mfa gene for placental development and skeletal patterning. EMBO J *17:* 6276–6288.

Kuznetsov S.A., A.J. Friedenstein, P.G. Robey (1997) Factors required for bone marrow stromal fibroblast colony formation in vitro. Br J Haematol *97:* 561–570.

Lane J.M., C.T. Brighton, H.R. Ottens, M. Lipton (1977) Joint resurfacing in the rabbit using an autologous osteochondral graft. J Bone Joint Surg Am *59:* 218–222.

Liang L., J.R. Bickenbach (2002) Somatic epidermal stem cells can produce multiple cell lineages during development. Stem Cells *20:* 21–31.

Loeffler M., I. Roeder (2002) Tissue stem cells: definition, plasticity, heterogeneity, self-organization and models – a conceptual approach. Cells Tissues Organs *171:* 8–26.

Long M.W. (2001) Osteogenesis and bone-marrow-derived cells. Blood Cells Mol Dis *27:* 677–690.

MacKay A.M., S.C. Beck, J.M. Murphy, F.P. Barry, C.O. Chichester, M.F. Pittenger (1998) Chondrogenic differentiation of cultured human mesenchymal stem cell from marrow. Tissue Eng *4:* 415–428.

Mackie E.J., I. Thesleff, R. Chiquet-Ehrismann (1987) Tenascin is associated with chondrogenic and osteogenic differentiation in vivo and promotes chondrogenesis in vitro. J Cell Biol *105:* 2569–2579.

Madry H., R. Padera, J. Seidel, R. Langer, L.E. Freed, S.B. Trippel, G. Vunjak-Novakovic (2002) Gene transfer of a human insulin-like growth factor I cDNA enhances tissue engineering of cartilage. Hum Gene Ther *1:* 1621–1630.

Majumdar M.K., M.A. Thiede, J.D. Mosca, M. Moorman, S.L. Gerson (1998) Phenotypic and functional comparisons of cultures of marrow-derived mesenchymal stem cells and stromal cells. J Cell Physiol *176:* 57–66.

Martin I., A. Muraglia, G. Campanile, R. Cancedda, R. Quarto (1997) Fibroblast growth factor-2 supports ex vivo expansion and maintenance of osteogenic precursors from human bone marrow. Endocrinology *138:* 4456–4462.

Martin I., R.F. Padera, G. Vunjak-Novakovic, L.E. Freed (1998) In vitro differentiation of chick embryo bone marrow stromal cells into cartilaginous and bone-like tissues. J Orthop Res *16:* 181–189.

Mason J.M., A.S. Breitbart, M. Barcia, D. Porti, R.G. Pergolizzi, D.A. Grande (2000) Cartilage and bone regeneration using gene-enhanced tissue engineering. Clin Orthop *379:* S171–S178.

Mason J.M., D.A. Grande, M. Barcia, R. Grant, R.G. Pergolizzi, A.S. Breitbart (1998) Expression of human bone morphogenic protein 7 in primary rabbit periosteal cells: potential utility in gene therapy for osteochondral repair. Gene Ther *5:* 1098–1104.

McMahon J.M., S. Conroy, M. Lyons, U. Greiser, C. O'shea, P. Strappe, L. Howard, M. Murphy, F. Barry, T. O'Brien (2006) Gene transfer into rat mesenchymal stem cells: a comparative study of viral and nonviral vectors. Stem Cells Dev *15:* 87–96.

Miller J., L. Schafer, J. Dominov (1999) Seeking muscle stem cells. Curr Topic Dev Biol *43:* 191–214.

Minas T., L. Peterson (1999) Advanced techniques in autologous chondrocyte transplantation. Clin Sports Med *18:* 13–44.

Minguell J.J., A. Erices, P.A. Conget (2001) Mesenchymal stem cells. Exp Biol Med *226:* 507–520.

Mizuno H. (2003) Versatility of adipose tissue as a source of stem cells. J Nippon Med Sch *70:* 428–431.

Moran J.M., D. Pazzano, L.J. Bonassar (2003) Characterization of polylactic acid-polyglycolic acid composites for cartilage tissue engineering. Tissue Eng *9:* 63–70.

Mosca, J.D., J.K. Hendricks, D. Buyaner, J. Davis-Sproul, L.C. Chuang, M.K. Majumdar, R. Chopra, F. Barry, M. Murphy, M.A. Thiede, U. Junker, R.J. Rigg, S.P. Forestell, E. Bohnlein (2000) Mesenchymal stem cells as vehicles for gene delivery. Clin Orthop *379(suppl): S*71–S90.

Moynihan D.P., D.A. Grande (2002) Gene therapy and cartilage repair: a review. Semin Arthroplasty *13:* 229–235.

Murphy J.M., K. Dixon, S. Beck, D. Fabian, A. Feldman, F. Barry (2002) Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. Arthritis Rheum *46:* 704–713.

Nakahara H., V.M. Goldberg, A.I. Caplan (1991) Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. J Orthop Res *9:* 465–476.

Niswander L., G.R. Martin (1992) Fgf-4 expression during gastrulation, myogenesis, limb and tooth development in the mouse. Development *114:* 755–768.

Nixon A.J., L.A. Fortier, J. Williams, H. Mohammed (1999) Enhanced repair of extensive articular defects by insulin-like growth factor-I-laden fibrin composites. J Orthop Res *17:* 475–487.

Noel D., D. Gazit, C. Bouquet, F. Apparailly, C. Bony, P. Plence, V. Millet, G. Turgeman, M. Perricaudet, J. Sany, C. Jorgensen (2004) Short-term BMP-2 expression is sufficient for in vivo osteochondral differentiation of mesenchymal stem cells. Stem Cells *22:* 74–85.

Noth U., A.M. Osyczka, R. Tuli, N.J. Hickok, K.G. Danielson, R.S. Tuan (2002) Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. J Orthop Res *20:* 1060–1069.

O'Connor W.J., T. Botti, S.N. Khan, J.M. Lane (2000) The use of growth factors in cartilage repair. Orthop Clin North Am *31:* 399–410.

O'Driscoll S.W., J.S Fitzsimmons (2001) The role of periosteum in cartilage repair. Clin Orthop *391(suppl):* 190–207.

Oreffo R.O., S. Bord , J.T. Triffitt (1998) Skeletal progenitor cells and ageing human populations. Clin Sci *94:* 549–555.

Oshima Y., N. Watanabe, K. Matsuda, S. Takai, M. Kawata, T. Kubo (2005) Behavior of transplanted bone marrow-derived GFP mesenchymal cells in osteochondral defect as a simulation of autologous transplantation. J Histochem Cytochem *53:* 207–216.

Osyczka A.M., U. Noth, K.G. Danielson, R.S. Tuan (2002) Different osteochondral potential of clonal cell lines derived from adult human trabecular bone. Ann N Y Acad Sci *961:* 73–77.

Palmer G.D., A. Steinert, A. Pascher, E. Gouze, J.N. Gouze, O. Betz, B. Johnstone, C.H. Evans, S.C. Ghivizzani (2005) Gene-induced chondrogenesis of primary mesenchymal stem cells in vitro. Mol Ther *12:* 219–228.

Pate, D.W., B.S. Southerland, D.A. Grande, H.E. Young, P.A. Lucas (1993) Isolation and differentiation of mesenchymal stem cells from rabbit muscle. Clin Res *41:* 374A.

Pechak D.G., M.J. Kujawa, A.I. Caplan (1986a) Morphological and histochemical events during first bone formation in embryonic chick limbs. Bone *7:* 441–458.

Pechak D.G., M.J. Kujawa, A.I. Caplan (1986b) Morphology of bone development and bone remodeling in embryonic chick limbs. Bone *7:* 459–472.

Pereira R.F., K.W. Halford, M.D. O'Hara, D.B. Leeper, B.P. Sokolov, M.D. Pollard, O. Bagasra, D.J. Prockop (1995) Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci USA *92:* 4857–4861.

Pereira R.F., M.D. O'Hara, A.V. Laptev, K.W. Halford, M.D. Pollard, R. Class, D. Simon, K. Livezey, D.J. Prockop (1998) Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc Natl Acad Sci USA *95:* 1142–1147.

Peterson L., M. Brittberg, I. Kiviranta, E.L. Akerlund, A. Lindahl (2002) Autologous chondrocyte transplantation. Biomechanics and long-term durability. Am J Sports Med *30:* 2–12.

Pittenger M.F., A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak (1999) Multilineage potential of adult human mesenchymal stem cells. Science *284:* 143–147.

Prockop D.J., C.A. Gregory, J.L. Spees (2003) One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci USA *100:* 11917–11923.

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

Qu Z., L. Balkir, J.C. van Deutekom, P.D. Robbins, R. Pruchnic, J. Huard (1998) Development of approaches to improve cell survival in myoblast transfer therapy. J Cell Biol *142:* 1257–1267.

Radice M., P. Brun, R. Cortivo, R. Scapinelli, C. Battaliard, G. Abatangelo (2000) Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. J Biomed Mater Res *50:* 101–109.

Redini F., P. Galera, A. Mauviel, G. Loyau, J.P. Pujol (1988) Transforming growth factor beta stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes. FEBS Lett *234:* 172–176.

Reyes M., C.M. Verfaille (1999a) Skeletal, smooth and cardiac muscle differentiation from single adult marrow-derived mesodermal progenitor cells (abstract). Blood *94(suppl 1):* 568a.

Reyes M., C.M. Verfaille (1999b) Turning marrow into brain: generation of glial and neuronal cells from adult bone marrow mesenchymal stem cells (abstract). Blood *94(suppl):* 377a.

Ringe J., C. Kaps, G. Burmester, M. Sittinger (2002) Stem cells for regenerative medicine: advances in the engineering of tissues and organs. Naturwissenschaften *89:* 338–351.

Rogers J.J., H.E. Young, L.R. Adkison, P.A. Lucas, A.C. Black Jr (1995) Differentiation factors induce expression of muscle, fat, cartilage, and bone in a clone of mouse pluripotent mesenchymal stem cells. Am Surg *61:* 231–236.

Romanov Y.A., V.A. Svintsitskaya, V.N. Smirnov (2003) Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells *21:* 105–110.

Saadeh P.B., B. Brent, B.J. Mehrara, D.S. Steinbrech, V. Ting, G.K. Gittes, M.T. Longaker (1999) Human cartilage engineering: chondrocytes extraction, proliferation, and characterization for construct development. Ann Plast Surg *42:* 509–513.

Saito T., J.E. Dennis, D.P. Lennon, R.G. Young, A.I. Caplan (1995) Myogenic expression of mesenchymal stem cells within myotubes of mdx mice in vitro and in vivo. Tissue Eng *4:* 327–342.

Saldanha V., D.A. Grande (2001) Extracellular matrix protein gene expression of bovine chondrocytes cultured on resorbable scaffolds. Biomaterials *21:* 2427–2431.

Seale P., M. Rudnicki (2000) A new look at the origin, function, and 'stem-cell' status of muscle satellite cells. Dev Biol *218:* 115–124.

Sellers R.S., D. Peluso, E.A. Morris (1997) The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. J Bone Joint Surg Am *79:* 1452–1463.

Shah M., D.M. Foreman, M.W. Ferguson (1995) Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J Cell Sci *108:* 985–1002.

Shao X., J.C. Goh, D.W. Hutmacher, E.H. Lee, G. Zigang (2006) Repair of large articular osteochondral defects using hybrid scaffolds and bone marrow-derived mesenchymal stem cells in a rabbit model. Tissue Eng *12:* 1539–1551.

Sherwood J.K., S.L. Riley, R. Palazzolo, S.C. Brown, D.C. Monkhouse, M. Coates, L.G. Griffith, L.K. Landeen, A. Ratcliffe (2002) A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials *23:* 4739–4751.

Steinert A., M. Weber, A. Dimmler, C. Julius, N. Schutze, U. Noth, H. Cramer, J. Eulert, U. Zimmermann, C. Hendrich (2003) Chondrogenic differentiation of mesenchymal progenitor cells encapsulated in ultrahigh-viscosity alginate. J Orthop Res *21:* 1090–1097.

Tanaka H., H. Mizokami, E. Shiigi, H. Murata, H. Ogasa, T. Mine, S. Kawai (2004) Effects of basic fibroblast growth factor on the repair of large osteochondral defects of articular cartilage in rabbits: dose-response effects and long-term outcomes. Tissue Eng *10:* 633–641.

Terada N, T. Hamazaki, M. Oka, M. Hoki, D.M. Mastalerz, Y. Nakano, E.M. Meyer, L. Morel, B.E. Petersen, E.W. Scott (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature *416:* 542–545.

Vacanti C.A., W. Kim, B. Schloo, J. Upton, J.P. Vacanti (1994) Joint resurfacing with cartilage grown in situ from cell-polymer structures. Am J Sports Med *22:* 485–488.

Vacanti C.A., R. Langer, B. Schloo, J.P. Vacanti (1991) Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast Reconstr Surg *88:* 753–759.

Wakitani S., T. Goto, S.J. Pineda, R.G. Young, J.M. Mansour, A.I. Caplan, V.M. Goldberg (1994) Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg *76A:* 579–592.

Wakitani S., T. Kimura, A. Hirooka, T. Ochi, M. Yoneda, N. Yasui, H. Owaki, K. Ono (1989) Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg Br *71:* 74–80.

Wakitani S., T. Saito (1995) Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve *18:* 1471–1426.

Watanabe N., Y. Tezuka, K. Matsuno, S. Miyatani, N. Morimura, M. Yasuda, R. Fujimaki, K. Kuroda, Y. Hiraki, N. Hozumi, K. Tezuka (2003) Suppression of differentiation and proliferation of early chondrogenic cells by Notch. J Bone Miner Metab *21:* 344–352.

Weng Y., Y. Cao, C.A. Silva, M.P. Vacanti, C.A. Vacanti (2001) Tissue-engineered composites of bone and cartilage for mandible condylar reconstruction. J Oral Maxillofac Surg *59:* 185–190.

West D.C., D.M. Shaw, P. Lorenz, N.S. Adzick, M.T. Longaker (1997) Fibrotic healing of adult and late gestation fetal wounds correlates with increased hyaluronidase activity and removal of hyaluronan. Int J Biochem Cell Biol *29:* 201–210.

Whitby D.J., M.W. Ferguson (1991a) Immunohistochemical localization of growth factors in fetal wound healing. Dev Biol *147:* 207–215.

Whitby D.J., M.W. Ferguson (1991b) The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development *112:* 651–668.

Wickham M.Q., G.R. Erickson, J.M. Gimble, T.P. Vail, F. Guilak (2003) Multipotent stromal stem cells derived from the infrapatellar fat pad of the knee. Clin Orthop *412:* 196–212.

Williams J.T., S.S. Southerland, J. Souza, A.F. Calcutt, R.G. Cartledge (1999) Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes. Am Surg *65;*22–26.

Williams C.G., T.K. Kim, A. Taboas, A. Malik, P. Manson, J. Elisseeff (2003) In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. Tissue Eng *9:* 679–688.

Winter A., S. Breit, D. Parsch, K. Benz, E. Steck, H. Hauner, R.M. Weber, V. Ewerbeck, W. Richter (2003) Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum *48:* 418–429.

Worster A.A., B.D. Brower-Toland, L.A. Fortier, S.J. Bent, J. Williams, A.J. Nixon (2001) Chondrocytic differentiation of mesenchymal stem cells sequentially exposed to transforming growth factor-beta1 in monolayer and insulin-like growth factor-I in a three-dimensional matrix. J Orthop Res *19:* 738–49.

Worster A.A., A.J. Nixon, B.D. Brower-Toland, J. Williams (2000) Effect of transforming growth factor beta1 on chondrogenic differentiation of cultured equine mesenchymal stem cells. Am J Vet Res *61:* 1003–1010.

Yajima Y., M. Sato, M. Sumida, S. Kawashima (2003) Mechanism of adult primitive mesenchymal ST-13 preadipocyte differentiation. Endocrinology *144:* 2559–2565.

Ying Q.L., J. Nichols, E.P. Evans, A.G. Smith (2002) Changing potency by spontaneous fusion. Nature *416:* 545–547.

Young, H.E., E.M. Ceballos, J.C. Smith, M.L. Mancini, R.P. Wright, B.L. Ragan, I. Bushell, P.A. Lucas (1993) Pluripotent mesenchymal stem cells reside within avian connective tissue matrices. In Vitro Cell Dev Biol Anim *29*A*:* 723–736.

Young H.E., T.A. Steele, R.A. Bray, K. Detmer, L.W. Blake, P.W. Lucas, A.C. Black (1999) Human pluripotent and progenitor cells display cell surface cluster differentiation markers CD10, CD13, CD56 and MHC class-I. Proc Soc Exp Biol Med *221:* 63–71.

Young H.E., T.A. Steele, R.A. Bray, J. Hudson, J.A. Floyd, K. Hawkins, K. Thomas, T. Austin, C. Edwards, J. Cuzzourt, M. Duenzl, P.A. Lucas, A.C. Black Jr (2001) Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. Anat Rec *264:* 51–62.

Young R.G., D.L. Butler, W. Weber, A.I. Caplan, S.L. Gordon, D.J. Fink (1998) Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res *16:* 406–413.

Zhao G.Q., X. Zhou, H. Eberspaecher, M. Solursh, B. de Crombrugghe (1993) Cartilage homeoprotein 1, a homeoprotein selectively expressed in chondrocytes. Proc Natl Acad Sci USA *90:* 8633–8637.

Zuk P.A., M. Zhu, H. Mizuno, J. Huang, JW Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng *7:* 211: 228.

Zvaifler N.J., L. Marinova-Mutafchieva, G. Adams, C.J. Edwards, J. Moss, J.A. Burger, R.N. Maini (2000) Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res *2:* 477–488.