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Review

Mesenchymal stem cells: clinical applications and biological characterization

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Abstract

Mesenchymal stem cells (MSCs) have been isolated from bone marrow, periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle and deciduous teeth. These cells have the capacity to differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle. A great deal has been learned in recent years about the isolation and characterization of MSCs, and control of their differentiation. These cells have generated a great deal of interest because of their potential use in regenerative medicine and tissue engineering and there are some dramatic examples, derived from both pre-clinical and clinical studies, that illustrate their therapeutic value. This review summarizes recent findings regarding the potential clinical use of MSCs in cardiovascular, neural and orthopaedic applications. As new methods are developed, there are several aspects to the implanted cell–host interaction that need to be addressed before we can fully understand the underlying mechanisms. These include the host immune response to implanted cells, the homing mechanisms that guide delivered cells to a site of injury and the differentiation in vivo of implanted cells under the influence of local signals.

Keywords: Mesenchymal stem cell; Tissue engineering; Allogeneic cell therapy; Bone marrow; Cell engraftment

Contents

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1. Introduction

Adult human stem cells have been isolated from a wide variety of tissues and, in general, their differentiation potential may reflect the local environment. They lack tissue-specific characteristics but under the influence of appropriate signals can differentiate into specialized cells with a phenotype distinct from that of the precursor. It may be that stem cells in adult tissues are reservoirs of reparative cells, ready to mobilize and differentiate in response to wound signals or disease conditions. Little information is currently available about the biology of endogenous stem cell populations in adults and their precise role in tissue repair or regeneration. This may be due in part to the lack of useful cell-specific markers. What is clear, however, is the ease with which these cells can be isolated and expanded in culture through many generations while retaining the capacity to differentiate. Recent progress in the isolation and characterization of these cells has led to the development and testing of therapeutic strategies in a variety of clinical applications.

Mesenchymal stem cells (MSCs), which reside within the stromal compartment of bone marrow were first identified in the pioneering studies of Friedenstein and Petrakova (1966), who isolated bone-forming progenitor cells from rat marrow. They have the capacity to differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle. In addition, they play a role in providing the stromal support system for haematopoietic stem cells in the marrow. MSCs represent a very small fraction, 0.001–0.01% of the total population of nucleated cells in marrow (Pittenger et al., 1999). However, they can be isolated and expanded with high efficiency, and induced to differentiate to multiple lineages under defined culture conditions. These cells have generated a great deal of interest because of their potential use in regenerative medicine and tissue engineering. Both pre-clinical and clinical studies offer dramatic examples that illustrate the therapeutic value of MSCs. While the therapeutic testing of these cells has progressed well, there are still many questions to be addressed concerning the role of endogenous populations of stem cells in the adult, and the function of various stem cell niches. In addition, there are several aspects to the implanted cell–host interaction that need to be addressed as we attempt to understand the mechanisms underlying these therapies. Firstly, host responses to allogeneic MSC therapy need to be defined. Secondly, little is known about the mechanisms that direct homing and engraftment of implanted cells and thirdly, the response of MSCs to local differentiation signals in vivo has not been clarified. This review will describe the characteristics of MSCs, as well as some of the many clinical applications that are currently being evaluated. In the context of cellular therapies, recent information regarding implanted cell–host interactions is discussed.

2. Isolation and characterization of adult MSCs

MSCs are generally isolated from an aspirate of bone marrow harvested from the superior iliac crest of the pelvis in humans (Digirolamo et al., 1999; Pittenger et al., 1999). MSCs have also been isolated from the tibial and femoral marrow compartments (Murphy et al., 2002; Oreffo, Bord, & Triffitt, 1988), and thoracic and lumbar spine (D'Ippolito, Schiller, Ricordi, Roos, & Howard, 1999). In larger animals (Kadiyala, Young, Thiede, & Bruder, 1997; Murphy, 2003; Ringe et al., 2002; Shake et al., 2002) marrow is often obtained from the same site, and in rodents it is generally harvested from the mid-diaphysis of the tibia or femur. While they represent a minor fraction of the total nucleated cell population in marrow, MSCs can be plated and enriched using standard cell culture techniques. Frequently, the whole marrow sample is subjected to fractionation on a density gradient solution such as Percoll, after which the cells are plated at densities ranging from 1×10^4 cells/cm^2 to 0.4×10^6 cells/cm^2 (Lodie et al., 2002; McBride, Gaupp, & Phinney, 2003; Pittenger et al., 1999). Cells are generally cultured in basal medium such as Dulbecco's modified Eagle's medium (high glucose) in the presence of 10% fetal bovine serum (FBS) (Pittenger et al., 1999). MSCs in culture have a fibroblastic morphology and adhere to the tissue culture substrate (Fig. 1). Primary cultures are usually maintained for 12–16 days, during which time the nonadherent haematopoietic cell fraction is depleted. The addition of growth factor supplements such as fibroblast growth factor-2 (FGF-2) to primary cultures of human MSCs was reported by Martin, Muraglia, Campanile, Cancedda, and Quarto (1997) to lead to

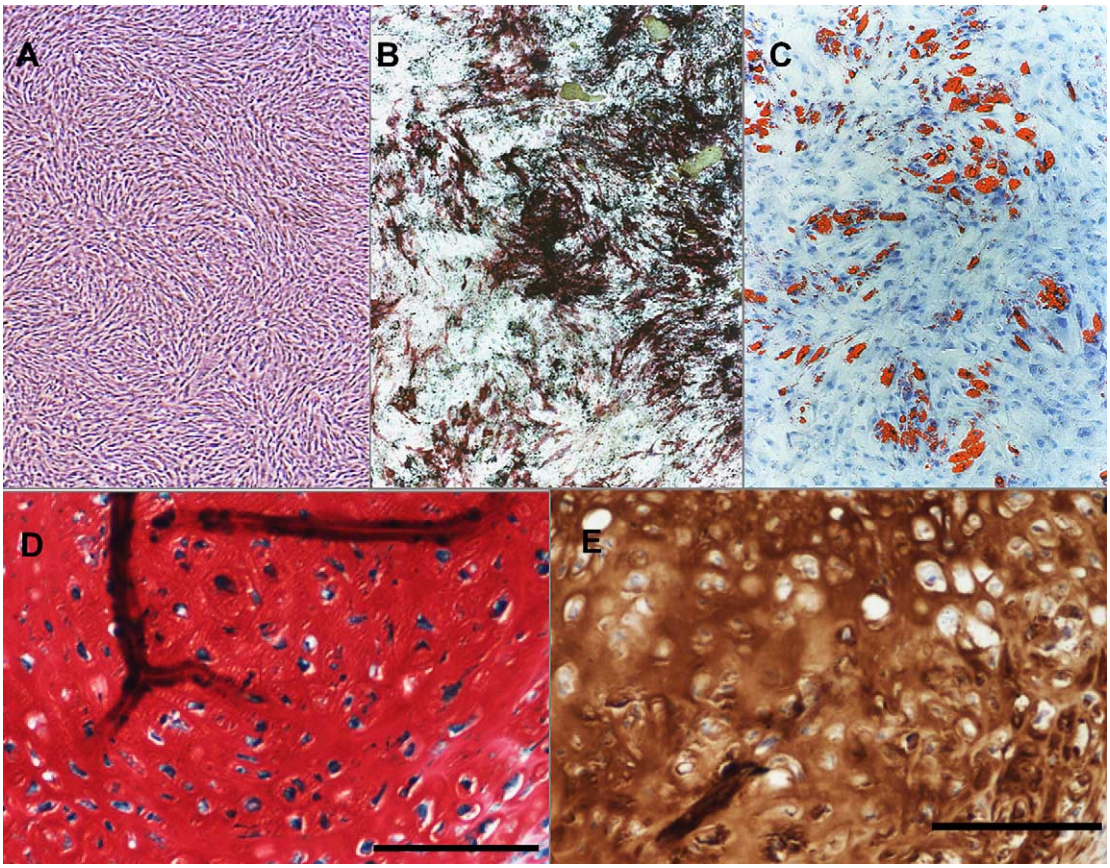

Fig. 1. Undifferentiated MSCs grown in monolayer culture (A) and after differentiation along the osteogenic (B), adipogenic (C) and chondrogenic pathways (D) and (E). Cell differentiation is these cultures was observed following staining with von Kossa (B), Nile red O (C), Safranin O (D) and by immunostaining with an antibody specific for type II collagen (E).

an enhanced osteogenic potential. Although this effect was not observed with murine MSCs (Phinney, Kopen, Isaacson, & Prockop, 1999) the addition of FGF-2 is associated with the selection of cells with increased telomere length (Bianchi et al., 2003). Optimal expansion of MSCs from marrow requires the pre-selection of FBS (Digirolamo et al., 1999; Pittenger et al., 1999). As MSCs are expanded in large-scale culture for human applications it will be important to identify defined growth media, without or with reduced FBS, to ensure more reproducible culture techniques and enhanced safety.

The property of plastic adherence itself is not sufficient to allow for the purification of MSCs, at least in the case of cells from mouse marrow. Phinney et al. (1999) reported substantial variation in the cell numbers and levels of expression of alkaline phosphatase in MSCs prepared from different strains of inbred mice. They also noted the persistence of $CD45^+$ and $CD11b^+$ pre-B-cell progenitors and granulocytic and monocytic precursors in these cultures. Nonetheless a fraction of these adherent cells represented true MSCs, as shown by osteogenic and adipogenic activity. These observations lead to the development of elegant methods involving CD34/CD45/CD11b immunodepletion to generate purified MSC preparations (Ortiz et al., 2003).

Further characterization of the conditions required for culturing progenitor cells from murine and rat bone marrow was performed by Jiang et al. (2002). These authors found that murine, but not human, cells required leukemia inhibitory factor (LIF) for expansion.

Further, they reported that rat cells required epidermal growth factor (EGF) and platelet-derived growth factor-BB (PDGF-BB) in addition to LIF, conditions similar to those required for embryonic stem cells. The cells, referred to as multipotent adult progenitor cells (MAPCs) were found to have the capacity to differentiate into cells with mesodermal, neuroectodermal and endodermal characteristics in vitro, and, when injected into an early blastocyst, gave rise to most somatic cell types. These observations indicated that the plasticity of cell populations in the marrow is greater than previously understood. In an attempt to understand the effect of different culture protocols on cell phenotype Lodie et al. (2002) carried out a systematic comparison of cells isolated from human marrow and cultured in either 10% FBS, 0.5% FBS supplemented with FGF-2, or 2% FBS supplemented with EGF and PDGF-BB. These authors reported little functional difference among the cells isolated by any protocol, in terms of surface marker expression and differentiation potential. Taken together, these results illustrate the complexity of subpopulations of bone marrow cells, the need to evaluate isolation techniques with care, and the need to identify new cell-specific markers.

3. Surface markers

Considerable effort has been expended on the identification of specific surface markers for selection, detection and testing of MSC preparations. Several monoclonal antibodies have been raised in an effort to provide reagents for the characterization and isolation of human MSCs. For instance, Stro-1 was identified as an antibody that reacted with non-haematopoietic progenitor bone marrow stromal cells (Simmons & Torok-Storb, 1991). The SB-10 antibody was shown to be reactive with an antigen present on undifferentiated MSCs, which disappeared once the cells embarked upon the osteogenic pathway and began to express cell surface alkaline phosphatase (Bruder, Horowitz, Mosca, & Haynesworth, 1997). The specific SB-10 antigen was identified as CD166 (activated leukocyte-cell adhesion molecule, ALCAM) (Bruder, Ricalton, et al., 1998), which may play a role in the progression of osteogenic differentiation, although the precise mechanism remains unclear. The SH-2 antibody (Haynesworth, Baber, & Caplan, 1992), also raised against human MSCs, reacts with an epitope present on the transforming growth factor-beta (TGF-β) receptor endoglin (CD105) (Barry, Boynton, Haynesworth, Murphy, & Zaia, 1999). This antibody has been used in immunomagnetic selection methods for human MSCs although CD105 is dominantly associated with endothelial cells (Cheifetz et al., 1992). Both the SH-3 and SH-4 antibodies (Haynesworth et al., 1992) apparently recognize distinct epitopes (Fig. 2) on the membrane-bound ecto-5′-nucleotidase (CD73) (Barry, Boynton, Murphy, Haynesworth, & Zaia, 2001). As was reported with SH-2, these antibodies do not react with haematopoietic cells or with osteocytes (Haynesworth et al., 1992).

The identification of the SB-10, SH-2, SH-3 and SH-4 antigens was carried out by immunoprecipitation of the antigens from a solubilized human MSC membrane fraction and purification by gel electrophoresis. Peptides derived from in-gel proteolysis were characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Barry et al., 1999, Barry, Boynton, Murphy, et al., 2001). Analysis of the peptide mass values facilitated identification of the antigen and this was confirmed by amino acid sequence analysis. The results were further confirmed by immunoblotting. This approach has been very successful in identifying these antigens and can certainly be extended to identify other molecules of interest, such as Stro-1. Unfortunately, all of the antigens identified by this method are expressed on a variety of other cell types and do not provide the specificity needed to extend these studies to in vivo evaluation of tissue-specific stem cells. This remains an obstacle in studying proliferation, mobilization and homing mechanisms of endogenous and implanted cells.

Much valuable information can also be gained from a systematic analysis of cell surface molecules on MSCs. Majumdar et al. (2003) determined that MSCs express a large spectrum of cell adhesion molecules of potential importance in cell binding and homing interactions. MSCs exhibit high expression of integrins α1, α5 and β1, low expression of α2, α3, α6, αV, β2 and β4, and no expression of α4, αL and β2. Human MSCs also express HLA-ABC and not HLA-DR but the latter is upregulated following treatment with interferon-γ. The results of this analysis point to several potentially key interactions in vivo between MSCs and other cell types. For instance, MSCs

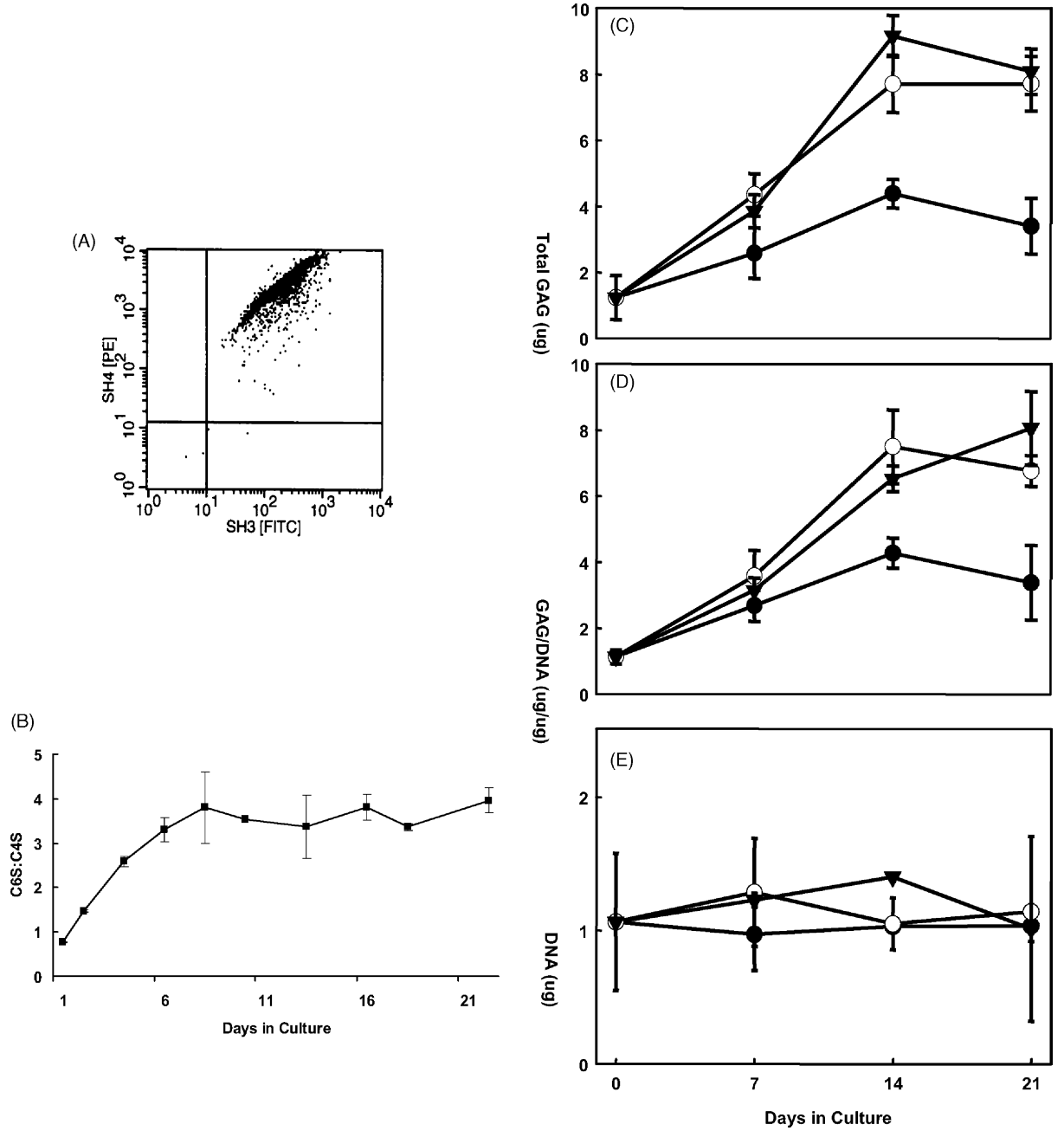

Fig. 2. Preparation and chondrogenic differentiation of MSCS derived from human bone marrow. (A) Analysis of cell preparations for expression of the surface antigens SH-3 and SH-4 by flow cytometry. (B) Changes in the ratio of 6- to 4-sulfated chondroitin sulfate during the early stages of chondrogenic differentiation (C)–(E) Accumulation of glycosaminoglycan in human MSC pellet cultures maintained in the presence of TGF-β1 (closed circles), β2 (open circles) or β3 (triangles) over 21 days in culture. The total amount of extracted DMMB-reactive glycosaminoglycan per pellet is shown (C) as well as the glycosaminoglycan synthesis per cell expressed as the amount of glycosaminoglycan as a function of DNA content in each pellet (D). Changes in the total DNA content of pellets are also shown (E). Data from Barry et al. (2001), reproduced with permission.

bound activated T lymphocytes with higher affinity than resting cells and in turn bound lymphocyte cell lines in preference to B lymphocytes or myeloid lineage cells (Le Blanc, Tammik, Rosendahl, Zetterberg, & Ringden, 2003). Expression of specific integrins by MSCs could also play a role in homing to sites of injury and binding to specific matrix molecules in the manner suggested by Bogenrieder and Herlyn (2003).

4. Tissue-specific stem cells

Recent reports have provided substantial new insights into stem cell populations in a variety of adult tissues, raising new questions about tissue-specific niches, stem cell mobilization and local differentiation cues. In addition to marrow, other sources of stem cells with mesenchymal potential include periosteum (Fukumoto et al., 2003; Nakahara et al., 1990; Zarnett & Salter, 1989; O'Driscoll, Saris, Ito, & Fitzimmons, 2001), trabecular bone (Noth et al., 2002; Sottile, Halleux, Bassilana, Keller, & Seuwen, 2002; Tuli et al., 2003) adipose tissue (De Ugarte et al., 2003; Dragoo et al., 2003; Gronthos et al., 2001; Wickham, Erickson, Gimble, Vail, & Guilak, 2003), synovium (De Bari, Dell'Accio, Tylzanowski, & Luyten, 2001), skeletal muscle (Jankowski, Deasy, & Huard, 2002), lung (Noort et al., 2002) and deciduous teeth (Miura et al., 2003). In all cases the cells have been shown to differentiate along several defined pathways. For instance, De Bari et al. (2001) showed that MSCs isolated from the synovium as an adherent cell population were capable of differentiation into chondrocytes, osteocytes and adipocytes. They also showed that these cells were capable of contributing to skeletal muscle regeneration in a nude mouse model and restored expression of dystrophin in the sarcolemma in dystrophic muscle of immunosuppressed *mdx* mice (De Bari et al., 2003). Stem cells from adipose tissue, variously referred to as processed lipoaspirate (PLA) cells (Dragoo et al., 2003) and adipose-derived adult stem (ADAS) cells (Gimble, 2003), have been shown to have similar differentiation potential. De Ugarte et al. (2003) suggest that there is little difference between cells from marrow and fat in terms of yield, growth kinetics, cell senescence, multi-lineage differentiation capacity, and gene transduction efficiency. The utility of these cells in therapeutic applications may then depend on the availability of tissue specimens and the ease of in vitro expansion.

5. Differentiation

The differentiation of MSCs into bone, cartilage and fat has been described and characterized by multiple laboratories (Barry, Boynton, Liu, & Murphy, 2001; Bruder, Kraus, Goldberg, & Kadiyala, 1998; Bruder, Kurth, et al., 1998; Digirolamo et al., 1999; Johnstone, Hering, Caplan, Goldberg, & Yoo, 1998; Muraglia, Cancedda, & Quarto, 2000; Pittenger et al., 1999). Osteogenic activation requires the presence of β-glycerol-phosphate, ascorbic acid-2-phosphate, dexamethasone and fetal bovine serum (Fig. 1B). When cultured in monolayer in the presence of these supplements the cells acquire an osteoblastic morphology with upregulation of alkaline phosphatase activity and deposition of a calcium-rich mineralized extracellular matrix.

Chondrogenic differentiation occurs when MSCs are grown under conditions that include (1) a three-dimensional culture format, (2) a serum-free nutrient medium and (3) the addition of a member of the TGF-β super-family (Fig. 1D and E). When these conditions are met the cells rapidly lose their fibroblastic morphology and begin to initiate expression of a number of cartilage-specific extracellular matrix components. This involves rapid biosynthesis of glycosaminoglycan (Fig. 2) and is accompanied by a dramatic alteration in cell morphology. During the early stage of chondrogenesis there is also a progressive change in the pattern of sulfation of chondroitin sulfate. In early cultures the ratio of 4- and 6-sulfated species is approximately equal and after 8 days there is a fourfold higher level of chondroitin-6-sulfate. Similar changes in sulfation pattern also occur during maturation of human articular cartilage (Bayliss, 1990). TGF-β1, 2 and 3 have the ability to induce this response and TGF-β2 and β3 are more effective than β1 in promoting chondrogenesis (Barry, Boynton, Liu, et al., 2001). This may relate to the abundance of these isoforms in bone, and their role in fracture callus formation and wound healing. During differentiation in the presence of TGF-β3 MSCs synthesize aggrecan, link protein, fibromodulin, cartilage oligomeric matrix protein, decorin, type II

collagen and chondroadherin, all components of the normal articular cartilage matrix (Barry, Boynton, Liu, et al., 2001). It appears that versican, fibromodulin and decorin form the earliest matrix components during chondrogenesis with aggrecan and biglycan being incorporated at a later time. Both fibromodulin and decorin play a role in the regulation of collagen fibrillogenesis and fibromodulin is expressed during

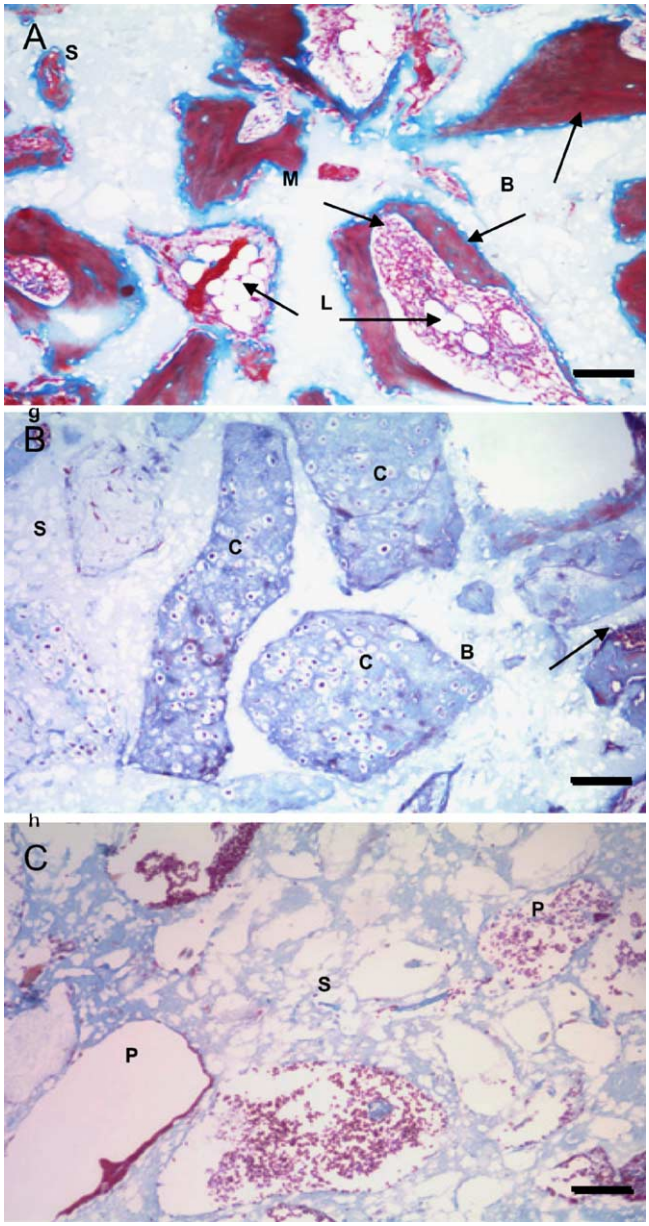

Fig. 3. Differentiation of caprine MSCs, loaded on a fibronectin-coated hydroxyapatite/tricalcium phosphate scaffold and implanted subcutaneously in nude mice. (A) (B) Areas of differentiation indicated by arrows, including bone (b), fat (l), marrow (m) and cartilage (c) were found after 6 weeks. (C), Unloaded scaffolds showed no evidence of mesenchymal differentiation and the pores (p) were empty or infiltrated with host hematopoietic cells. Bars represent 100 μM.

early limb chondrogenesis in mice, where it precedes joint cavitation (Archer, Morrison, Bayliss, & Ferguson, 1996; Murphy, Heinegard, McIntosh, Sterchi, & Barry, 1999). Both molecules also play a role in TGF-β binding and this may influence their early expression in the process of matrix assembly.

In addition to these in vitro systems differentiation of human MSCs in vivo has been observed when the cells are loaded on a fibronectin-coated hydroxyapatite cube and implanted subcutaneously in nude mice (Fig. 3A and B). In this type of application areas of bone and cartilage formation are observed as well as lipid-containing elements. Unloaded cubes, in contrast, show no evidence of differentiation (Fig. 3C). That these areas of differentiation arise from the implanted cells can be confirmed by labeling studies.

MSCs cultured in monolayer in the presence of isobutylmethylxanthine become adipocytes with the production of large lipid-filled vacuoles (Fig. 1C). Adipogenic differentiation of MSCs is induced by the nuclear receptor and transcription factor, peroxisome proliferator-activated receptor-gamma (PPAR-γ) as well as fatty acid synthetase. Both IL-1 and TNF-α suppress adipogenesis, and this is mediated through NF-κB activated by the TAK1/TAB1/NF-κB induction kinase cascade (Suzawa et al., 2003). The effect of inhibition by these cytokines is to direct differentiation towards osteogenesis.

5-Azacytidine induction of myogenesis was reported by Taylor and Jones for embryonic and adult cells (Taylor & Jones, 1982) and by Wakitani et al. for rat stromal cells (Wakitani, Saito, & Caplan, 1995). Phinney et al. found that exposure of mouse MSCs to amphotericin B, but not 5-azacytidine, resulted in the formation of multinucleated fibers resembling myotubes (Phinney et al., 1999).

Induction of mouse, rat and human MSCs along the neurogenic pathway has been described (Deng, Obrocka, Fischer, & Prockop, 2001; Kohyama et al., 2001; Sanchez-Ramos, 2002; Sanchez-Ramos et al., 2000; Woodbury, Reynolds, & Black, 2002; Woodbury, Schwarz, Prockop, Black, 2000). Treatment of MSCs with isobutylmethylxanthine and dibutyryl cyclic AMP induced expression of early markers of neuronal differentiation (Deng et al., 2001), as did EGF or brain-derived neurotrophic factor (BDNF) in a neuronal growth medium (Sanchez-Ramos et al., 2000). Transdifferentiation of mouse marrow stromal-derived mature osteoblasts and the stromal cells themselves to a neural phenotype was achieved by treatment with 5-azacytidine in the presence of nerve growth factor, BDNF and neurotrophin-3 (Kohyama et al., 2001). Treatment of rat cells with DMSO/butylated hydroxyanisole in the presence of bFGF and PDGF was also successful in inducing a neural phenotype (Woodbury et al., 2000, 2002).

The potential role of MSCs in blood vessel formation has also been evaluated. Enhanced neovascularigenesis has been associated with regeneration of infracted myocardium by bone marrow-derived stem cells (Fuchs et al., 2001; Jackson et al., 2001; Kobayashi et al., 2000; Orlic et al., 2001; Tomita et al., 1999). Autologous bone marrow stromal cells also improve blood flow in a chronic limb ischemia model (Al-Khaldi, Al-Sabti, Galipeau, & Lachapelle, 2003). More recently, mesenchymal progenitor cells as well as MSCs were shown to differentiate to an endothelial phenotype and enhance vascularization (Davani et al., 2003; Gojo et al., 2003). In the latter study MSCs differentiated to cardiomyocytes, endothelial cells, pericytes and smooth muscle cells after direct injection into the adult heart (Gojo et al., 2003).

6. Therapeutic applications

Stem cell therapy involves the transplantation of autologous or allogeneic stem cells into patients, either through local delivery or systemic infusion. There is a precedent in haematopoietic stem cell transplantation, which has been used for some years in the treatment of leukemia and other cancers (Tabbara, Zimmerman, Morgan, & Nahleh, 2002). Some striking examples of the therapeutic use of marrow-derived MSCs have been reported recently. These address a broad spectrum of indications, including cardiovascular repair, treatment of lung fibrosis, spinal cord injury and bone and cartilage repair. Orlic et al. (2001) showed that locally delivered bone marrow cells can generate de novo myocardium, indicating that stem cell therapy can be useful in treating coronary artery disease. Stamm et al. (2003) demonstrated the practical utility of this approach in a study involving the delivery of bone marrow cells into the infarct zone in patients

following myocardial infarction. The result of this treatment was a dramatic improvement in global heart function. Deb et al. (2003) have also shown engraftment of bone marrow-derived cardiomyocytes in the adult heart following bone marrow transplantation. Saito, Kuang, Bittira, Al-Khaldi, and Chiu (2002) demonstrated that MSCs are tolerated in a xenogeneic environment while retaining their ability to be recruited to the injured myocardium and undergo differentiation to a cardiac phenotype.

In vivo differentiation of MSCs to a skeletal muscle phenotype has also been demonstrated. Gussoni et al. (1999) showed that murine MSCs, injected into the quadriceps muscle of *mdx* mice, expressed dystrophin in association with the muscle fiber sarcolemma, and pointed towards a potential therapy for muscular dystrophy. Toma, Pittenger, Cahill, Byrne, and Kessler (2002) injected β-galactosidase-expressing human MSCs into the left ventricle of CB17 SCID/*beige* adult mice, and found the labeled cells dispersed throughout the myocardium and expressing desmin, cardiac-specific troponin T, α-actinin and phospholamban, all indicative of differentiation of the engrafted cells to a mature myocardial phenotype. MSCs have also been shown by Ortiz et al. (2003) to engraft at high levels in lung tissue following exposure to bleomycin, and to offer protection against bleomycin-induced lung injury, including inflammation and collagen deposition. These observations have broad implications in the area of lung disease associated with environmental damage.

Stem cells with the ability to differentiate into neurons, astrocytes and oligodendrocytes have been isolated from rat spinal cord (Shihabuddin, Horner, Ray, & Gage, 2000), and implantation of neural stem cells in an adult rat model of spinal cord injury resulted in long-term functional improvement (Teng et al., 2002). Embryonic stem cells are capable of forming dopamine neurons in an animal model of Parkinson's Disease (Kim et al., 2002). The ability of bone marrow-derived stem cells to differentiate into neural lineages in vitro and after transplantation in both mice and rats has been evaluated by Sanchez-Ramos (2002) leading to the conclusion that they may be useful in the treatment of stroke, traumatic injury and Parkinson's Disease. Furthermore, it was recently demonstrated by Mezey that adult human bone marrow cells could enter the brain and generate neurons after transplantation (Mezey et al., 2003). These, and other equally dramatic observations underlie much of the current excitement and optimism about the use of stem cell therapy in the treatment of neuronal injury.

In the area of orthopedic medicine there are also many examples of applications involving local delivery of marrow stem cells. These include spine fusion (Muschler et al., 2003), the repair of segmental bone defects (Quarto et al., 2001) and craniotomy defects (Krebsbach, Mankani, Satomura, Kuznetsov, & Robey, 1998). Similar approaches have also been described for the repair of focal defects in articular cartilage (Ponticiello, Schinagl, Kadiyala, & Barry, 2000; Solchaga et al., 2002) and tendon (Young et al., 1998). In an animal model of osteoarthritis involving injury to the meniscus delivery of stem cells by intraarticular injection resulted in engraftment of those cells on the meniscus, fat pad and synovium (Fig. 4) with regeneration of meniscal tissue and protection of the cartilage (Murphy, Fink, Hunziker, & Barry, 2003). The chondroprotective effects seen in these studies apparently derive from the regenerated meniscus since there is no evidence of direct engraftment of the implanted cells on the fibrillated cartilage (Fig. 4). Table 1 summarizes some of the therapeutic applications of MSCs in animal models.

There is accumulating evidence of the hypoimmunogenic nature of MSCs and this has broad implications in terms of allogeneic therapy, or the delivery to a recipient of cells derived from an unmatched donor. There are several reports describing the clinical use of allogeneic donor-mismatched cells with little evidence of host immune rejection or GVHD. For example, allogeneic bone marrow transplantation in children with Osteogenesis Imperfecta resulted in engraftment of donor-derived MSCs and an increase in new bone formation (Horwitz et al., 1999). Infusion of allogeneic MSCs into patients with Hurler's syndrome or metachromatic leukodystrophy showed no evidence of alloreactive T cells and no incidence of graft-versus-host disease (GVHD) (Koc et al., 2002). Engraftment of allogeneic MSCs has also been demonstrated in a patient with severe idiopathic aplastic anaemia with improvement of marrow stromal function (Fouillard et al., 2003). Table 2 summarizes the current status of therapeutic applications of MSCs.

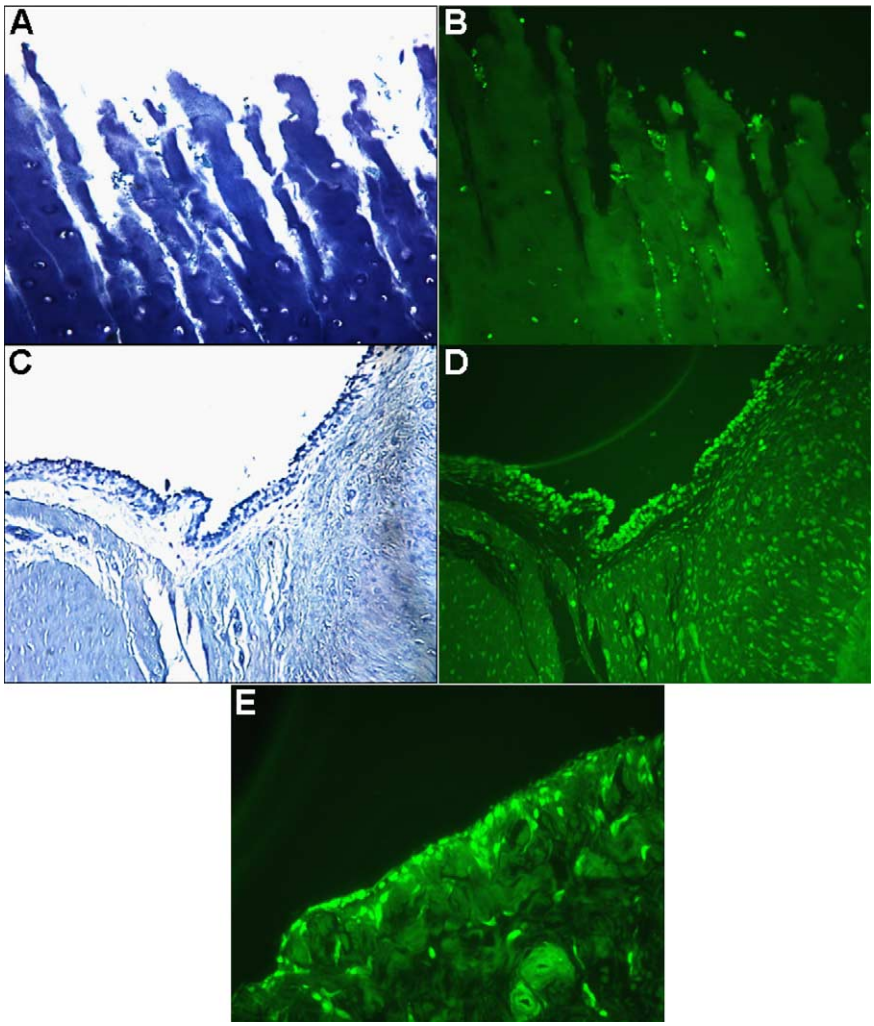

Fig. 4. Engraftment of MSCs in the caprine osteoarthritic joint. GFP-expressing MSCs delivered to the caprine knee joint following medial meniscectomy did not show engraftment at the fibrillated cartilage surface. Serial sections were stained with Toluidine blue (A) and viewed by fluorescence microscopy (B). In contrast the synovial lining in the intercondylar area showed evidence of marked engraftment. Sections were stained with Toluidine blue (C) and viewed under fluorescent microscopy (D), (E). Original magnification 200× (A)–(D) and 400× (E).

7. Implanted cell–host interactions

The question of the host response to implanted MSCs is critical and receiving attention as these cells are being considered in a variety of clinical applications. There are several aspects to the implanted cell–host interaction that need to be addressed as we attempt to understand the mechanisms underlying stem cell therapies. These are (1) the host immune response to implanted cells, (2) the homing mechanisms that guide delivered cells to a site of injury and (3) differentiation of implanted cells under the influence of local signals.

7.1. Host immune response

This topic has been the subject of some recent studies which have demonstrated that MSCs are capable of suppressing mixed lymphocyte reactions (MLRs) involving autologous or allogeneic T cells or

Table 1
Therapeutic application of MSCs in animal models

Indication	Animal model/route of delivery	MSC source	Result	Reference
Myocardial infarction	Mouse/direct injection	$Lin^{-}c\text{-}kit^{+}$ bone marrow cells	De novo myocardium	Orlic et al. (2001)
Myocardial infarction	Immunocompetent Lewis rats/IV injection 1 week pre-infarction	C57B1/6 mouse MSCs	Donor-derived cardiomyocytes and angiogenesis	Saito et al. (2002)
Muscular dystrophy	*mdx* mouse/IV injection	Normal mouse muscle-derived MSCs	Partial restoration of dystrophin expression in affected muscle	Gussoni et al. (1999)
Lung fibrosis	Bleomycin (BLM)-sensitive C57BL/6 mouse/IV injection	BLM-resistant BALB/c mouse	Reduced inflammation and collagen deposition	Ortiz et al. (2003)
Spine fusion	Canine bone marrow-derived cells/cancellous bone matrix	Autologous	Improved bone grafting	Muschler et al. (2003)
Segmental bone defects	Athymic rat/ceramic carrier	Human MSCs	Enhanced bone formation and improved biomechanics	Bruder, Kurth, et al. (1998)
	Canine/ceramic carrier	Autologous MSCs	Enhanced bone formation	Bruder, Kraus, et al. (1998)
	Canine/ceramic carrier	Allogeneic MSCs	Enhanced bone formation	Rombouts & Ploemacher (2003)
Craniotomy defect	Immunocompromised mouse/gelatin sponge	Alloplastic transgenic mouse marrow stromal cells	>99% repair within 2 weeks	Krebsbach et al. (1998)
Tendon defect	Rabbit/contracted collagen gel	Autologous MSCs	Improved tendon biomechanics, structure and function	Young et al. (1998)
Meniscus	Caprine/intraarticular injection	Autologous MSCs	Enhanced tissue formation and reduced osteoarthritis	Murphy et al. (2003)

Table 2
Therapeutic application of MSCs

Indication	Source/route of delivery	Result	Reference
Myocardial infarction	AC133+ bone marrow cells/direct injection	Function enhanced in 4/6 and tissue perfusion improved strikingly in 5/6 patients	Al-Khaldi et al. (2003)
Osteogenesis imperfecta	Allogeneic bone marrow transplantation/infusion	New dense bone formation and engraftment of donor-derived cells in three patients	Horwitz et al. (1999)
Large bone defect	Autologous bone marrow stromal cells/scaffold	Enhanced bone repair in 1/1 patient	Quarto et al. (2001)
Metachromatic leukodystrophy (MLD) and Hurler syndrome	Allogeneic MSCs/infusion	Significant improvements in nerve conduction velocities in 4/6 MLD patients; no GVDH	Koc et al. (2002)
Severe idiopathic aplastic anemia	Allogeneic MSCs/infusion	Improved stroma in 1/1 patient	Fouillard et al. (2003)

dendritic cells. Di Nicola et al. (2002) found that human T-cell proliferation, stimulated by the addition of irradiated allogeneic peripheral blood lymphocytes, dendritic cells or phytohaemaglutinin, was greatly suppressed when the cultures also contained MSCs. They also found that this effect was reversed by the addition of monoclonal antibodies that had a neutralizing effect on TGF-β1 and hepatocyte growth factor (HGF). This effect represents a specific suppression of MLR and is not due to apoptosis. Indeed a recent study by Kuroiwa et al. (2001) shows that, in a murine model of allogeneic bone marrow transplantation, treatment with rhHGF strongly reduces the incidence of GVHD. More recently, Tse et al. found that the suppressive activity of MSCs on T-cell proliferation could not be accounted for by production of interleukin-10, TGF-β1 or prostaglandin E2 (Tse, Pendleton, Beyer, Egalka, & Guinan, 2003). These authors suggest that MSCs actively inhibit T-cell proliferation. Krampera et al. also suggest that MSCs inhibited both naive and memory T-cell responses and may function to physically hinder T-cell contact with antigen presenting cells in a noncognate fashion (Krampera et al., 2003). Djouad et al. postulate that a soluble factor released by splenocyte-activated MSCs is involved in the immunosuppression and suggest that CD8+ regulatory cells are involved in the inhibition of allogeneic lymphocyte proliferation by MSCs (Djouad et al., 2003).

Of course, these issues are central to the use of allogeneic MSCs in therapeutic applications. There is some convincing evidence, as discussed above, that human MSCs, by virtue of their distinct immunophenotype, associated with the absence of HLA Class II expression, as well as low expression of co-stimulatory molecules (Majumdar et al., 2003), may be nonimmunogenic or hypoimmunogenic. HLA Class II expression is also absent from the surface of differentiated MSCs and these cells did not elicit an alloreactive lymphocyte proliferative response (Le Blanc et al., 2003). The use of allogeneic MSCs in therapeutic applications has many advantages, not the least of which is delivery in an acute setting, for instance following myocardial infarction. The disadvantage of an allogeneic approach relates to the potential risk of disease transmission from donor to recipient.

7.2. Homing mechanisms

Transplantation of human MSCs into fetal sheep resulted in long-term engraftment of the cells to various tissues, even after development of immunocompetence (Liechty et al., 2000). It also seems clear that MSCs, when delivered by intravenous infusion, are capable of specific migration to a site of injury. This extraordinary ability of implanted cells to seek out the site of tissue damage has been demonstrated in the case of bone fracture, myocardial infarction (Shake et al., 2002) and ischaemic cerebral injury (Wang et al., 2002). In addition, MSCs, delivered as a free suspension by intraarticular injection to the knee joint

following traumatic injury, are capable of specific engraftment to and repair of damaged meniscus and cartilage (Murphy et al., 2003). The mechanisms that guide homing of implanted cells are unclear, but in one study Wang et al. (2002) showed that the chemokine monocyte chemoattractant protein-1 (MCP-1) in cerebral ischaemic tissue promotes migration of infused MSCs to the site of injury. They showed that MCP-1, not present in normal brain, is rapidly upregulated following middle cerebral artery occlusion in rats, and that it is chemotactic for MSCs. Homing and expansion of MSCs to the injured host was also elegantly demonstrated by Rombouts and Ploemacher (2003). They showed that in an irradiated host there was both migration and expansion of GFP-expressing syngeneic MSCs in the marrow and spleen. This was not the case with un-irradiated animals, again supporting the concept that these cells are specifically attracted to a wound environment. Interestingly, these authors also noted that the efficiency of homing of these cells was decreased following long-term culture, an effect that will influence the preparation of these cells for therapeutic use.

7.3. In vivo differentiation

The fundamental principle of stem cell therapy is that undifferentiated cells, following delivery to the injured host and migration to the site of injury, will, under the influence of local signals, differentiate to cells of the appropriate phenotype. These differentiated cells then contribute to the repair of the injured tissue. There is evidence to indicate that this is the case, but little or no data concerning the specific signals that give rise to differentiation in situ. For instance, cells implanted in an osseous defect, such as a large segmental gap in the femur, stimulate formation of new bone that can be assessed both radiologically and histologically (Arinzeh et al., 2003; Bruder, Kraus, et al., 1998; Bruder, Kurth, et al., 1998). Similarly, Ponticiello et al. (2000) showed that scaffolds loaded with MSCs and implanted in an osteochondral lesion on the medial femoral condyle give rise to both cartilage and bone cells. Several reports have also demonstrated that the delivery of murine MSCs to dystrophic *mdx* mice resulted in the implanted cells contributing dystrophin to the muscle fiber sarcolemma (Ferrari & Mavilio, 2002).

7.4. Stem cells in disease

There is another perspective on the role of adult stem cells in disease, and that is the concept that certain degenerative conditions, where there is progressive tissue damage and an inability to repair, may be due to the fact that stem cell populations are depleted or functionally altered. This has been considered in the case of osteoarthritis, a disease of the joints where there is progressive and irreversible loss of cartilage, with changes also in the underlying bone. In a study described by Murphy et al. (2002) MSCs were prepared from marrow taken from patients with end-stage OA undergoing joint replacement surgery. The marrow samples were harvested both from the site of surgery (either the hip or the knee) and also from the iliac crest. It was found that the proliferative capacity of the cells was substantially reduced in the osteoarthritic patients, and this was independent of the site of harvest. In addition, the chondrogenic and adipogenic activity of the cells was also significantly reduced, again independent of the site of marrow harvest. These effects were apparently disease-related, and not age-related, but additional studies will be necessary to confirm these preliminary observations. However, the data suggest that susceptibility to OA and perhaps other degenerative diseases may be due to the reduced mobilization or proliferation of stem cells. In addition, successfully recruited cells may have a limited capacity to differentiate, leading to defective tissue repair. Alternatively, the altered stem cell activity may be in response to the elevated levels of inflammatory cytokines seen in OA (Fernandes, Martel-Pelletier, & Pelletier, 2002; Goldring, 2001).

8. Conclusions

Although early pre-clinical and clinical data demonstrate the safety and effectiveness of MSC therapy there are still many questions to be answered surrounding the mechanism of action. Additional information is required concerning the therapeutic efficacy of transplanted cells and the mechanisms of engraftment, homing and in vivo differentiation. There is also a need to carry out appropriately designed toxicology studies to demonstrate the long-term safety of these therapies. The widespread use of stem cell therapy will

also depend upon the availability of validated methods for large-scale culture, storage and distribution. In addition, there is a need for novel engineered devices for tissue-specific delivery of cells, such as cell-coated stents and catheter-based delivery in cardiovascular applications, and arthroscopic delivery in the treatment of joint disease. As these areas are addressed new applications will be developed leading to novel therapeutic opportunities. Much has been learned about stem cell therapy in the past few years, and much remains to be learned.

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