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Fat tissue: an underappreciated source of stem cells for biotechnology

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Adipose tissue can be harvested in large amounts with minimal morbidity. It contains numerous cells types, including adipocytes, preadipocytes, vascular endothelial cells and vascular smooth muscle cells; it also contains cells that have the ability to differentiate into several lineages, such as fat, bone, cartilage, skeletal, smooth, and cardiac muscle, endothelium, hematopoietic cells, hepatocytes and neuronal cells. Cloning studies have shown that some adipose-derived stem cells (ADSCs) have multilineage differentiation potential. ADSCs are also capable of expressing multiple growth factors, including vascular endothelial growth factor and hepatocyte growth factor. Early, uncontrolled, non-randomized clinical research, applying fresh adipose-derived cells into a cranial defect or undifferentiated ADSCs into fistulas in Crohn's disease, has shown healing and an absence of side effects. The combination of these properties, and the large quantity of cells that can be obtained from fat, suggests that this tissue will be a useful tool in biotechnology.

Introduction

Adipose tissue has a remarkable ability to undergo considerable changes in volume during the lifespan of an individual. Although relatively small increases in volume can be accommodated by changes in the amount of lipid stored in individual adipocytes (hypertrophy), larger changes are mediated by the generation of new adipocytes (hyperplasia) accompanied by coordinated expansion and remodeling of the adipose vasculature [1,2]. These changes are mediated by a population (or populations) of stem and progenitor cells within adipose tissue. Thus, for many years researchers studied the adipogenic potential of preadipocytes within the stromal vascular fraction of adipose tissue [3–5]. Subsequently, we, and others, have shown that in addition to committed adipogenic and vascular cells, such as smooth muscle cells and endothelium, adipose tissue contains a multipotent cell population with properties that are similar, although not identical, to those of marrow-derived mesenchymal stem cells (MSCs) [6–11]: we refer to these cells as adipose-derived stem cells (ADSCs). Other names used in the literature for multipotent cells derived from adipose tissue include adipose-derived adult stem cells (ADAS), human multipotent adipose-derived stem cells (hMADS), processed lipoaspirate cells (PLA) and adipose tissue-derived stromal cells. In addition, Folkman *et al.* showed that the vascular plasticity of adipose tissue derives from the maintenance of microvascular endothelial cells (and their progenitors) in a relatively immature state [1] – a finding that is consistent with the presence of endothelial progenitor cells within adipose tissue [12]. In this review we discuss the biology of these stem and progenitor cell populations and examine the potential role of adipose tissue as a source of cells for biotechnology and, in particular, for regenerative medicine.

Adipose tissue as a stem cell source

The most important features of adipose tissue as a cell source might be the relative expendability of this tissue and the consequent ease with which it can be obtained in relatively large quantities with minimal risk. Liposuction is a common surgical procedure: 478 251 elective liposuction surgeries were performed in the USA during 2004 [13]. It is also safe: an American Society for Dermatologic Surgery study of outpatient cosmetic liposuction performed between 1994 and 2000 showed zero deaths on 66 570 procedures and a serious adverse event rate of 0.68 per 1000 cases [14].

Several groups have estimated the frequency and yield of MSCs in bone marrow by applying clonogenic assays for either fibroblastoid-like colonies (CFU-F) or colonies expressing alkaline phosphatase (CFU-AP) [15–18]. Results generally indicate a frequency in the whole bone marrow in skeletally mature adults of between ~1 in 50 000 and 1 in 100 000, which corresponds to a yield of a few hundred MSCs per milliliter of marrow. By contrast, our data suggest that once adipose tissue is digested with collagenase, the major contaminating cell type, the mature adipocyte, is easily removed on the basis of its buoyancy, yielding a cell population in which the frequency of CFU-F and CFU-AP is in the order of 1 in 100 – some 500-fold more than that found in marrow (Figure 1). This substantially greater frequency is reflected in the observation that to generate a culture that is confluent within ~5–7 days, it is typical to plate in the range of 20 000–400 000 freshly isolated bone marrow cells per square centimeter [19,20]. By comparison, confluence is achieved in the same time frame with cells obtained from adipose tissue digestion by plating as few as 3500 cells cm^2 [21,22].

The frequency and yield of ADSCs and MSCs might be different but their biology, although not identical, is similar. In culture, these cells express cell-surface

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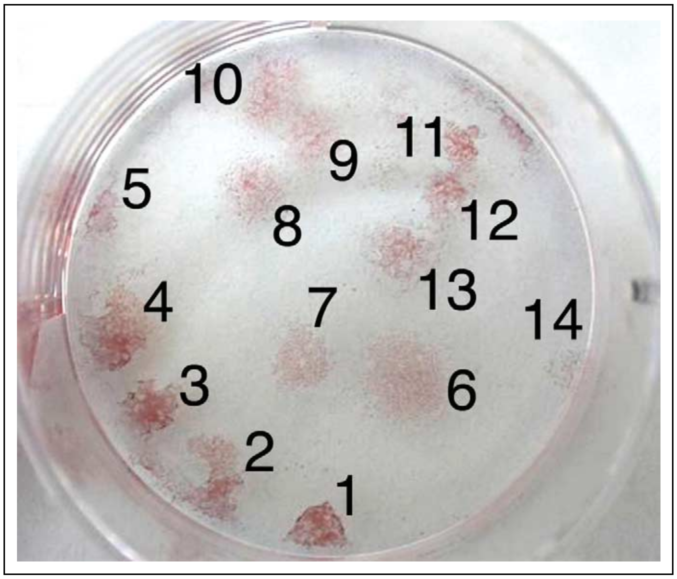

Figure 1. Digestates of human adipose tissue contain a high frequency of CFU-F. Adipose tissue was digested with collagenase and centrifuged to separate the buoyant adipocytes from the non-buoyant cells. The non-buoyant fraction was plated in 24-well plates at 1000 cells/plate in DMEM/F12 medium supplemented with 20% fetal calf serum, and fed twice weekly for two weeks. At the end of culture the medium was removed and colonies of cells were immunostained with an antibody to CD105 (a marker expressed on MSCs and ADSCs). At least 14 colonies are evident, demonstrating a CFU-F frequency of 1.4% for this donor. We typically observe between 0.3% and 4% plating efficiency for adipose-derived cells in this, and related, assays.

markers that are similar to those expressed by MSCs, including CD105, SH3, Stro-1, CD90, and CD44, but they do not express the hematopoietic marker CD45 nor the endothelial marker CD31 [6,9,11,21,22]. However, in studies in which both marrow and adipose tissue were obtained from the same individuals we detected some differences in protein expression: ADSCs express CD49d and not CD106, whereas MSCs express CD106 but not CD49d [6]. The significance of this finding is not clear but it is interesting that these molecules represent part of a receptor–ligand pair that has an important role in hematopoietic stem cell homing to, and mobilization from, bone marrow [23,24]. There is some controversy in the literature regarding expression of CD34 by ADSCs. CD34 is a widely used marker of hematopoietic stem and progenitor cells [25,26] but is also highly expressed in vascular endothelial cells and their precursors [27,28]. Our own data suggest that expression of this molecule is low-to-absent in cultured ADSCs [7,22] but, by contrast, Gronthos *et al.* and Festy *et al.* have both reported higher levels of CD34 expression [21,29]. This might reflect differences in the epitope recognized by the anti-CD34 antibody or the duration of culture [30].

ADSCs and MSCs both possess the ability to suppress a mixed lymphocyte reaction in a dose-dependent and time-dependent fashion [31,32]. Furthermore, Rodriguez *et al.* [11] have demonstrated that clonally derived, multipotent cells from adipose tissue are immunoprivileged, both *in vitro* and *in vivo*. This suggests that, similar to MSCs, ADSCs might have potential as immunoprivileged universal donor cells with the capacity to be used in the allogeneic setting and to reduce graft-versus-host disease [32].

The differentiation capacity of adipose-derived stem cells (ADSCs)

Adipose-derived cells differentiate into several cell types (Table 1). It is not clear if a single adipose-derived cell can differentiate into all of these lineages; however, we, and others, have generated ADSC clones expressing four cell lineages (adipo-, chondro-, osteo-, and neuro-) [7,10,33], thereby demonstrating the presence of multipotent and oligopotent cells within adipose tissue.

Osteogenesis

ADSCs differentiate *in vitro* into osteoblast-like cells that are capable of depositing a mineralized extracellular matrix in much the same fashion, and under the same general conditions, as MSCs. Thus, ADSCs cultured for between 14 and 21 days in low concentrations of ascorbic acid, β-glycerophosphate (βGP) and dexamethasone and/or vitamin D analogs will express multiple markers of osteogenesis, including cbfa-1, alkaline phosphatase, osteopontin, osteocalcin and collagen I [7,34].

As with MSCs, transduction of ADSCs with the gene encoding bone morphogenic protein 2 (BMP2) results in the generation of ectopic bone production *in vivo* [35]. Thus, ADSCs transduced with adenovirus–BMP2 and seeded into standard collagen type I sponges will form a radio-opaque, bone-like material when implanted, intramuscularly, into SCID mice [35]. Furthermore, Cowan *et al.* demonstrated the ability of native (non-transduced and undifferentiated) ADSCs to form bone in an orthotopic site [36]. In this study the authors delivered ADSCs on an apatite-coated, resorbable scaffold into a critical size (4mm) murine calvarial defect (a full-thickness bone defect in the skull exhibiting minimal spontaneous healing), and demonstrated in the range of 70–90% closure of the defect within 12 weeks, with radio-opacity equivalent to 90% of uninjured bone at 8 weeks. Fluorescence *in situ* hybridization studies in these sex mismatched transplants demonstrated that between 92 and 99% of cells within the new bone were of donor (adipose) origin [36].

These findings have led to a case report in which ADSCs, generated under good manufacturing conditions, were applied, safely, in a clinical case of calvarial reconstruction [37].

Cartilage

Several groups have applied systems similar to those used with MSCs to demonstrate the ability of ADSCs to undergo chondrocytic differentiation [8,38–40]. These systems incorporate high cell-density (micromass)

Table 1. Differentiation potential of adipose-derived cells

Cell type	Refs
Adipose	[7,8,66,67]
Bone	[7,8,36,68,69]
Cartilage	[38–40,70]
Skeletal Muscle	[11,43,44]
Cardiac Muscle	[45–47]
Neuronal Cells	[54,57,71]
Hematopoietic Cells	[72]
Endothelial Cells	[12]
Hepatocytes	[73]

cultures supplemented with transforming growth factor-β1 (TGF-ß1) to generate proteoglycan-rich spheroids that express collagen II, aggrecan, decorin and other markers consistent with chondrogenesis.

One group presented data suggesting that the chondrogenic capacity of ADSCs is less complete than that of MSCs [38]. Specifically, comparison of the chondrogenic gene expression patterns of ADSCs and MSCs grown in monolayers showed similar patterns; however, when MSCs were grown in three-dimensional constructs, the pattern of gene expression more closely approximated that of mature cartilage than that observed with ADSCs. In particular, the authors noted the absence of expression of certain genes, including aggrecan and Col2A, in three-dimensional cultures of ADSCs. These data contrast with those described above, in which detection of these markers in ADSCs induced to undergo chondrogenic differentiation in alginate discs was observed at 12 weeks. Furthermore, *in vivo* development of mature cartilage from ADSCs has been demonstrated in a rabbit osteochondral defect model [41]. In this study, although the no treatment group showed repair of cartilage to native levels of function, injuries treated with ADSC-derived grafts had better function, at each time point assessed, than those receiving conventional osteochondral grafts. These findings suggest that the observations of Winter *et al.* might reflect issues associated with cell culture rather than an inherent defect of ADSCs. Discrepancies of this nature raise an important point: multipotent cells from different sources can respond differently to different stimuli, such that conditions optimal for MSC differentiation might not be so for ADSCs and vice versa. Recently, Dicker *et al.* described a specific example of this behavior in a study exposing the subtleties in the biology of adipocytes generated, *in vitro*, from different stem and/or progenitor cell sources [42].

Muscle

We have shown that culture of ADSCs in the presence of dexamethasone and hydrocortisone results in a time-dependent increase in the expression of muscle-specific markers (MyoD1 and myosin), expression of transcription factors (myf5, myf6, and myogenin) and the appearance of elongated, multinucleated structures that closely resemble myotubes [7,8,43]. Furthermore, Bacou *et al.* showed that three-day-old primary cultures of adipose-derived cells transduced with *LacZ* and injected into crush-injured anterior tibialis muscles were incorporated into the myofibers within 15 days [44]. Two months after the injection, the ADSC-treated muscles showed increased mass, increased myotube fiber diameter and increased force generation compared with the untreated controls. Interestingly, extending the initial culture period to seven days resulted in the loss of the ability of cells to induce repair. This contrasts with the observation of long-term *in vivo* expression of human dystrophin following injection of extensively cultured hMADS into the tibialis anterior muscle of the immunocompetent mdx mouse (a model of Duschenne muscular dystrophy) [11].

Three groups have now presented data demonstrating the ability of rodent ADSCs to undergo differentiation into cardiac myocytes [45–47]. The most compelling data was generated by Planat-Bernard *et al.* [47]. They observed spontaneous differentiation of murine adipose-derived cells placed in methylcellulose-based cultures supplemented with recombinant hematopoietic growth factors. After three weeks, clusters of spontaneously beating cells, which possessed electrophysiological, pharmacological and electron micrographic properties consistent with differentiation into cardiac myocytes, were detected. We have shown, recently, that intravascular injection of adipose-derived cells into mice immediately following cryoinjury of the left ventricle results in detection of donor-derived cells at two weeks. These cells express the cardiac-specific transcription factor Nkx2.5, troponin I and myosin heavy chain, consistent with *in vivo* differentiation of ADSCs into cardiac myocytes [48].

Endothelial cells

Miranville *et al.* performed a series of studies using $CD34^{+}/CD31^{-}$ cells isolated from human adipose tissue, and demonstrated the ability of these cells to undergo *in vitro* and *in vivo* endothelial differentiation [12]. In these studies cultures of selected cells, grown in low serum medium supplemented with vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1), formed network-like structures that expressed endothelial markers – no such structures were observed in the absence of supplementation. By contrast, we showed that these structures can be generated in the absence of growth factor supplements by eliminating the process of cell selection, for example, the culture of adipose-derived cells in Alpha medium and 10% fetal calf serum results in creation of a monolayer of ADSCs, upon which network structures of cells expressing endothelial markers formed. This difference might reflect the ability of ADSCs to express VEGF and other angiogenic factors [49].

Interestingly, Miranville *et al.* [12] and Rehman *et al.* [49] showed that delivery of adipose-derived cells accelerates restoration of perfusion following surgical induction of severe hind limb ischemia. In the former study this finding was associated with incorporation of donor cells into the microvasculature; however, reperfusion might also have been promoted by the expression of anti-apoptotic (hepatocyte growth factor; HGF) and angiogenic (VEGF) growth factors by ADSCs [49]. One author described these two molecules as the ‘dynamic duo’ for revascularization [50], suggesting that dual-gene therapy with these factors might overcome the limitations encountered with single-gene therapy for angiogenesis. Rehman *et al.* demonstrated up-regulation of these factors by ADSCs in response to hypoxia [49], suggesting that the delivery of ADSCs to ischemic tissues might result in induction of angiogenesis in a more physiologically relevant fashion than with gene therapy.

Neuronal cells

In any study of multipotent cell differentiation, the integrity of the conclusions drawn is limited by the specificity of the endpoints evaluated to define differentiation. When the lineage into which the cells are apparently differentiating is one that is unanticipated, healthy skepticism requires that claims of differentiation are supported by the detection of several robust markers. For this reason studies indicating neuronal differentiation of MSCs and ADSCs need to be reviewed with particular care. Early studies showed that treatment of cells with the reducing agent beta-mercaptoethanol induced rapid (8 h) changes in cell shape – the cells assumed a neuronal morphology with a small cell body and multiple neurite-like projections [7,51]. This is accompanied by the expression of early neuronal markers, including nestin, neuron-specific enolase (NSE) and neuron-specific nuclear protein (NeuN). However, others have challenged such findings, suggesting that they represent disordered gene expression and cell morphology, in response to a toxic agent [52,53]. Other groups have used more physiologic inducers that act over a longer time-frame to detect inducible expression of neuronal genes in the absence of observable toxicity. For example, Ashjian *et al.* [54] showed that ADSCs treated with insulin, indomethacin and IBMX showed increased expression of NSE, NeuN and the NGF receptor, trk-A. However, to date, no published study has demonstrated the ability of either ADSCs or MSCs to acquire the key functional attributes of neuronal cells – formation of synaptic junctions and the generation or transmission of an action potential – although Ashjian *et al.* have described voltage-dependent outward channels similar to those found in mammalian nodes of Ranvier.

To test the potential for ADSCs and MSCs to differentiate into neuronal cells, these cells have been administered to small-animal models of ischemic stroke or traumatic brain injury, and the majority of studies have detected improved outcome with both stem cell types [55–57]. However, follow-up studies, examining the mechanism by which this benefit is derived, indicate that the primary mechanisms appear to be mediated through stem cell-mediated recruitment of endogenous neural precursors [58,59] and the elaboration of angiogenic and anti-apoptotic growth factors by the donor cells [60–62]. This results in the preservation of existing neuronal cells rather than *de novo* generation of neurons from the stem cells themselves.

Summary

In conclusion, adipose tissue appears to contain cells with the ability to act as functional and vascular building blocks for several tissues. One group has sought to harness this potential to heal fistulas in Crohn's disease patients [63,64]. In a four-patient clinical trial of the use of ADSCs in this context, full healing was observed in 6 out of 8 lesions, and partial healing in the remainder. No side effects were observed in this study, in which all patients were followed for at least 12 months. It should be noted that no mechanistic data were obtained nor was the degree or longevity of engraftment of the ADSCs measured; therefore, it is not clear if the positive outcome was the result of stem cell differentiation, paracrine expression of angiogenic or anti-apoptotic factors, local immunosuppression, a combination of these, or other effects. The relative ease with which ADSCs can be infected by adenoviral, oncoretroviral, and lentiviral vectors [65] provides an opportunity to introduce markers and/or to knock-out or knock-down gene expression and, thereby, evaluate this, and related questions, in animal models. In conclusion, because of the frequency of multipotent cells within adipose tissue, combined with the relative ease with which this tissue can be harvested in large quantities, we believe that adipose-derived cells will be a valuable resource in biotechnology.

References

1 Rupnick, M.A. *et al.* (2002) Adipose tissue mass can be regulated through the vasculature. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10730–10735

2 Hausman, D.B. *et al.* (2001) The biology of white adipocyte proliferation. *Obes. Rev.* 2, 239–254

3 Ailhaud, G. *et al.* (1992) Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* 12, 207–233

4 Dixon-Shanies, D. *et al.* (1975) Observatons on the growth and metabolic functions of cultured cells derived from human adipose tissue. *Proc. Soc. Exp. Biol. Med.* 149, 541–545

5 Hausman, G.J. and Martin, R.J. (1989) The influence of human growth hormone on preadipocyte development in serum-supplemented and serum-free cultures of stromal–vascular cells from pig adipose tissue. *Domest. Anim. Endocrinol.* 6, 331–337

6 De Ugarte, D.A. *et al.* (2003) Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol. Lett.* 89, 267–270

7 Zuk, P.A. *et al.* (2002) Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell* 13, 4279–4295

8 Zuk, P.A. *et al.* (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 7, 211–228

9 Wickham, M.Q. *et al.* (2003) Multipotent stromal cells derived from the infrapatellar fat pad of the knee. *Clin. Orthop. Relat. Res.* 412, 196–212

10 Rodriguez, A.M. *et al.* (2005) The human adipose tissue is a source of multipotent stem cells. *Biochimie* 87, 125–128

11 Rodriguez, A.M. *et al.* (2005) Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. *J. Exp. Med.* 201, 1397–1405

12 Miranville, A. *et al.* (2004) Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation* 110, 349–355

13 American Society for Aesthetic Plastic Surgery. (2005) Cosmetic surgery quick facts. (http://surgery.org/press/procedurefacts-asqf.php)

14 Housman, T.S. *et al.* (2002) The safety of liposuction: results of a national survey. *Dermatol. Surg.* 28, 971–978

15 Castro-Malaspina, H. *et al.* (1984) Human bone marrow fibroblast colony-forming units (CFU-F). *Prog. Clin. Biol. Res.* 154, 209–236

16 D'Ippolito, G. *et al.* (1999) Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *J. Bone Miner. Res.* 14, 1115–1122

17 Oreffo, R.O. *et al.* (1998) Skeletal progenitor cells and ageing human populations. *Clin. Sci. (Lond.)* 94, 549–555

18 Muschler, G.F. *et al.* (2001) Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. *J. Orthop. Res.* 19, 117–125

19 Jaiswal, N. *et al.* (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. *J. Cell. Biochem.* 64, 295–312

20 Wexler, S.A. *et al.* (2003) Adult bone marrow is a rich source of human mesenchymal stem cells but umbilical cord and mobilized adult blood are not. *Br. J. Haematol.* 121, 368–374

21 Gronthos, S. *et al.* (2001) Surface protein characterization of human adipose tissue-derived stromal cells. *J. Cell. Physiol.* 189, 54–63

22 De Ugarte, D.A. *et al.* (2003) Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* 174, 101–109

23 Papayannopoulou, T. *et al.* (1998) Anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the kit/mkit ligand pathway. *Blood* 91, 2231–2239

24 Kronenwett, R. *et al*. (2000) The role of cytokines and adhesion molecules for mobilization of peripheral blood stem cells. *Stem Cells* 18, 320–330

25 Trischmann, T.M. *et al*. (1993) Measurement of $CD34^+$ cells in bone marrow by flow cytometry. *J. Hematother.* 2, 305–313

26 Bensinger, W. *et al*. (1993) Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte-stimulating factor. *Bone Marrow Transplant.* 11, 369–374

27 Asahara, T. *et al*. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275, 964–967

28 Rafii, S. *et al*. (1994) Isolation and characterization of human bone marrow microvascular endothelial cells: hematopoietic progenitor cell adhesion. *Blood* 84, 10–19

29 Festy, F. *et al*. (2005) Surface protein expression between human adipose tissue-derived stromal cells and mature adipocytes. *Histochem. Cell Biol.* 124, 113–121

30 Sengenes, C. *et al*. (2005) Preadipocytes in the human subcutaneous adipose tissue display distinct features from the adult mesenchymal and hematopoietic stem cells. *J. Cell. Physiol.* 205, 114–122

31 Puissant, B. *et al*. (2005) Immunomodulatory effects of human adipose tissue-derived stem cells: comparison with bone marrow mesenchymal stem cells. *Br. J. Haematol.* 129, 118–129

32 Tse, W.T. *et al*. (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75, 389–397

33 Guilak, F. *et al*. (2006) Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. *J. Cell. Physiol.* 206, 229–237

34 Halvorsen, Y.D. *et al*. (2001) Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived stromal cells. *Tissue Eng.* 7, 729–741

35 Dragoo, J.L. *et al*. (2003) Bone induction by BMP-2-transduced stem cells derived from human fat. *J. Orthop. Res.* 21, 622–629

36 Cowan, C.M. *et al*. (2004) Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat. Biotechnol.* 22, 560–567

37 Lendeckel, S. *et al*. (2004) Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J. Craniomaxillofac. Surg.* 32, 370–373

38 Winter, A. *et al*. (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

39 Erickson, G.R. *et al*. (2002) Chondrogenic potential of adipose tissue-derived stromal cells *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.* 290, 763–769

40 Dragoo, J.L. *et al*. (2003) Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads. *J. Bone Joint Surg. Br.* 85, 740–747

41 Nathan, S. *et al*. (2003) Cell-based therapy in the repair of osteochondral defects: a novel use for adipose tissue. *Tissue Eng.* 9, 733–744

42 Dicker, A. *et al*. (2005) Differential function of the alpha2A-adrenoceptor and phosphodiesterase-3B in human adipocytes of different origin. *Int. J. Obes.* 29, 1413–1421

43 Mizuno, H. *et al*. (2002) Myogenic differentiation by human processed lipoaspirate cells. *Plast. Reconstr. Surg.* 109, 199–209

44 Bacou, F. *et al*. (2004) Transplantation of adipose tissue-derived stromal cells increases mass and functional capacity of damaged skeletal muscle. *Cell Transplant.* 13, 103–111

45 Rangappa, S. *et al*. (2003) Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann. Thorac. Surg.* 75, 775–779

46 Gaustad, K.G. *et al*. (2004) Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. *Biochem. Biophys. Res. Commun.* 314, 420–427

47 Planat-Benard, V. *et al*. (2004) Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. *Circ. Res.* 94, 223–229

48 Strem, B.M. *et al*. (2005) Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. *Cytotherapy* 7, 282–291

49 Rehman, J. *et al*. (2004) Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109, 1292–1298

50 Gerritsen, M.E. (2005) HGF and VEGF: a dynamic duo. *Circ. Res.* 96, 272–273

51 Woodbury, D. *et al*. (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. *J. Neurosci. Res.* 61, 364–370

52 Neuhuber, B. *et al*. (2004) Re-evaluation of *in vitro* differentiation protocols for bone marrow stromal cells: disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. *J. Neurosci. Res.* 77, 192–204

53 Lu, P. *et al*. (2004) Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation or artifact? *J. Neurosci. Res.* 77, 174–191

54 Ashjian, P.H. *et al*. (2003) *In vitro* differentiation of human processed lipoaspirate cells into early neural progenitors. *Plast. Reconstr. Surg.* 111, 1922–1931

55 Lu, D. *et al*. (2001) Intra-arterial administration of marrow stromal cells in a rat model of traumatic brain injury. *J. Neurotrauma* 18, 813–819

56 Mahmood, A. *et al*. (2003) Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. *Neurosurgery* 53, 697–702

57 Kang, S.K. *et al*. (2003) Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. *Exp. Neurol.* 183, 355–366

58 Kang, S.K. *et al*. (2003) Interactions between human adipose stromal cells and mouse neural stem cells *in vitro*. *Brain Res. Dev. Brain Res.* 145, 141–149

59 Mahmood, A. *et al*. (2004) Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. *Neurosurgery* 55, 1185–1193

60 Garcia, R. *et al*. (2004) Bone marrow stromal cells produce nerve growth factor and glial cell line-derived neurotrophic factors. *Biochem. Biophys. Res. Commun.* 316, 753–754

61 Chen, J. *et al*. (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ. Res.* 92, 692–699

62 Li, Y. *et al*. (2002) Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 59, 514–523

63 Garcia-Olmo, D. *et al*. (2003) Autologous stem cell transplantation for treatment of recto–vaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int. J. Colorectal Dis.* 18, 451–454

64 Garcia-Olmo, D. *et al*. (2005) A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis. Colon Rectum* 48, 1416–1423

65 Morizono, K. *et al*. (2003) Multilineage cells from adipose tissue as gene delivery vehicles. *Hum. Gene Ther.* 14, 59–66

66 Deslex, S. *et al*. (1987) Differentiation of human adipocyte precursors in a chemically defined serum-free medium. *Int. J. Obes.* 11, 19–27

67 Rodriguez, A.M. *et al*. (2004) Adipocyte differentiation of multipotent cells established from human adipose tissue. *Biochem. Biophys. Res. Commun.* 315, 255–263

68 Huang, J.I. *et al*. (2002) Rat extramedullary adipose tissue as a source of osteochondrogenic progenitor cells. *Plast. Reconstr. Surg.* 109, 1033–1041

69 Hicok, K.C. *et al*. (2004) Human adipose-derived adult stem cells produce osteoid *in vivo*. *Tissue Eng.* 10, 371–380

70 Awad, H.A. *et al*. (2004) Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate and gelatin scaffolds. *Biomaterials* 25, 3211–3222

71 Safford, K.M. *et al*. (2002) Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem. Biophys. Res. Commun.* 294, 371–379

72 Cousin, B. *et al*. (2003) Reconstitution of lethally irradiated mice by cells isolated from adipose tissue. *Biochem. Biophys. Res. Commun.* 301, 1016–1022

73 Seo, M.J. *et al*. (2005) Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.* 328, 258–264