Adult bone-marrow stem cells and their potential in medicine

H T Hassan MD PhD M El-Sheemy MS PhD

J R Soc Med 2004;**97**:465–471

An area of research that today generates great optimism is the use of stem cells for therapy of human diseases. Much of the excitement centres on embryonic stem cells, but this approach remains controversial for ethical reasons; moreover, routine clinical application of this strategy is many years away. By contrast, haematopoietic stem cells from adult bone marrow are well characterized and have long been used therapeutically.[1] An adult weighing 70 kg has a functional haematopoietic marrow volume of about 1.75 L and upon increased demands such as infection or haemorrhage it can increase sixfold.[1,2] No moral controversy surrounds the use of these cells since they are either autologous or collected from a consenting donor. The potential applications of adult bone marrow cells have gained momentum with discoveries relating to the mesenchymal stem cell.

MESENCHYMAL STEM CELLS

Adult bone-marrow-derived mesenchymal stem cells (MSC) are capable of differentiation along several lineages (Box 1).[3–15] They are positive for CD29, CD44, CD105 and CD166, have a doubling time of about two days, expand in culture up to sixfold and their biological functions are not altered by ageing.[3,15] Box 2 lists some of the cytokine receptors expressed by these cells and the cytokines produced. Their features and properties are closely similar to those of counterpart cells isolated from fetal blood, liver and bone in the first and second trimesters, from amniotic fluid and umbilical cord blood, and from adult peripheral blood, compact bone and adipose tissue.[21–27] Moreover, a CD133-positive subpopulation of these cells, which can be expanded under defined conditions for more than one hundred population doublings without telomere shortening or karyotypic abnormality, has proved capable of differentiation not only into mesenchymal cell types (osteoblasts, chondrocytes, adipocytes, myocytes) but also into endothelium and cells with neuroectodermal phenotype and function.[28–30] Previously, adult marrow-derived stem cells were believed to yield a limited number of cell types whereas embryonic cells were totipotent. The discovery of these multipotent adult stem cells has clearly narrowed the gap: they offer a very promising and much more abundant potential resource for therapy of inherited or degenerative diseases and for repair of tissues such as cartilage, bone and myocardium.

Box 1 **Differentiation potential of adult bone marrow mesenchymal stem cells** (from refs 3–15)

Adipocyte	Myofibroblast
Osteoblast	Endothelial cell
Chondrocyte	Neural cell
Cardiac myocyte	Hepatic cell
Skeletal myocyte	Renal tubular cell
Tenocyte	

ADULT STEM CELL PLASTICITY

What is the mechanism of stem cell differentiation? When the phenomenon was first explored, the possibility of cell fusion was mooted—that is, hybridization with other cells rather than true plasticity. Indeed, embryonic stem cells were seen to hybridize with brain cells to form tetraploid cells with pluripotent character.[31] However, *in-vitro* and *in-vivo* studies of adult bone marrow stem cells suggest a

Box 2 **Cytokine expression and production of human adult bone marrow mesenchymal stem cells** (Refs 3, 15–20)

Cytokine receptor expression	*Cytokine production*
c-kit (stem cell factor)	Stem cell factor
gp130	Interleukin-1, -6, -7, -8, -11, -12, -14, -15
Interleukin-1, -3, -4, -7	FLT-3 ligand
Leukaemia inhibitory factor	Leukaemia inhibitory factor
G-CSF	G-CSF, GM-CSF and M-CSF
TNF-alpha	
TGF-beta	
Basic fibroblast growth factor	
Platelet derived growth factor	
Epidermal growth factor	
Nerve growth factor (p75)	

G-CSF=granulocyte colony stimulating factor; TNF=tumour necrosis factor; TGF=transforming growth factor; GM-CSF=granulocyte macrophage CSF; FLT=tyrosine kinase receptor

Institute of Medical Sciences, University of Lincoln, UK

Correspondence to: Professor H T Hassan, Director, Institute of Medical Sciences, University of Lincoln, Brayford Pool, Lincoln LN6 7TS, UK

E-mail: hhassan@lincoln.ac.uk

rate of cell fusion too low to account for the transdifferentiation.[32] Moreover, single euploid bone marrow MSC, never co-cultured with tissue-specific cells or embryonic cells, have been seen to differentiate into cells of the three germ layers;[33] *in vivo*, the use of bone marrow cells selectively expressing the enhanced green fluorescent protein ruled out fusion as a mechanism for the generation of functional pancreatic islet beta cells;[34] and hepatocytes, cardiomyocytes, and pancreatic and endothelial cells have been described as physiologically either diploid or polyploid.[35–37] Certain cytokines, including interleukins (IL) 1, 4, and 13, tumour necrosis factor alpha and interferon gamma, are involved in the generation of normal multinucleated cells such as osteoclasts and Langhans giant cells;[38–40] thus, observations suggesting fusion of bone marrow cells with, for example, Purkinje neurons, cardiomyocytes and hepatocytes[41] may instead simply reflect physiological polyploidy.

BIOLOGY OF ADULT MARROW MESENCHYMAL STEM CELLS

The direction in which bone marrow MSC differentiate is heavily influenced by cytokines (Table 1). For example, bone morphogenetic protein 6 (BMP-6) not only influences differentiation towards chondrogenesis or osteogenesis but may also serve to regulate the bone marrow environment via the effects of IL-6 on haematopoiesis and osteogenesis.[50] Two possible mechanisms have been proposed for a regulatory role of BMP-6 in the human bone marrow microenvironment: (i) it might enhance the osteoblastic differentiation of human MSC; or (ii) it might reduce the osteoclastic differentiation of haematopoietic marrow cells by decreasing interleukin-6 production in bone marrow stroma. MSC coexpressing CD133 and fetal liver kinase 1 generated endothelial cells in the presence of vascular endothelial growth factor, and functional hepatocytes in the presence of fibroblast growth factor-4 and hepatocyte growth factor.[29,30] Also, MSC coexpressing CD133, CD172 and nestin differentiated along a neural pathway in the presence of fibroblast growth factor or retinoic acid plus nerve growth factor.[51,54] An MSC side-population with high efflux of DNA binding dye and expressing CD90 (Thy1) differentiated into mesangial renal cells.[55]

MIGRATION/MOBILIZATION OF ADULT MARROW STEM CELLS

In animal models, transplanted bone marrow cells have been detected in skeletal and cardiac muscle,[56–58] vascular endothelium,[58,59] liver,[60–62] lung, gut and skin epithelia,[62] pancreatic beta cell islets,[34,63] renal glomeruli,[14,55] and neural tissue.[33,64–69] When bone-marrow-derived MSC were injected intracerebrally in acid-sphingomyelinase-deficient mice, the onset of neurological abnormalities was delayed and the animals lifespan was extended.[70] Local transplantation of such cells is also reported to have regenerated bone[71–73] and myocardium.[74,75] It is noteworthy that no donor-derived tumours have been seen in these animal models—whereas with transplantation of undifferentiated embryonic stem cells teratoma development has been reported.[76] The results also differ from those of undifferentiated embryonic stem cell transplantation in that engraftment and tissue-specific differentiation are achieved without pretransplantation measures to induce differentiation down the lineage desired. The ability of marrow-derived cells to populate numerous body tissues—bone, liver, cardiac muscle, colon, skin—is well shown in patients who have received cells from gender-mismatched donors (Table 2).[77–84] A post-mortem study revealed donor-derived neurons in the hippocampus and cerebral cortex of brain samples from women who had received bone marrow transplants from men.[85] Deductions from such findings must be qualified by the observation that women who have carried male fetuses may show long-term mosaicism with male cells;

Table 1 ***In-vitro*** **differentiation conditions of human adult bone marrow mesenchymal stem cell**

Tissue-type generated	*Cytokines, reagents and conditions*	*Ref.*
Chondrogenic cells	TGF-beta and dexamethasone	42
	Bone morphogenetic protein-6 (BMP-6)	43
	Prolactin	44
Osteogenic cells	Ascorbate, β-glycerophosphate and dexamethasone	45,46
	Parathyroid hormone vitamin D3 $\pm$ BMP-6	3
Endothelial cells	Vascular endothelial growth factor	29
Functional hepatocytes	FGF-4 and hepatocyte growth factor on Matrigel	30
Neuronal cells	FGF and retinoic acid $\pm$ NGF on fibronectin	51–54
Mesangial renal cells	Side population cell CD90+subset of MSC	13,55

FGF=fibroblast growth factor; NGF=nerve growth factor; TGF=transforming growth factor; MSC=mesenchymal stem cell

Table 2 **Migration of human adult bone marrow stem cells in gender-mismatched bone marrow transplantation patients**

Donor male cells in female recipient	*Frequency*	*Ref.*
Osteoblasts	2%	77
Hepatocytes	2%	78
Colon mucosal epithelial cells	13%	79
Hepatocytes, gut and skin epithelia	7%	80*
Buccal epithelial cells	–	81
Purkinje cells	–	82
Myocardial cells	10%	83,84
Cerebral (neural) cells	–	85

*Stem cells mobilized with granulocyte colony stimulating factor

nevertheless, the weight of the evidence is that donor bone-marrow-derived cells can migrate and give rise to tissues belonging to all three germ-cell layers.[77–85] It is noteworthy that, in the transdifferentiation of these adult marrow stem cells, there was no evidence of cell fusion.[77–85] Lately, work in mice indicated that such cells participate in skin regeneration and reconstitution and promote wound healing;[86–88] and one research group reports a pilot study in three patients indicating that locally applied autologous bone marrow cells enhanced dermal building and closure of long-term non-healing wounds.[89]

CLINICAL STUDIES

In animal models of myocardial infarction, stem cells were reported to participate in repair whether injected locally or stimulated in bone marrow by use of stem cell factor (SCF) and G-CSF.[90] In man, a randomized placebo-controlled study revealed increased coronary collateral flow in patients treated with intracoronary GM-CSF (molgramostim) followed by two weeks of subcutaneous administration.[91]

In the past decade the use of G-CSF (filgrastim) has transformed the treatment of cancer by facilitating marrow reconstitution after myeloablative therapy. We must hope for a similar breakthrough in the management of coronary heart disease.

In allogeneic transplantation, mesenchymal stem cells in bone marrow play a key part in immunomodulation and the induction of tolerance. MSC suppress the proliferation of T-lymphocytes induced by cellular or non-specific mitogenic stimuli[92] and negatively influence B-cell lymphopoiesis.[93] Allogeneic/xenogeneic MSC transplants engraft in immunocompetent sheep and non-human primates.[94–97] When a patient was treated, after myeloablation, with both haematopoietic stem cells and cultured MSC from a mismatched donor, only grade I graft-versus-host disease (GvHD) was observed.[98] That MSC can not only reduce GvHD but also facilitate haematopoietic engraftment is evidenced by the rapid haematopoietic recovery of patients with breast cancer who received autologous blood stem cells together with culture-expanded MSC after high-dose chemotherapy.[99] In both clinical trials, MSC transplantation was well tolerated.

Osteogenesis imperfecta has been the focus of two studies in children. Allogeneic MSC transplantation, leading to successful osteoblast engraftment in 3 of 5 children with type III osteogenesis imperfecta, was associated with a 44–77% increase in bone mineral content, improved linear growth and reduced fracture frequency.[77,100] In another cohort of 6 children with type III osteogenesis imperfecta who had received earlier bone marrow transplantation, MSC infusions from the original donor resulted in a 50% improvement in their growth velocity.[101] Similar improvements were observed in children with metachromatic leukodystrophy and Hurler's syndrome after repeated allogeneic marrow MSC infusions.[102]

Ten clinical studies have been reported on the effects of autologous bone marrow stem cell transplantation in patients with myocardial infarction or ischaemic heart failure (Table 3).[103–112] In three pilot studies, two of them randomized controlled trials, bone marrow cells infused via a coronary catheter a few days after acute myocardial infarction led to significant improvement in coronary flow reserve and left ventricular ejection fraction.[104,105,111] In the remaining seven, marrow cells injected directly into the myocardium of patients with chronic ischaemic heart disease yielded benefits in ejection fraction and also angina score.[103,106–110,112]

Despite the impressive safety record of all these pilot clinical trials, the possibility of undesired differentiation into other tissues must be borne in mind in monitoring of future studies.

THE FUTURE

In the next decade, the approaches discussed above will clearly be developed and refined. Further avenues will open up. For example, bone-marrow-derived cells expressing stem cell factor have been shown to initiate endogenous pancreatic tissue regeneration in mice.[113] If such cells could be used as pancreatic beta islet cell progenitors, there would be scope for autologous transplantation in patients with diabetes, avoiding the need for the immunosuppression necessary after allotransplantation and circumventing the scarcity of allogeneic material. Whereas the multipotent adult dermal stem cells from human scalp skin have shown mainly neural differentiation, suggesting a possible therapeutic role in neurodegenerative diseases,[114,115] the bone marrow MSC show strong orientation towards bone, cartilage, endothelium and cardiac muscle.

In conclusion, the existing medical uses of bone marrow are likely to expand greatly with exploitation of the

Table 3 **Clinical trials of adult bone marrow autotransplantation in ischaemic heart disease**

No.	Bone marrow (BM) cells	Route of administration	Clinical outcome	Follow-up (months)	Ref.
Acute myocardial infarction					
10 (5–9 days after injury)	Mononuclear BM cell aspirate 2.1% CD34+, 0.6% CD133+ total 18 million CD34+ cells	Intracoronary, 6–7 injections of 2–3 mL containing1.5–4 million CD34+ cells each	Increased contractility; reduced hypokinetic area	3	104
20 (4 days after injury) versus 11 control	Mononuclear BM cell aspirate (*n*=9) 1–7 million CD34+ or blood progenitor cells (*n*=11) 245 blood million cells	Intracoronary catheter 3 injections of 3 mL each	Increased global LVEF; reduced end-systolic; improved coronary flow reserve	4	105
30 (3–6 days after injury) versus 30 control	Mononuclear BM cell aspirate 4% CD34+, total 3–15 million CD34+ cells	Intracoronary, 4–5 injections of 5 mL each containing 0.6–3 million CD34+ cells	Increased global LVEF; increased systolic wall motion	6	111
Chronic ischaemic heart disease					
5	Mononuclear BM cell aspirate	With coronary artery bypass, 11 transendocardial injections of 0.1 mL	Improved coronary perfusion	12	103
6	Selected CD133+ BM cells; total 1.5 million CD133+ cells	With coronary artery bypass, 10 transendocardial injections of 0.2 mL	Improved coronary perfusion; improved global LVEF	6	109
14	Mononuclear BM cell aspirate	With coronary artery bypass	Improved global LVEF; increased cardiac function	10	110
12	Selected CD133+ BM cells; total 1.5 million CD133+ cells	With coronary artery bypass, 10 transendocardial injections of 0.2 mL	Improved local perfusion; improved global LVEF	12	112
10	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Improved LVEF	3	106
14	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Improved LVEF; reduced end systolic volume	2–4	107
8	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Increased wall motion	3	106

LVEF=left ventricular ejection fraction

therapeutic potential of adult mesenchymal stem cells, with their capacity for many lines of differentiation. The next stage is to isolate the various subsets and investigate their mechanisms of differentiation and homing to tissues. This work has vast implications for human wellbeing, through cell and gene therapies, through tissue engineering and through immunotherapy.

REFERENCES

1 Hassan HT, Gutensohn K, Zander AR, Kuhnl P. CD34 positive cell sorting and enrichment: applications in bloodbanking and transplantation. In: Recktenwald D, Radbruch A, eds. *Cell Separation: Methods and Applications*. New York: Marcel Dekker, 1998:283–92

2 Hassan HT, Zander AR. Stem cell factor as a survival and growth factor in human normal and malignant hematopoiesis. *Acta Haematol* 1996;**95**:257–62

3 Sammons J, Ahmed N, El-Sheemy M, Hassan HT. The role of BMP-6, IL-6 and BMP-4 in mesenchymal stem cells-dependent bone development; effects on osteoblastic differentiation induced by parathyroid hormone and vitamin D_3. *Stem Cells Devel* 2004;**13**:273–80

4 Awad HA, Butler DL, Boivin GP, *et al*. Autologous mesenchymal stem cell-mediated repair of tendon. *Tissue Eng* 1999;**5**:267–77

5 Direkze NC, Forbes SJ, Brittan M, *et al*. Multiple organ engraftment by bone marrow-derived myelofibroblasts and fibroblasts in bone marrow-transplanted mice. *Stem Cells* 2003;**21**:514–19

6 Ryden M, Dicker A, Gotherstrom C, *et al*. Functional characterisation of human mesenchymal stem cell-derived adipocytes. *Biochem Biophys Res Commun* 2003;**311**:391–7

7 MacKay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pettenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* 1998;**4**:415–28

8 Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 1995;**18**:1417–26

9 Sanchez-Ramos J, Song S, Cardozo-Pelaez F, *et al*. Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp Neurol* 2002;**174**:11–20

10 Schwartz RE, Reyes M, Koodie L, *et al*. Multipotent adult progenitor cell from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002;**109**:1291–302

11 Reyes M, Dubek A, Jahagirdar B, Koodie L, Marker PH, Verfaille CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;**109**:337–46

12 Woodbury D, Schwartz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurones. *J Neurosci Res* 2000;**61**:364–70

13 Imai E, Ito T. Can bone marrow differentiate into renal cells? *Pediatr Nephrol* 2002;**17**:790–4

14 Lin F, Cordes K, Li L, *et al*. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia–reperfusion injury in mice. *J Am Soc Nephrol* 2003;**14**:1188–99

15 Suva D, Garavaglia G, Menetrey J, *et al*. Non-haematopoietic human bone marrow contains long-lasting pluripotential mesenchymal stem cells. *J Cell Physiol* 2004;**198**:110–18

16 Erices A, Conget P, Rojas C, Minguell JJ. Gp130 activation by soluble interleukin-6 receptor/interleukin-6 enhances osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells. *Exp Cell Res* 2002;**280**:24–32

17 Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 2001;**7**:259–67

18 De Ugarte DA, Alfonso Z, Zuk PA, *et al*. Differential expression of stem cell mobilisation-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett* 2003;**89**:267–71

19 Roufosse CA, Direkze NC, Otto WR, Wright NA. Circulating mesenchymal stem cells. *Int J Biochem Cell Biol* 2004;**36**:585–97

20 Bonnet D. Biology of human bone marrow stem cells. *Clin Exp Med* 2003;**3**:140–9

21 Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono T, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver and bone marrow. *Blood* 2001;**98**:2396–402

22 Zvaifler NJ, Marinova-Mutafchieva L, Adams G, *et al*. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2000;**2**:477–88

23 Zuk PA, Zhu M, Ashjian P, Benhaim P, Henrick MH, Fraser JK. Human adipose tissue as source of multipotent stem cells. *Mol Cell Biol* 2002;**13**:4279–95

24 Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* 2003;**21**:105–10

25 Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey SA. Circulating skeletal stem cells. *J Cell Biol* 2001;**153**:1133–40

26 in't Anker PS, Noort WA, Scherjon SA, *et al*. Mesenchymal stem cells in human second trimester bone marrow, liver, lung and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differential potential. *Haematologica* 2003;**88**:845–52

27 in't Anker PS, Scherjon SA, Kleijburg-van der Keur C, *et al*. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood* 2003;**102**:1548–9

28 Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 2001;**98**:2615–25

29 Reyes M, Dubek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;**109**:337–46

30 Schwartz RE, Reyes M, Koodie L, *et al*. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002;**109**:1291–302

31 Ying QY, Nicols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. *Nature* 2002;**416**:545–8

32 O'Malley K, Scott EW. Stem cell fusion confusion. *Exp Hematol* 2004;**32**:131–4

33 Jiang Y, Balkrishna N, Jahagirdar R, *et al*. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;**418**:41–9

34 Janus A, Holz GG, Theise ND, Hussain MA. *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003;**111**:843–50

35 Seglen PO. DNA ploidy and autophagic protein degradation as determinants of hepatocellular growth and survival. *Cell Biol Toxicol* 1997;**13**:301–15

36 Brodsky V, Chernyaev AL, Vasilyeba IA. Variability of the cardiomyocytes ploidy in normal human hearts. *Virchow Arch B Cell Pathol* 1991;**61**:289–94

37 Lee GM, Rasch EM, Thornthwaite JT. Cytophotometric comparisons of DNA levels in neuronal and glial cells of the cerebellum: a comparative study. *Cell Biochem Funct* 1984;**2**:225–36

38 Anderson JM. Multi-nucleated giant cells. *Curr Opin Hematol* 2000;**7**:40–7

39 Merkel KD, Erdmann JM, McHugh KP, Abu-Amer Y, Ross FP, Teitelbaum SL. TNF-alpha mediates orthopaedic implant osteolysis. *Am J Pathol* 1999;**154**:203–10

40 Jimi E, Akiyama S, Tsurukai T, *et al*. Osteoclast differentiation factor acts as a multifunctional regulator in murine osteoclast differentiation and function. *J Immunol* 1999;**163**:434–42

41 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, *et al*. Fusion of bone marrow-derived cells with purkinje neurones, cardiomyocytes and hepatocytes. *Nature* 2003;**425**:768–73

42 Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM. The chondrogenic potential of human bone marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg* 1998;**80A**:1745–67

43 Sekiya I, Colter DC, Prockop DJ. BMP-6 enhances chondrogenesis in a subpopulation of human marrow stromal cells. *Biochem Biophys Res Comm* 2001;**284**:411–18

44 Ogueta S, Munoz J, Obregon E, Delgado-Baeza E, Garcia-Ruiz JP. Prolactin is a component of the human synovial liquid and modulates the growth and chondrogenic differentiation of bone marrow derived mesenchymal stem cells. *Mol Cell Endocrinol* 2002;**190**:51–63

45 Coelho MJ, Fernandes MH. Human bone cell cultures in compatibility testing. Part II: effect of ascorbic acid, beta-glycerophosphate and dexamethasone on osteoblastic differentiation. *Biomaterials* 2000;**21**:1095–102

46 Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. *J Cell Biochem* 1997;**64**:295

47 Sammons J, Ahmed N, Khokher MA, Hassan HT. Mechanisms mediating the inhibitory effect of retinoic acid on haemopoietic stem cells in human long-term bone marrow cultures. *Stem Cells* 2000;**18**:214–19

48 Ahmed N, Khokher MA, Hassan HT. Cytokine-induced expansion of human CD34 positive stem cell and CD34+CD41+ early megakaryocytic marrow cells cultured on normal osteoblasts. *Stem Cells* 1999;**17**:92–9

49 Ahmed N, Sammons J, Carson RJ, Khokher MA, Hassan HT. Effect of bone morphogenetic protein-6 on haematopoietic stem cells and cytokine production in normal human bone marrow stroma. *Cell Biol Int* 2001;**25**:429–35

50 Mbalaviele G, Jaiswal N, Meng A, Cheng L, Van Den Bos C, Thiede M. Human mesenchymal stem cells promote human osteoclast differentiation from CD34+ bone marrow hematopoietic progenitors. *Endocrinology* 1999;**140**:3736–3

51 Kim BJ, Seo JH, Bubien JK, Oh YS. Differentiation of adult bone marrow stem cells into neuroprogenitor *in vitro*. *NeuroReport* 2002;**13**:1185–8

52 Vogel W, Grunebach F, Messam CA, Kanz L, Brugger W, Buhring HJ. Heterogeneity among human bone marrow-derived mesenchymal stem cells and neural progenitor cells. *Haematologica* 2003;**88**:126–33

53 Sanchez-Ramos JR, Song S, Cardoz-Paleaz F, *et al*. Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp Neurol* 2000;**164**:247–56

54 Reyes M, Verfaillie CM. Characterisation of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. *Ann NY Acad Sci* 2001;**938**:231–5

55 Ito T, Suzuki A, Imai E, Okabe M, Hori M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodelling. *J Am Soc Nephrol* 2001;**12**:2625–35

56 Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;**279**:528–30

57 Orlic D, Kajstura J, Chimenti S, *et al*. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;**410**:701–5

58 Jackson KA, Majka SM, Wang H, *et al*. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;**107**:1395–402

59 Asahara T, Takahashi T, Masuda H, *et al*. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neo-vascularisation. *Circulation Res* 1999;**85**:221–8

60 Petersen BE, Bowen WC, Patrene KD, *et al*. Bone marrow as potential source of hepatic oval cells. *Science* 1999;**284**:1168–70

61 Theise ND, Nimmakayalu M, Gardner R, *et al*. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 2000;**31**:235–40

62 Krause DS, Theise ND, Collector MI, *et al*. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;**105**:369–77

63 Lee VM, Stoffel M. Bone marrow: an extra-pancreatic hideout for the elusive pancreatic stem cell? *J Clin Invest* 2003;**111**:799–801

64 Brazelton TR, Rossi FMV, Keshet GI, Blau HE. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 2000;**290**:1775–9

65 Kopen G, Prockop D, Phinney D. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci USA* 1999;**96**:10711–16

66 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 2000;**290**:1779–82

67 Priller J, Persons DA, Klett FF, Kempermann G, Kreutzberg SW, Dirnagl U. Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells *in vivo*. *J Cell Biol* 2001;**26**:733–8

68 Azizi SA, Stokes D, Augelli BJ, Digirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocyte grafts. *Proc Natl Acad Sci USA* 1998;**95**:3908–13

69 Schwarz EJ, Alexander GM, Prockop DJ, Azizi SA. Multi-potential marrow stromal cells transduced to produce L-DOPA: engraftment in a rat model of Parkinson's disease. *Hum Gene Therapy* 1999;**10**:2539–49

70 Jin HK, Carter JE, Huntley GW, Schuchman EH. Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. *J Clin Invest* 2002;**109**:1183–91

71 Pereira RF, O'Hara MD, Laptev AV, *et al*. Marrow stromal cells as a source of progenitor cells for non-hematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1998;**95**:1142–7

72 Niedzwiedzki T, Dabrowski Z, Miszta H, Pawlikowski M. Bone healing after bone marrow stromal cell transplantation to the bone defect. *Biomaterials* 1993;**14**:115–21

73 Kadiyala S, Jaiswal N, Bruder SP. Culture-expanded bone marrow-derived mesenchymal stem cells regenerate a critical-sized bone defect. *Tissue Eng* 9197;**3**:173–85

74 Pak HN, Qayyum M, Kim D, *et al*. Mesenchymal stem cell injection induces cardiac nerve sprouting and increased tenascin expression in a Swine model of myocardial infarction. *J Cardiovasc Electrophysiol* 2003;**14**:841–8

75 Shake JG, Gruber PJ, Baumgartner WA, *et al*. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg* 2002;**73**:1919–25

76 Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al*. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;**282**:1145–7

77 Horwitz EM, Prockop DJ, Fitzpatrick LA, *et al*. Transplantability and therapeutic effects of bone marrow-derived mesenchymal stem cells in children with osteogenesis imperfecta. *Nat Med* 1999;**5**:309–13

78 Theise ND, Nimmakayalu M, Gardner R, *et al*. Liver from bone marrow in humans. *Hepatology* 2000;**32**:11

79 Okamoto R, Yajima T, Yamazaki M, *et al*. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. *Nat Med* 2002;**8**:1011–17

80 Korbling M, Ktz RL, Khanna A, *et al*. Hepatocytes and epithelial cells of donor origin in recipients of peripheral blood stem cells. *N Eng J Med* 2002;**346**:738–6

81 Tran SD, Pillemer SR, Dutra A, *et al*. Differentiation of human bone marrow-derived cells into buccal epithelial cells *in vivo*: a molecular analytical study. *Lancet* 2003;**361**:1084–8

82 Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. Contribution of transplanted bone marrow cells to Purkinje neurones in human adult brains. *Proc Natl Acad* Sci USA 2003;**100**:2088–93

83 Caplice NM, Bunch TJ, Stallboerger PG, *et al*. Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. *Proc Natl Acad Sci USA* 2003;**100**,4754–9

84 Deb A, Wang S, Skelding KA, *et al*. Bone marrow derived cardiomyocytes are present in adult human heart: a study of gender mismatched bone marrow transplantation patients. *Circulation* 2003;**107**:1247–9

85 Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. Transplanted bone marrow generates new neurons in human brains. *Proc Natl Acad Sci USA* 2003;**100**:1364–9

86 Kataoka K, Medina RJ, Kageyama T, *et al*. Participation of adult mouse bone marrow cells in reconstitution of skin. *Am J Pathol* 2003;**163**:1227–31

87 Badiavas EV, Abedi M, Butmarc J, Flanga V, Quesenberry P. Participation of bone marrow derived cells in cutaneous wound healing. *J Cell Physiol* 2003;**196**:245–50

88 Stepanovic V, Awad O, Jiao C, Dunnwald M, Schatteman GC. Leprdb diabetic mouse bone marrow cells inhibit skin wound vascularisation but promote wound healing. *Circulation Res* 2003;**92**:1247–53

89 Badiavas EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. *Arch Dermatol* 2003;**139**:510–16

90 Orlic D, Kajstura J, Chimenti S, *et al*. Mobilised bone marrow cells repair in infarcted heart, improving function and survival. *Proc Natl Acad Sci USA* 2001;**98**:10344–9

91 Seiler JG, Pohl T, Wustmann K, *et al*. Promotion of collateral growth by GM-CSF in patients with coronary artery disease: a

randomised double blind placebo-controlled study. *Circulation* 2001;**104**:2012–15

92 Di Nicola M, Carlo-Stella C, Magni M, *et al*. Human bone marrow stromal cells suppress T-lymphoctye proliferation induced by cellular or non-specific mitogenic stimuli. *Blood* 2002;**99**:3838–43

93 Shoham T, Parameswaran R, Shav-Tal Y, Brda-Saad M, Zipori D. The mesenchymal stroma negatively regulates B cell lymphopoiesis through the expression of Activin A. *Ann N Y Acad Sci* 2003;**996**:245–60

94 Bartholomew A, Sturgeon C, Siatskas M, *et al*. Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. *Exp Hematol* 2002;**30**:42–8

95 Liechty KW, MacKenzie TC, Shaaban AF, *et al*. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after *in utero* transplantation in sheep. *Nat Med* 2000;**6**:1282–6

96 Devine S, Bartholomew AM, Mahmud N, *et al*. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol* 2001;**29**:244–5

97 Devine S, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion in non-human primates. *Blood* 2003;**101**:2999–3001

98 Lee ST, Jang JH, Cheong JW, *et al*. Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T-cell depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype. *Br J Haematol* 2002;**118**:1128–31

99 Koc ON, Gerson SL, Cooper BW, *et al*. Rapid haematopoietic recovery after co-infusion of autologous blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high dose chemotherapy. *J Clin Oncol* 2000;**18**:307–16

100 Horwitz EM, Prockop DJ, Gordon PL, *et al*. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 2001;**97**:1227–31

101 Horwitz EM, Gordon PL, Koo WK, *et al*. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci USA* 2002;**99**:8932–7

102 Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome. *Bone Marrow Transplant* 2002;**30**:215–22

103 Hamano K, Nishida M, Hirata K, *et al*. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischaemic heart disease: clinical trial and preliminary results. *Jpn Circulation J* 2001;**65**:845–7

104 Strauer BE, Brehm M, Zeus T, *et al*. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;**106**:1913–18

105 Assmus B, Schachinger V, Teupe C, *et al*. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002;**106**:3009–17

106 Fuchs S, Satler LF, Komowski R, *et al*. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. *J Am Coll Cardiol* 2003;**41**:1721–4

107 Perin EC, Dohmann HF, Borojlevic R, *et al*. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;**107**:2294–302

108 Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;**361**:47–9

109 Stamm C, Westphal B, Kleine HD, *et al*. Autologous bone marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;**361**:45–6

110 Galinanes M, Loubani M, Davies J, Chin D, Pasi J, Bell PR. Autotransplantation of unmanipulated bone marrow into scarred myocardium is safe and enhances cardiac function in humans. *Cell Transplant* 2004;**13**:7–13

111 Wollert KC, Meyer GP, Lotz J, *et al*. Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004;**364**: 141–8

112 Stamm C, Kleine HD, Westphal B, *et al*. CABG and bone marrow stem cell transplantation after myocardial infarction. *J Thorac Cardiovasc Surg* 2004;**52**:152–8

113 Hess D, Li L, Martin M, *et al*. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 2003;**21**:763–70

114 Toma JG, Akhavan M, Fernandes KJL, *et al*. Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 2001;**3**:778–84

115 Joannides A, Gaughwin P, Schwiening C, *et al*. Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. *Lancet* 2004; **364**:172–8