■ **REVIEW ARTICLE**

Exploring the application of mesenchymal stem cells in bone repair and regeneration

M. Griffin,
S. A. Iqbal,
A. Bayat

From the University of Manchester, Manchester, United Kingdom

Failure of bone repair is a challenging problem in the management of fractures. There is a limited supply of autologous bone grafts for treating nonunions, with associated morbidity after harvesting. There is need for a better source of cells for repair. Mesenchymal stem cells (MSCs) hold promise for healing of bone because of their capacity to differentiate into osteoblasts and their availability from a wide variety of sources. Our review aims to evaluate the available clinical evidence and recent progress in strategies which attempt to use autologous and heterologous MSCs in clinical practice, including genetically-modified MSCs and those grown on scaffolds. We have compared various procedures for isolating and expanding a sufficient number of MSCs for use in a clinical setting.

There are now a number of clinical studies which have shown that implantation of MSCs is an effective, safe and durable method for aiding the repair and regeneration of bone.

The healing of fractures depends on the interacting triad of the osteogenic cell, the osteoinductive stimulus and the osteoconductive matrix scaffold.[1]

Various natural or synthetic biomaterial grafts have been developed with excellent osteoconductive properties. There may be local morbidity after harvesting autologous bone graft from the iliac crest and a limited supply of bone is available.[2,3] These limitations have encouraged the use of allografts. However, despite their availability and the decreased rates of complication, there remains the possible risk of transmission of disease, diminished biological and mechanical properties in comparison with autologous bone, and higher costs.[4] In recent years, interest has risen in the use of deproteinated and defatted xenografts which give reduced immune responses, but these grafts have reduced osteoconductive properties.[5] Synthetic substitutes include ceramics, mixes of collagen and ceramic, coral derivatives and bioactive glass.[5] These are potentially attractive alternatives, but because of varied properties of restorability, higher costs and variation in their osteoconductive, osteoinductive and mechanical properties, they are considered to be less useful than allografts.[5]

Even after grafting, healing is not guaranteed unless fresh cells are recruited to the fracture site. Osteoinductive molecules are responsible for supporting the cellular migration and differentiation of the new progenitor cells required for the formation of new bone.[6] One of the important types of cell which are influenced by these osteoinductive molecules is the mesenchymal stem cell (MSC), a vital component in the natural process of new bone formation. Because of a lack of an adequate supply of autologous bone grafts and the unsuitability of allografts, there has been some impetus to use MSCs, one of the body's own versatile progenitor cells, to encourage repair.

The aims of our review are to describe the role of MSCs in bone repair and regeneration, to evaluate the techniques used to apply MSCs in clinical studies and to discuss what further studies are required to expand their use.

MSCs and bone repair

After a fracture, a haematoma forms which prevents excessive extravasation.[7] The platelets, inflammatory cells and macrophages arriving at the site of injury secrete cytokines and growth factors, including platelet-derived-growth factor, bone-morphogenetic proteins (BMPs), vascular-endothelial-growth-factor and interleukin-1 to -6.[7] This cellular response triggers the invasion of the MSCs which differentiate into osteoblasts and chondrocytes in order to complete the repair.[7] MSCs from the periosteum, bone marrow, circulating blood and the surrounding soft tissues have been shown to contribute to bone repair in rodent models.[8-11] The management of a defect in the

■ M. Griffin, MRes, Postgraduate Student
■ S. A. Iqbal, PhD, Research Associate
■ A. Bayat, MD, PhD, Plastic Surgeon
Plastic and Reconstructive Surgery Research
Manchester Interdisciplinary Biocentre, School of Translational Medicine, University of Manchester, Princess Street, Manchester M1 7DN, UK.

Correspondence should be sent to Dr A. Bayat; e-mail: ardeshir.bayat@manchester.ac.uk

doi:10.1302/0301-620X.93B4.25249 $2.00

J Bone Joint Surg [Br] 2011;93-B:427-34.

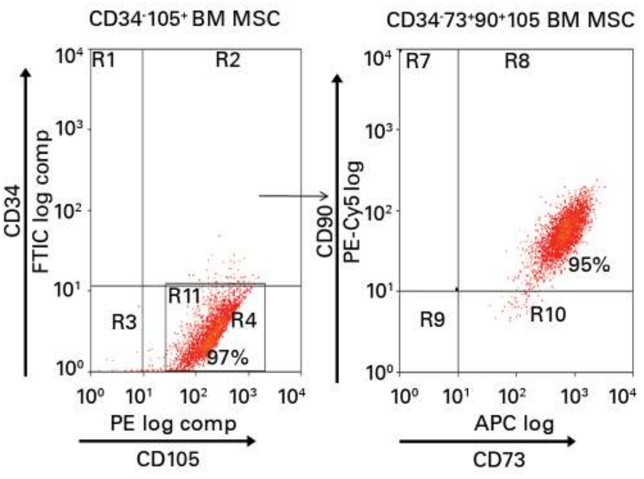


Fig. 1a

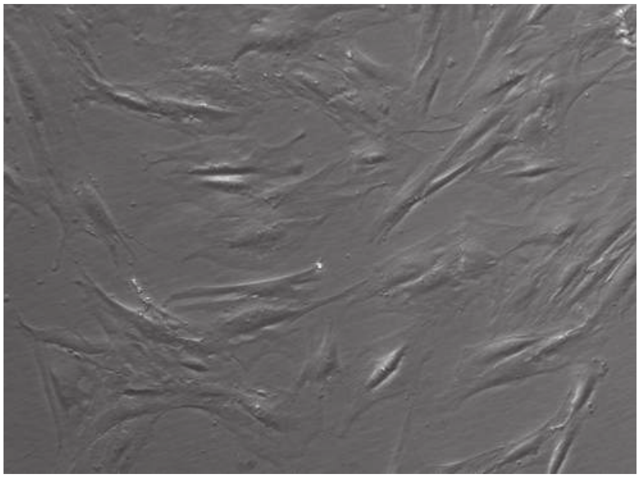


Fig. 1b

Figure 1a – Flourescence Activated Cell Sorting (FACS) analysis of bone-marrow-derived mesenchymal stem cells (MSCs) which can be used to purify them further. Live CD34-105+ cells were gated for CD90 and CD73 positive cells. More than 90% of the cells were CD34-73+90+105+, and hence were considered as MSCs according to the definition of the Mesenchymal and Tissue Stem Cell Committee of the International Society.[62] Figure 1b – light photomicrograph of MSCs which have been expanded using two-dimensional culture systems after isolation from bone marrow (× 20 magnification).

femur of athymic rats showed that a ceramic scaffold loaded with expanded MSCs gave significantly more new bone formation at 12 weeks and stronger bone than a scaffold without MSCs. This study confirmed that the MSCs formed bone by differentiating into osteoblasts.[8]

MSCs are non-immunogenic. They do not express major histocompatibility class II and co-stimulatory molecules, including CD40, CD80, and CD86.[12] Hence, allogeneic transplantation of MSCs should not require immunosuppression of the host. Most importantly, MSCs do not induce the proliferation of lymphocytes.[13] They have immunosuppressive properties and suppress the proliferation of T-cells and cytokine production in response to alloantigens or insignificant mitogens, as well as inhibiting the function of B cells,[14] dendritic cells[15] and the natural killer cells.[16]

MSCs show homing potential which is affected by numerous cytokines and growth factors, including stromal cell derived growth factor-1 (SDF-1) and its receptor CXCR4, which have been shown to act as a potential homing signal for MSCs in bone healing. In a mouse model of a femoral defect, Kitaori et al[17] showed that bone formation was mediated by expression of SDF-1 in the periosteum, causing recruitment of MSCs to the bone lesion. However, some studies have reported low levels of CXCR4 expressed by MSCs and have found that blocking CXCR4 had no effect on the migration of MSCs, suggesting that other molecules may be involved. There is no consensus on the mode of migration of MSCs since some studies have shown them in the circulation,[18] whereas others have found no evidence of this.[19]

Isolation and expansion of MSCs

MSCs are frequently isolated from the marrow of the superior iliac crest, but MSCs from the femur and the tibia during hip and knee replacements are a suitable alternative. However, since only 0.001% to 0.01% of mononuclear cells from bone marrow are MSCs,[20] an efficient method of isolation is required. This is usually achieved by density gradient centrifugation using Ficoll or Percoll.[21] When cells are layered over Ficoll or Percoll and centrifuged, layers of red blood cells, fat cells and mononuclear cells (MNCs) are formed according to their densities. The MNCs containing potential MSCs which form the middle layer can be aspirated, purified (Fig. 1a) and expanded (Fig. 1b) in a short period of time. With optimal conditions they can be cultured up to passage 30, although the proliferation and differentiation potentials of MSCs are reduced because of senescence, the Heyflick effect and telomere shortening.[22,23] Early passage cells (< 10) are considered to be most useful and their senescence can be circumvented by the addition of growth factors or by expanding them in a three-dimensional bioreactor which mimics the environment *in vivo*.[24] An important barrier to the use of *ex vivo* expanded MSCs is the risk of introducing pathogens and xeno-immunisation because of the use of fetal bovine serum for their culture. Increasingly, serum-free media are used for culture thus making it possible to use them for clinical applications.[25] The main therapeutic usefulness of MSCs is their ability to differentiate into osteocytes, chondrocytes and adipocytes in the *ex vivo* environment (Fig. 2). They can be differentiated *in vitro* into osteoblasts by the addition of dexamethasone and ascorbic acid, although no study has compared whether differentiated or undifferentiated MSCs differ in their ability to aid bone regeneration.

Application of stem cells in bone regeneration

After MSCs are expanded *ex vivo* they are either introduced by systemic infusion, or growth on a scaffold and

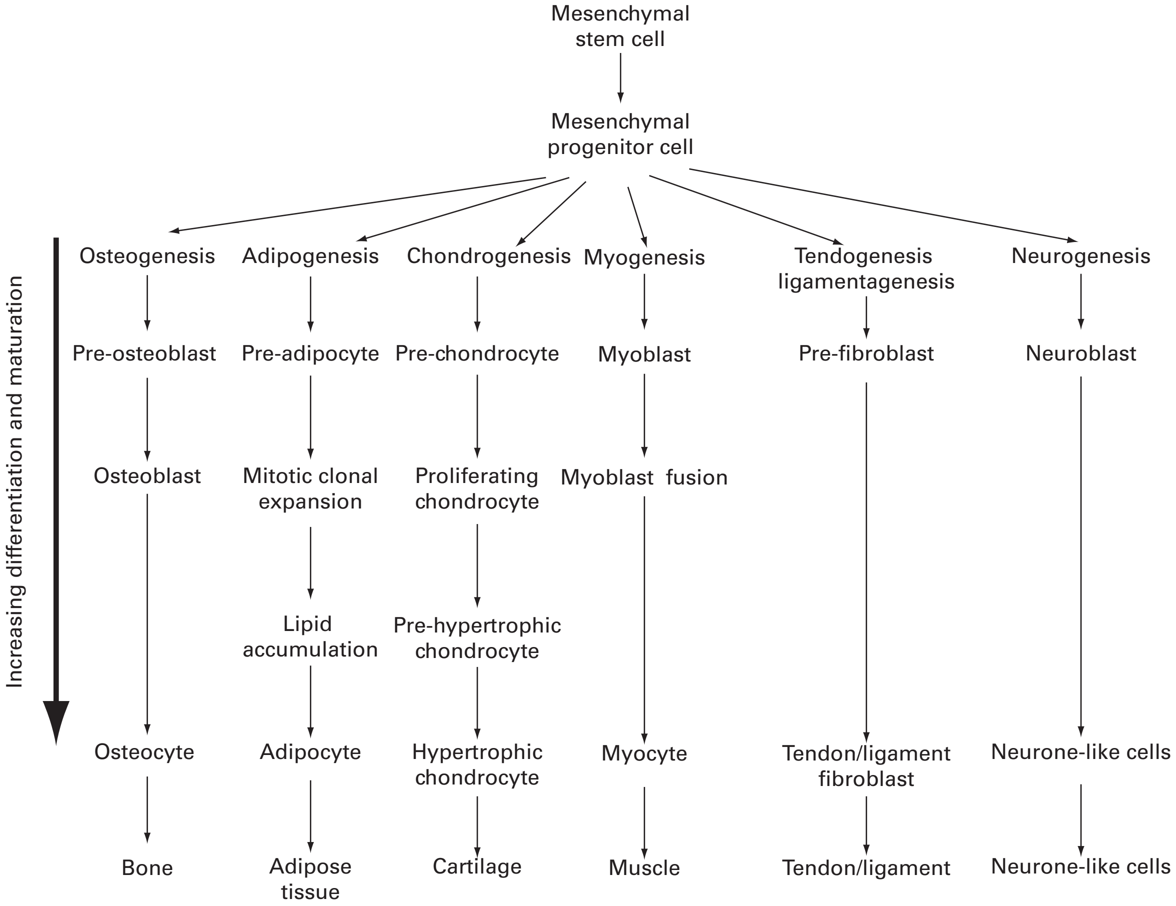


Fig. 2

Diagram showing the multi-differentiation potential of mesenchymal stem cells. They can differentiate into osteocytes, chondrocytes, adipocytes, myocytes, neurone-like cells and fibroblasts.

applied directly to the site of the lesion, or genetically modified before being used in a scaffold.

Expanded MSCs introduced by systemic infusion. Animal models have shown that MSCs can migrate to the bone marrow after peripheral injection and remain there for an extended duration.[26] Studies have shown the successful infusion of *ex vivo* expanded MSCs into human volunteers[27,28] indicating that this is feasible and well tolerated. Systemic infusion of MSCs for bone regeneration has been successfully used by Horwitz et al[29] in treating osteogenesis imperfecta. Six children with severe osteogenesis imperfecta received two infusions of allogeneic MSCs. Five children showed homing of MSCs into one or more sites including bone, skin and marrow stroma with an acceleration of bone growth of between 60% and 94% compared with matched unaffected children.[29] However, this technique has not been used in the repair of fractures. This may be because of reports[30,31] showing that most of the infused MSCs became trapped in the lungs with only a few migrating to the site of injury. Direct application of MSCs to the fracture is deemed to be more practical,[32] with research into systemic infusion of MSCs being more academically driven.

Application of MSCs grown on scaffolds. Scaffolds serve as carriers for cultured MSCs before implantation. Scaffolds need to mimic the natural environment of the bone matrix and should be safe to be used in clinical practice (Fig. 3).[33-35] The synergistic effect of using composites of scaffolds with growth factors has been shown to increase the formation and vascularisation of bone.[36,37] Numerous scaffolds have been investigated in pre-clinical studies, although hydroxyapatite (HA) and calcium phosphate seem to be favoured because of their excellent osteoconductive properties.[38] HA provides good strength but is not resorbed, while beta-tricalcium phosphate (β-TCP) is fragile but has a greater capacity for resorption.[39] Hence, a combination of HA and β-TCP, biphasic calcium phosphate, is typically used.[40] HA also has poor mechanical properties and bone formed using an HA composite cannot maintain the mechanical loading needed for remodelling.[41] In order to overcome this, HA can be combined with biodegradable polymer/bioceramic composites, including polylactic-co-glycolic acid, which allows for better control over shaping micro- and macrostructure composites for bone regeneration.[42]

Cell

Contain pores of appropriate scale to allow integration, vascularistion and angiogenesis

Produced from biocompatible materials with good biogradability or biocompatibility so that new bone will replace scaffold

Surface that allows attachment, proliferation and differentiation

Good mechanical strength that matches the bone lesion site

Surgeon

Be easily moulded into a variety of shapes and sizes

Be safe and easy to handle

Patient

Have no adverse reaction or tumourgenic effect

Be cost-effective and approved for use by the Federal Drug Administration or the National Institute for Clinical Excellence

Fig. 3

Diagram showing the scaffold requirements for both the patient and surgeon for mesenchymal stem cells to be used in clinical practice.

The fibres of the extracellular matrix (ECM), their interconnecting pores and HA crystals making up the bone tissue all have nano-scale dimensions (< 100 nm in one dimension).[35] Therefore manufacturing nano-composite materials is of interest since it provides three-dimensional (3D) constructs which fit the size of the surrounding matrix, promoting cell adhesion and matrix interactions.[16] More recently, mechanical stability has been identified as an important factor in the repair of fractures. Three-dimensional polymer scaffolds with dimensions of 150 μm to 500 μm have been shown to have excellent stability.[43] *Ex vivo* mechanical loading has an additive effect on BMP2-induced osteogenesis in genetically-engineered MSC-like cells,[44] and mechanical stimulation of MSCs has shown them to differentiate into adipocytes, chondrocytes and osteocytes.[45]

Quarto et al[46] expanded bone-marrow-derived stem cells for three weeks and seeded them on to macroporous HA scaffolds to treat nonunion. At seven months the three treated patients showed good integration of the implant. Angiographic evaluation after seven years showed vascularisation of the grafted zone, which is vital for the survival and future stability of the graft. Before such scaffolds can be used in clinical practice, a resorbable ceramic scaffold is needed since ceramic masks the newly-formed bone, making radiological follow-up difficult.[46] Marcacci et al[47] also showed that *in vitro* expanded MSCs loaded on to porous HA ceramic scaffolds can heal diaphyseal defects with good integration when followed up after five to seven years.

Warnke et al[48] described a new bone-muscle-flap technique for the treatment of a mandibular defect. The outer scaffold replacement was designed using computer software, placed in an external titanium mesh loaded with HA blocks coated with BMP-7 and MSCs and then implanted into latissimus dorsi for growth of blood vessels and bone. After seven weeks, the constructed mandible was removed and fixed to the stumps of the original mandible. The patient regained full function of his jaw by four weeks after operation. Since BMP-7 and MSCs were used together, it was difficult to determine whether the results were solely due to the presence of the MSCs. Furthermore, because of ethical issues a tissue sample from the jaw was not available to demonstrate complete healing of the mandible. However, this is a promising approach to avoid donor-site morbidity associated with bone transplantation with no risk of aesthetic disturbance.[48]

The *ex vivo* differentiation of MSCs into osteoblasts is another technique which has emerged. Morishita et al[49] used an HA scaffold to differentiate MSCs *ex vivo* into osteoblasts to heal the defect in a patient after curettage of a tumour, which illustrated that tissue-engineered osteogenic ceramics may be an alternative to autologous bone grafting. A tissue-engineered prosthesis has been used successfully in three patients with osteoarthritis of the ankle with no adverse reactions and high clinical scores.[50] Kitoh et al[51] injected differentiated bone-marrow-derived stem cells with platelet-rich plasma without a scaffold into three femora and two tibiae in two patients undergoing distraction osteogenesis to obtain the target lengths without major complications.

MSCs have been successfully used in the treatment of osteonecrosis of the femoral head. Kawate et al[52] cultured MSCs and applied MSCs/beta-TCP composite granules for steroid-induced osteonecrosis of the femoral head and found that this treatment prevented progression and showed early bone regeneration at 34 months, but it was not useful in the presence of pre-operative collapse.

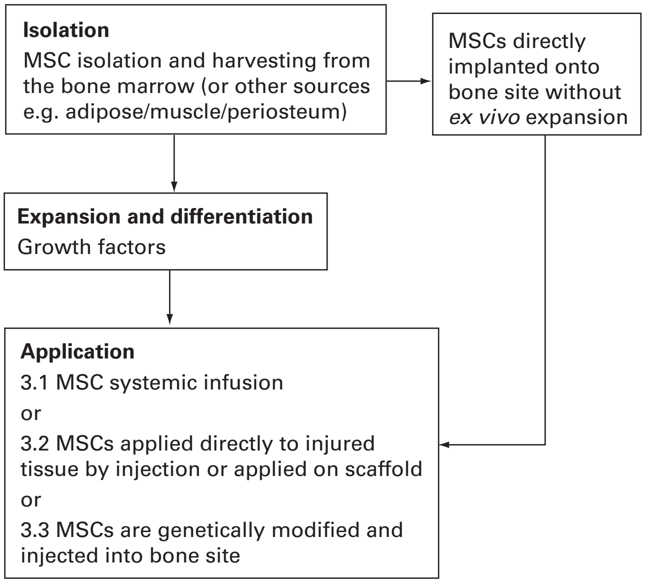


Fig. 4

Diagram showing the process of applying mesenchymal stem cells (MSCs) for bone repair. 1. These are first isolated from the bone marrow or other sources including adipose tissue/muscle/umbilical cord. 2. They are either expanded to increase the number or directly applied to the bone site after centrifugation. Growth factors can be added if expanded *ex vivo* to increase the yield and to differentiate towards different lineages. 3. After expansion the MSCs are then either applied through a scaffold or percutaneously injected at the site to aid regeneration of bone.

Allograft bone chips containing bone-marrow-derived cells have been used for spinal fusion.[53] Expanded MSCs have also been used in spinal fusion using porous β-TCP scaffolds seeded with MSCs. Of 41 patients, fusion was demonstrated in 95%.[54] With limited evidence supporting the role of MSCs in spinal fusion, further investigation using randomised control trials is required.

Genetically modified MSCs. Although the combination of growth factors and scaffolds remains a promising approach, there are limitations in the long-term release of growth factors to promote the proliferation and maintenance of MSCs. Therefore genetic modification of MSCs to express growth factors, which involves either transfection of MSCs through viral vectors or by the use of non-viral vectors, is a suitable alternative. Viral vectors have been shown to elicit immune reactions and have variable ability to transfect dividing and non-dividing cells efficiently.[55] However, compared with non-viral vectors, they show better expression of the desired protein and their efficiency in transfection is better. Viral vectors are often the optimal choice for gene delivery in MSCs.

Numerous osteoinductive growth factors have been used to modify MSCs and have been shown to give successful bone induction *in vivo*. BMP-2-transfected MSCs showed bone formation in mouse hindlimbs and in bony union of critical-sized mouse radial defects.[56] In another study, Lin et al[57] compared adipose- and bone-marrow-derived stem cells (ADSC/BMSCs) which had been genetically modified with BMP-4 to repair defects in the calvarial bone in rabbits and found no significant difference in bone regeneration. Furthermore, *in vitro* studies showed that deposition of ECM was significantly higher in differentiated ADSCs than in BMSCs.[57] Fat was detected in ADSC-seeded ECM, thus requiring further investigation of their use in bone defects especially in those bearing load.[57] To date, no clinical studies have applied *ex vivo*-expanded genetically-modified MSCs because of the need to identify the optimal growth factor and the vector to ensure effective, safe and consistent treatment.

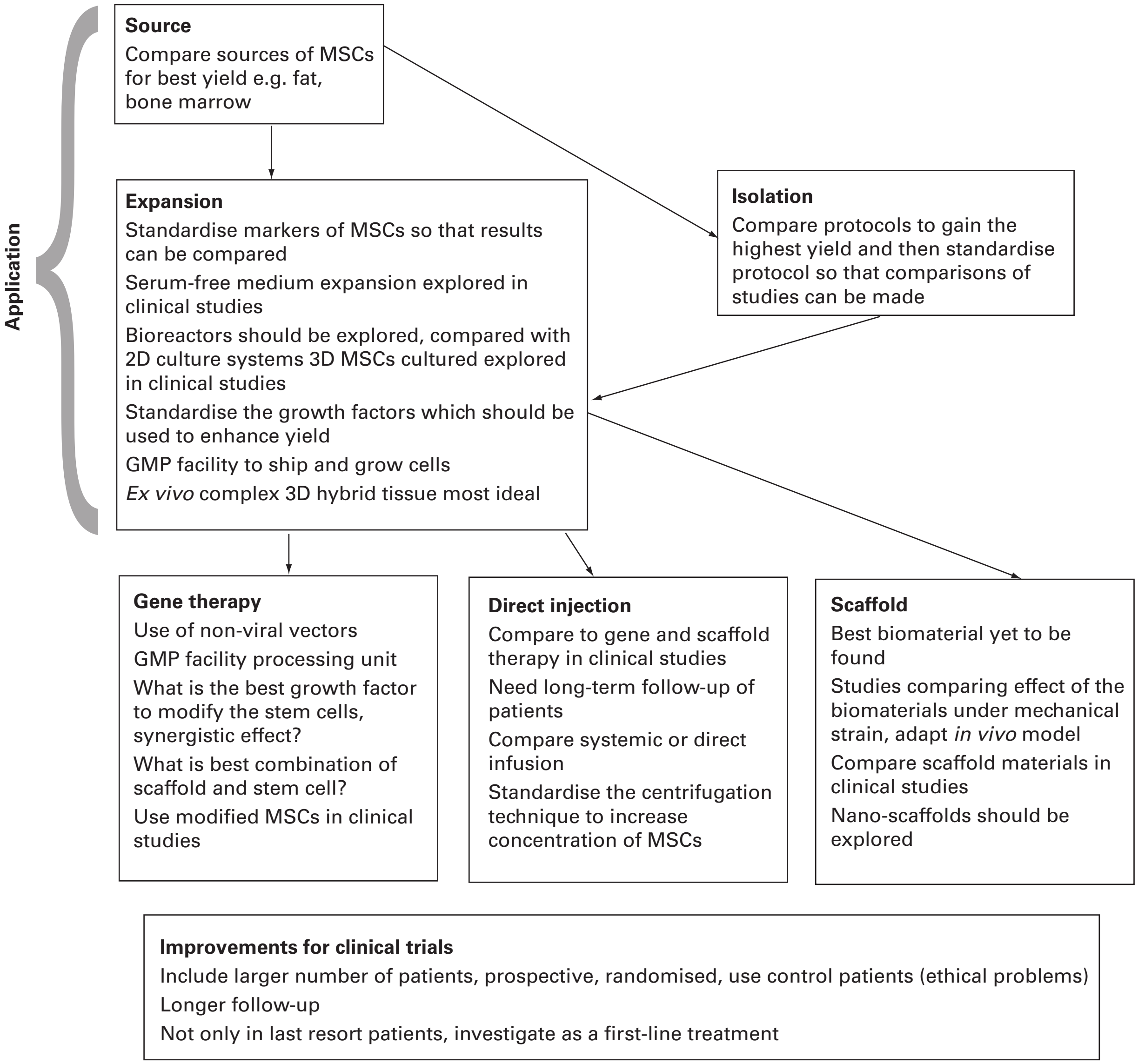
Application of non-expanded MSCs for bone regeneration

MSCs can be applied for bone regeneration without expansion *ex vivo* in order to avoid cost and time. After the preliminary work by Herzog[58] in 1951 which demonstrated the procedure of percutaneous bone grafting, many clinical studies have successfully applied MSCs for bone regeneration. In 1995 Connolly[59] demonstrated in a series of 100 skeletal healing problems, including delayed unions and nonunions of fractures, arthrodeses, and bone defects that MSCs were effective in bone repair when applied in this way. However, this and earlier studies did not report the number of MSCs needed to give optimum healing.

Hernigou et al[60] showed that bone healing depended on the number and concentration of transplanted MSCs. They found that seven of 60 patients with defects of the shaft of the tibia did not achieve union.[60] In these patients the mean number of MSCs in the graft was < 1000 cells per cm^3 and < 30 000 cells in total. Both the mean concentration and the mean number in patients who had not achieved union were significantly lower ($p < 0.01$) than in those in whom union was successful.[60] Therefore they considered that a graft needed to contain at least > 1000 MSCs per cm^3 to achieve union. This has implications for the technique used to isolate MSCs since the aspirate is not guaranteed to contain the required total cell count.[50] However, recent studies by Wongchuensoontorn et al[61] have demonstrated two techniques for increasing the volume of bone marrow applied to a bone defect. First, small volumes preferably within the range of 2 ml to 4 ml should be aspirated from the site since larger volumes dilute the bone marrow with blood, and secondly, the concentration of MSCs should be increased by centrifuging the aspirate before injection.[61] In contrast to the classic laboratory procedure, separation by density gradient does not require training and allows the processing of stem cells at the bedside.[61] Although Hernigou et al[60] demonstrated an approximate estimate for the number of MSCs required for bone regeneration, the number of viable cells after implantation into man has not yet been analysed.

The future of MSCs for clinical practice

The process of harvesting MSCs is a simple, stepwise process as illustrated in Figure 4. It is clear from the various



Source
Compare sources of MSCs for best yield e.g. fat, bone marrow

Expansion
Standardise markers of MSCs so that results can be compared
Serum-free medium expansion explored in clinical studies
Bioreactors should be explored, compared with 2D culture systems 3D MSCs cultured explored in clinical studies
Standardise the growth factors which should be used to enhance yield
GMP facility to ship and grow cells
Ex vivo complex 3D hybrid tissue most ideal

Isolation
Compare protocols to gain the highest yield and then standardise protocol so that comparisons of studies can be made

Gene therapy
Use of non-viral vectors
GMP facility processing unit
What is the best growth factor to modify the stem cells, synergistic effect?
What is best combination of scaffold and stem cell?
Use modified MSCs in clinical studies

Direct injection
Compare to gene and scaffold therapy in clinical studies
Need long-term follow-up of patients
Compare systemic or direct infusion
Standardise the centrifugation technique to increase concentration of MSCs

Scaffold
Best biomaterial yet to be found
Studies comparing effect of the biomaterials under mechanical strain, adapt *in vivo* model
Compare scaffold materials in clinical studies
Nano-scaffolds should be explored

Improvements for clinical trials
Include larger number of patients, prospective, randomised, use control patients (ethical problems)
Longer follow-up
Not only in last resort patients, investigate as a first-line treatment

Fig. 5

Diagram showing details of future mesenchymal stem cell (MSC) studies which are required for bone healing. There are aspects of MSC protocol which could be investigated further to aid the use of MSCs in clinical practice (2D, two-dimensional; 3D, three-dimensional).

studies discussed above that the application of MSCs to bone defects enhances bone formation without any adverse reactions to the patients. However, the success is limited so far because of the small numbers in the trials, the lack of controls and the short follow-up. Furthermore, most studies have investigated the application of MSCs in ‘worst-case’ clinical situations. Future studies need to explore the use of MSCs as a first-line treatment for bone defects, after the acquisition of adequate data to verify their effectiveness in bone repair and regeneration.

The transition of MSCs to clinical practice is developing fast as evidenced by major advances in studies performed on animals by introducing scaffolds and gene therapy. However, there are aspects of the application of MSCs which need further investigation (Fig. 5). Despite the advances in using MSCs on scaffolds, few studies have applied this technique in clinical trials. Studies in man can evolve by comparative studies to discover the optimal scaffold and by expansion into nano-scaffolds, particularly under strain models to determine mechanical stability. Genetic modification of MSCs has not been addressed in human bone healing because further studies are required to find the optimal and best combination of growth factors along with finding optimal non-viral vectors.

The future of stem cells looks promising as advances in tissue engineering, biomaterials and cell biology converge to enable stem cells to play a major role in the repair and regeneration of bone.

Support was obtained from NIHR, GATT family foundation and Eumedic Ltd.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

1. **Giannoudis PV, Einhorn TA, Marsh D.** Fracture healing: the diamond concept. *Injury* 2007;38(Suppl 4):S53-6.
2. **Cypher TJ, Grossman JP.** Biological principles of bone graft healing. *J Foot Ankle Surg* 1996;35:413-17.
3. **Finkemeier CG.** Bone-grafting and bone-graft substitutes. *J Bone Joint Surg [Am]* 2002;84-A:454-64.
4. **Parikh SN.** Bone graft substitutes: past, present, future. *J Postgrad Med* 2002;48:142-8.
5. **Bauer TW, Muschler GF.** Bone graft materials: an overview of the basic science. *Clin Orthop* 2000;371:10-27.
6. **Simpson AH, Mills L, Noble B.** The role of growth factors and related agents in accelerating fracture healing. *J Bone Joint Surg [Br]* 2006;88-B:701-5.
7. **Schindeler A, McDonald MM, Bokko P, Little DG.** Bone remodeling during fracture repair: the cellular picture. *Semin Cell Dev Biol* 2008;19:459-66.
8. **Bruder SP, Kurth AA, Shea M, et al.** Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. *J Orthop Res* 1998;16:155-62.
9. **Shang Q, Wang Z, Liu W, et al.** Tissue-engineered bone repair of sheep cranial defects with autologous bone marrow stromal cells. *J Craniofac Surg* 2001;12:586-93.
10. **Bruder SP, Kraus KH, Goldberg VM, Kadiyala S.** The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg [Am]* 1998;80-A:985-96.
11. **Ohgushi H, Goldberg VM, Caplan AI.** Repair of bone defects with marrow cells and porous ceramic: experiments in rats. *Acta Orthop Scand* 1989;60:334-9.
12. **Javazon EH, Beggs KJ, Flake AW.** Mesenchymal stem cells: paradoxes of passaging. *Exp Hematol* 2004;32:414-25.
13. **Klyushnenkova E, Mosca JD, Zernetkina V, et al.** T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci* 2005;12:47-57.
14. **Asari S, Itakura S, Ferreri K, et al.** Mesenchymal stem cells suppress B-cell terminal differentiation. *Exp Hematol* 2009;37:604-15.
15. **Jiang XX, Zhang Y, Liu B, et al.** Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood* 2005;105:4120-6.
16. **Spaggiari GM, Capobianco A, Abdelrazik H, et al.** Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* 2008;111:1327-33.
17. **Kitaori T, Ito H, Schwarz EM, et al.** Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. *Arthritis Rheum* 2009;60:813-23.
18. **Kassis I, Zangi L, Rivkin R, et al.** Isolation of mesenchymal stem cells from G-CSF-mobilized human periperal blood using fibrin microbeads. *Bone Marrow Transplant* 2006;37:967-76.
19. **Lazarus HM, Haynesworth E, Gerson SL, Caplan AI.** Human bone marrow-derived mesenchymal (stromal) progenitor cells (MPCs) cannot be recovered from peripheral blood progenitor cell collections. *J Hematother* 1997;6:447-55.
20. **D'Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA.** Age-related osteogenic potential of mesenchymal stromal cells from human vertebral bone marrow. *J Bone Miner Res* 1999;14:1115-22.
21. **Nunes SP, Galembeck F.** Percoll and Ficoll self-generated density gradients by low-speed osmocentrifugation. *Anal Biochem* 1985;146:58-51.
22. **Bonab MM, Alimoghaddam K, Talebian F, et al.** Aging of mesenchymal stem cell in vitro. *BMC Cell Biol* 2006;7:14.
23. **Stenderup K, Justesen J, Clausen C, Kassem M.** Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone* 2003;33:919-26.
24. **Abdallah BM, Kassem M.** Human mesenchymal stem cells: from basic biology to clinical applications. *Gene Ther* 2008;15:109-16.
25. **Schallmoser K, Rohde E, Bartmann C, et al.** Platelet-derived growth factors for GMP-compliant propagation of mesenchymal stromal cells. *Biomed Mater Eng* 2009;19:271-6.
26. **Devine SM, 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;29:244-55.
27. **Lazarus HM, Haynesworth SE, Gerson SI, Rosenthal NS, Caplan AI.** Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant* 1995;16:557-64.
28. **Liu L, Sun Z, Chen B, et al.** Ex vivo expansion and in vivo infusion of bone marrow-derived Flk-1+CD31-CD34- mesenchymal stem cells: feasibility and safety from monkey to human. *Stem Cells Dev* 2006;15:349-57.
29. **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.
30. **Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI.** The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusions. *Cells Tissues Organs* 2001;169:12-20.
31. **Schrepter S, Deuse T, Reichenspurner H, et al.** Stem cell transplantation: the lung barrier. *Transplant Procs* 2007;39:573-6.
32. **Jones E, McGonagle D.** Human bone marrow mesenchymal stem cells in vivo. *Rheumatology (Oxford)* 2008:47:126-31.
33. **Hutmacher DW.** Scaffold design and fabrication technologies for engineering tissues: state of the art and future perspectives. *J Biomater Sci Polym Ed* 2001;12:107-24.
34. **Frenkel SR, Di Cesare PE.** Scaffolds for articular cartilage repair. *Ann Biomed Eng* 2004;32:26-34.
35. **Brekke JH, Toth JM.** Principles of tissue engineering applied to programmable osteogenesis. *J Biomed Mater Res* 1998;43:380-98.
36. **Hou R, Chen F, Yang Y, et al.** Comparative study between coral-mesenchymal stem cells-rhBMP-2 composite and auto-bone-graft in rabbit critical-sized cranial defect model. *J Biomed Mater Res A* 2007;80:85-93.
37. **Leach JK, Kaigler D, Wang Z, Krebsbach PH, Mooney DJ.** Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration. *Biomaterials* 2006;27:3249-55.
38. **Shikinami Y, Okazaki K, Saito M, et al.** Bioactive and bioresorbable cellular cubic-composite scaffolds for use in bone reconstruction. *J R Soc Interface* 2006;3:805-21.
39. **Kim SS, Sun Park M, Jeon O, Yong Choi C, Kim BS.** Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* 2006;27:1399-409.
40. **Wang M.** Developing bioactive composite materials for tissue replacement. *Biomaterials* 2003;24:2133-51.
41. **Lin Y, Wang T, Wu L, et al.** Ectopic and in situ bone formation of adipose tissue-derived stromal cells in biphasic calcium phosphate nanocomposite. *J Biomed Mater Res A* 2007;81:900-10.
42. **Arinzeh TL, Tran T, McAlary J, Daculsi G.** A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem-cell-induced bone formation. *Biomaterials* 2005;26:3631-8.
43. **Gil-Albarova J, Salinas AJ, Bueno-Lozano AL, et al.** The in vivo behaviour of a sol-gel glass and a glass-ceramic during critical diaphyseal bone defects healing. *Biomaterials* 2005;26:4374-82.
44. **Kimelman-Bleich N, Seliktar D, Kallai I, et al.** The effect of ex vivo dynamic loading on the osteogenic differentiation of genetically engineered mesenchymal stem cell model. *J Tissue Eng Regen Med* 2010;Epub.
45. **Pittenger MF, Mosca JD, McIntosh KR.** Human mesenchymal stem cells: progenitor cells for cartilage, bone, fat and stroma. *Curr Top Microbiol Immunol* 2000;251:3-11.
46. **Quarto R, Mastrogiacomo M, Cancedda R, et al.** Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 2001;344:385-6.
47. **Marcacci M, Kon E, Moukhachev V, et al.** Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng* 2007;13:947-55.
48. **Warnke PH, Springer IN, Wiltfang J, et al**. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet* 2004;364:766-70.
49. **Morishita T, Honoki K, Ohgushi H, et al.** Tissue engineering approach to the treatment of bone tumors: three cases of cultured bone grafts derived from patients' mesenchymal stem cells. *Artif Organs* 2006;30:115-18.
50. **Ohgushi H, Kotobuki N, Funaoka H, et al.** Tissue engineered ceramic artificial joint: ex vivo osteogenic differentiation of patient mesenchymal cells on total ankle joints for treatment of osteoarthritis. *Biomaterials* 2005;26:4654-61.
51. **Kitoh H, Kitakoji T, Tsuchiya H, et al.** Transplantation of marrow-derived mesenchymal stem cells and platelet-rich plasma during distraction osteogenesis: a preliminary result of three cases. *Bone* 2004;35:892-8.
52. **Kawate K, Yajima H, Ohgushi H, et al.** Tissue-engineered approach for the treatment of steroid-induced osteonecrosis of the femoral head: transplantation of autologous mesenchymal stem cell cultured with beta-tricalcium phosphate ceramics and free vascularized fibula. *Artif Organs* 2006;30:960-2.
53. **Muschler GF, Matsukara Y, Nitto H, et al.** Selective retention of bone marrow-derived cells to enhance spinal fusion. *Clin Orthop* 2005;432:242-51.
54. **Gan Y, Dai K, Zhang P, et al.** The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion. *Biomaterials* 2008;29:3973-82.
55. **Gamradt SC, Lieberman JR.** Genetic modification of stem cells to enhance bone repair. *Ann Biomed Eng* 2004;32:136-47.

56. **Gamradt SC, Abe N, Bahamonde ME, et al.** Tracking expression of virally mediated BMP-2 in gene therapy for bone repair. *Clin Orthop* 2006;450:238-45.
57. **Lin L, Shen Q, Wei X, et al.** Comparison of osteogenic potentials of BMP4 transduced stem cells from autologous bone marrow and fat tissue in a rabbit model of calvarial defects. *Calcif Tissue Int* 2009;85:55-65.
58. **Herzog K.** Verlangerung osteotomie unter Verwendung des Percutan gezielt verwendung des percutan gezielt verriegelten Markangels. *Unfallheikunde* 1951;42:26 (in German).
59. **Connolly JF.** Injectable bone marrow preparations to stimulate osteogenic repair. *Clin Orthop* 1995;313:8-18.
60. **Hernigou P, Poignard A, Beaujean F, Rouard H.** Percutaneous autologous bone-marrow grafting for nonunions: influence of the number and concentration of progenitor cells. *J Bone Joint Surg [Am]* 2005;87-A:1430-7.
61. **Wongchuensoontorn C, Liebehenschel N, Schwarz U, et al.** Application of a new chair-side method for the harvest of mesenchymal stem cells in a patient with nonunion of a fracture of the atrophic mandible: a case report. *J Craniomaxillofac Surg* 2009;37:155-61.
62. **Dominici M, Le Blanc K, Mueller I, et al.** Minimal criteria for defining multipotent mesenchymal stromal cells: the International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-17.