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REVIEW

Stem cells for repair of cartilage and bone: the next challenge in osteoarthritis and rheumatoid arthritis

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Over the last few years, immunotherapy targeting proinflammatory cytokines has been the main goal of research into rheumatoid arthritis (RA), and recently the new anti-(tumour necrosis factor) blocking agents have dramatically improved the course of the disease by stabilising the symptoms.¹ However, this treatment has no effect on regeneration of articular cartilage damaged during the inflammatory process. The next challenge is thus to ensure cartilage repair through cell therapy and tissue engineering (fig 1). Tissue in the body is replaced by two main mechanisms. One is self repair by fully differentiated cells (healing), and

the second is replacement with newly differentiated cells derived from stem cells. Recently, the regenerative potential of mesenchymal stem cells (MSCs) has been under intense investigation because of their ability for self renewal and differentiation to reconstitute muscle, cartilage, or bone.²

Articular cartilage is a complex tissue consisting of cartilage matrix and the chondrocytes that have produced the matrix during development. The matrix is composed of collagens and proteoglycan aggregates (aggrecan) which provide the architectural structure and biomechanical strength of the tissue. During RA pathogenesis, proinflammatory cytokines present in the rheumatoid synovial fluid activate metalloproteinase (MMP) secretion by chondrocytes and increase pericellular matrix degradation of these cells. Some of them (interleukin 1 β and 6 (IL1 β and IL6)) even display the synergistic ability to decrease aggrecan chondral synthesis and hyaluronan/aggrecan ratio in the extracellular matrix.^{3,4} However, proteoglycan depletion may be partially inhibited by IL1 receptor antagonist.⁵ Cartilage and bone degeneration in RA is also the result of the action of MMPs secreted into the synovial fluid by the synoviocytes. MMPs constitute a family of 18 endopeptidases, the secretion of which is stimulated by cytokines of the IL6 family (IL11, IL6, oncostatin M) through a protein called the extracellular matrix metalloproteinase inducer. This 58 kDa glycoprotein is expressed by activated monocytes and initiates the MMP cascade.⁶ Simultaneously, serine proteases are released by polymorphonuclear cells present in the inflamed joints. Joint destruction in RA thus results from an imbalance between the activity of proteases and their inhibitors which are also released: tissue inhibitors of metalloproteinase for MMPs, and secretory leucocyte proteinase inhibitor for the serine protease.⁷ Cartilage

Figure 1 Different strategies for cartilage repair.

degradation is also inhibited by other factors: either anti-inflammatory cytokines such as IL10, which act indirectly by counteracting the effects of proinflammatory cytokines, or growth factors of the transforming growth factor β (TGF β) family (TGF β 1 and bone morphogenetic protein (BMP)-2), which stimulate synthesis of collagen type II and proteoglycan by chondral cells.⁸ These chondrocyte stimulating factors, in particular BMP-2, are secreted by synovial cells in the presence of IL1 in a dose dependent manner.⁹ Thus some of the factors of cartilage healing are present in arthritic joints, but probably not in sufficient amount to inhibit the catabolic activities of proinflammatory cytokines.

Growth factors for cartilage repair

The TGF β superfamily consists of more than 40 polypeptide growth factors which share high homology with seven conserved cysteine residues in their C-terminal region.¹⁰ These peptides bind to a cell surface receptor complex that activates the Smad proteins. The Smads then assemble multisubunit complexes in the nucleus, where they act as transcription factors.¹¹ TGF β related molecules or BMPs regulate the morphogenetic events during embryonic development and are important signalling molecules during the development of the skeleton in vertebrates.¹² BMP is also involved in bone healing as well as in cartilage metabolism in adults. After bone fracture, healing proceeds through endogenous cartilage formation followed by blood vessel invasion delivering to the wound both chondroclasts and osteoblasts, which are responsible for resorption of cartilage and its replacement by bone (endochondral ossification). Therefore the use of growth factors that are able to stimulate regeneration of depleted cartilage could be of therapeutic value.

MORPHOGENETIC PROTEINS FOR BONE REPAIR

Bone healing may be achieved through local implantation of a biodegradable matrix impregnated with recombinant human BMP-2 (rhBMP-2), as shown in a 5 mm femoral defect created in Lewis rats.¹³ By week 12, 80% of the union was achieved with good mechanical properties compared with 38% when a bone graft was used or 47% when bone marrow alone was used. This formation of new bone is due to the recruitment of bone marrow MSCs induced by the chemoattractant properties of BMP-2. In addition, BMP-2 has proliferative and osteocyte differentiating properties.¹⁴ Pluripotent stem cells may also be recruited from the muscle, as suggested by endochondral bone formation after intramuscular injection of an adenoviral vector expressing human BMP-2 in nude rats.¹⁵ In these experiments, histological observations disclosed the presence of chondrocytes in the muscle by day 9, which then secreted a cartilaginous matrix on day 12. Four days later, osteoblasts could be observed, and mineralisation of the matrix occurred. Bone had undergone remodelling three months later, and showed evidence of a mature stroma heavily populated with cells.

MORPHOGENETIC PROTEINS FOR CARTILAGE REPAIR

In the joint, BMP-2 induces synthesis of extracellular matrix by chondrocytes and therefore cartilage growth. Injection of rhBMP-2 into the joint induced proteoglycan synthesis in a dose dependent manner, up to three times the initial value.¹⁶ Compared with TGF β 1, human BMP-2 stimulated proteoglycan synthesis more efficiently, but its biological effect was more time restricted. In the same way, implantation of collagen sponges impregnated with rhBMP-2 into osteochondral defects of rabbit knees enabled regeneration of the impaired tissue after four weeks.¹⁷ Safranin O and collagen type II staining showed the presence of histologically normal cartilage at 24 weeks. However, four of the 12 defects showed a persistent gap at the margins of the surrounding host cartilage, and the regenerated tissue remained 70% thinner and irregular at its surface. These studies did not evaluate the mechanical properties of the treated joints, and long term follow up (after 24 weeks) results were not available. Finally, cartilage repair in induced arthritis in rabbit was not evaluated. Thus the importance of inflammation has been shown in experiments with arthritic mice by rhBMP-2 injection into joints unable to induce proteoglycan synthesis. This study shows that stimulation of proteoglycan synthesis with rhBMP-2 did not inhibit IL1 α induced proteoglycan depletion.¹⁸

OTHER CARTILAGE MORPHOGENIC FACTORS

In contrast with BMP-2, BMP-7 or osteogenic protein (OP-1) was effective in restoring proteoglycan synthesis by chondrocytes in culture despite the presence of IL1 β .¹⁹ It should, however, be noted that persistent stimulation with BMP-2 or OP-1 could induce bone formation. Other members of the TGF β family, such as CDMP-1 or CDMP-2 (GDF-5 and GDF-6 respectively) seem to be more specific differentiation factors of cartilaginous tissue and could be useful for cartilage repair without inducing further ossification of the regenerative tissue.²⁰ Several additional factors involved in chondrogenesis during embryogenesis have also been identified. Of these, Indian Hedgehog, parathyroid hormone related protein, fibroblast growth factor, insulin-like growth factor, and Noggin regulate chondrogenesis. The nuclear factor of activated T cells (NFATp) is critical for cartilage morphogenesis in the adult animal and controls differentiation of MSCs into cartilage as shown in NFATp $^{-/-}$ mice, which develop ectopic formations of cartilage and show extensive destruction of peripheral joints after 6 months of age.²¹ However, to obtain effective cartilage repair, chondral specific factors have to be combined with cell therapy.

Cells for cartilage repair

To obtain cartilage repair in arthritic joints, both downregulation of inflammatory cytokines and introduction of cartilage-producing cells must be achieved. In contrast, cartilage injuries caused by trauma can be

managed by allogenic cartilage transplantation or implantation of differentiated chondrocytes into the cartilage defect.

AUTOLOGOUS CHONDROCYTES

The first approach uses osteochondral shell allograft resurfacing of the knee, with 76% good outcome with a 75 month follow up.²² The second approach, used in a study by Brittberg *et al*,²³ is autologous chondrocyte transplantation into the articular surface of injured knee joints. A clinical benefit was obtained in 14 out of 16 patients, and normal cartilage was observed after a two year follow up. However, in this clinical trial, the patients were less than 50 years of age with presumably healthy chondrocytes. Moreover, in the therapeutic procedure, a periosteal flap was sutured over the cartilage defect. As this tissue is rich in osteoprogenitor cells, participation of stem cells in the repair pathway cannot be excluded. This method will probably not be applicable in chronic inflammatory diseases such as RA, in which injured chondrocytes are often observed in the cartilage. Chondrocytes present in inflamed joints lose their differentiation, and up to 3% of the cells undergo apoptosis.²⁴

BONE MARROW

Another strategy uses autologous cells that can differentiate into chondrocytes. These cartilage potent stem cells may arise from synovial tissue or migrate from the bone marrow. In fact, formation of new cartilage was observed in synovial explants cultured with TGF β 1 in either agarose or aggregate culture.²⁵ Without TGF β 1, chondrocyte differentiation was not observed, suggesting the need for continuous TGF β 1 secretion. Total bone marrow transplantation has also been used. Patients with osteogenesis imperfecta (a genetic disorder caused by a mutation in the type I collagen gene and characterised by generalised osteopenia) have been treated by systemic injection of allogeneic bone marrow. The treatment resulted in osteoblast engraftment (1.5–2% of donor cells) at three months and an increase in total body bone mineral content and skeletal growth.²⁶ Total bone marrow has also been used to fill cartilage defects experimentally created in the distal femoral condyle of New Zealand White rabbits. In these experiments, six weeks after transplantation, cartilaginous cells were observed in the defect, which were then rapidly replaced by bone and covered by a fibrous layer at the top of the defect.²⁷ By 12 weeks, bone had completely filled the defect, and there was no cartilage left. These results prompted a search for autologous pluripotent stem cells in bone marrow with the ability to differentiate into chondrocytes and their possible use for regeneration of tissue of mesenchymal origin.

MSCS FOR BONE REPAIR

MSCs are pluripotent cells present in the bone marrow in low quantity (1 out of 10⁴–10⁵ mononuclear cells), which are capable of differentiating into chondrocytes, osteocytes, myocytes, and adipocytes.²⁸ These stem cells

support the growth of haematopoietic progenitors by secreting a number of haematopoietic cytokines (GM-CSF, IL6, IL7, IL8, IL11) and stem cell factors such as SDF-1. They are distinct from haematopoietic stem cells as they are not progenitors for the haematopoietic lineages and they do not express CD34, CD45, CD14 or T or B cell markers.²⁹

MSCs can be used in two different therapeutic strategies. The first uses MSCs as a cell factory for protein delivery by introducing *ex vivo* the gene coding for a secreted protein and reimplanting the cells back into the animal. In experimental SCID/hu models, MSCs engineered to produce IL3 were seeded into ceramic cubes which were then subcutaneously implanted into mice. MSC differentiation inside the cubes resulted in bone formation, and human IL3 was detected in the systemic circulation for at least 12 weeks.³⁰ Similarly, implantation of MSCs genetically engineered to express soluble tumour necrosis factor receptor II into NOD/SCID mice resulted in detection of the cytokine in the serum, and its functionality was shown by decreased serum levels of mouse tumour necrosis factor α .³¹

The second strategy uses MSCs as pluripotent cells which will differentiate *in vivo* to repair non-functional tissue or generate new tissue.³² This approach was successfully used in a breast cancer phase I trial for patients receiving high dose chemotherapy. Rapid haematopoietic recovery was obtained after co-infusion of autologous expanded human MSCs and peripheral blood progenitor cells.³³ This strategy should be particularly useful for the repair of bone and cartilage in articular pathologies and fracture healing. Primary bone marrow cells were not able to heal femoral defects after two months whereas engineered cells expressing BMP-2 induced complete bone fusion in 22 out of 24 treated animals.³⁴ Moreover, BMP-producing cells induced robust trabecular bone, compared with defective bone when injected with recombinant BMP-2. In Lewis rats, implantation of bone marrow in polylactic acid/polyglycolic acid carrier resulted in less than 50% of bone fusion by 12 weeks, whereas rhBMP-2 and bone marrow together achieved 100% union as early as six weeks.¹³ In rabbit, Boden *et al*³⁵ did not obtain bone fusion using bone marrow seeded in ceramic without BMP stimulation for the four weeks of follow up. However, when bone marrow and osteoinductive rhBMP-2 were combined in an inert polylactic acid carrier, a synergistic effect leading to bone formation was observed. All femur defects in the rabbit were filled and united at six weeks. It should be noted that, in these experiments, rhBMP-2 in the absence of bone marrow achieved 80% union by week 12, probably through recruitment of local stem cells. Thus, a composite bone graft using osteoprogenitor cells, osteoinductive factors, and a biodegradable matrix showed synergy and superiority over a simple bone graft.

To enhance the quality of bone repair further, several groups have developed MSCs originating from the bone marrow. C3H/10T1/2 is a murine mesenchymal stem cell line

which has often been used both in vitro and in vivo. Engineered C3H/10T1/2 cells expressing human BMP-2 spontaneously differentiate in vitro into osteogenic cells and induce segmental defect repair by four to eight weeks after transplantation in syngeneic mice.³⁶ In this case, cell mediated treatment offers the advantages of recruiting stem cells from the recipient (paracrine effect) and inducing new endochondral bone formation (autocrine effect). Adenovirus mediated transfer of human BMP-2 gene into C3H/10T1/2 cells resulted in bone formation after intramuscular injection in nude mice.¹⁵ In these experiments, human BMP-2 induced both cell proliferation and differentiation in vitro.

MSCS FOR CARTILAGE REPAIR

Urist *et al*^{37, 38} reported many years ago that MSCs may be used as chondrocyte progenitor cells for cartilage healing after stimulation by cell-cell contact and soluble factors such as TGFβ1 and human BMP-2. As early as day 5 a cellular matrix containing type II collagen was present.³⁹ Caplan *et al*²⁷ showed that, after implantation into the femoral condyle, rabbit MSCs enclosed in a highly hydrated type I collagen matrix filled the cartilage defects after four weeks. The quality of the regenerated cartilage at six months was impressive, but a discontinuity between the host tissue and the new one was observed and 20–100% of the edges were not physically integrated to the cartilage. This could be partially solved by a short exposure of the cartilage surface to trypsin, which alters the type II collagen scaffolding and favours infiltration of host cartilage by the expanding new tissue. MSCs arising from bone marrow or periosteum were compared with respect to their capacity for repair of articular cartilage in rabbit.⁴⁰ In these experiments, cartilage defects were filled with 10⁶ cells included in a collagen matrix, and, as early as two weeks, MSCs were differentiated into chondrocytes which were subsequently replaced by bone. At 12 weeks, the subchondral bone was completely repaired without any loss of articular cartilage at the surface. This repair process was limited by progressive thinning of the repaired tissue, which was clearly discernible at 24 weeks. Moreover, with the periosteal derived MSCs, the repaired surface tended to become fibrillate, whereas, with the bone marrow derived MSCs, the articular surface remained smooth. In fact, despite normal histological appearance, the mechanical properties of the repaired tissue did not return to normal (average compliance 5.2 compared with 11 in normal cartilage) and a gap between it and the adjacent cartilage appeared in most of the treated condyles. Thus MSCs from bone marrow have useful applications for cartilage repair, but the methodology requires improvement in the area of both cartilage thickness and mechanical properties. Engineered MSCs may improve the quality of the repair through continuous secretion of a chondral differentiating factor.

Conclusion

Regeneration of damaged cartilage in different pathological situations is a major goal which could be achieved through cell and/or gene therapy. Although autologous chondrocytes have been successfully used in traumatic pathologies, they do not have the qualities required for cartilage repair in cases of chronic inflammation. In the same way, transplantation of total bone marrow is limited by the low number of progenitor cells present in bone marrow aspirates. MSCs seem to be the best candidates for cell therapy for regeneration of injured tissue.² They are easily isolated from bone marrow and can be rapidly amplified up to 2000-fold over about 10 days.⁴¹ When these cells are implanted into cartilage defects, they appear to be able to proliferate and differentiate into chondrocytes. This procedure is of considerable relevance for the regeneration of large areas of cartilage lesions. However, complete healing is difficult to obtain and requires integration of newly regenerated tissue with the surrounding host tissue and true differentiation through pathways involved in embryonic development. This goal may be achieved through the combination of MSC mediated therapy and gene transfer of selective differentiating cytokines.

1 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, *et al*. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor, a monoclonal antibody, combined with low dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998;41:1552–63.

2 Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science* 2000;287:1442–6.

3 Dsouza AL, Masuda K, Otten LM, Knudson W, Thonar EJ. Differential effect of IL1 on hyaluronan and proteoglycan metabolism in two compartments of the matrix formed by articular chondrocytes maintained in alginate. *Arch Biochem Biophys* 2000;374:59–65.

4 Jikko A, Wakisaka T, Iwamoto M, Hiranuma H, Kato Y, Maeda T, Fujishita M, *et al*. Effects of IL6 on proliferation and proteoglycan metabolism in articular chondrocyte culture. *Cell Biol Int* 1998;22:615–21.

5 Muller-Ladner U, Roberts CR, Franklin BN, Gay RE, Robbins PD, Evans CH, *et al*. Human IL-1Ra gene transfer into human synovial fibroblasts is chondroprotective. *J Immunol* 1997;158:3492–8.

6 Kontinen Y T, Li T, Mandelin J, Liljestrom M, Sorsa T. Increased expression of extracellular matrix metalloproteinase inducer in rheumatoid synovium. *Arthritis Rheum* 2000;43:275–81.

7 Song XY, Zeng L, Jin W, Thompson J, Mizel DE, Lei KJ. Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. *J Exp Med* 1999;190:535–42.

8 Vanbeuningen HM, Glansbeek HL, Vander Kraan PM, Van Den Berg WB. Osteoarthritis like change in the murine knee joint resulting from intraarticular TGFβ injections. *Osteoarthritis Cartilage* 2000;8:25–33.

9 Fowler MJ, Neff MS, Borghaei RC, Pease EA, Mochan E, Thornton RD. Induction of BMP-2 by IL1 in human fibroblasts. *Biochem Biophys Res Commun* 1998;248:450–3.

10 Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. *Clin Orthop* 1998;346:26–37.

11 Massague J, Wotton D. Transcriptional control by the TGF-β/Smad signaling system. *EMBO J* 2000;19:1745–54.

12 Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 1999;400:687–92.

13 Lane JM, Yasko AW, Tomin E, Cole BJ, Waller S, Browne M, *et al*. Bone marrow and recombinant human bone morphogenetic protein-2 is osseous repair. *Clin Orthop* 1999;361:216–27.

14 Alden TD, Pittman DD, Hankins GR, Beres EJ, Engh JA, Das S, *et al*. In vivo endochondral bone formation using a bone morphogenetic protein 2 adenoviral vector. *Hum Gene Ther* 1999;10:2245–53.

15 Lou J, Xu F, Merkel K, Manske P. Gene therapy: adenovirus-mediated human bone morphogenetic protein-2 gene transfer induces mesenchymal progenitor

- cell proliferation and differentiation in vitro and bone formation in vivo. *Orthop Res* 1999;17:43–50.
- 16 Vanbeuningen HM, Glansbeek HL, Vander Kraan PM, Van Den Berg WB. Differential effects of local application of BMP-2 or TGFβ1 on both articular cartilage composition and osteophyte formation. *Osteoarthritis Cartilage* 1998;6:306–17.
- 17 Sellers RS, Peluso D, Morris EA. The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1997;79:1452–63.
- 18 Glansbeek HL, Van Beuningen HM, Vitters EL, Morris EA, Van Der Kraan PM, Van Den Berg WB. Bone morphogenetic protein 2 stimulates articular cartilage proteoglycan synthesis in vivo but does not counteract interleukin-1α effects on proteoglycan synthesis and content. *Arthritis Rheum* 1997;40:1020–8.
- 19 Lietman SA, Yanagishita M, Campath TK, Beddi H. Stimulation of proteoglycan synthesis in explants of porcine articular cartilage by recombinant osteogenic protein-1 (bone morphogenetic protein-7). *J Bone Joint Surg Am* 1997;79:1132–7.
- 20 Erlacher L, Ng CK, Ullrich R. Presence of cartilage-derived morphogenetic proteins in articular cartilage and enhancement of matrix replacement in vitro. *Arthritis Rheum* 1998;41:263–73.
- 21 Ranger AM, Gerstenfeld LC, Wang J, Kon T, Bae H, Gravallese EM, *et al.* The nuclear factor of activated T cells (NFAT) transcription factor NFATp (NFATc2) is a repressor of chondrogenesis. *J Exp Med* 2000;191:9–21.
- 22 Chu CR, Convery FR, Akeson W, Meyers M, Amiel D. Articular cartilage transplantation. *Clin Orthop* 1999;360:159–68.
- 23 Brittberg M, Anders L, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;331:889–95.
- 24 Kim HA, Song YW. Apoptotic chondrocyte death in rheumatoid arthritis. *Arthritis Rheum* 1999;42:1528–37.
- 25 Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B. Chondroprogenitor cells of synovial tissue. *Arthritis Rheum* 1999;42:2631–7.
- 26 Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, *et al.* Transplantability and therapeutic effects of bone marrow derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999;5:309–13.
- 27 Caplan AI, Elyaderani M, Mochizuki Y, Wakitani S, Goldberg VM. Principles of cartilage repair and regeneration. *Clin Orthop* 1997;342:254–69.
- 28 Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
- 29 Gerson SL. Mesenchymal stem cells: no longer second class marrow citizens. *Nat Med* 1999;5:262–4.
- 30 Allay J, Dennis JE, Haynesworth S, Majumdar M, Clapp DW, Shultz L, *et al.* LacZ and IL3 expression in vivo after retroviral transduction of marrow derived human osteogenic mesenchymal progenitors. *Hum Gene Ther* 1999;8:1417–27.
- 31 Liu L, Wang G, Lee K, Hoppa N, Buyaner D, Hendricks K, *et al.* Expression of soluble TNF-RII from transduced human mesenchymal stem cells: *in vitro* and *in vivo* efficacy [abstract]. *Blood* 1999:94.
- 32 Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276:71–4.
- 33 Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE. Rapid hematopoietic recovery after coinfusion of autologous culture expanded human mesenchymal stem cells and PBPC in breast cancer patients receiving high dose chemotherapy [abstract]. *Blood* 1998;92:274A.
- 34 Scaduto A, Lieberman JR. Gene therapy for osteoinduction. *Orthop Clin North Am* 1999;30:625–33.
- 35 Boden SC, Martin GJ, Morone M, Ugbo JL, Titus L, Hutton WC. The use of coralline hydroxyapatite with bone marrow, autogenous bone graft, or osteoinductive bone protein extract for posterolateral lumbar spine fusion. *Spine* 1999;24:320–7.
- 36 Gazit D, Turgeman G, Kelley P, Wang E, Jalenak M, Zilberman Y, *et al.* Engineered pluripotent mesenchymal cells integrate and differentiate in regenerating bone : a novel cell-mediated gene therapy. *Journal of Gene Medicine* 1999;1:121–33.
- 37 Nogami H, Urist MR. A substratum of bone matrix for differentiation of mesenchymal cells into chondro-osseous tissues in vitro. *Exp Cell Res* 1970;63:404–10.
- 38 Urist MR, Nogami H. Morphogenic substratum for differentiation of cartilage tissues in culture. *Nature* 1970;225:1051–2.
- 39 Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, *et al.* The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg [Am]* 1998;80:1745–57.
- 40 Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, *et al.* Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg [Am]* 1994;76:579–92.
- 41 Colter DC, Class R, Digirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proc Natl Acad Sci USA* 2000;97:3213–18.