Technology Insight: adult mesenchymal stem cells for osteoarthritis therapy

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SUMMARY

Despite the high prevalence and morbidity of osteoarthritis (OA), an effective treatment for this disease is currently lacking. Restoration of the diseased articular cartilage in patients with OA is, therefore, a challenge of considerable appeal to researchers and clinicians. Techniques that cause multipotent adult mesenchymal stem cells (MSCs) to differentiate into cells of the chondrogenic lineage have led to a variety of experimental strategies to investigate whether MSCs instead of chondrocytes can be used for the regeneration and maintenance of articular cartilage. MSC-based strategies should provide practical advantages for the patient with OA. These strategies include use of MSCs as progenitor cells to engineer cartilage implants that can be used to repair chondral and osteochondral lesions, or as trophic producers of bioactive factors to initiate endogenous regenerative activities in the OA joint. Targeted gene therapy might further enhance these activities of MSCs. Delivery of MSCs might be attained by direct intra-articular injection or by graft of engineered constructs derived from cell-seeded scaffolds; this latter approach could provide a three-dimensional construct with mechanical properties that are congruous with the weight-bearing function of the joint. Promising experimental and clinical data are beginning to emerge in support of the use of MSCs for regenerative applications.

KEYWORDS articular cartilage, biomaterial scaffold, gene delivery, osteoarthritis, mesenchymal stem cell

REVIEW CRITERIA

We searched for full-text, English-language articles in the PubMed database up to December 2007 using the term "stem cells" in combination with "osteoarthritis", "articular cartilage", "biomaterial scaffold" and "intra-articular injection", as well as the combination of "stem cells and cartilage and gene therapy". We also searched the reference lists of identified articles for additional published reports.

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INTRODUCTION

Osteoarthritis (OA), the most common form of joint disease, is characterized by degeneration of the articular cartilage and, ultimately, joint destruction.[1] Currently, OA is a major cause of disability in the elderly; the prevalence of this disease is expected to increase dramatically over the next 20 years with an increasingly aged population.[2] The burden of OA is exacerbated by the inadequacies of current therapies. Nonpharmacologic and pharmacologic treatments are used for early and moderately early cases of OA, but protection of articular cartilage has so far not been convincingly shown.[3,4] Surgical intervention is often indicated when the symptoms cannot be controlled and the disease progresses.[5] Whether arthroscopic lavage and/or debridement can provide symptomatic relief is unclear.[6] Methods for the repair of articular cartilage lesions include the transplantation of osteochondral grafts, microfracturing, and autologous chondrocyte implantation, with or without the assistance of a scaffold matrix to deliver the cells;[7–12] however, all of these techniques are limited to the repair of focal lesions.[13] Consequently, patients with OA are currently excluded from these treatments. In the case of joint malalignment,[14] osteotomy can provide pain relief for several years, until the new weight-bearing articular cartilage erodes, but this tactic merely buys time until a total knee replacement becomes necessary. The challenge for researchers to develop disease-modifying OA treatments is, therefore, of paramount importance.

Adult mesenchymal stem cells (MSCs), which have the ability to differentiate into cells of the chondrogenic lineage, have emerged as a candidate cell type with great potential for cell-based articular cartilage repair technologies. MSCs can be isolated from a variety of adult tissues, readily culture-expanded without losing their multilineage differentiation potential, and have been induced to undergo chondrogenic differentiation *in vitro* and *in vivo*.[15–17] Unlike chondrocytes,

the use of MSCs is not hindered by the limited availability of healthy articular cartilage or an intrinsic tendency of the cells to lose their phenotype during expansion. The use of MSCs also obviates the need for a cartilage biopsy and, thereby, avoids morbidity caused by damage to the donor-site articular surface.

In this Review, we will discuss current MSC-based strategies for the treatment of OA. We first address the etiopathophysiology of OA and the mechanisms responsible for breakdown of the cartilage extracellular matrix. We then discuss the potential of MSCs for articular cartilage repair in patients with OA, with particular respect to the chondrogenic differentiation potential of MSCs, and review the currently used experimental strategies (intra-articular injection, matrix-guided technologies, and gene therapy). An example of the repair of articular cartilage defects by use of a hydrogel seeded with MSCs is presented, to highlight the current strategies, limitations and perspectives of using MSCs to treat OA.

ETIOPATHOPHYSIOLOGY OF OA

The late stage at which OA is diagnosed, difficulties in studying the disease in humans, and inadequacies in animal models of OA account for (or contribute to) the poor understanding of this disease. Much research into the pathophysiology of OA has focused on the loss of articular cartilage, caused by mechanical and oxidative stresses, aging or apoptotic chondrocytes.[18] Articular chondrocytes within diseased cartilage synthesize and secrete proteolytic enzymes, such as matrix metalloproteinases and aggrecanases, which degrade the cartilaginous matrix. The proinflammatory cytokine interleukin 1 (IL-1) is the most powerful inducer of these enzymes and of other mediators of OA in articular chondrocytes. The induction of these factors leads to matrix depletion through a combination of accelerated breakdown and reduced synthesis.[18] Other proinflammatory cytokines, such as tumor necrosis factor, are also involved in cartilage breakdown and, together with biomechanical factors implicated in OA etiopathophysiology,[19,20] contribute to induction of the disease. Despite the considerable efforts put into development of inhibitors of these molecules for use in treating OA, clinical success with respect to the prevention of further cartilage matrix breakdown or cartilage restoration in OA remains elusive.[21,22]

POTENTIAL OF MESENCHYMAL STEM CELLS TO AID CARTILAGE RESTORATION

Some of the various OA pathologies might be obviated by the application of cell-based treatments. MSCs are multilineage progenitors that can be stimulated to differentiate along specific pathways, including chondrogenesis.[15] In contrast to mature chondrocytes, which must be surgically harvested from a limited supply of non-weight-bearing articular cartilage, MSCs can be readily harvested from bone marrow or other tissues of mesenchymal origin, and will maintain their multilineage potential even with extended passage, which enables their considerable expansion in culture.[16,17] MSCs are commonly isolated by adherence to cell-culture plastic or by density-gradient fractionation and, therefore, represent a heterogeneous population of cells.[16,17] Although no definitive marker(s) for MSCs has been identified, an immunophenotype that is positive for STRO-1, CD73, CD146, CD105, CD106, and CD166, and negative for CD11b, CD45, CD34, CD31 and CD117 has been shown to be the most reliable means of characterizing the MSC population.[16,17]

For the purpose of cartilage regeneration, extensive analyses of microenvironments that promote chondrogenesis in MSCs *in vitro* have been performed. Conditioning the culture medium with growth factors such as fibroblast growth factor 2 or transforming growth factor β during monolayer expansion enhances positive selection for chondroprogenitor cells.[23] The development of effective methods to maintain an articular cartilage phenotype without hypertrophy, ossification or fibrinogenesis, and a delivery system to localize the cells within a lesion without compromising their chondrogenic differentiation or the integrity of the repair tissue[13] are additional requirements for the use of MSCs in articular cartilage regeneration.

EXTENDING THE APPLICATION OF MESENCHYMAL STEM CELLS TO OA CARTILAGE

Despite the promising features of MSCs and their potential to reverse some of the pathology associated with OA, cartilage defects that arise from an underlying disease process (such as occurs in OA) are distinct from focal cartilage lesions that result from acute injury or osteochondrosis dissecans, and this difference must be taken into consideration. Specifically, acute cartilage injury and osteochondrosis dissecans often

occur in an otherwise healthy joint; the patient might be young, and the focal defect will probably require localized treatment. By contrast, patients with OA are likely to be elderly, and often the entire articulating surface will require treatment. Repair of lesions might provide symptomatic relief and delay the progression of OA symptoms, but without effective treatment of the underlying disease, any improvement is likely to be short-lived.

Some researchers have suggested that tissue damage in progressive, degenerative, joint diseases might be related to the depletion or functional alteration of MSC populations.[24] Of importance, when considering the potential application of MSCs in OA treatment, researchers should ascertain whether MSCs obtained from the patient with OA differ functionally from those of healthy individuals, in terms of their chondrogenic capacity and longevity. The proliferative, chondrogenic and adipogenic capacities of MSCs obtained from patients with OA are reportedly reduced.[25] Perhaps the altered activity status of these MSCs is related to their exposure to elevated levels of proinflammatory cytokines and/or anti-inflammatory drugs. Whether susceptibility to OA might result from reduced mobilization or proliferation of MSCs remains to be ascertained.[24] Another factor associated with OA is advanced age; several studies have described an age-dependent reduction in the number of progenitor cells isolated from human bone marrow,[26,27] although others could not find any such inverse relationship between age and MSC numbers.[25,28] Also, an age-dependent decline in the differentiation capability of MSCs has been reported by several investigators.[25,27–29] In this context, however, researchers and clinicians should note that sufficient numbers of MSCs with adequate chondrogenic differentiation potential can be isolated from patients with OA, irrespective of their age or the etiology of their disease.[23,30,31] These results, therefore, suggest that the therapeutic use of MSCs for the regeneration of cartilage in patients with OA is feasible.

DELIVERY MODES FOR MESENCHYMAL STEM CELLS

A crucial requirement for MSC-based OA therapy is the delivery of the cells to the defect site. Direct intra-articular injection might be possible in early stages of the disease when the defect is restricted to the cartilage layer, whereas a scaffold or matrix of some kind would be required to support the MCSs in cases where the subchondral bone is exposed over large areas.

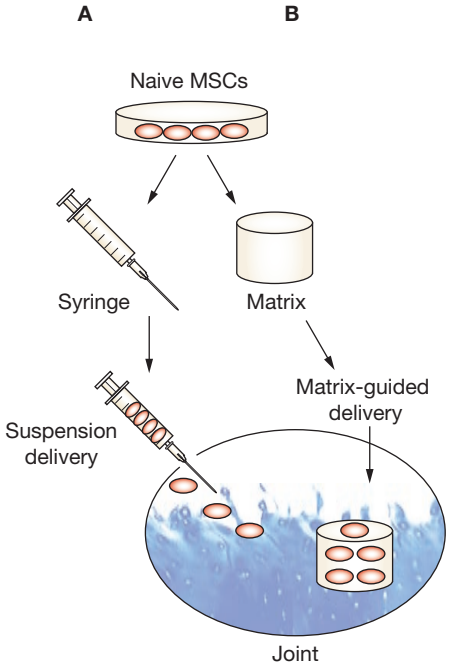

Figure 1 Delivery of MSCs to diseased cartilage in patients with osteoarthritis. (**A**) Direct intra-articular injection of naive MSCs. After harvest from an appropriate source, MSCs can be delivered in suspension to the joint space, where they encounter all intra-articular tissues. (**B**) Matrix-guided application of naive MSCs. Restoration of the deep cartilage defects that occur in osteoarthritis might require MSCs to be seeded into a biodegradable scaffold, which enables their controlled, local application to damaged areas of cartilage. Abbreviation: MSC, mesenchymal stem cell.

Direct intra-articular injection of MSCs

Direct intra-articular injection of MSCs is, technically, the simplest approach to their use in OA therapy (Figure 1A). Following injection, MSCs would be distributed throughout the joint space, and would interact with any available receptive cells and surfaces. The highly cellular synovium lines all the internal surfaces of the joint space, except for the cartilage and meniscus, so it is likely to be a primary tissue for MSC interaction.

Direct intra-articular injection of MSCs has only been carried out a few times. In one study, autologous MSCs in a dilute solution of sodium hyaluronan (hyaluronic acid) were directly injected into the knee joints of goats, in which

OA had been induced by a total medial meniscectomy and resection of the anterior cruciate ligament.[32] Joints exposed to MSCs showed evidence of marked regeneration of the medial meniscus, and implanted cells were detected in the newly formed tissue. Articular cartilage degeneration, osteophytic remodeling, and subchondral sclerosis were also reduced in the treated joints. There was no evidence of repair of the ligament in any of the joints.[32] Whether the changes observed in MSC-treated joints resulted from direct tissue repair by the transplanted cells or from their interaction with host synovial fibroblasts at the site of injury is still unclear.

In another study, a freshly created, partial-thickness cartilage defect in the knee joints of mini-pigs was also treated by direct intra-articular injection of MSCs suspended in hyaluronic acid.[33] The cell-treated group of animals showed improved cartilage healing compared with the control group. The authors postulated that hyaluronic acid might facilitate the migration and adherence of MSCs or MSC-like cells—probably derived from the synovium—to the defect, which might explain the occurrence of partial healing at 6 weeks in animals that were treated with hyaluronic acid alone. The repair tissue in animals treated with hyaluronic acid alone was of inferior quality, however (possibly because an insufficient number of endogenous MSCs were recruited to the injury site), and was shown to deteriorate further by 12 weeks.

The exact mechanisms that guide homing of implanted or mobilized MSCs are not known, but it is clear that these cells secrete a broad spectrum of bioactive molecules that have immunoregulatory[34,35] and/or regenerative activities.[36] Bioactive factors secreted by MSCs have been shown to inhibit tissue scarring, suppress apoptosis, stimulate angiogenesis, and enhance mitosis of tissue-intrinsic stem or progenitor cells. The complex, multifaceted effects that result from the secretory activity of MSCs have been referred to as 'trophic activity'. Of note, the trophic activity of MSCs is distinct from their capacity to differentiate.[37]

Matrix-guided application of MSCs

Compared with direct intra-articular injection, MSC application to eroded cartilage surfaces via a scaffold offers more control (Figure 1B). Seeding MSCs into a scaffold, such as a biodegradable template, for proliferation and matrix production offers the advantage of providing an accessible, easy-to-manipulate, self-renewing source of progenitor cells (which would otherwise be of limited availability). The ideal scaffold should be biocompatible and biodegradable upon tissue healing, highly porous so as to permit cell penetration and tissue impregnation, sufficiently permeable to allow nutrient delivery and gas exchange, and adaptable to the mechanical environment. Also, the scaffold should have a surface that is conducive to cell attachment and migration, and permits appropriate extracellular matrix formation and the transmission of signaling molecules.[13,17,38,39] Various biomaterials have been utilized as vehicles to deliver MSCs for articular cartilage repair. However, few—if any—of the currently available scaffolds fulfill all of the requirements described above,[40] and further developments in biomaterial design are clearly needed to achieve optimal neocartilage formation with the use of cell–scaffold constructs.

Synthetic scaffolds

Synthetic scaffolds can be designed to offer optimal fiber diameter, pore size, degradation time and reproducibility in production. Many synthetic scaffolds commonly used in cartilage repair are fabricated using α-hydroxy polyesters, including polyglycolic acid, poly-L-lactic acid, the copolymer poly-DL-lactic-co-glycolic acid, and poly-ε-caprolactone.[41–43] The topography and material properties of these scaffolds are important in their ability to support MSC differentiation—for example, a nanofibrous scaffold of biodegradable polymers has demonstrated enhanced support of MSC proliferative and multilineage differentiative activities.[39,42]

Natural scaffolds

Native biomaterials, including collagen type I, hyaluronan, chitosan and alginate,[44,45] present a more natural microenvironment for MSCs than synthetic scaffolds do. Collagen type I hydrogels have several advantages: these matrices are biodegradable, can be metabolized by MSCs via the action of endogenous collagenases, elicit minimal, if any, inflammation, and surround the MSCs in three dimensions. The material properties of collagen hydrogels are similar to those of hyaline cartilage. Collagen gels can also be adapted as desired to most defect shapes. Compared with meshes or fleeces, in which cell seeding is often limited to superficial regions of the scaffold

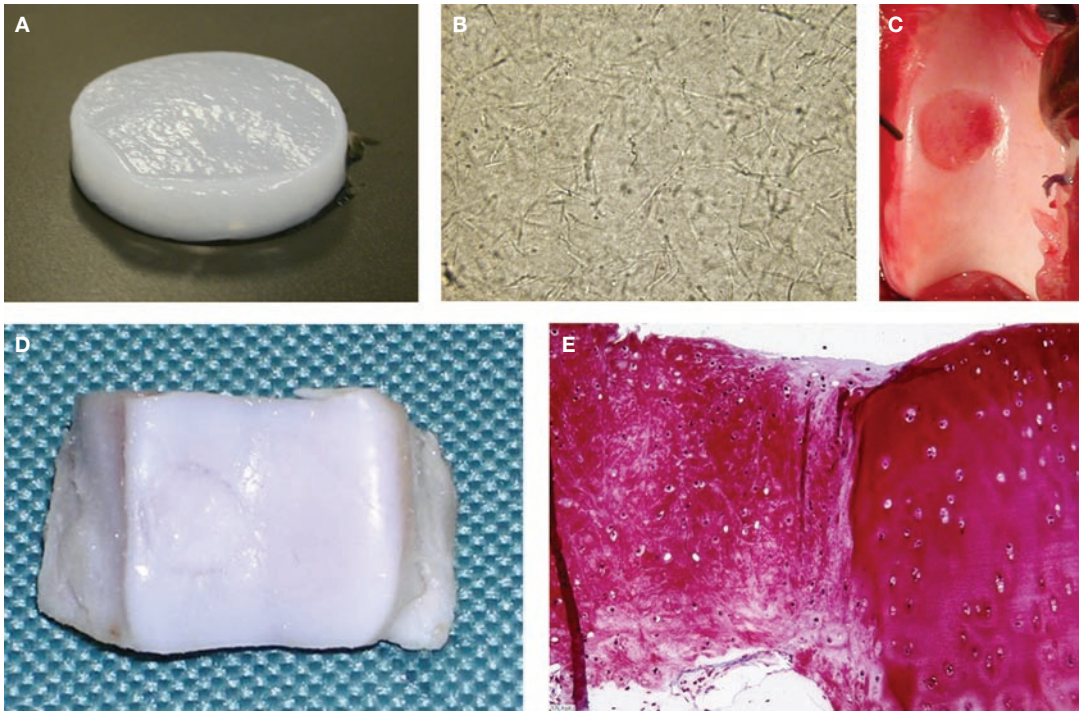

Figure 2 MSCs embedded in a collagen type I hydrogel can be used for tissue engineering of cartilage (U Nöth, unpublished data). (**A**) The collagen type I hydrogel used for matrix-based MSC transplantation was fabricated from rat-tail collagen (Arthro Kinetics, Esslingen, Germany). The implant (3 mm high and 7 mm wide) was seeded with MSCs and used to treat a cartilage defect in the trochlea of the mini-pig. (**B**) Magnified view of the MSC-containing collagen type I hydrogel, after 10 days of culture *in vitro* with Dulbecco's Modified Eagle's Medium plus 10% serum. The seeded cells are homogeneously distributed within the gel and show a fibroblast-like phenotype (×20 magnification). (**C**) Isolated chondral defect of the trochlea in a 6-month-old mini-pig. (**D**) Macroscopic appearance of the chondral defect 6 months after treatment with autologous MSCs seeded in a collagen type I hydrogel. (**E**) Immunohistochemical staining of the cartilage graft shows a cartilaginous, collagen type II-rich extracellular matrix, which contains chondrocytes that differentiated from MSCs. Bonding of the implanted gel to the host cartilage tissue was evident (×40 magnification). Abbreviation: MSC, mesenchymal stem cell.

material, hydrogels permit a more even distribution of seeded MSCs, which promotes homogeneous production of extracellular matrix.[46] Matrix-based implantation of autologous chondrocytes uses a collagen type I hydrogel for cell delivery.[47] Similarly, collagen hydrogel seeded with MSCs and implanted in mini-pig knee joints showed a homogeneous cell and extracellular matrix distribution 6 months after implantation (Figure 2).

Clinical studies of MSC implantation in collagen hydrogels

The first results for use of transplanted MSCs seeded within collagen type I hydrogels to repair isolated, full-thickness, cartilage defects in humans were reported by Wakitani *et al.*[48] Two patients with a patellar defect were treated with collagen gels containing MSCs, which were covered with a periosteal flap. Fibrocartilaginous filling of the defects was found after 1 year, and both patients showed significantly improved clinical outcomes in their respective follow-ups after 1, 4, and 5 years. The same group[49] has also used this protocol to treat another patient with a full-thickness cartilage defect in the weight-bearing area of the medial femoral condyle. The patient's clinical symptoms had improved significantly 1 year after surgery. Histologically, the defect was filled with a hyaline-like type of cartilage tissue that stained positively with safranin O, which indicated that the transplanted MSCs had differentiated into chondrocytes.

These pilot studies have been performed on isolated or focal articular cartilage defects in an otherwise healthy joint. The loss of joint

Table 1 Vectors used for *ex vivo* intra-articular gene delivery.

Vector	Efficiency of transgene expression	Duration of transgene expression	Features	DNA capacity (kb)	Host range
Nonviral	Weak	Transient	Inflammatory Used in many clinical trials of RA	>20	Broad
Adenovirus	High	Transient	Inflammatory Approved for use in clinical trials	8–28	Broad
AAV	Moderate	Transient	Cause no known disease in humans Used in clinical trials of RA	4	Broad
HSV	High	Transient	Cytotoxic	40	Broad
Retrovirus	Moderate	Stable	Risk of insertional mutagenesis Used in clinical trials of RA	8	Dividing cells
Lentivirus	High	Stable	Risk of insertional mutagenesis Safety concerns	8	Broad
Spumavirus	Moderate	Stable	Cause no known disease in humans	>8	Broad

Abbreviations: AAV, adeno-associated virus; HSV, herpes simplex virus; kb, kilobases; RA, rheumatoid arthritis.

homeostasis in OA creates a very different microenvironment, which will influence MSC engraftment and tissue differentiation. The potential outcome of matrix-based cell transplantation in an OA joint is still unclear.[45] Generally, cartilage lesions in OA are usually large, unconfined, and affect more than one location—opposed (or 'kissing') lesions are common. In the knee joint, kissing lesions are regularly seen, and are frequently accompanied by a varus or valgus deformity or patella maltracking. The direct contact between opposed matrices bearing the transplanted cells creates a high probability that implanted matrices will be rapidly worn down as a result of joint articulation. Consequently, we must point out that current biological and technological developments do not indicate sufficient retention of cell-loaded scaffolds in OA lesions.

MESENCHYMAL STEM CELLS AS VEHICLES FOR GENE DELIVERY

MSCs seem to be receptive to transduction with various viral vectors, including adenovirus, adeno-associated virus, retrovirus, herpes simplex virus, lentivirus and spumavirus (also termed foamyvirus) (Table 1), so it is conceivable that some of the aforementioned limitations of current OA therapies might be overcome by adaptation of MSC-based gene-transfer technologies.[50] This approach will involve isolation of MSCs, *ex vivo* genetic modification of the MSCs, and transplantation of the modified cells into the diseased joint.

Generally, *ex vivo* gene-delivery approaches are more invasive, expensive and time-consuming than *in vivo* approaches (in which therapeutic vectors are applied directly into the body), but they do permit control of the transduced cells and safety testing before reimplantation.[51] In particular, use of MSCs should allow the development of techniques for delivering genes that encode proteins that might reverse some of the major pathologies of OA (Table 2).[13,51] Analogous to the delivery approaches described above for native MSCs (Figures 1A and 1B), genetically modified MSCs can be delivered to joints either as a cell suspension to counteract the inflammatory and matrix degradation processes, or via matrix-based strategies to induce formation of neocartilage tissue (Figure 3).

Delivery by cell suspension

Following delivery of cell suspensions, the aim is for transduced MSCs to release therapeutic proteins that interact with all available tissues, including cartilage. Considerable progress has been made towards defining the parameters that prolong intra-articular transgene expression, an approach that was originally developed for the treatment of rheumatoid arthritis (RA).[52] Current research suggests that immunologically compatible vector systems allow sustained intra-articular transgene expression.[53] In a phase I clinical study, IL-1 receptor antagonist complementary DNA was successfully retrovirally delivered by an *ex vivo* strategy to the metacarpophalangeal joints of individuals with

Table 2 Classes of gene products used to augment MSC-based therapy for OA.

Potential therapeutic targets	Gene product class	Examples
Chondrocyte induction and protection		
Chondrogenic differentiation	Anabolic growth factors Signal-transduction molecules Transcription factors	TGF-β, BMP, Wnt Smad4, Smad5 SOX, brachyury
Osteogenic inhibition	Osteogenic inhibitors Inhibitors of chondrocyte terminal differentiation Signal-transduction molecules	Noggin, chordin PTHrP, IHH, SHH, DHH Smad6, Smad7, mLAP-1
Apoptosis inhibition	Caspase inhibitors Agents that block FasL Inhibitors of NO-induced apoptosis TNF, TRAIL inhibition	Bcl-2, Bcl-XL Anti-FasL antibodies Akt, PI3K NFκB
Senescence inhibition	Inhibitors of telomere erosion Free-radical antagonists	hTERT NO antagonists, SOD
Cartilage matrix induction and protection		
Cartilage matrix synthesis	Anabolic growth factors Extracellular matrix components Enzymes for glycosaminoglycan synthesis	TGF-β, BMPs, IGF-I Collagen type II GlcAT-1
Inhibition of inflammation	Cytokine antagonists Proteinase inhibitors Anti-inflammatory cytokines Enzymes that inhibit IL-1	IL-1Ra, sIL-1R, sTNFR, anti-TNF antibodies TIMP1, TIMP2 IL-4, IL-10, IL-11, IL-13 GFAT

Abbreviations: Akt, protein kinase B; Bcl-2, B-cell chronic lymphocytic leukemia and/or lymphoma 2; Bcl-XL, B-cell chronic lymphocytic leukemia and/or lymphoma apoptosis regulator; BMP, bone morphogenetic protein; DHH, Desert hedgehog; FasL, Fas ligand or CD178; GFAT, glutamine fructose 6 phosphate amidotransferase; GlcAT-1 glucuronosyltransferase I; hTERT, human telomerase reverse transcriptase; IGF-I, insulin-like growth factor I; IHH, Indian hedgehog; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; mLAP-1, murine latency-associated peptide 1; MSC, mesenchymal stem cell; NF-κB, nuclear factor κB; NO, nitric oxide; OA, osteoarthritis; PI3K, phosphatidylinositol 3 kinase; PTHrP, parathyroid-hormone-related protein; sIL-1R, soluble IL-1 receptor; sTNFR, soluble TNF receptor; TGF-β, transforming growth factor β; SHH, Sonic hedgehog; Smad, mothers against decapentaplegic homolog 1; SOD, superoxide dismutase; SOX, sex-determining region Y-box-containing proteins; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; Wnt, wingless-type mouse mammary tumor virus integration site family member.

RA.[54] This study shows that genes can indeed be delivered safely to human joints, and highlights the clinical utility of *ex vivo* gene transfer as a treatment for arthritis.[55] Data are beginning to emerge on the potential of such an approach for treating OA; encouraging results have been reported for IL-1 receptor antagonist adenovirally delivered to the joints of horses with experimental OA.[56] Furthermore, insulin-like growth factor 'administered' by intra-articular delivery partially reversed matrix degradation in OA.[51,57,58] Other cell types were initially investigated, but MSCs have the potential to be at least as beneficial when used in *ex vivo* approaches.[13,16,59]

A growing body of literature indicates that many of the pleiotropic gene products considered necessary for cartilage repair and regeneration are compatible with intra-articular delivery in suspension. However, delivery of transforming growth factor β1 or bone morphogenetic protein 2 to the synovium resulted in severe swelling, fibrosis, and osteophyte formation within joints.[60,61] Candidate complementary DNAs for synovial gene transfer should, therefore, be carefully chosen, safety-tested and validated (Table 2).

Delivery within a matrix

The above-mentioned anti-inflammatory treatments for RA and OA are, in principle, useful for preventing disease progression, but might not be able to restore damaged cartilage. An alternative strategy uses genetically modified MSCs in matrix-guided approaches to cartilage regeneration.[59,62] MSCs are first stimulated to undergo chondrogenic differentiation, stabilized as chondrocytes, then introduced on a matrix to the defect site, with the aim of establishing a cartilage phenotype without progression to hypertrophy or dedifferentiation.[13] A number of *in vitro* systems that use various transgenes (Table 2) demonstrate that MSCs can undergo chondrogenesis efficiently in defined, three-dimensional, serum-free, culture conditions.[44] Data indicating that delivery and expression

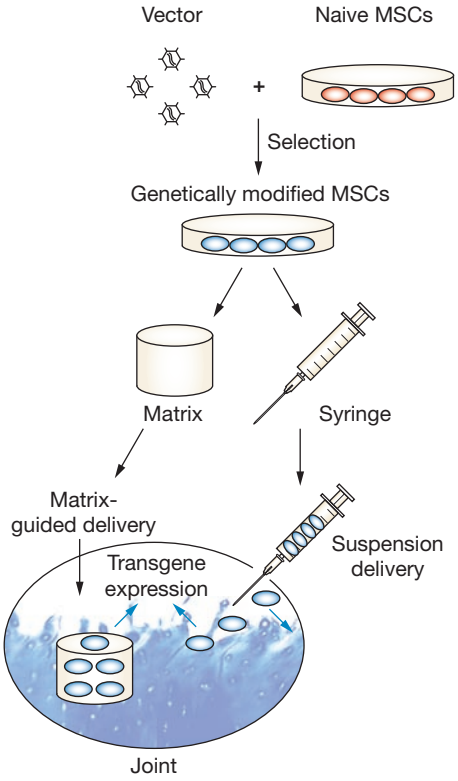

Figure 3 MSCs can be used as vehicles for *ex vivo* gene delivery. Cell-based approaches to osteoarthritis therapy might be augmented by use of genetically modified MSCs, which would involve gene transduction of culture-expanded MSCs. Successfully transduced cells would be isolated and applied to the joint space either as a cell suspension, or seeded within a biological matrix that can be implanted in a cartilage defect. Depending on which delivery approach is chosen, ubiquitous or local transgene expression is induced by the genetically modified MSCs, and the gene products could beneficially influence osteoarthritis pathology. Abbreviation: MSC, mesenchymal stem cell.

of certain genes might bias the repair response towards the synthesis of normal articular cartilage *in vivo* are beginning to emerge.[59] As already mentioned, however, this approach has been used mainly to treat focal cartilage defects. Future studies will show whether such technology will be suitable for repairing large areas of eroded cartilage, as occurs in advanced OA.[63]

CONCLUSIONS

OA is associated with the loss of homeostasis in joint tissues, particularly in the articular cartilage and the underlying bone. An insufficient repair response in articular cartilage, which results from a reduction in cell number and the loss of phenotypic stability, is a major contributor to disease progression. Further investigation will determine whether the titers of existing MSCs—both locally and throughout the body—as well as the quality of these cells might be important in the rate and extent of the repair of the damaged tissue.

The delivery of an appropriate MSC population is currently being investigated in the search for new therapeutic approaches to treat OA. The principal attraction of MSCs lies in their proliferative and chondrodifferentiation abilities, since articular chondrocytes are in limited supply. Understanding the biological activities and mechanisms of action of MSCs is crucial for a rational approach to their clinical application; specifically, conditions must be optimized to maintain MSC-derived chondrocytes in a stable, hyaline, chondrocyte-like state, without hypertrophy. Although MSC-based approaches might be developed and adapted for the treatment of both localized cartilage lesions and diseased or degenerate cartilage, as in OA, these states should be recognized as different entities.

Although direct intra-articular injection of cells is considered a technically simple approach to treatment of advanced OA, whether this approach can elicit beneficial effects (such as minimizing further cartilage damage) in human OA joints remains to be seen—and, if so, to what extent and under which conditions. The engineering design of matrix and scaffold material for cell-based articular cartilage repair has taken substantial strides, but the ideal scaffold material is still being sought, particularly for OA joints. Defects such as kissing lesions necessitate the design and engineering of new biomaterials that can be seeded with cells and can withstand significant mechanical loads. The use of MSCs in combination with bioactive substrates, natural or synthetic, also has significant clinical potential and is likely to be important in future, MSC-based, cartilage-repair technologies. In this context, MSCs might also offer promise in the future as vehicles for therapeutic gene delivery. In the long term, we hope that MSC-based technologies will permit the engineering of cartilage not only for repair of focal lesions but also as a treatment option for OA joints, to realize the ultimate goal of a fully biological prosthesis.

KEY POINTS

- Osteoarthritis (OA), the most common joint disease, is characterized by degeneration of the articular cartilage that ultimately leads to joint destruction
- Current treatment strategies for OA are inadequate
- Delivery of an appropriate mesenchymal stem cell (MSC) population is currently being investigated in the search for new therapies for OA
- MSCs could be used as trophic producers of bioactive factors to initiate endogenous regenerative activities in the OA joint; their activities might be further enhanced via targeted gene therapy
- Delivery of MSCs might be achieved either by direct intra-articular injection or by implantation of engineered constructs derived from MSC-seeded scaffolds
- In the long term, MSC-based technologies could permit the engineering and repair of cartilage as a treatment option for OA joints

References

1 Elders MJ (2000) The increasing impact of arthritis on public health. *J Rheumatol Suppl* **60:** 6–8
2 Brooks PM (2002) Impact of osteoarthritis on individuals and society: how much disability? Social consequences and health economic implications. *Curr Opin Rheumatol* **14:** 573–577
3 Hochberg MC *et al*. (1995) Guidelines for the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. American College of Rheumatology. *Arthritis Rheum* **38:** 1541–1546
4 Gerwin N *et al*. (2006) Intraarticular drug delivery in osteoarthritis. *Adv Drug Deliv Rev* **58:** 226–242
5 Gunther KP (2001) Surgical approaches for osteoarthritis. *Best Pract Res Clin Rheumatol* **15:** 627–643
6 Moseley JB *et al*. (2002) A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med* **347:** 81–88
7 Bartlett W *et al*. (2005) Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. *J Bone Joint Surg Br* **87:** 640–645
8 Bentley G *et al*. (2003) A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br* **85:** 223–230
9 Hangody L and Fules P (2003) Autologous osteochondral mosaicplasty for the treatment of full-thickness defects of weight-bearing joints: ten years of experimental and clinical experience. *J Bone Joint Surg Am* **85A (Suppl 2):** 25–32
10 Henderson I *et al*. (2005) Autologous chondrocyte implantation for treatment of focal chondral defects of the knee—a clinical, arthroscopic, MRI and histologic evaluation at 2 years. *Knee* **12:** 209–216
11 Peterson L *et al*. (2003) Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. *J Bone Joint Surg Am* **85A (Suppl 2):** 17–24
12 Knutsen G *et al*. (2004) Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am* **86A:** 455–464
13 Steinert AF *et al*. (2007) Major biological obstacles for persistent cell-based regeneration of articular cartilage. *Arthritis Res Ther* **9:** 213
14 Bert JM and Gasser SI (2002) Approach to the osteoarthritic knee in the aging athlete: debridement to osteotomy. *Arthroscopy* **18:** 107–110
15 Pittenger MF *et al*. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* **284:** 143–147
16 Kolf CM *et al*. (2007) Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. *Arthritis Res Ther* **9:** 204
17 Chen FH *et al*. (2006) Technology insight: adult stem cells in cartilage regeneration and tissue engineering. *Nat Clin Pract Rheumatol* **2:** 373–382
18 Aigner T *et al*. (2007) Mechanisms of disease: role of chondrocytes in the pathogenesis of osteoarthritis—structure, chaos and senescence. *Nat Clin Pract Rheumatol* **3:** 391–399
19 Buckwalter JA *et al*. (2006) Perspectives on chondrocyte mechanobiology and osteoarthritis. *Biorheology* **43:** 603–609
20 Martin JA *et al*. (2004) Chondrocyte senescence, joint loading and osteoarthritis. *Clin Orthop Relat Res* **427 (Suppl):** S96–S103
21 Verbruggen G (2006) Chondroprotective drugs in degenerative joint diseases. *Rheumatology (Oxford)* **45:** 129–138
22 Deschner J *et al*. (2003) Signal transduction by mechanical strain in chondrocytes. *Curr Opin Clin Nutr Metab Care* **6:** 289–293
23 Im GI *et al*. (2006) Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. *Tissue Eng* **12:** 527–536
24 Barry FP (2003) Biology and clinical applications of mesenchymal stem cells. *Birth Defects Res C Embryo Today* **69:** 250–256
25 Murphy JM *et al*. (2002) Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis Rheum* **46:** 704–713
26 Muschler GF *et al*. (2001) Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. *J Orthop Res* **19:** 117–125
27 Quarto R *et al*. (1995) Bone progenitor cell deficits and the age-associated decline in bone repair capacity. *Calcif Tissue Int* **56:** 123–129
28 Leskela HV *et al*. (2003) Osteoblast recruitment from stem cells does not decrease by age at late adulthood. *Biochem Biophys Res Commun* **311:** 1008–1013
29 De Bari C and Dell'Accio F (2007) Mesenchymal stem cells in rheumatology: a regenerative approach to joint repair. *Clin Sci (Lond)* **113:** 339–348
30 Kafienah W *et al*. (2007) Three-dimensional cartilage tissue engineering using adult stem cells from osteoarthritis patients. *Arthritis Rheum* **56:** 177–187
31 Scharstuhl A *et al*. (2007) Chondrogenic potential of human adult mesenchymal stem cells is independent of age or osteoarthritis etiology. *Stem Cells* **25:** 3244–3251
32 Murphy JM *et al*. (2003) Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* **48:** 3464–3474
33 Lee KB *et al*. (2007) Injectable mesenchymal stem cell therapy for large cartilage defects—a porcine model. *Stem Cells* **25:** 2964–2971
34 Chen X *et al*. (2006) Mesenchymal stem cells in immunoregulation. *Immunol Cell Biol* **84:** 413–421

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35 Uccelli A *et al*. (2007) Mesenchymal stem cells: a new strategy for immunosuppression? *Trends Immunol* **28:** 219–226
36 Kan I *et al*. (2007) Autotransplantation of bone marrow-derived stem cells as a therapy for neurodegenerative diseases. *Handb Exp Pharmacol* **180:** 219–242
37 Caplan AI and Dennis JE (2006) Mesenchymal stem cells as trophic mediators. *J Cell Biochem* **98:** 1076–1084
38 Raghunath J *et al*. (2007) Biomaterials and scaffold design: key to tissue-engineering cartilage. *Biotechnol Appl Biochem* **46:** 73–84
39 Li WJ *et al*. (2005) Application of nanofibrous scaffolds in skeletal tissue engineering. *J Biomed Nanotechnol* **1:** 1–17
40 Mouw JK *et al*. (2005) Variations in matrix composition and GAG fine structure among scaffolds for cartilage tissue engineering. *Osteoarthritis Cartilage* **13:** 828–836
41 Nöth U *et al*. (2002) *In vitro* engineered cartilage constructs produced by press-coating biodegradable polymer with human mesenchymal stem cells. *Tissue Eng* **8:** 131–144
42 Li WJ *et al*. (2006) Chondrocyte phenotype in engineered fibrous matrix is regulated by fiber size. *Tissue Eng* **12:** 1775–1785
43 Terada S *et al*. (2005) Hydrogel optimization for cultured elastic chondrocytes seeded onto a polyglycolic acid scaffold. *J Biomed Mater Res A* **75:** 907–916
44 Kuo CK *et al*. (2006) Cartilage tissue engineering: its potential and uses. *Curr Opin Rheumatol* **18:** 64–73
45 Nesic D *et al*. (2006) Cartilage tissue engineering for degenerative joint disease. *Adv Drug Deliv Rev* **58:** 300–322
46 Nöth U *et al*. (2007) Chondrogenic differentiation of human mesenchymal stem cells in collagen type I hydrogels. *J Biomed Mater Res A* **83:** 626–635
47 Nöth U *et al*. (2006). Matrix-based autologous chondrocyte transplantation for the treatment of large osteochondral defects. In: *European Musculoskeletal Review 2006*, 62–64. London: Touch Briefings
48 Wakitani S *et al*. (2004) Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transplant* **13:** 595–600
49 Kuroda R *et al*. (2007) Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage* **15:** 226–231
50 Evans CH *et al*. (2006) Will arthritis gene therapy become a clinical reality? *Nat Clin Pract Rheumatol* **2:** 344–345
51 Evans CH *et al*. (2004) Osteoarthritis gene therapy. *Gene Ther* **11:** 379–389
52 Robbins PD *et al*. (2003) Gene therapy for arthritis. *Gene Ther* **10:** 902–911
53 Gouze E *et al*. (2007) Transgene persistence and cell turnover in the diarthrodial joint: implications for gene therapy of chronic joint diseases. *Mol Ther* **15:** 1114–1120
54 Evans CH *et al*. (1996) Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. *Hum Gene Ther* **7:** 1261–1280
55 Evans CH *et al*. (2005) Gene transfer to human joints: progress toward a gene therapy of arthritis. *Proc Natl Acad Sci USA* **102:** 8698–8703
56 Frisbie DD *et al*. (2002) Treatment of experimental equine osteoarthritis by *in vivo* delivery of the equine interleukin-1 receptor antagonist gene. *Gene Ther* **9:** 12–20
57 Haupt JL *et al*. (2005) Dual transduction of insulin-like growth factor-I and interleukin-1 receptor antagonist protein controls cartilage degradation in an osteoarthritic culture model. *J Orthop Res* **23:** 118–126
58 Nixon AJ *et al*. (2005) Gene-mediated restoration of cartilage matrix by combination insulin-like growth factor-I/interleukin-1 receptor antagonist therapy. *Gene Ther* **12:** 177–186
59 Trippel SB *et al*. (2004) Gene-based approaches for the repair of articular cartilage. *Gene Ther* **11:** 351–359
60 Mi Z *et al*. (2003) Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees. *Arthritis Res Ther* **5:** 132–139
61 Gelse K *et al*. (2003) Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. *Arthritis Rheum* **48:** 430–441
62 Tuli R *et al*. (2003) Current state of cartilage tissue engineering. *Arthritis Res Ther* **5:** 235–238
63 Hollander AP *et al*. (2006) Maturation of tissue engineered cartilage implanted in injured and osteoarthritic human knees. *Tissue Eng* **12:** 1787–1798