Gene Therapy for Cartilage Repair

Henning Madry1, Patrick Orth1, and Magali Cucchiarini1

Abstract

The concept of using gene transfer strategies for cartilage repair originates from the idea of transferring genes encoding therapeutic factors into the repair tissue, resulting in a temporarily and spatially defined delivery of therapeutic molecules to sites of cartilage damage. This review focuses on the potential benefits of using gene therapy approaches for the repair of articular cartilage and meniscal fibrocartilage, including articular cartilage defects resulting from acute trauma, osteochondritis dissecans, osteonecrosis, and osteoarthritis. Possible applications for meniscal repair comprise meniscal lesions, meniscal sutures, and meniscal transplantation. Recent studies in both small and large animal models have demonstrated the applicability of gene-based approaches for cartilage repair. Chondrogenic pathways were stimulated in the repair tissue and in osteoarthritic cartilage using genes for polypeptide growth factors and transcription factors. Although encouraging data have been generated, a successful translation of gene therapy for cartilage repair will require an ongoing combined effort of orthopedic surgeons and of basic scientists.

Keywords

gene therapy, cartilage repair, osteoarthritis, meniscal lesions, clinical trials

Introduction

Articular cartilage defects and meniscal lesions have a reduced capacity for regeneration. The concept of using gene transfer strategies for cartilage repair originates from the idea of transferring genes encoding therapeutic factors into the repair tissue, resulting in a temporarily and spatially defined delivery of the therapeutic molecule. In this review, we will focus on gene therapy approaches for the repair of articular cartilage and meniscal fibrocartilage, including articular cartilage defects resulting from acute trauma, osteochondritis dissecans, osteonecrosis, and osteoarthritis. Possible applications for meniscal repair will be described for meniscal lesions, meniscal sutures, and meniscal transplantation. As a discussion of cartilage damage resulting from rheumatoid arthritis is beyond the scope of this review, we refer to the many reviews already published on this subject.1-9

Principles of Gene Therapy

Gene transfer is the introduction of foreign genes or gene sequences into different types of cells. Gene therapy is the treatment of diseases using gene transfer techniques. Gene transfer via nonviral vectors is termed transfection; gene transfer using viral vectors is termed transduction. The foreign genetic material enters the cell and is next transferred towards the nucleus, where it either integrates into the host genome or remains extrachromosomally as an episome that generally allows only for transient transgene expression. For therapeutic applications, gene transfer into a sufficiently high number of target cells is essential for the secretion of relevant concentrations of the transgene product. Current vectors available for use in gene therapy include nonviral approaches (naked DNA, physical and chemical methods) and various viral (adenoviral, HSV, retroviral, lentiviral, rAAV) vehicles (Table 1).

Among the nonviral systems, chemical methods of complexing DNA to various macromolecules include cationic lipids and liposomes,10-12 polymers,13 polyamines and polyethylenimines,14,15 and nanoparticles,16 but also calcium phosphate coprecipitates17 are mainly used. Nonviral systems avoid the risk of acquiring replication competence inherent to viral vectors, can be repeatedly administered, have the capacity to carry large therapeutic genes, are relatively easy to produce on a large scale, and do not elicit a detectable immune response. Nevertheless, their efficacy is often inferior to those of viral vectors. Moreover, the fact that they stay as episomal forms in the target cells often

1Saarland University, Homburg, Germany

Corresponding Author:
Henning Madry, Saarland University, Kirrbergerstrasse 1, Homburg, 66424 Germany
Email: henning.madry@uks.eu
results in short-term transgene expression. To avoid low gene transfer efficacy in vivo, nonviral gene transfer strategies are often based on the transplantation of ex vivo–modified cells to cartilage defects.

Viral vectors utilize natural entry pathways in human cells. Adenoviral vectors have been among the most employed gene vehicles for cartilage repair in the past.\textsuperscript{18-22} They allow for high transduction efficiencies and transgene expression in a variety of cells, enabling direct approaches in vivo. However, serious concerns about their clinical safety were raised after the death of Jesse Gelsinger, a patient included in a gene therapy trial employing adenoviral vectors. Moreover, transgene expression via adenoviral delivery is limited for about 1 to 2 weeks as the transgenes remain episomal and due to the development of host immune responses against transduction with most of the constructs derived from these viruses.

An advantage of retroviruses is their ability to integrate in the genome of the target, allowing for the replication and maintenance of the transgene over extended periods of time. Yet, this might lead to insertional mutagenesis, with the potential for activating tumor genes. Also, retroviral vectors do not transduce nondividing cells and have a restricted host range. As for nonviral systems, ex vivo approaches with selection of transduced cells are usually required with retroviral vectors\textsuperscript{23-27} because they are produced only at relatively medium titers and do not exhibit very high efficiencies. Instead, lentiviral vectors, a subclass of retroviruses derived from the human immunodeficiency virus (HIV), can integrate in the genome of nondividing cells.\textsuperscript{28} Therefore, such vectors might be good alternatives to the use of retroviruses, as they show also higher levels of transduction in vivo and avoid the need for cell division.\textsuperscript{29,30}

Yet, there are common concerns associated with their application, including the potential for insertional mutagenesis and the psychological problem of introducing genetic material carrying HIV sequences.

Herpes simplex virus (HSV)–derived vectors are large vehicles that can deliver long transgenes to almost all known cell types, including nondividing cells. Although first-generation vectors induced high levels of cytotoxicity, recent work has demonstrated that second-generation HSV were less deleterious, in particular for cartilage repair.\textsuperscript{31}

One problem remains the transient nature of transgene expression mediated by this family of vectors.

In any case, the direct application of viral vectors raises legitimate safety concerns, as potentially infectious agents or sequences (especially lentiviral vectors) might be intro-
duced *per se* in the body. This is of particular importance for the treatment of cartilage and meniscal lesions that are not life-threatening disorders. In this regard, adeno-associated viral vectors (AAV), which are based on the nonpathogenic, replication-defective human parvovirus AAV, might prove more adequate in direct gene therapy settings. Vectors based on AAV (rAAV) are produced by complete removal of the viral gene coding sequences, making them less immunogenic than adenoviral vectors and less toxic than HSV. Also, the latter vectors generally mediate only short-term expression of the transgenes they carry, whereas rAAV can be transcribed for months to years due to the stabilization of the episomal transgene cassettes by concatamer formation. Cell division and integration are not required for expression of the foreign material delivered, in marked contrast with retroviral vectors. Redosing of vectors is practicable with rAAV, based on the manipulation of various available serotypes of the virus. For these reasons, rAAV became a preferred gene transfer method for experimental settings *in vivo* and for clinical applications.

The greatest obstacle to develop efficient gene transfer protocols targeting sites of articular cartilage and meniscal fibrocartilage damage so far has been the restrained accessibility of the lesions to a treatment. Therefore, the following experimental approaches are currently employed to transfer genes to sites of interest *in vivo* (Fig. 1):

1. intra-articular injection of the therapeutic formulation, and
2. administration of the therapeutic formulation to the defect via arthrotomy:
   2.1. direct application of a gene vector to the repair tissue,
   2.2. application of biomaterials carrying a gene vector, and
   2.3. matrix-supported application of *ex vivo* genetically modified cells.

The target cells in which genes may be transferred include the following:

1. progenitor cells (e.g., resulting from marrow-stimulating techniques or transplanted cells),
2. isolated articular chondrocytes or meniscal fibrochondrocytes that are transplanted into the defect, and
3. cells of the tissues adjacent to the defect:
   3.1. articular cartilage: articular chondrocytes from the adjacent cartilage, osteoblasts, and osteocytes from the subchondral bone; and
   3.2. meniscal tissue: meniscal fibrochondrocytes, synoviocytes from the synovial lining, and fibroblasts from the joint capsule.

*Figure 1.* Therapeutic genes may be transferred to sites of articular cartilage damage or to meniscal lesions *in vivo* via intra-articular injection or by direct application into the lesion. Intra-articular injection (upper panel) of the therapeutic formulation (most often a viral vector) results in a nonselective transduction of many intra-articular tissues. Direct administration of the therapeutic formulation (lower panel) to the target lesion (e.g., an articular cartilage defect) can be achieved by directly applying a gene vector to the repair tissue in the defect (left), by matrix-supported application (e.g., alginate) of target cells (e.g., articular chondrocytes, meniscal fibrochondrocytes, progenitor cells) that were previously genetically modified *ex vivo* (middle), or by application of a gene vector attached to a biomaterial (right). *In vivo*, it often includes an arthrotomy.
Articular Cartilage

Introduction

Anatomy, Function, and Pathophysiology. Adult hyaline articular cartilage is avascular and aneural and does not possess a lymphatic drainage. Its major function is to allow for smooth gliding of the articulating surfaces of a joint and to protect the subchondral bone from mechanical stress. Hyaline articular cartilage is structured in several laminar zones and formed by chondrocytes that are surrounded by an intricate network of extracellular matrix. This cartilaginous matrix is rich in proteoglycans and collagen fibrils composed of type II collagen but also contains types VI, IX, XI, and XIV collagens and a number of additional macromolecules. Normal hyaline articular cartilage contains about 70% to 80% water, which is mainly bound to proteoglycans. Articular chondrocytes synthesize and degrade the extracellular matrix, thereby regulating the structural and functional properties according to the applied loads.

The integrity of articular cartilage can be disrupted as a result of mainly 4 different etiologies. These include focal articular cartilage defects resulting from an acute trauma, osteoarthritis, osteonecrosis, and osteochondritis dissecans. The resulting articular cartilage defect is characterized as being either chondral, involving only the cartilaginous zones, or osteochondral, reaching further into the subchondral bone. Although a chondral defect may be in part repopulated by cells from the synovial membrane, it usually remains and may expand over time. An osteochondral defect is filled with a blood clot that forms if the bone marrow communicates with the defect. The pluripotent, undifferentiated mesenchymal cells of the blood clot differentiate into chondrocytes and osteoblasts that later form the cartilaginous repair tissue and the new subchondral bone. However, over time, this repair tissue increasingly exhibits characteristics of fibrocartilage, such as an increased type I and a decreased type II collagen content and may degenerate after several years. If left untreated, secondary osteoarthritis of the joint may result.

Chondrogenic Therapeutic Factors. Strategies for enhancing chondrogenesis in an articular cartilage defect aim at improving the differentiation of mesenchymal cells into chondrocytes for cartilage repair and osteoblasts for the repair of the subchondral bone, the production and maintenance of a new cartilaginous matrix rich in type II collagen and proteoglycans, at increasing the cellularity of the repair tissue to prevent the hypertrophic differentiation of chondrocytes, and at inhibiting articular cartilage degeneration.

Growth and transcription factors are good candidates for these approaches. The therapeutic efficacy of polypeptide has a plasma half-life of less than 1 hour and is cleared in some hours after intra-articular administration. To overcome this problem, the idea of applying the gene encoding for a particular therapeutic protein has gained attraction.

Candidate factors to support chondrogenesis include members of the transforming growth factor beta (TGF-β) superfamily such as TGF-β1 and TGF-β2, bone morphogenetic protein 2 (BMP-2), BMP-7, members of the fibroblast growth factor family such as the basic fibroblast growth factor (FGF-2), growth/differentiation factor 5 (GDF-5), and the parathyroid hormone–related protein (PTHrP). Cell proliferation is promoted, among others, by FGF-2 and the insulin-like growth factor I (IGF-I). Particularly potent candidates to stimulate matrix synthesis include IGF-1 and BMP-2 and BMP-7, and the cartilage-derived morphogenetic proteins (CDMP).

Transcription factors directly modulate the expression of genes involved in chondrogenesis, such as type II collagen or aggrecan. Experimental models have demonstrated the chondrogenic properties of transcription factors, such as SOX9, Cbfa-1/Runx-2, Cart-1, the Ets family members, and various signaling molecules as well as extracellular matrix glycoproteins themselves. Another attractive approach is to inhibit degenerative pathways within the repair tissue. Potential targets include cytokines that mediate catabolic events, in particular the members of the interleukin-1 (IL-1), IL-17, and tumor necrosis factor (TNF) families. These strategies are based on the inhibition of the production of matrix-degrading enzymes, proinflammatory mediators, as well as apoptotic mechanisms.

Traumatic Articular Cartilage Defects

Intra-articular Injection. Intra-articular injection is a convenient way to target the joint space and has been studied using naked DNA, adenoviral, retroviral, HSV, lentivirus, rAAV, and nonviral vectors. In 1998, Ikeda et al. injected adenoviral vectors encoding for the TGF-β1 gene into the joints of guinea pigs and reported elevated TGF-β1 levels in the synovial fluid for 2 weeks following gene delivery. The effectiveness of a direct intra-articular gene therapy approach in combination with a marrow stimulation technique has been shown by Morisset et al. Full-thickness chondral defects in equine stifle and knee joints were treated by microfracturing, followed by intra-articular application of adenoviral vectors carrying the genes for interleukin-1 receptor antagonist protein (IL-1Ra) and IGF-I. Sixteen weeks postoperatively, articular cartilage defects treated with IL-1Ra and IGF-I showed increased proteoglycan content and type II collagen expression compared with defects treated using a marrow-stimulating technique alone. Yet, articular cartilage defects cannot be specifically
targeted with this approach since the transgene is expressed mainly in cells of the synovial membrane and gene transfer into articular cartilage defect is a very rare event. Therefore, many of the gene-based approaches have focused on direct gene vector delivery into a defect exposed by arthrotomy (Table 2).

**Arthrotomy**

**Direct application of a gene vector in vivo.** The direct delivery of therapeutic genes into cartilage defects in depth has long been arduous due to the reduced capability of nonviral and various viral vectors to penetrate the dense extracellular cartilaginous matrix. Following arthrotomy and gene vector application to cartilage defects, limited transgene expression was observed only in the superficial cartilage layers. 80 With the implementation of rAAV vectors, direct gene transfer to cells within defects and adjacent cartilage has met success. Reporter gene studies demonstrated efficient transgene expression in normal and osteoarthritic human articular chondrocytes within their native matrix *in situ* to depths relevant for clinical applications. 87 Moreover, transgene expression was also present in chondral and osteochondral articular cartilage defects *in vivo* for at least 4 months. 87 RAAV vectors have been manipulated recently to deliver therapeutic genes such as FGF-2 directly into osteochondral cartilage defects. 36 Cartilage repair was significantly enhanced 4 months after vector application. 36

**Application of biomaterials carrying a gene vector into defects.** In order to avoid a dilution of the therapeutic agents, gene vectors or modified cells can be delivered in conjunction with biomaterials such as fibrin, collagen, gelatin, carbohydrate-based polymers (polyactic acid/polyglycolic acid, hyaluronan, agarose, alginate, chitosan), and artificial polymers (dacron, teflon, carbon fibers, polyestherurethane, polybutyric acid, polyethylmethacrylate, hydroxyapatite). 45,88 When preparations of adenoviral vectors carrying a marker gene were adsorbed onto type II collagen-glycosaminoglycan matrices and implanted into osteochondral defects, transgene expression was present until day 21. 89

**Application of ex vivo genetically modified cells.** The direct transplantation of cells genetically modified *ex vivo* involves their isolation, genetic modification, and reimplantation into articular cartilage defects. These modified cells can be applied without (e.g., as coagulated bone marrow aspirate) or with supportive matrices. Such components include alginate,90-92 agarose,93,94 fibrin or type I collagen gels without95,97 or with a periosteal flap,98,99 and synthetic biodegradable scaffolds.100-102 Kang et al. were the first to transplant genetically modified cells into an articular cartilage defect *in vivo*.103 In this study, chondrocytes were transduced with a retroviral vector. Other studies used nonviral,104-106 adenoviral,89,96,107 retroviral,103,108-111 and rAAV vectors112 to deliver marker genes in defects via *ex vivo*–modified cells. Although engineered chondrocytes are generally transplanted,21,96,103,104,106,111,113 fibroblasts,27,114 perichondrial,105 periosteal,108,112 or muscle-derived cells109 have been also applied. The data from these studies showed that transgenes can be expressed in cartilage defects via *ex vivo* strategies, remaining active for about 1 month. This is significantly longer compared with the application of recombinant proteins (Table 2). Figure 2 depicts improvements in the repair of osteochondral defects following combined gene transfer of IGF-I and FGF-2 compared with the application of a marker gene (*lacZ*) to NIH 3T3 fibroblasts.114

Periosteal cells transduced by a BMP-7 retroviral vector and attached to a polyglycolic acid scaffold improved cartilage repair at 8 and 12 weeks *in vivo*. Interestingly, this was the first study in which a growth factor gene was transferred into a focal defect.25 Since, many reports described the use of a variety of therapeutic genes like BMP-2, BMP-7, IGF-I, FGF-2, and TGF-β.22,90,91,114-120 Significant improvement in articular cartilage repair was noted in these reports (Table 2). Although most of the evaluations were carried out in small animal models, Hidaka et al.21 and, more recently, Goodrich et al.121 performed arthroscopic implantation of chondrocytes genetically engineered by adenoviral transduction with the BMP-711 or IGF-1121 gene in horses.

On the basis of such encouraging data, cartilage repair was addressed by matrix-supported implantation of genetically engineered mesenchymal stem cells (MSC). Kuroda et al.122

![Figure 2](image-url)
| Gene          | Route     | Vector | Cells               | Support | Defect          | Size (mm) | Animal Model | Joint Location          | Period of Evaluation | Ref. |
|--------------|-----------|--------|--------------------|---------|----------------|-----------|--------------|-------------------------|----------------------|------|
| BMP-2        | Ex vivo   | Retroviral | Chondrocytes       | Fibrin  | Osteochondral  | 3.6 Ø     | Rabbit       | Knee Patellar groove    | Min 4 Max 12         | 120  |
| BMP-2        | Ex vivo   | Adenoviral | Fat/muscle grafts  | −/−     | Osteochondral  | 3.0 Ø     | Rabbit       | Knee Patellar groove, medial femoral condyle | Min 6 Max 6         | 124  |
| BMP-2, IGF-I | Ex vivo   | Adenoviral | Perichondrial cells | Fibrin  | Chondral       | <1.0 Ø    | Rat          |                         | Min 3 Max 8          | 19   |
| BMP-7        | Ex vivo   | Retroviral | Periosteal cells   | PGA     | Osteochondral  | 3.0 Ø     | Rabbit       | Knee Lateral trochlear ridge | Min 4 Max 12         | 25   |
| BMP-7        | Ex vivo   | Adenoviral | Chondrocytes       | Fibrin  | Osteochondral  | 15.0 Ø    | Horse        | Knee Lateral trochlear ridge | Min 4 Max 36         | 21   |
| IGF-I        | Ex vivo   | FuGENE 6 | Chondrocytes       | Alginate| Osteochondral  | 3.2 Ø     | Rabbit       | Knee Patellar groove    | Min 3 Max 14         | 91   |
| IGF-I        | Ex vivo   | Adenoviral | Chondrocytes       | Fibrin  | Chondral       | 15.0 Ø    | Horse        | Knee Lateral trochlear ridge | Min 4 Max 32         | 121  |
| IL-1RA + IGF-I | Intra-articularly | Adenoviral | −/−                | −/−     | Chondral (with microfracture) | 10.0 × 10.0 □ | Horse | Knee, Distal radial carpal bone, medial femoral condyle | Min 16 Max 16       | 86   |
| IGF-I + FGF-2 | Ex vivo   | FuGENE 6 | NIH 3T3            | Alginate| Osteochondral  | 3.2 Ø     | Rabbit       | Knee Patellar groove    | Min 3 Max 3          | 114  |
| FGF-2        | Ex vivo   | FuGENE 6 | Chondrocytes       | Alginate| Osteochondral  | 3.2 Ø     | Rabbit       | Knee Patellar groove    | Min 3 Max 14         | 90   |
| FGF-2        | In vivo   | rAAV    | −/−                | −/−     | Osteochondral  | 3.2 Ø     | Rabbit       | Knee Patellar groove    | Min 1 Max 18         | 36   |
| FGF-2        | In vivo   | rAAV    | Chondrocytes       | −/−     | Osteochondral  | 5.0 Ø     | Rabbit       | Knee Patellar groove    | Min 4 Max 12         | 99   |
| FGF-2        | In vivo   | rAAV    | −/−                | −/−     | Osteochondral  | 5.0 Ø     | Rabbit       | Knee Patellar groove    | Min 4 Max 12         | 163  |
| TGF-β        | Ex vivo   | Retroviral | NIH3T3            | −/−     | Chondral       | 3.0 × 6.0 □ | Rabbit | Knee           | Min 1 Max 6          | 27   |
| TGF-β        | Ex vivo   | rAAV    | hMSC               | −/−     | Osteochondral  | 1.5 Ø     | Rat (athymic) | Knee Patellar groove    | Min 4 Max 12         | 38   |
| TGF-β1       | Ex vivo   | Adenoviral | Bone marrow aspirate | −/−     | Chondral       | 6.2 Ø     | Sheep        | Knee Medial femoral condyle | Min 26 Max 26       | 125  |
| CDMP1 (GDF-5)| Ex vivo   | FuGENE 6 | MSC                | Type I collagen | Osteochondral | 4.0 Ø     | Rabbit       | Knee Patellar groove    | Min 2 Max 8          | 66   |

Note: PGA = polyglycolic acid; MSC = mesenchymal stem cells; Ø = cylindrical defect; □ = rectangular defect.
implanted BMP-4–transduced MSCs using fibrin glue in full-thickness cartilage defects in the trochlear groove of rabbit femurs. After 24 weeks, histological scoring of the defects revealed significantly better cartilage repair in the BMP-4 treatment group compared with defects receiving lacZ-transduced MSCs. Guo et al. seeded TGF-β1–engineered MSCs onto poly-L-lysine–coated polylactide scaffolds in vitro and allografted them into full-thickness defects in rabbits. This resulted in improved joint repair with regard to extracellular matrix formation, reconstitution of the subchondral bone, and inhibition of inflammatory immune responses. Repair of osteochondral defects was also enhanced by transplantation of MSCs transfected with the CDMP1 gene, applying a lipofection method.

A novel method of gene therapy for the repair of osteochondral defects has recently been published by Evans et al. Rather than genetically modifying isolated cells, this technique describes gene transfer to biopsies of muscle and fat. An adenovirus vector carrying cDNA encoding human BMP-2 was used for genetic engineering of tissues. These gene-activated muscle or fat pads were transplanted into osteochondral defects in rabbits. Histological analysis after 6 weeks revealed the formation of a proteoglycan-rich articular surface with subchondral bone beneath and good union with the adjacent cartilage.

Ivkovic et al. used autologous bone marrow, transduced ex vivo, with adenoviral vectors containing the cDNA for TGF-β1. Implantation of the marrow clot improved the histological, biochemical, and biomechanical parameters of partial-thickness chondral defects in sheep at 6 months.

**Osteoarthritis**

Osteoarthritis (OA) is the leading, most disabling human condition and prevalent form of arthritis (80%), impairing the quality of life of millions of people worldwide. OA is a chronic disorder of diarthrodial joints, mainly characterized by a slow, gradual deterioration of the articular cartilage that remains without effective treatment to date. OA not only affects the cartilage but also the subchondral bone and, to a minor degree, the synovial lining, ligaments, tendons, and muscles. Current options to manage OA, such as pharmacological therapy and reconstructive surgical interventions, do not allow for the restoration of a native cartilage. OA is a complex disorder characterized by an activation of inflammatory cascades at the molecular level, leading ultimately to cartilage breakdown, associated with alterations of the phenotype of chondrocytes and a loss of the major components of the cartilage matrix. Under mechanical or biochemical stress (presence of IL-1 and TNF-α, NO, prostaglandins, matrix degradation products), the chondrocytes undergo pathological changes in their gene expression patterns that lead to an impairment of the overall homeostasis, with diminished production of normal cartilage matrix molecules (proteoglycans, type II collagen), enhanced production of matrix-degrading enzymes (MMPs and adamalysins, including ADAMs and ADAMTs), and decreased responsiveness to reparative stimuli, ultimately leading to the degradation of the matrix and cell senescence and apoptosis (NO, Fas/FasL signaling) by alteration of cell viability.

**Gene Transfer In Vitro**

Target cells in the joint include cells of the synovial lining, chondrocytes, chondroprogenitor cells, and surrounding tissues (bone, muscle, tendons, ligaments, meniscus). Application of nonviral, adenoviral, or retroviral vectors has been achieved in these cell types with more or less success. Instead, RAAV vectors are potent alternatives as they can efficiently and durably transduce synoviocytes, chondrocytes, MSCs, and cells of surrounding tissues. Regeneration of a normal structural and functional cartilage might be achieved by the following:

---

**Figure 3.** Direct rAAV-mediated gene transfer to rabbit meniscus explants in vitro using an rAAV-lacZ (left panel) or rAAV-hFGF-2 vector (right panel) (50 mL each vector). Persistent transgene expression after 10 days in vitro in meniscal explants following immunohistochemical detection of lacZ (A), while no signal is present in the control (B). Direct transduction of a rabbit meniscal explant with rAAV-hFGF-2 results in an increased cell density (D) compared with the control (C), indicative of the mitogenic effect of FGF-2 on meniscal fibrochondrocytes. (C, D) hematoxylin and eosin/fast green. All magnifications, 20x.
Inhibition of catabolic pathways has been achieved in vitro by expressing inhibitors of matrix-degrading enzymes (tissue inhibitor of metalloproteinases, i.e., TIMP), inhibitors of proinflammatory cytokines (IL-1Ra, the soluble receptors sIL-1R or Soluble Tumor Necrosis Factor Receptor), and chondroprotective cytokines (IL-4, IL-10). Activation of anabolic processes in vitro has been noted by single or combined administration of components of the cartilage matrix or of the enzymes that synthesize them of growth factors and receptors (IGF-I, FGF-2, BMPs, TGF-β), and of transcription factors (SOX family of DNA-binding proteins, i.e., SOX5, SOX6, SOX9). Restoration of cell vitality and activation of proliferation in vitro have been achieved by application of IGF-1 and FGF-2, telomerase (hTERT), inhibitors of apoptosis (bcl-2), or of HSP70. Interestingly, approaches that influence several of these processes have been also successfully attempted, like combining the transfer of inhibitors of catabolism pathways and of activators of anabolic events (IGF-I/IL-1Ra or IGF-I/IL-4), as well as that of activators of anabolic and proliferative processes (FGF-2/SOX9 or FGF-2/IGF-I).

In Vivo Indirect Gene Transfer. The key issue in establishing an efficient therapy against OA is the accessibility of the targets to the treatment when they reside in the joint cavity. The following approaches have been developed to deliver a molecular composition:

1. systemic delivery, and
2. intra-articular administration (via injection or arthroscopy).

Systemic approaches are better suited to target diseases that are systemic in nature like rheumatoid arthritis (RA). Local administration of components might be preferable in the case of OA that affects only a limited number of joints without major extra-articular or systemic manifestations. The foreign material may be delivered directly (gene vector preparation) or indirectly (genetically modified cells).

Several lines of evidence have demonstrated that intra-articular injection of most vector types leads to a preferential transduction of the synovium, being more suited for strategies aiming at inhibiting inflammatory and catabolic pathways and a common approach employed against experimental RA. Successful attempts towards these goals have been reported by direct application of vectors coding for IL-4, IL-10, sTNFR alone or combined with IL-10, IL-1Ra alone or combined with sTNFR, antagonists and inhibitors of TGF-β and of the BMPs, HSP70, gene expression silencers, and kallistatin or thrombospondin.

Yet, even if cartilage breakdown can be contained, this will not be sufficient to fully compensate for the loss of matrix elements and cells noted during the disease progression. In this regard, increased synthesis of cartilage matrix components has been documented following injection of vectors carrying genes for anabolic factors (IGF-I).

Ex Vivo Indirect Gene Transfer. Although more complex, ex vivo gene therapy is considered safer because no free vector particles are introduced in the body. Modified cells can be extensively controlled, tested, and selected while maintained in culture. Administration of cells is also a means to increase the cellularity like needed for severe OA.

Synoviocytes have been predominantly employed to deliver inhibitors of inflammatory and catabolic processes. Such pathways could be regulated by injecting synoviocytes transduced to overexpress an IL-1Ra alone or combined with IL-10. Also, dermal fibroblasts have been modified for this purpose to overexpress an IL-1Ra, sTNFR, or a combination of both.

Reduced severity of the induced arthritis was associated with a decrease in cartilage breakdown, but complete resurfacing was not achieved. Successful attempts to promote the formation of new cartilage have been made by administrating dermal fibroblasts modified to express BMP-2.

Still, preparation of terminally differentiated cells from unaffected sites remains invasive, with a limited supply, and represents an additional burden for the patient. Also, committed cells generally undergo major phenotypic changes upon passaging in culture, especially chondrocytes. Multipotent cells might be more suited for transplantation purposes, possibly leading to the production of a cartilage surface of enhanced quality compared with committed cells that lead to the formation of a poorly differentiated fibrous cartilage. Progenitor cells can be easily isolated from multiple tissues (bone marrow, peristeme, perichondrium, muscle, fat, subdermis, cartilage, bone, synovial membrane, ligaments), even in OA patients, maintaining a multilineage potential with a reliability for differentiation and a capacity for expansion. Indeed, injection of muscle-derived stem cells modified by combined gene transfer of BMP-4 with sFlt1 (a vascular endothelial growth factor (VEGF) antagonist) allowed for cartilage repair in a rat model of OA.

Osteonecrosis
Osteonecrosis (ON) is primarily a disease of the subchondral bone that secondarily affects the articular cartilage.
Initially, a vascular insult is thought to cause an interference of the microcirculation of the subchondral bone, resulting in an edema that leads to an increased intraosseous pressure. This leads to ON of the affected segment of the subchondral bone, which may result in a subchondral insufficiency fracture, destabilizing the overlying articular cartilage and eventually resulting in its collapse and the creation of an osteochondral defect. Treatment options consist of conservative therapy in early stages. Precollapse lesions can be treated with retrograde core decompression, while later-stage lesions presenting with osteochondral defects require osteochondral transplants and/or osteotomies, or ultimately, partial or total knee arthroplasty.\textsuperscript{214}

Possible experimental gene therapy approaches need to be stage dependent, focusing on early stages (when the articular cartilage is not compromised) at the revascularization of the necrotic bone, while at the stage of osteochondral lesion, only gene-enhanced osteochondral transplants might be useful. Katsube \textit{et al}.\textsuperscript{214} applied gene transfer of VEGF, to accelerate revascularization of the necrotic bone. Using an adenoviral vector encoding for VEGF, endothelial cells of the rabbit saphenous arteries were transduced. These gene-modified arteries were then placed with its venae comitantes into necrotic iliac crest bone \textit{in vivo}. Angiogenesis in the necrotic bone was quantified by bone blood flow measurement and assessment of vessel density following microangiography. The extent of neoangiogenesis was significantly greater in the VEGF group than the control group, reflected in an increased capillary density, length of newly formed capillaries, and increased bone blood flow at 1 week postoperatively. While this study was restricted to the bone of the iliac crest, it might serve as a paradigm for the treatment of ON in a subchondral location. Such a therapy may allow the healing of avascular necrosis before fracture and subchondral collapse occur, preventing the articular cartilage from damage. More studies with time points longer than the 1-week evaluation are needed, preferentially performed in animal models of subchondral ON, such as the femoral condyles of the knee joint, its second most common location.

\textbf{Osteochondritis Dissecans}

Osteochondritis dissecans (OCD) usually affects children and young adults and occurs mainly in the knee joint, characteristic in the lateral aspect of the medial femoral condyle. Possible etiological factors beside a genetic predisposition include ischemia and epiphyseal abnormalities with subsequent necrosis. For example, disruption of epiphyseal plate vessels may lead to localized avascular necrosis. Its revascularization usually occurs with the formation of a scar tissue, absorption of necrotic fragments, intertrabecular osteoid deposition, and remodeling with new bone formation. When revascularization is delayed, an OCD lesion can occur. Clinical treatment principles focus on stimulation of revascularization or removal of necrotic subchondral bone together with its restoration (e.g., using autologous bone transplants), beside the surgical fixation of an unstable osteochondral fragment.\textsuperscript{214}

So far, no experimental gene-based treatment has been proposed for the treatment of OCD. In theory, the same principles apply for the revascularization of necrotic subchondral bone, as already outlined for ON with subsequent articular cartilage defects. It may be also possible to enhance the surgical fixation of an osteochondral fragment by applying osteoinductive genes such as the BMPs to the subchondral bone–osteochondral fragment interface to improve integration of the osteochondral fragment. It is unclear whether the integration of a chondral fragment may be achieved, a rare indication currently favored only for surgical refixation of large fragments in juvenile patients.\textsuperscript{215} Likewise, gene-modified osteochondral transplants may be applied at later stages of deep osteochondral defects.

\textbf{Meniscal Fibrocartilage}

\textit{Anatomy, Function, and Pathophysicsiology}

The menisci are semilunar fibrocartilage structures that transmit weightbearing forces and increase stability, facilitate nutrition and provide lubrication for the articular cartilage, and promote knee proprioception.\textsuperscript{216,217} As the medial meniscus is less mobile during joint motion,\textsuperscript{218,219} injuries are much more common compared to the lateral meniscus.\textsuperscript{220} Type I collagen is the predominant collagen of the meniscal tissue.\textsuperscript{221} It is arranged with a circumferential orientation with interspersed radially oriented fibers.\textsuperscript{222} The central parts of the menisci are mainly constituted of fibrochondrocytes, whereas fibroblasts are the predominant cell type in the peripheral regions.\textsuperscript{223} Meniscal blood supply is restricted to the peripheral 10% to 25% of the meniscal tissue.\textsuperscript{224,225} Nourishment in the central area is provided only by diffusion of the synovial fluid,\textsuperscript{226} perhaps playing a role in the poor healing capacity of central lesions.\textsuperscript{225-227} Gene transfer strategies may be applied for the following:

1. meniscal repair, and
2. meniscal reconstruction, using
   2.1. meniscal substitutes, and
   2.2. meniscal allografts.

\textbf{Meniscal Repair}

Meniscal tears are common\textsuperscript{228,229} and predispose the affected joint to develop secondary OA.\textsuperscript{230} Tears of the meniscus in the vascularized peripheral parts can be
repaired by sutures, while tears of the central avascular parts are treated by arthroscopic partial meniscectomy.

**Gene Transfer Strategies: In Vitro Studies.** Gene transfer strategies for the repair of meniscal tears focus on the delivery of therapeutic agents, for example, growth factors, to the site of the meniscal lesion. This can be performed either via direct application of gene vectors or by transplantation of genetically modified cells overexpressing therapeutic genes. Treatment of meniscal fibrochondrocytes with recombinant growth factor proteins such as the platelet-derived growth factor AB (PDGF-AB), FGF-2, TGF-β1, TGF-β3, or TGF-β3 has been shown to improve the phenotypical and biochemical properties of the cells in vitro. Fibrochondrogenesis of stem cells is enhanced by incubation with growth factors such as TGF-β1 or TGF-β3 in combination with BMP-4. The possible application of gene transfer strategies in meniscal repair has first been investigated by Goto et al. The lacZ marker gene was transferred to meniscal cell cultures using retroviral and adenoviral vectors. In a next step, the marker gene was applied to human meniscal fragments and whole lapine menisci using direct adenoviral gene transfer and transplantation of meniscal fibrochondrocytes transduced with a retroviral vector. Transgene expression was detected in meniscal explants following ex vivo gene transfer for at least 20 weeks. Successful transfer of the lacZ marker gene was also achieved by rAAV-mediated transfer into human and lapine fibrochondrocytes in vitro. Encouraged by these findings, in 2000, the group of Chris Evans transferred the gene encoding for human IGF-I to meniscal fibrochondrocytes, yielding accelerated proliferation and differentiation of the modified cells. Recently, we tested the hypothesis that overexpression of FGF-2 through rAAV vectors leads to detectable metabolic changes in human meniscal fibrochondrocytes and inside defects of human meniscal explants. Application of the rAAV-vector allowed for enhanced cell proliferation and survival in vitro (Figure 3). The idea of applying gene therapy protocols to deliver fibrochondrogenic agents to meniscal tears was supported by a significant reduction of the amplitude of meniscal tears after FGF-2 treatment in this study.

**Gene Therapy: In Vivo Studies.** Only few reports have evaluated the feasibility of gene therapy strategies to enhance the repair of meniscal tears in vivo. Experimental studies have shown that repair in the central part of the meniscus can be promoted by various chemotherapeutic and mitogenic stimuli delivered by an autologous fibrin clot or a free graft of synovium in vivo. In a sheep model, longitudinal tears of the anterior horn of the medial meniscus were sutured using VEGF-coated sutures. Interestingly, meniscal repair was not enhanced in the VEGF treatment group. In 1999, methods of direct and indirect gene transfer to meniscal lesions were compared. In a lapine model, a suspension of adenoviral vectors carrying the lacZ marker gene was mixed with whole blood, and the clot was inserted into 2-mm-long incisions in the medial meniscus. In the same study, using a canine model, retrovirally transduced allogenic meniscal fibrochondrocytes carrying the lacZ gene were embedded in collagen gels and transferred to partial-thickness circular defects (depth, 3 mm; diameter, 2 mm) in the medial meniscus. Gene expression persisted for at least 3 weeks in the lapine model but for 6 weeks within the transplanted meniscal fibrochondrocytes in the canine model. In another animal study, longitudinal incisions were created in the avascular zone of the medial meniscus of rabbits. When rAAV-lacZ constructs were injected intralesionally, X-Gal staining was present by day 20 postoperatively, the longest time point evaluated.

**Meniscal Reconstruction**

**Meniscal Substitutes.** Meniscal substitutes have been proposed as a means to overcome problems associated with meniscal allografts and to promote meniscal repair of segmental defects, for example, resulting from a partial meniscectomy. Meniscal substitutes already in clinical use are based on porous matrices of type I collagen/glycosaminoglycan (Menaflex, ReGen Biologics, Hackensack, NJ) or polyurethane (Actifit, Orteq, London, UK). The feasibility of genetic engineering of meniscal fibrochondrocytes has already been described above. However, in the treatment of circumscribed meniscal defects, direct gene vector administration into injured knee joints may be difficult to achieve because a loss of the bradytrophic meniscal tissue may hardly be restored by local cells, even after administration of mitogenic and anabolic genes. Therefore, gene therapy in the treatment of meniscal defects may need to be used in combination with the transplantation of modified cells or tissues.

Tissue engineering involves the combination of cells, engineered extracellular matrices, and biologically active molecules for tissue regeneration. Over the last 2 decades, numerous tissue engineering strategies have emerged for the replacement of meniscal tissue. In general, 2 basic approaches for meniscal replacement can be distinguished:

1. application of acellular matrices versus
2. application of cell-seeded matrices.

Several concepts for treating circumscribed meniscal defects concentrate on meniscal replacement by acellular matrices, avoiding possible risks associated with transplantation of human allografts (e.g., failure rate,
immunoreaction,\textsuperscript{270} disease transmission\textsuperscript{271}). Different types of meniscal substitutes, such as decellularized allogenic and xenogenic grafts,\textsuperscript{262-265,272-273} collagen grafts,\textsuperscript{253,274} permanent synthetic scaffolds,\textsuperscript{251} and biodegradable scaffolds based on small intestine submucosa,\textsuperscript{275-277} poly-lactic acid (PLA), or poly-glycolic acid (PGA),\textsuperscript{279,282} have been used in experimental and clinical studies. However, after transplantation of acellular meniscal constructs into defects, the transplants are populated by synovial fibroblasts, resulting in a scar tissue with poor biomechanical properties.\textsuperscript{245,283} Therefore, some tissue engineering approaches focus on additional cell-seeding techniques prior to transplantation.\textsuperscript{251,284} Meniscal cells,\textsuperscript{282,285} articular chondrocytes,\textsuperscript{286,287} synovial fibroblasts,\textsuperscript{288} and MSC\textsuperscript{289} have been proposed as potential cell sources and have been cultivated \textit{in vivo} and \textit{in vitro} on various matrices.\textsuperscript{207} In addition, different environmental factors such as growth factors have been used to optimize cell proliferation \textit{in vitro}.\textsuperscript{290}

Gene therapy may aid to further enhance the fibrochondrogenic potential of tissue-engineered transplants. In 2002, Hidaka \textit{et al.}\textsuperscript{291} applied a gene transfer protocol to enhance the vascularization and blood supply of cell-seeded bioengineered meniscus transplants. Bovine meniscal cells overexpressing hepatocyte growth factor (HGF) were seeded onto PGA scaffolds and transplanted subcutaneously in athymic nude mice for 8 weeks. Ink injection studies showed that HGF-treated meniscal cells formed a tissue that contained significantly more blood vessels than the controls. In another preliminary \textit{ex vivo} study, Steinert \textit{et al.}\textsuperscript{152} transduced primary meniscus cells and bone marrow–derived MSCs with adenoviral vectors encoding for marker genes or TGF-β1. Modified cells were seeded in type I collagen-glycosaminoglycan (GAG) matrices and transplanted into defects of bovine menisci explants. \textit{In vitro}, the vectors efficiently transduced meniscal cells and MSCs, and transgene expression remained elevated after incorporation of the cells into matrices. Transfer of TGF-β1 increased the fibrochondrogenic potential of modified cells, and transplantation of the TGF-β1–transduced constructs resulted in satisfactory filling of the lesions \textit{ex vivo} (Table 3).

A recent \textit{in vivo} work on the use of gene transfer to enhance meniscal repair has been published by Zhang \textit{et al.}\textsuperscript{292} Following an indirect gene therapy approach without tissue engineering features, the authors created full-thickness meniscal defects in the avascular area of the anterior horn of the medial meniscus in a goat model. Bone marrow stromal cells were transfected with the gene encoding for human IGF-I using a nonviral transfection system (FuGENE 6) and suspended in calcium alginate prior to injection into the meniscal defects. After 16 weeks, the resulting repair tissue was improved according to MRI and histological and biochemical evaluation and compared with the controls (Table 3).

\textbf{Meniscal Allografts}. Meniscal reconstitution with allografts\textsuperscript{293-305} is a therapeutic option especially for young and symptomatic patients with a history of lateral meniscectomy in a normally aligned, stable joint without severe degenerative changes of the articular cartilage. A recent review\textsuperscript{206} suggests that meniscal allograft transplantation improves pain and function in the short and intermediate term.

Application of gene-based strategies has been suggested to improve remodeling of meniscal allografts.\textsuperscript{307} Martinek \textit{et al.}\textsuperscript{308} studied the feasibility of gene transfer in lapine meniscal allografts \textit{ex vivo} using a retroviral vector encoding the marker gene \textit{lacZ}. Subsequently, unilateral meniscal replacements were performed with these engineered allografts. Transduced fibrochondrocytes migrated into the depth of the graft, while transgene expression persisted for up to 8 weeks. This investigation suggests potential promise for growth factor delivery in autografts and allografts prior to implantation.

\section{Clinical Gene Therapy Trials}

Preclinical data, as those described above, have encouraged the initiation of human clinical trials originally for arthritis. The first studies were based on the \textit{ex vivo} retroviral gene transfer of a human IL-1Ra sequence in synoviocytes from patients with end-stage RA followed by reinjection of the modified cells in the metacarpophalangeal joint.\textsuperscript{23,82,309} The aim of these studies was to evaluate the possibility of transferring genes to human joints and expressing them intra-articularly in a safe fashion acceptable to the patients. The use of these protocols has permitted extensive testing of the cells prior to reimplantation, demonstrating successful expression of the transgene locally vis-à-vis control joints, without adverse events related to the treatment but with clinical improvements in some of the patients, encouraging the implementation of phase II studies (pending).\textsuperscript{1,5,30,310-315}

Another protocol has been initiated for \textit{intra-articular} plasmid\textsuperscript{316} delivery of the HSV thymidine kinase gene to the synovial lining of RA patients followed by administration of ganciclovir to achieve synovial ablation,\textsuperscript{1,5,309,311-314} but this protocol has been closed because of a failure to recruit. A new phase I trial for RA involved the direct \textit{in vivo} intra-articular injection of an AAV vector carrying the sequence for a fusion protein as sTNFR on an immunoglobulin molecule (tgAAC94 protocol).\textsuperscript{317} As the study revealed that the treatment was safe and well tolerated in subjects without use of concurrent systemic TNF-α antagonist,\textsuperscript{1,311,312,314,317} a phase I/II trial was subsequently started\textsuperscript{118} with the possibility to include patients who were already taking systemic TNF blockers and the administration of a second injection of tgAAC94. As one of the participants who was simultaneously being treated with systemic TNF antagonist and other immunosuppressive medications died after receiving the second injection, the trial was placed on hold by the U.S. Food and Drug Administration (FDA) to investigate, in parallel with the Recombinant DNA Advisory Committee
| Gene     | Strategy | Vector | Cells                          | Support                  | Experimental Model | Period of Evaluation | Major Findings                                                                                       | Ref. |
|----------|----------|--------|-------------------------------|--------------------------|--------------------|---------------------|---------------------------------------------------------------------------------------------------|------|
| IGF-I    | In vitro | Liposome (FuGENE 6) | Meniscal fibrochondrocytes (human) | −/−                      | In vitro           | 0 h 10 d            | Transfection efficiency 16% ± 1.2% No cytotoxicity Decrease in PDT from 52.6 to 40.2 h           | 134  |
| IGF-I    | In vitro | Liposome (FuGENE 6) | Bone marrow stromal cells (goat) | Calcium alginate gel     | In vitro           | 4 h 10 d            | Transfection efficiency 22.0% ± 2.4% Elevated IGF-I secretion (3.28 v. 1.67 ng/mL by untransfected fibrochondrocytes) | 292  |
|          | In vivo  |         |                               |                          | In vivo            | 3 d 16 wk          | Macrosopically and histologically improved repair tissue Elevated GAG content (14.2 x 13.7 mg/g by untransfected BMSCs) Improved aspect of repair tissue on MRI |     |
| HGF      | In vitro | Adenoviral | Meniscal cells (calf) | PGA (subcutaneous pouch) | In vitro           | 48 h                | Transduction efficiency N.D. No enhanced proliferation of HGF-transduced cells            | 291  |
|          | In vivo  |         |                               |                          | In vivo            | 3 d 8 wk            | HGF expression detectable for ≥2 weeks Fibrocartilage with structural limitations (presence and organization of collagen fibrils) Enhanced vascularization of engineered constructs No improved biomechanical properties (compression testing) |     |
| FGF-2    | In vitro | rAAV   | Meniscal fibrochondrocytes (human) | −/− (direct vector injection) | In vitro           | 0 d 21 d           | Transduction efficiency 53%-59% Efficient FGF-2 transgene expression Enhanced cell proliferation and survival | 177  |
|          | Ex vivo  |         |                               |                          | Ex vivo            | 5 d 15 d            | Enhanced contractile markers (α-SMA) Reduction of meniscal tear amplitude in depth and width (up to 2.4-fold) No stimulation of extracellular matrix components (type II collagen, PG) |     |
| TGF-β1   | In vitro | Adenoviral | Primary meniscal cells (calf); bone marrow–derived MSCs (calf) | Type I collagen-GAG matrix | In vitro           | 3 d 3 wk           | Transduction efficiency >75% Increased cellularity and GAG/DNA synthesis Enhanced proteoglycan and type II collagen staining Enhanced meniscal gene expression (COL I, COL II, DCN, BCN) | 152  |
|          | Ex vivo  |         |                               |                          | Ex vivo            |                    | Formation of highly cellular repair tissue; no differences between treatment and control group |     |
| TGF-β1   | In vitro | Retroviral | Meniscal cells (human and canine) | −/−                      | Ex vivo            | 3 wk                | Transduction efficiency N.D. Increased transgene expression Enhanced collagen and proteoglycan synthesis (up to 15-fold) | 158  |

Note: PDT = population doubling time; GAG = glycosaminoglycan; BMSC = bone marrow stromal cell; HGF = hepatocyte growth factor; PGA = polyglycolic acid; α-SMA = alpha-smooth muscle actin; PG = proteoglycan; COL I = type I A2 collagen (537 bp); COL II = type II A1 collagen (580 bp); DCN = decorin (400 bp); BCN = biglycan (165 bp); MSC = mesenchymal stem cells; N.D. = not determined. FGF = fibroblast growth factor; TGF = transforming growth factor.
Recombinant DNA Advisory Committee (RAC), the circumstances of the demise of the patient. The death was apparently due to a disseminated infection with Histoplasma capsulatum, a fungus endemic in the region of origin of the volunteer, and to an immunosuppression. Indeed, known serious complications of the particular TNF antagonist are susceptibility to H. capsulatum. The most probable explanation is that the subject was already infected with the fungus when receiving the second injection of tgAAC94. As the committee felt that the gene therapy protocol was very unlikely to have played any significant role in the event based on a large body of data from the independent investigations and since rAAV has been used safely in 47 previous human gene therapy clinical trials, the evaluation has been reopened with some modifications (exclusion of patients with elevated temperature, localized symptoms, fatigue, or with history of opportunistic infection), requiring additional monitoring (repeated blood counts, serum chemistry, vector DNA and transgene product titration, analysis of T-cell responses to vector genomes in the blood at the highest vector dose). Regarding OA, a phase I protocol is currently ongoing, based on an ex vivo approach using the retroviral transfer of TGF-β.

**Gene Doping**

Although the previously discussed gene-based approaches may have potential value for the treatment of articular cartilage defects and meniscal lesions, some of the therapeutic genes used in these studies have been also implicated for gene doping, a term referring to the potential misuse of gene therapy for the purposes of enhancing athletic performance. Possible genes with such potential include, but are not limited to, growth hormone and IGF-I, erythropoietin (Epo), VEGF, FGF-2, and endorphins.

IGF-I, the prime target of growth hormone action, is a potential candidate gene. A number of studies have shown that upregulation of IGF-I stimulates muscle growth and improves muscle function. Interestingly, this increase in muscle volume is not reflected by detectable increases in circulating IGF-I. While favorable responses have been obtained in animal studies, the transfer of such techniques to humans with the goal of a higher performance still presents many technical challenges.

The hormone Epo is produced by the peritubular capillary endothelial cells in the kidney. Under hypoxic conditions, Epo is produced and secreted, increasing the production of red blood cells. Eero Mäntyranta, a Finnish cross-country skier who won 2 gold medals in the 1964 Olympics, was born with a mutation in the Epo receptor gene that allowed his blood to carry significantly more oxygen than an average person. Recombinant Epo has been used already as a performance-enhancing drug. Because of differences in its peptide sequence compared with the endogenous protein, it may be detected in blood. Recently, a viral vector for the release of Epo in response to low oxygen concentrations has been developed under the trade name Repoxygen (Oxford BioMedica, Oxford, UK). The viral vector of undisclosed origin carries the human Epo gene under the control of a hypoxia control element (HRE). At low oxygen concentrations, HRE switches on the expression of the transgene. The vector is designed to be delivered by a simple intramuscular injection, resulting in the synthesis of recombinant Epo by muscle cells, rather than by cells of the liver or kidneys. Initially developed to treat anemia, there have been speculations in the media that it has been already applied for doping purposes.

Recently, genetically engineered mice have been created with an alteration in energy metabolism based on overexpression of the gene for phosphoenolpyruvate carboxykinases (PEPCK-C). PEPCK-C is an enzyme of the lyase family that plays a role in the metabolic pathway of gluconeogenesis, converting oxaloacetate into phosphoenolpyruvate and carbon dioxide. These transgenic PEPCK-C mice carry a chimeric gene in which a copy of the cDNA for PEPCK-C is placed under control of the skeletal actin gene promoter, directing overexpression of PEPCK-C exclusively to skeletal muscle. PEPCK-C mice were more active, could run longer and faster, and used fatty acids more efficiently and produced far less lactate than control animals. Whether these data can be corroborated by studies in large animals remains to be determined.

Taken together, there is an emerging body of results from a number of transgenic and somatic gene transfer studies that suggest the principle of gene transfer may find application to enhance athletic performance. Many of the genes are already cloned in functional vectors, and some of them are being evaluated in clinical trials for the treatment of diseases. However, therapeutic gene transfer to humans is still technically challenging, and no clear evidence has been given that athletes have been using gene technology to enhance their performance. For antidoping authorities, the challenge will be to detect these endogenously produced gene products because of the homology between the transferred cDNA, the homology of the endogenously produced protein, and the limited specificity of indirect detection procedures. Further studies in this field are needed since a possible uncontrolled use of these gene vectors imposes potential high risks for both the athlete and the general public.

**Outlook**

Despite these encouraging data, application of gene transfer approaches in the treatment of articular cartilage and meniscal lesion tears is still in its infancy. Although the use
of gene therapy holds great promise, issues that need to be addressed include the duration of transgene expression, further studies in clinically relevant animal models of articular cartilage and meniscal lesions, the benefit of using ex vivo genetically modified cells versus direct gene transfer approaches, and the identification of (an) optimal therapeutic factor(s) for each particular clinical problem. Future studies will also have to shed light on the safety of these approaches regarding the nonlethal nature of these diseases. A successful application of gene therapy for cartilage repair requires the combined effort of orthopedic surgeons continuing to ask clinically relevant questions and of basic scientists further improving the currently available gene transfer systems.

Acknowledgments and Funding
The authors received no financial support for the research and/or authorship of this article.

Declaration of Conflicting Interests
The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

References
1. Evans CH, Ghivizzani SC, Herndon JH, Robbins PD. Gene therapy for the treatment of musculoskeletal diseases. J Am Acad Orthop Surg. 2005;13(4):230-42.
2. Nakajima A. Application of cellular gene therapy for rheumatoid arthritis. Mod Rheumatol. 2006;16(5):269-75.
3. Madry H, Kohn D, Cucchiari M. [Gene therapy in orthopaedic surgery]. Orthopade. 2006;35(11):1193-202.
4. Ivkovic A, Pascher A, Hudetz D, Jelic M, Haspl M, Windhager R, et al. Current concepts in gene therapy of the musculoskeletal system. Acta Chir Orthop Traumatol Cech. 2006;73(2):115-22.
5. Robbins PD, Evans CH, Chernajovsky Y. Gene therapy for arthritis. Gene Ther. 2003;10(10):902-11.
6. Abramson SB, Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. Rheumatology (Oxford). 2002;41(9):972-80.
7. Evans CH, Ghivizzani SC, Kang R, Muzzoni R, Wasko MC, Herndon JH, et al. Gene therapy for rheumatic diseases. Arthritis Rheum. 1999;42(1):1-16.
8. Vervoordeldonk MJ, Tak PP. Gene therapy in rheumatic diseases. Best Pract Res Clin Rheumatol. 2001;15(5):771-88.
9. Burstein H. Gene therapy for rheumatoid arthritis. Curr Opin Mol Ther. 2001;3(4):362-74.
10. Felghner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A. 1987;84(21):7413-7.
11. Schwendener RA. Liposomes in biology and medicine. Adv Exp Med Biol. 2007;620:117-28.
12. Orth P, Weiner A, Kaul G, Kohn D, Cucchiari M, Madry H. Analysis of novel nonviral gene transfer systems for gene delivery to cells of the musculoskeletal system. Mol Biotechnol. 2008;38(2):137-44.
13. Hudde T, Rayner SA, Comer RM, Weber M, Isaacs JD, Waldmann H, et al. Activated polyamidoamine dendrimers, a nonviral vector for gene transfer to the corneal endothelium. Gene Ther. 1999;6(5):939-43.
14. Godbey WT, Wu KK, Hirasaki GJ, Mikos AG. Improved packing of poly(ethylenimine)/DNA complexes increases transfection efficiency. Gene Ther. 1999;6(8):1380-8.
15. Chemin I, Moradpour D, Wieland S, Offensperger WB, Walter E, Behr JP, et al. Liver-directed gene transfer: a linear polyethlenimine derivative mediates highly efficient DNA delivery to primary hepatocytes in vitro and in vivo. J Viral Hepat. 1998;5(6):369-75.
16. Ravi Kumar M, Hellermann G, Lockey RF, Mohapatra SS. Nanoparticle-mediated gene delivery: state of the art. Expert Opin Biol Ther. 2004;4(8):1213-24.
17. Graham FL, van der Eb AJ. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology. 1973;52(2):456-67.
18. Frisbie DD, Ghivizzani SC, Robbins PD, Evans CH, McIlwraith CW. Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther. 2002;9(1):12-20.
19. Gelse K, von der Mark K, Aigner T, Park J, Schneider H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum. 2003;48(2):430-41.
20. Ghivizzani SC, Lechman ER, Kang R, Tio C, Kolls J, Evans CH, et al. Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor alpha soluble receptors to rabbit knees with experimental arthritis has local and distal antiarthritic effects. Proc Natl Acad Sci U S A. 1998;95(8):4613-8.
21. Hidaka C, Goodrich LR, Chen CT, Warren RF, Crystal RG, Nixon AJ. Acceleration of cartilage repair by genetically modified chondrocytes over expressing bone morphogenetic protein-7. J Orthop Res. 2003;21(4):573-83.
22. Park J, Gelse K, Frank S, von der Mark K, Aigner T, Schneider H. Transgene-activated mesenchymal cells for articular cartilage repair: a comparison of primary bone marrow-, perichondrium/periosteum- and fat-derived cells. J Gene Med. 2006;8(1):112-25.
23. Evans CH, Robbins PD, Ghivizzani SC, Herndon JH, Kang R, Bahnson AB, et al. 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. 1996;7(10):1261-80.
24. Grande DA, Mason J, Light E, Dines D. Stem cells as platforms for delivery of genes to enhance cartilage repair. J Bone Joint Surg Am. 2003;85-A(Suppl 2):111-6.
25. Mason JM, Breitbart AS, Barcia M, Porti D, Pergolizzi RG, Grande DA. Cartilage and bone regeneration using gene-enhanced tissue engineering. Clin Orthop Relat Res. 2000;(379 Suppl):S171-8.
26. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum. 2003;48(12):3464-74.

27. Lee KH, Song SU, Hwang TS, Yi Y, Oh IS, Lee JY, et al. Regeneration of hyaline cartilage by cell-mediated gene therapy using transforming growth factor beta 1-producing fibroblasts. Hum Gene Ther. 2001;12(14):1805-13.

28. Gouze E, Pawliuk R, Gouze JN, Pilapil C, Fleet C, Palmer GD, et al. Lentiviral-mediated gene delivery to synovium: potent intra-articular expression with amplification by inflammation. Mol Ther. 2003;7(4):460-6.

29. Gouze E, Pawliuk R, Pilapil C, Gouze JN, Fleet C, Palmer GD, et al. In vivo gene delivery to synovium by lentiviral vectors. Mol Ther. 2002;5(4):397-404.

30. Evans CH, Ghivizzani SC, Robbins PD. The 2003 Nicolas Andry Award: orthopaedic gene therapy. Clin Orthop Relat Res. 2004;429:316-29.

31. Oligino T, Ghivizzani S, Wolfe D, Lechman E, Kirsy D, Mi Z, et al. Intra-articular delivery of a herpes simplex virus IL-1Ra gene vector reduces inflammation in a rabbit model of arthritis. Gene Ther. 1999;6(10):1713-20.

32. Berns KJ, Linden RM. The cryptic life style of adeno-associated virus. Bioessays. 1995;17(3):237-45.

33. Flotte TR, Afione SA, Conrad C, McGrath SA, Solow R, Oka H, et al. Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. Proc Natl Acad Sci U S A. 1993;90(22):10613-7.

34. Pan RY, Chen SL, Xiao X, Liu DW, Peng HJ, Tsao YP. Therapy and prevention of arthritis by recombinant adeno-associated virus vector with delivery of interleukin-1 receptor antagonist. Arthritis Rheum. 2000;43(2):289-97.

35. Watanabe S, Imagawa T, Boivin GP, Gao G, Wilson JM, Hirsch R. Adeno-associated virus mediates long-term gene transfer and delivery of chondroprotective IL-4 to murine synovium. Mol Ther. 2000;2(2):147-52.

36. Cucchiariini M, Madry H, Ma C, Thurn T, Zurakowski D, Menger MD, et al. Improved tissue repair in articular cartilage defects in vivo by rAAV-mediated overexpression of human fibroblast growth factor 2. Mol Ther. 2005;12(2):229-38.

37. Evans CH, Ghivizzani SC, Smith P, Shuler FD, Mi Z, Robbins PD. Using gene therapy to protect and restore cartilage. Clin Orthop Relat Res. 2000;(379 Suppl):S214-9.

38. Pagnotto MR, Wang Z, Karpie JC, Ferretti M, Xiao X, Chu CR. Adeno-associated viral gene transfer of transforming growth factor-beta1 to human meniscal stem cells improves cartilage repair. Gene Ther. 2007;14(10):804-13.

39. Mease PJ, Wei N, Fuhrman EJ, Kivitz AJ, Schechtman J, Trapp RG, et al. Safety, tolerability, and clinical outcomes after intraarticular injection of a recombinant adeno-associated vector containing a tumor necrosis factor antagonist gene: results of a phase 1/2 study. J Rheumatol. 2010;37(4):692-703.

40. O'Driscoll SW. The healing and regeneration of articular cartilage. J Bone Joint Surg Am. 1998;80(12):1795-812.

41. Hunziker EB, Michel M, Studer D. Ultrastructure of adult human articular cartilage matrix after cryotechnical processing. Microsc Res Tech. 1997;37(4):271-84.

42. Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):419-33.

43. Pape D, Filardo G, Kon E, van Dijk CN, Madry H. Disease-specific clinical problems associated with the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):448-62.

44. Noyes FR, Stabler CL. A system for grading articular cartilage lesions at arthroscopy. Am J Sports Med. 1989;17(4):505-13.

45. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage. 2002;10(6):432-63.

46. Furukawa T, Eyre DR, Koide S, Glimcher MJ. Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. J Bone Joint Surg Am. 1980;62(1):79-89.

47. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg Am. 1993;75(4):532-53.

48. Jackson DW, Lalor PA, Aberman HM, Simon TM. Spontaneous repair of full-thickness defects of articular cartilage in a goat model: a preliminary study. J Bone Joint Surg Am. 2001;83-A(1):53-64.

49. Rogachevsky RA, Dean DD, Howell DS, Altman RD. Treatment of canine osteoarthritis with insulin-like growth factor-1 (IGF-1) and sodium pentosan polysulfate. Osteoarthritis Cartilage. 1993;1(2):105-14.

50. Shida J, Jingushi S, Iwami T, Yewaki S, Sugioka Y. Basic fibroblast growth factor stimulates articular cartilage enlargement in young rats in vivo. J Orthop Res. 1996;14(2):265-72.

51. 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(10):1452-63.

52. Joyce ME, Roberts AB, Sporn MB, Bolander ME. Transforming growth factor-beta and the initiation of chondrogenesis and osteogenesis in the rat femur. J Cell Biol. 1990;116(6):2195-207.

53. Hanada K, Solchaga LA, Caplan AL, Hering TM, Goldberg VM, Yoo JU, et al. BMP-2 induction and TGF-beta1 modulation of rat periosteal cell chondrogenesis. J Cell Biochem. 2001;81(2):284-94.

54. Asahina I, Sampath TK, Hauschka PV. Human osteogenic protein-1 induces chondroblastic, osteoblastic, and/or adipocytic differentiation of clonal murine target cells. Exp Cell Res. 1996;222(1):38-47.

55. Klein-Nulend J, Louwerse RT, Heyligers IC, Wuisman PI, Semeins CM, Goei SW, et al. Osteogenic protein (OP-1, BMP-7) stimulates cartilage differentiation of human and goat perichondrium tissue in vitro. J Biomed Mater Res. 1998;40(4):614-20.
I, Gejo R, like growth factor-I-laden fibrin composites. J Orthop Res. Enhanced repair of extensive articular defects by insulin-embryonic development. Mech Dev. 1994;48(3):245-54.

The gene for the homeodomain-containing protein Cart-1 is expressed in cells that have a chondrogenic potential during deficient mice. Dev Dyn. 1999;214(4):279-90.

M, cells derived from bone marrow. Rheumatology (Oxford). bits using CDMP1 gene-transfected autologous mesenchymal

Rheumatol Suppl. 1995;43:129-32.

Schoenfeld D, Doctrow SR. Regulation of growth-plate chondrocytes. J Bone Joint Surg Am. 1993;75(2):177-89.

Ohara T, Inada CM, Kola R, Sumarsono Zhao T, Quach J, Lotz M. Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J Biol Chem. 1998;273(42):27467-73.

Pelletier JP, Martel-Pelletier J. [Role of synovial inflammation cytokines and IGF-1 in the physiopathology of osteoarthritis]. Rev Rhum Ed Fr. 1994;61(9 Pt 2):103S-8S.

Blanco FJ, Ochs RL, Schwarz H, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Pathol. 1995;146(1): 75-85.

Niita I, Ghivizzani SC, Galea-Lauri J, Bandara G, Georgescu HI, Robbins PD, et al. Direct gene delivery to synovium: an evaluation of potential vectors in vitro and in vivo. Arthritis Rheum. 1996;39(5):820-8.

Ikeda T, Kubo T, Arai Y, Nakanishi T, Kobayashi K, Takahashi K, et al. Adenovirus mediated gene delivery to the joints of guinea pigs. J Rheumatol. 1998;25(9):1666-73.

Ghivizzani SC, Lechman ER, Tio C, Mule KM, Chada S, McCormack JE, et al. Direct retrovirus-mediated gene transfer to the synovium of the rabbit knee: implications for arthritis gene therapy. Gene Ther. 1997;4(9):977-82.

Wehling P, Reinecke J, Baltzer AW, Granrath M, Schulitz KP, Schultz C, et al. Clinical responses to gene therapy in joints of two subjects with rheumatoid arthritis. Hum Gene Ther. 2009;20(2):97-101.

Pan RX, Xiaox, Chen SL, Li J, Lin LC, Wang JH, et al. Disease-inducible transgene expression from a recombinant adenovirus-infected virus vector in a rat arthritis model. J Virol. 1999;73(4):3410-7.

Watanabe S, Kim KN, Imagawa T, Thornton S, Grom A, Hirsch R. On the mechanism of protection of distal joints after local gene transfer in collagen-induced arthritis. Hum Gene Ther. 2000;11(5):751-8.

Tomita T, Hashimoto H, Tomita N, Morishita R, Lee SB, Hayashida K, et al. In vivo direct gene transfer into articular cartilage.
cartilage by intraarticular injection mediated by HVJ (Sendai virus) and liposomes. Arthritis Rheum. 1997;40(5):901-6.
86. Morisset S, Frisbie DD, Robbins PD, Nixon AJ, McIlwraith CW. LL-1ra/IGF-1 gene therapy modulates repair of microfractured chondral defects. Clin Orthop Relat Res. 2007;462:221-8.
87. Madry H, Cucchiarini M, Terwilliger EF, Trippel SB. Recombinant adeno-associated virus vectors efficiently and persistently transduce chondrocytes in normal and osteoarthritic human articular cartilage. Hum Gene Ther. 2003;14(4):393-402.
88. Coutts RD, Healey RM, Ostrander R, Sah RL, Goomer R, Amiel D. Matrices for cartilage repair. Clin Orthop Relat Res. 2001;(391 Suppl):S271-9.
89. Pascher A, Palmer GD, Steinert A, Oligino T, Gouze E, Gouze JN, et al. Gene delivery to cartilage defects using coagulated bone marrow aspirate. Gene Ther. 2004;11(2):133-41.
90. Kaul G, Cucchiarini M, Arntzen D, Zurakowski D, Menger MD, Kohn D, et al. Local stimulation of articular cartilage repair by transplantation of encapsulated chondrocytes overexpressing human fibroblast growth factor 2 (FGF-2) in vivo. J Gene Med. 2006;8(1):100-11.
91. Madry H, Kaul G, Cucchiarini M, Stein U, Zurakowski D, Remberger K, et al. Enhanced repair of articular cartilage defects in vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). Gene Ther. 2005;12(15):1171-9.
92. Fragonas E, Valente M, Pozzi-Mucelli M, Tofani R, Rizzo R, Silvestri F, et al. Articular cartilage repair in rabbits by using suspensions of allogenic chondrocytes in alginate. Biomaterials. 2000;21(8):795-801.
93. Rahfoth B, Weisser J, Sternkopf F, Aigner T, von der Mark K, Brauer R. Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits. Osteoarthritis Cartilage. 1997;5(2):139-43.
94. Madry H, Cucchiarini M, Stein U, Remberger K, Menger MD, Kohn D, et al. Sustained transgene expression in cartilage defects in vivo after transplantation of articular chondrocytes modified by lipid-mediated gene transfer in a gel suspension delivery system. J Gene Med. 2003;5(6):502-9.
95. Goomer RS, Deftos L, Terkeltaub R, Maris T, Lee MC, Harwood FL, et al. High-efficiency non-viral transfection of primary chondrocytes and perichondrial cells for ex-vivo gene therapy to repair articular cartilage defects. Osteoarthritis Cartilage. 2001;9(3):248-56.
96. Ueblacker P, Wagner B, Kruger A, Vogt S, DeSantis G, Kernerknecht E, et al. Inducible nonviral gene expression in the treatment of osteochondral defects. Osteoarthritis Cartilage. 2004;12(9):711-9.
97. Baragi VM, Renkiewicz RR, Jordan H, Bonadio J, Hartman JW, Roessler BJ. Transplantation of transduced chondrocytes protects articular cartilage from interleukin 1-induced extracellular matrix degradation. J Clin Invest. 1995;96(5):2454-60.
98. Mason JM, Grande DA, Barcia M, Grant R, Pergolizzi RG, Breitbart AS. Expression of human bone morphogenic protein 7 in primary rabbit periosteal cells: potential utility in gene therapy for osteochondral repair. Gene Ther. 1998;5(8):1098-104.
99. Adachi N, Sato K, Usas A, Fu FH, Ochi M, Han CW, et al. Muscle derived, cell based ex vivo gene therapy for treatment of full thickness articular cartilage defects. J Rheumatol. 2002;29(9):1920-30.
100. Hirschmann F, Verhoeyen E, Wirth D, Bauwens S, Hauser H, Rudert M. Vital marking of articular chondrocytes by retroviral infection using green fluorescence protein. Osteoarthritis Cartilage. 2002;10(2):109-18.
101. Mierisch CM, Wilson HA, Turner MA, Milbrandt TA, Berthoux L, Hammerskjold ML, et al. Chondrocyte transplantation into articular cartilage defects with use of calcium alginate: the fate of the cells. J Bone Joint Surg Am. 2003;85-A(9):1757-67.
102. Kobayashi N, Koshino T, Uesugi M, Yokoo N, Xin QK, Okuda K, et al. Repair of articular cartilage defect by autologous transplantation of basic fibroblast growth factor gene-transduced chondrocytes with adeno-associated virus vector. Arthritis Rheum. 2005;52(1):164-70.
113. Baragi VM, Renkiewicz RR, Qiu L, Brammer D, Riley JM, Sigler RE, et al. Transplantation of adenovirally transduced allogeneic chondrocytes into articular cartilage defects in vivo. Osteoarthritis Cartilage. 1997;5(4):275-82.

114. Madry H, Orth P, Kaul G, Zurakowski D, Menger MD, Kohn D, et al. Acceleration of articular cartilage repair by combined gene transfer of human-insulin-like growth factor I and fibroblast growth factor-2 in vivo. Arch Orthop Trauma Surg. 2010;130(10):1311-22.

115. Turgeman G, Pittman DD, Muller R, Kurkalli BG, Zhou S, Pelleg G, et al. Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy. J Gene Med. 2001;3(3):240-51.

116. Cucchiari M, Madry H. Gene therapy for cartilage defects. J Gene Med. 2005;7(12):1495-509.

117. Che JH, Zhang ZR, Li GZ, Tan WH, Bai XD, Qu FJ. Application of tissue-engineered cartilage with BMP-7 gene to repair knee joint cartilage injury in rabbits. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):496-503.

118. Gelse K, Muhle C, Franke O, Park J, Jehle M, Durst K, et al. Cell-based resurfacing of large cartilage defects: long-term evaluation of grafts from autologous transgene-activated periosteal cells in a porcine model of osteoarthritis. Arthritis Rheum. 2008;58(2):475-88.

119. Gysin R, Wergedal JE, Sheng MH, Kasukawa Y, Miyakoshi N, Chen ST, et al. Ex vivo gene therapy with stromal cells transduced with a retroviral vector containing the BMP4 gene completely heals critical size calvarial defect in rats. Gene Ther. 2002;9(15):991-9.

120. Vogt S, Wexel G, Tischer T, Schillinger U, Ueblacker P, Wagner B, et al. The influence of the stable expression of BMP2 in fibrin clots on the remodelling and repair of osteochondral defects. Biomaterials. 2009;30(12):2385-92.

121. Goodrich LR, Hidaka C, Robbins PD, Evans CH, Nixon AJ. Genetic modification of chondrocytes with insulin-like growth factor-1 enhances cartilage healing in an equine model. J Bone Joint Surg Br. 2007;89(5):672-85.

122. Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, et al. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. Arthritis Rheum. 2006;54(2):433-42.

123. Guo X, Zheng Q, Yang S, Shao Z, Yuan Q, Pan Z, et al. Repair of full-thickness articular cartilage defects by cultured mesenchymal stem cells transfected with the transforming growth factor beta1 gene. Biomed Mater. 2006;1(4):206-15.

124. Evans CH, Liu FJ, Glatt V, Hoyland JA, Kirker-Head C, Walsh A, et al. Use of genetically modified muscle and fat grafts to repair defects in bone and cartilage. Eur Cell Mater. 2009;18:96-111.

125. Ivkovic A, Pascher A, Hudetz D, Maticic D, Jelic M, Dickinson S, et al. Articular cartilage repair by genetically modified bone marrow aspirate in sheep. Gene Ther. 2010;17(6):779-89.

126. Gerich TG, Lobenhoffer HP, Fu FH, Robbins PD, Evans CH. [Vira ally mediated gene transfer in the patellar tendon: an experimental study in rabbits]. Unfallchirurg. 1997;100(5):354-62.

127. Madry H, Zurakowski D, Trippel SB. Overexpression of human insulin-like growth factor-I promotes new tissue formation in an ex vivo model of articular chondrocyte transplantation. Gene Ther. 2001;8(19):1443-9.

128. Goater JJ, O’Keefe RJ, Rosier RN, Puzas JE, Schwarz EM. Efficacy of ex vivo OPG gene therapy in preventing wear debris induced osteolysis. J Orthop Res. 2002;20(2):169-73.

129. Taniyama Y, Tachibana K, Hiraoka K, Aoki M, Yamamoto S, Matsumoto K, et al. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Ther. 2002;9(6):372-80.

130. Tsuchiya H, Kito H, Sugiu F, Ishiguro N. Chondrogenesis enhanced by overexpression of sox9 gene in mouse bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun. 2003;301(2):338-43.

131. Madry H, Emkey G, Zurakowski D, Trippel SB. Overexpression of human fibroblast growth factor 2 stimulates cell proliferation in an ex vivo model of articular chondrocyte transplantation. J Gene Med. 2004;6(2):238-45.

132. Grossin L, Cournil-Henriquet N, Pinzano A, Gaborit N, Dumas D, Etienne S, et al. Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis. FASEB J. 2006;20(1):65-75.

133. Yeh LC, Lee JC. Co-transfection with the osteogenic protein (OP)-I gene and the insulin-like growth factor (IGF)-I gene enhanced osteoblastic cell differentiation. Biochem Biophys Acta. 2006;1763(1):57-63.

134. Zhang HN, Leng P, Wang YZ, Zhang J. Treating human meniscal fibrochondrocytes with hIGF-1 gene by liposome. Clin Orthop Relat Res. 2009;467(12):3175-82.

135. Manning K, Rachakonda PS, Rai MF, Schmidt MF. Co-expression of insulin-like growth factor-1 and interleukin-4 in an in vitro inflammatory model. Cytokine. 2010;50(3):297-305.

136. Li Y, Tew SR, Russell AM, Gonzalez KR, Harding RE, Hawkins RE. Transduction of passaged human articular chondrocytes with adenoviral, retroviral, and lentiviral vectors and the effects of enhanced expression of SOX9. Tissue Eng. 2004;10(3-4):575-84.

137. Attur MG, Dave MN, Leung MY, Cipolletta C, Mesecik M, Woo SL, et al. Functional genomic analysis of type II IL-beta decoy receptor: potential for gene therapy in human arthritis and inflammation. J Immunol. 2002;168(4):2001-10.

138. Smith P, Shuler FD, Georgescu HI, Ghivizzani SC, Johnstone H, Madry H, Emkey G, Zurakowski D, Trippel SB. Overexpression of human fibroblast growth factor 2 stimulates cell proliferation in an ex vivo model of articular chondrocyte transplantation. Gene Ther. 2001;8(19):1443-9.

139. Goiter JJ, O’Keefe RJ, Rosier RN, Puzas JE, Schwarz EM. Efficacy of ex vivo OPG gene therapy in preventing wear debris induced osteolysis. J Orthop Res. 2002;20(2):169-73.

140. Taniyama Y, Tachibana K, Hiraoka K, Aoki M, Yamamoto S, Matsumoto K, et al. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Ther. 2002;9(6):372-80.

141. Tsuchiya H, Kito H, Sugiu F, Ishiguro N. Chondrogenesis enhanced by overexpression of sox9 gene in mouse bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun. 2003;301(2):338-43.

142. Madry H, Emkey G, Zurakowski D, Trippel SB. Overexpression of human fibroblast growth factor 2 stimulates cell proliferation in an ex vivo model of articular chondrocyte transplantation. J Gene Med. 2004;6(2):238-45.

143. Grossin L, Cournil-Henriquet N, Pinzano A, Gaborit N, Dumas D, Etienne S, et al. Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis. FASEB J. 2006;20(1):65-75.

144. Yeh LC, Lee JC. Co-transfection with the osteogenic protein (OP)-I gene and the insulin-like growth factor (IGF)-I gene enhanced osteoblastic cell differentiation. Biochem Biophys Acta. 2006;1763(1):57-63.

145. Zhang HN, Leng P, Wang YZ, Zhang J. Treating human meniscal fibrochondrocytes with hIGF-1 gene by liposome. Clin Orthop Relat Res. 2009;467(12):3175-82.

146. Manning K, Rachakonda PS, Rai MF, Schmidt MF. Co-expression of insulin-like growth factor-1 and interleukin-4 in an in vitro inflammatory model. Cytokine. 2010;50(3):297-305.

147. Li Y, Tew SR, Russell AM, Gonzalez KR, Harding RE, Hawkins RE. Transduction of passaged human articular chondrocytes with adenoviral, retroviral, and lentiviral vectors and the effects of enhanced expression of SOX9. Tissue Eng. 2004;10(3-4):575-84.

148. Attur MG, Dave MN, Leung MY, Cipolletta C, Mesecik M, Woo SL, et al. Functional genomic analysis of type II IL-beta decoy receptor: potential for gene therapy in human arthritis and inflammation. J Immunol. 2002;168(4):2001-10.

149. Smith P, Shuler FD, Georgescu HI, Ghivizzani SC, Johnstone H, Madry H, Emkey G, Zurakowski D, Trippel SB. Overexpression of human fibroblast growth factor 2 stimulates cell proliferation in an ex vivo model of articular chondrocyte transplantation. Gene Ther. 2001;8(19):1443-9.

150. Goiter JJ, O’Keefe RJ, Rosier RN, Puzas JE, Schwarz EM. Efficacy of ex vivo OPG gene therapy in preventing wear debris induced osteolysis. J Orthop Res. 2002;20(2):169-73.
140. Gerich TG, Kang R, Fu FH, Robbins PD, Evans CH. Gene transfer to the rabbit patellar tendon: potential for genetic enhancement of tendon and ligament healing. Gene Ther. 1996;3(12):1089-93.

141. Lou J, Kubota H, Hotokezaka S, Ludwig FJ, Manske PR. In vivo gene transfer and overexpression of focal adhesion kinase (p125 FAK) mediated by recombinant adenovirus-induced tendon adhesion formation and epitenon cell change. J Orthop Res. 1997;15(6):911-8.

142. Mehrara BJ, Saadeh PB, Steinbrech DS, Dudziak M, Specter JA, Greenwald JA, et al. Adenovirus-mediated gene therapy of osteoblasts in vitro and in vivo. J Bone Miner Res. 1999;14(8):1290-301.

143. Nixon AJ, Brower-Toland BD, Bent SJ, Saxer RA, Wilke MJ, Robbins PD, et al. Insulin-like growth factor-I gene therapy applications for cartilage repair. Clin Orthop Relat Res. 2000;(379 Suppl):S201-13.

144. Shuler FD, Georgescu HI, Niyibizi C, Studer RK, Mi Z, Johnstone B, et al. Increased matrix synthesis following adenoviral transfer of a transforming growth factor beta1 gene into articular chondrocytes. J Orthop Res. 2000;18(4):585-92.

145. Brower-Toland BD, Saxer RA, Goodrich LR, Mi Z, Robbins PD, Evans CH, et al. Direct adenovirus-mediated insulin-like growth factor I gene transfer enhances transplant chondrocyte function. Hum Gene Ther. 2001;12(2):117-29.

146. Gelse K, Jiang QJ, Aigner T, Ritter T, Wagner K, Poschl E, et al. Fibroblast-mediated delivery of growth factor complementary DNA into mouse joints induces chondrogenesis but avoids the disadvantages of direct viral gene transfer. Arthritis Rheum. 2001;44(8):1943-53.

147. Saxer RA, Bent SJ, Brower-Toland BD, Mi Z, Robbins PD, Evans CH, et al. Gene mediated insulin-like growth factor-I delivery to the synovium. J Orthop Res. 2001;19(5):759-67.

148. Musgrave DS, Prunich R, Bosch P, Ziran BH, Whalen J, Huard J. Human skeletal muscle cells in ex vivo gene therapy to deliver bone morphogenetic protein-2. J Bone Joint Surg Br. 2002;84(1):120-7.

149. Ikeda T, Kamekura S, Mabuchi A, Kou I, Seki S, Takato T, et al. The combination of SOX5, SOX6, and SOX9 (the SOX trio) provides signals sufficient for induction of permanent cartilage. Hum Gene Ther. 2004;50(11):3561-73.

150. Haupert JL, Frisbie DD, McIlwraith CW, Robbins PD, Ghivizzani S, Evans CH, et al. 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. 2005;23(1):118-26.

151. Nixon AJ, Haupert JL, Frisbie DD, Morisset SS, McIlwraith CW, Robbins PD, et al. Gene-mediated restoration of cartilage matrix by combination insulin-like growth factor-I/interleukin-1 receptor antagonist therapy. Gene Ther. 2005;12(2):177-86.

152. Steinert AF, Palmer GD, Capito R, Hofstaetter JG, Pilapil C, Ghivizzani SC, et al. Genetically enhanced engineering of meniscus tissue using ex vivo delivery of transforming growth factor-beta 1 complementary deoxyribonucleic acid. Tissue Eng. 2007;13(9):2227-37.

153. Steinert AF, Weber M, Kunz M, Palmer GD, Noth U, Evans CH, et al. In situ IGF-1 gene delivery to cells emerging from the injured anterior cruciate ligament. Biomaterials. 2008;29(7):904-16.

154. Steinert AF, Proffen B, Kunz M, Hendrich C, Ghivizzani SC, Noth U, et al. Hypertrophy is induced during the in vitro chondrogenic differentiation of human mesenchymal stem cells by bone morphogenetic protein-2 and bone morphogenetic protein-4 gene transfer. Arthritis Res Ther. 2009;11(5):R148.

155. Roessler BJ, Hartman JW, Vallance DK, Latta JM, Janich SL, Davidson BL. Inhibition of interleukin-1-induced effects in synoviocytes transduced with the human IL-1 receptor antagonist cDNA using an adenoviral vector. Hum Gene Ther. 1995;6(3):307-16.

156. Baltzer AW, Whalen JD, Muzzzonegro T, Georgescu HI, Robbins PD, Evans CH. [In vitro transduction of human osteoblast cell populations with retroviral vectors]. Z Rheumatol. 1999;58(2):88-94.

157. Hildebrand KA, Deie M, Allen CR, Smith DW, Georgescu HI, Evans CH, et al. Early expression of marker genes in the rabbit medial collateral and anterior cruciate ligaments: the use of different viral vectors and the effects of injury. J Orthop Res. 1999;17(1):37-42.

158. Goto H, Shuler FD, Niyibizi C, Fu FH, Robbins PD, Evans CH. Gene therapy for meniscal injury: enhanced synthesis of proteoglycan and collagen by meniscal cells transduced with a TGFbeta(1)gene. Osteoarthritis Cartilage. 2000;8(4):266-71.

159. Tew SR, Li Y, Pothacharoen P, Tweats LM, Hawkins RE, Hardingham TE. Retroviral transduction with SOX9 enhances re-expression of the chondrocyte phenotype in passaged osteoarthritic human articular chondrocytes. Osteoarthritis Cartilage. 2005;13(1):80-9.

160. Jennings K, Miyamae T, Traister R, Marinov A, Katakura S, Sowders D, et al. Proteasome inhibition enhances AAV-mediated transgene expression in human synoviocytes in vitro and in vivo. Mol Ther. 2005;11(4):600-7.

161. Goater J, Muller R, Kollias G, Firestein GS, Sanz I, O’Keefe RJ, et al. Empirical advantages of adenoviral associated viral vectors in vivo gene therapy for arthritis. J Rheumatol. 2000;27(4):983-9.

162. Zhang HG, Xie J, Yang P, Wang Y, Xu L, Liu D, et al. Adeno-associated virus production of soluble tumor necrosis factor receptor neutralizes tumor necrosis factor alpha and reduces arthritis. Hum Gene Ther. 2000;11(17):2431-42.

163. Hiraiade A, Yokoo N, Xin KQ, Okuda K, Mizukami H, Ozawa K, et al. Repair of articular cartilage defect by intraarticular administration of basic fibroblast growth factor gene, using adeno-associated virus vector. Hum Gene Ther. 2005;16(12):1413-21.
by an adeno-associated virus: application to experimental arthritis. Hum Gene Ther. 2002;13(10):1179-88.

165. Arai Y, Kubo T, Fushiki S, Mazda O, Nakai H, Iwaki Y, et al. Gene delivery to human chondrocytes by an adeno associated virus vector. J Rheumatol. 2000;27(4):979-82.

166. Ulrich-Vinther M, Maloney MD, Goater JJ, Soballe K, Goldring MB, O’Keefe RJ, et al. Light-activated gene transduction enhances adeno-associated virus vector-mediated gene expression in human articular chondrocytes. Arthritis Rheum. 2002;46(8):2095-104.

167. Cucchiarini M, Thurn T, Weimer A, Kohn D, Terwilliger EF, Madry H. Restoration of the extracellular matrix in human osteoarthritic articular cartilage by overexpression of the transcription factor SOX9. Arthritis Rheum. 2007;56(1):158-67.

168. Cucchiarini M, Terwilliger EF, Kohn D, Madry H. Remodelling of human osteoarthritic cartilage by FGF-2, alone or combined with Sox9 via rAAV gene transfer. J Cell Mol Med. 2009;13(8B):2476-88.

169. Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD, Pace JM, et al. Gene targeting in stem cells from individuals with osteogenesis imperfecta. Science. 2004;303(5661):1198-201.

170. Ito H, Goater JJ, Tiyapatanaputi P, Rubery PT, O’Keefe RJ, Schwarz EM. Light-activated gene transduction of recombinant adeno-associated virus in human mesenchymal stem cells. Gene Ther. 2004;11(1):34-41.

171. Basile P, Dadali T, Jacobson J, Hasslund S, Ulrich-Vinther M, Soballe K, et al. Freeze-dried tendon allografts as tissue-engineering scaffolds for Gdf5 gene delivery. Mol Ther. 2008;16(3):466-73.

172. Arsic N, Zacchigna S, Zentlin L, Ramirez-Correa G, Patarini L, Salvi A, et al. Vascular endothelial growth factor stimulates skeletal muscle regeneration in vivo. Mol Ther. 2004;10(5):844-54.

173. Madry H, Cucchiarini M, Kaul G, Kohn D, Terwilliger EF, Trippel SB. Menisci are efficiently transduced by recombinant adeno-associated virus vectors in vitro and in vivo. Am J Sports Med. 2004;32(8):1860-5.

174. Ito H, Koeboe M, Tiyapatanaputi P, Gromov K, Goater JJ, Carmouche J, et al. Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. Nat Med. 2005;11(3):291-7.

175. Wang XT, Liu PY, Xin KQ, Tang JB. Tendon healing in vitro: bFGF gene transfer to tenocytes by adeno-associated viral vectors promotes expression of collagen genes. J Hand Surg Am. 2005;30(6):1255-61.

176. Tang JB, Cao Y, Zhu B, Xin KQ, Wang XT, Liu PY. Adeno-associated virus-2-mediated bFGF gene transfer to digital flexor tendons significantly increases healing strength: an in vivo study. J Bone Joint Surg Am. 2008;90(5):1078-89.

177. Cucchiarini M, Schetting S, Terwilliger EF, Kohn D, Madry H. rAAV-mediated overexpression of FGF-2 promotes cell proliferation, survival, and alpha-SMA expression in human meniscal lesions. Gene Ther. 2009;16(11):1363-72.
chronic disease in a streptococcal cell wall-induced arthritis model. J Clin Invest. 1998;101(12):2615-21.

191. Roessler BJ, Allen ED, Wilson JM, Hartman JW, Davidson BL. Adenoviral-mediated gene transfer to rabbit synovium in vivo. J Clin Invest. 1993;92(2):1085-92.

192. Kim SH, Evans CH, Kim S, Oligino T, Ghivizzani SC, Robbins PD. Gene therapy for established murine collagen-induced arthritis by local and systemic adenovirus-mediated delivery of interleukin-4. Arthritis Res. 2000;2(4):293-302.

193. Lechman ER, Jaffurs D, Ghivizzani SC, Gambotto A, Kovesdi I, Mi Z, et al. Direct adenoviral gene transfer of viral IL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral control knees. J Immunol. 1999;163(4):2202-8.

194. Keravala A, Lechman ER, Nash J, Mi Z, Robbins PD. Human, viral or mutant human IL-10 expressed after local adenovirus-mediated gene transfer are equally effective in ameliorating disease pathology in a rabbit knee model of antigen-induced arthritis. Arthritis Res Ther. 2006;8(4):R91.

195. Kim KN, Watanabe S, Ma Y, Thornton S, Giannini EH, Hirsch R. Viral IL-10 and soluble TNF receptor act synergistically to inhibit collagen-induced arthritis following adenovirus-mediated gene transfer. J Immunol. 2000;164(3):1576-81.

196. Fernandes J, Tartif G, Martel-Pelletier J, Lascau-Coman V, Dupuis M, Moldovan F, et al. In vivo transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: prevention of osteoarthritis progression. Am J Pathol. 1999;154(4):1159-69.

197. Frisbie DD, McIlwraith CW. Evaluation of gene therapy as a treatment for equine traumatic arthritis and osteoarthritis. Clin Orthop Relat Res. 2000;(379 Suppl):S273-87.

198. Schrastuhl A, Vitters EL, van der Kraan PM, van den Berg WB. Reduction of osteophyte formation and synovial thickening by adenoviral overexpression of transforming growth factor beta/bone morphogenetic protein inhibitors during experimental osteoarthritis. Arthritis Rheum. 2003;48(12):3442-51.

199. Chen LX, Lin L, Wang HJ, Wei XL, Fu X, Zhang JY, et al. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. Osteoarthritis Cartilage. 2008;16(2):174-84.

200. Hsieh JL, Shen PC, Shiau AL, Jou IM, Lee CH, Teo ML, et al. Adenovirus-mediated kallistatin gene transfer ameliorates disease progression in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Hum Gene Ther. 2009;20(2):147-58.

201. Hsieh JL, Shen PC, Shiau AL, Jou IM, Lee CH, Wang CR, et al. Intraarticular gene transfer of thrombospondin-1 suppresses the disease progression of experimental osteoarthritis. J Orthop Surg. 2010;28(10):1300-6.

202. Mi Z, Ghivizzani SC, Lechman ER, Jaffurs D, Glorioso JC, Evans CH, et al. Adenovirus-mediated gene transfer of insulin-like growth factor 1 stimulates proteoglycan synthesis in rabbit joints. Arthritis Rheum. 2000;43(11):2563-70.

203. Bandara G, Mueller GM, Galea-Laury J, Tindal MH, Georgescu HI, Suchanek MK, et al. Intraarticular expression of biologically active interleukin 1-receptor-antagonist protein by ex vivo gene transfer. Proc Natl Acad Sci U S A. 1993;90(22):10764-8.
217. Gray JC. Neural and vascular anatomy of the menisci of the human knee. J Orthop Sports Phys Ther. 1999;29(1):23-30.
218. Messner K, Gao J. The menisci of the knee joint: anatomical and functional characteristics, and a rationale for clinical treatment. J Anat. 1998;193(Pt 2):161-78.
219. Chivers MD, Howitt SD. Anatomy and physical examination of the knee menisci: a narrative review of the orthopedic literature. J Can Chiropr Assoc. 2009;53(4):319-33.
220. Lengsfeld M, Rudig L, von Issendorff WD, Koebke J. [Significance of shape differences between medial and lateral knee joint menisci for functional change of position]. Unfallchirurgie. 1991;17(6):309-15.
221. Bullough PG, Munuera L, Murphy J, Weinstein AM. The strength of the menisci of the knee as it relates to their fine structure. J Bone Joint Surg Br. 1970;52(3):564-7.
222. McDevitt CA, Webber RJ. The ultrastructure and biochemistry of meniscal cartilage. Clin Orthop Relat Res. 1990;(252):8-18.
223. Verdonk PC, Forsyth RG, Wang J, Almqvist KF, Verdonk R, Veys EM, et al. Characterisation of human knee meniscus cell phenotype. Osteoarthritis Cartilage. 2005;13(7):548-60.
224. Arnoczky SP, Warren RF. Microvascularity of the human meniscus. Am J Sports Med. 1982;10(2):90-5.
225. Cooper DE, Arnoczky SP, Warren RF. Meniscal repair. Clin Sports Med. 1991;10(3):529-48.
226. Arnoczky SP, Warren RF. The microvasculature of the meniscus and its response to injury: an experimental study in the dog. Am J Sports Med. 1983;11(3):131-41.
227. Englund M, Guermazi A, Roemer FW, Aliabadi P, Yang M, Lewis CE, et al. Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons: the Multicenter Osteoarthritis Study. Arthritis Rheum. 2009;60(3):831-9.
228. Englund M, Lohmander LS. Patellofemoral osteoarthritis coexistent with tibiofemoral osteoarthritis in a meniscectomy population. Ann Rheum Dis. 2005;64(12):1721-6.
229. Beaufils P, Hulet C, Dhenain M, Nizard R, Nourissat G, Bullough PG, Munuera L, Murphy J, Weinstein AM. The strength of the menisci of the knee as it relates to their fine structure. J Bone Joint Surg Br. 1970;52(3):564-7.
230. McDevitt CA, Webber RJ. The ultrastructure and biochemistry of meniscal cartilage. Clin Orthop Relat Res. 1990;(252):8-18.
231. Verdonk PC, Forsyth RG, Wang J, Almqvist KF, Verdonk R, Veys EM, et al. Characterisation of human knee meniscus cell phenotype. Osteoarthritis Cartilage. 2005;13(7):548-60.
232. Arnoczky SP, Warren RF. Microvascularity of the human meniscus. Am J Sports Med. 1982;10(2):90-5.
233. Cooper DE, Arnoczky SP, Warren RF. Meniscal repair. Clin Sports Med. 1991;10(3):529-48.
234. Arnoczky SP, Warren RF. The microvasculature of the meniscus and its response to injury: an experimental study in the dog. Am J Sports Med. 1983;11(3):131-41.
235. Englund M, Guermazi A, Roemer FW, Aliabadi P, Yang M, Lewis CE, et al. Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons: the Multicenter Osteoarthritis Study. Arthritis Rheum. 2009;60(3):831-9.
248. Jitsuki J, Ochi M, Ikuta Y. Meniscal repair enhanced by an interpositional free synovial autograft: an experimental study in rabbits. Arthroscopy. 1994;10(6):659-66.
249. Petersen W, Pufe T, Starke C, Fuchs T, Kopf S, Neumann W, et al. The effect of locally applied vascular endothelial growth factor on meniscus healing: gross and histological findings. Arch Orthop Trauma Surg. 2007;127(4):235-40.
250. Petersen W, Pufe T, Starke C, Fuchs T, Kopf S, Raschke M, et al. Locally applied angiogenic factors: a new therapeutic tool for meniscal repair. Ann Anat. 2005;187(5-6):509-19.
251. van Tienen TG, Hannink G, Buma P. Meniscus replacement using synthetic materials. Clin Sports Med. 2009;28(1):143-56.
252. Martinek V, Imhoff A. Das künstliche meniskusimplantat. Arthroskopie. 2008;21:266-70.
253. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold: analysis of preliminary data. J Bone Joint Surg Am. 1997;79(12):1770-7.
254. Rodkey WG, DeHaven KE, Montgomery WH 3rd, Baker CL Jr, Beck CL Jr, Horelm SE, et al. Comparison of the collagen meniscus implant with partial meniscectomy: a prospective randomized trial. J Bone Joint Surg Am. 2008;90(7):1413-26.
255. de Groot JH, de Vrijer R, Pennings AJ, Klompmaker J, Veth RP, Jansen HW. Use of porous polyurethanes for meniscal reconstruction and meniscal prostheses. Biomaterials. 1996;17(2):163-73.
256. Welsing RT, van Tienen TG, Ramrattan N, Heijkants R, Schouten AJ, Veth RP, et al. Effect on tissue differentiation and articular cartilage degradation of a polymer meniscus implant: a 2-year follow-up study in dogs. Am J Sports Med. 2008;36(10):1978-89.
257. Langer R, Vacanti JP. Tissue engineering. Science. 1993;260(5110):920-6.
258. Nerem RM. Cellular engineering. Ann Biomed Eng. 1991;19(5):529-45.
259. Buma P, Ramrattan NN, van Tienen TG, Veth RP. Tissue engineering of the meniscus. Biomaterials. 2004;25(9):1523-32.
260. Sweigart MA, Athanasiou KA. Toward tissue engineering of the meniscus. Biomaterials. 2009;30(22):3749-56.
261. Stapleton TW, Ingram J, Katta J, Knight R, Korossis S, Fisher J, et al. Development and characterization of an acellular porcine medial meniscus for use in tissue engineering. Tissue Eng Part A. 2008;14(4):505-18.
262. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Meniscal regeneration with copolymeric collagen scaffolds: in vitro and in vivo studies evaluated clinically, histologically, and biochemically. Am J Sports Med. 1992;20(2):104-11.
263. Gastel JA, Muirhead WR, Lifrak JT, Fadale PD, Hulstyn MJ, Labrador DP. Meniscal tissue regeneration using a collagenous biomaterial derived from porcine small intestine submucosa. Arthroscopy. 2001;17(2):151-9.
264. Welch JA, Montgomery RD, Lenz SD, Plouhar P, Shelton WR. Evaluation of small-intestinal submucosal implants for repair of meniscal defects in dogs. Am J Vet Res. 2002;63(3):427-31.
265. Cook JL, Fox DB, Malaviya P, Tomlinson JL, Kuroki K, Cook CR, et al. Long-term outcome for large meniscal defects treated with small intestinal submucosa in a dog model. Am J Sports Med. 2006;34(1):32-42.
266. Adams SB Jr, Randolph MA, Gill TJ. Tissue engineering for meniscal repair. J Knee Surg. 2005;18(1):25-30.
267. Armoczky SP. Building a meniscus: biologic considerations. Clin Orthop Relat Res. 1999;(367 Suppl):S244-53.
268. Baker BM, Gee AO, Sheth NP, Huffman GR, Sennett BJ, Schaefer TP, et al. Meniscus tissue engineering on the nanoscale: from basic principles to clinical application. J Knee Surg. 2009;22(1):45-59.
269. Steadman JR, Rodkey WG. Tissue-engineered collagen meniscus implants: 5- to 6-year feasibility study results. Arthroscopy. 2005;21(5):515-25.
270. Rodeo SA, Seneviratne A, Suzuki K, Felker K, Wickiewicz TL, Warren RF. Histological analysis of human meniscal allografts: a preliminary report. J Bone Joint Surg Am. 2000;82-A(8):1071-82.
271. Sohn DH, Toth AP. Meniscus transplantation: current concepts. J Knee Surg. 2008;21(2):163-72.
272. Elderd BD, Eleswarapu SV, Athanasiou KA. Extraction techniques for the decellularization of tissue engineered articular cartilage constructs. Biomaterials. 2009;30(22):3749-56.
273. Stapleton TW, Ingram J, Katta J, Knight R, Korossis S, Fisher J, et al. Development and characterization of an acellular porcine medial meniscus for use in tissue engineering. Tissue Eng Part A. 2008;14(4):505-18.
274. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Meniscal regeneration with copolymeric collagen scaffolds: in vitro and in vivo studies evaluated clinically, histologically, and biochemically. Am J Sports Med. 1992;20(2):104-11.
275. Gastel JA, Muirhead WR, Lifrak JT, Fadale PD, Hulstyn MJ, Labrador DP. Meniscal tissue regeneration using a collagenous biomaterial derived from porcine small intestine submucosa. Arthroscopy. 2001;17(2):151-9.
276. Welch JA, Montgomery RD, Lenz SD, Plouhar P, Shelton WR. Evaluation of small-intestinal submucosal implants for repair of meniscal defects in dogs. Am J Vet Res. 2002;63(3):427-31.
277. Cook JL, Fox DB, Malaviya P, Tomlinson JL, Kuroki K, Cook CR, et al. Long-term outcome for large meniscal defects treated with small intestinal submucosa in a dog model. Am J Sports Med. 2006;34(1):32-42.
278. Cook JL, Tomlinson JL, Kreeger JM, Cook CR. Induction of meniscal regeneration in dogs using a novel biomaterial. Am J Sports Med. 1999;27(5):658-65.
279. Klompmaker J, Jansen HW, Veth RP, de Groot JH, Nijenhuis AJ, Pennings AJ. Porous polymer implant for repair of meniscal lesions: a preliminary study in dogs. Biomaterials. 1991;12(9):810-6.
280. Tienen TG, Heijkants RG, de Groot JH, Pennings AJ, Schouten AJ, Veth RP, et al. Replacement of the knee meniscus by a porous polymer implant: a study in dogs. Am J Sports Med. 2006;34(1):64-71.
282. Kang SW, Son SM, Lee JS, Lee ES, Lee KY, Park SG, et al. Regeneration of whole meniscus using meniscal cells and polymer scaffolds in a rabbit total meniscectomy model. J Biomed Mater Res A. 2006;78(3):659-71.

283. Arnoezky SP, DiCarlo EF, O’Brien SJ, Warren RF. Cellular repopulation of deep-frozen meniscal autografts: an experimental study in the dog. Arthroscopy. 1992;8(4):428-36.

284. Marsano A, Vunjak-Novakovic G, Martin I. Towards tissue engineering of meniscus substitutes: selection of cell source and culture environment. Conf Proc IEEE Eng Med Biol Soc. 2006;1:3656-8.

285. Chiari C, Koller U, Kapeller B, Dorotka R, Bindreiter U, Nehrer S. Different behavior of meniscal cells in collagen II/I,III and Hyaff-11 scaffolds in vitro. Tissue Eng Part A.

286. Gunja NJ, Athanasiou KA. Effects of co-cultures of meniscus cells and articular chondrocytes on PLLA scaffolds. Biotechnol Bioeng. 2009;103(4):808-16.

287. Kon E, Chiari M, Delcogliano M, Salter DM, Martin I, et al. Tissue engineering for total meniscal substitution: animal study in sheep model. Tissue Eng Part A. 2008;14(8):1295-304.

288. Tan Y, Zhang Y, Pei M. Meniscus reconstruction through coculturing meniscus cells with synovium-derived stem cells on small intestine submucosa: a pilot study to engineer meniscus tissue constructs. Tissue Eng Part A. 2010;16(1):67-79.

289. Yamasaki T, Deie M, Shimomiyi R, Yasunaga Y, Yanada S, Ochi M. Transplantation of meniscus regenerated by tissue engineering with a scaffold derived from a rat meniscus and small intestine submucosa: a pilot study to engineer meniscus tissue constructs. Cell Tissue Res. 2008;333(3):439-47.

290. Hidaka C, Ibarra C, Hannafin JA, Torzilli PA, Quitoriano M, Jen SS, et al. Formation of vascularized meniscal tissue by combining gene therapy with tissue engineering. Tissue Eng. 2002;8(1):93-105.

291. Zhang H, Leng P, Zhang J. Enhanced meniscal repair by overexpression of hIGF-1 in a full-thickness model. Clin Orthop Relat Res. 2009;467(12):3165-74.

292. Arnoezky SP, Warren RF, McDevitt CA. Meniscal replacement using a cryopreserved allograft: an experimental study in the dog. Clin Orthop Relat Res. 1990;(252):121-8.

293. Cole BJ, Carter TR, Rodeo SA. Allograft meniscal transplantation: background, techniques, and results. Instr Course Lect. 2003;52:383-96.

294. Dienst M, Greis PE, Ellis BJ, Bachus KN, Burks RT. Effect of lateral meniscal allograft sizing on contact mechanics of the lateral tibial plateau: an experimental study in human cadaveric knee joints. Am J Sports Med. 2007;35(1):34-42.

295. Garrett JC, Steensen RN. Meniscal transplantation in the human knee: a preliminary report. Arthroscopy. 1991;7(1):57-62.

296. Greis PE, Holmstrom MC, Bardana DD, Burks RT. Meniscal injury. II: management. J Am Acad Orthop Surg. 2002;10(3):177-87.

297. Kohn D, Verdonk R, Aagaard H, Seil R, Dienst M. Meniscal substitutes: animal experience. Scand J Med Sci Sports. 1999;9(3):141-5.

298. Messner K. Meniscal regeneration or meniscal transplantation? Scand J Med Sci Sports. 1999;9(3):162-7.

299. Packer JD, Rodeo SA. Meniscal allograft transplantation. Clin Sports Med. 2009;28(2):259-83, viii.

300. Rodeo SA. Meniscal allografts: where do we stand? Am J Sports Med. 2001;29(2):246-61.

301. Siegel MG, Roberts CS. Meniscal allografts. Clin Sports Med. 1993;12(1):59-80.

302. Verdonk PC, Verstraete KL, Almqvist KF, De Cuyper K, Veys EM, Verbruggen G, et al. Meniscal allograft transplantation: long-term clinical results with radiological and magnetic resonance imaging correlations. Knee Surg Sports Traumatol Arthrosc. 2006;14(8):694-706.

303. Verdonk R. Meniscal transplantation. Acta Orthop Belg. 2002;68(2):118-27.

304. Verdonk R, Almqvist KF, Huyse W, Verdonk PC. Meniscal allografts: indications and outcomes. Sports Med Arthrosc. 2007;15(3):121-5.

305. Crook TB, Ardolino A, Williams LA, Barlow IW. Meniscal allograft transplantation: a review of the current literature. Ann R Coll Surg Engl. 2009;91(5):361-5.

306. Huard J, Li Y, Peng H, Fu FH. Gene therapy and tissue engineering for sports medicine. J Gene Med. 2003;5(2):93-108.

307. Martinek V, Usas A, Pelinkovic D, Robbins P, Fu FH, Huard J. Genetic engineering of meniscal allografts. Tissue Eng. 2002;8(1):107-17.

308. Evans CH, Ghiwizzani SC, Herndon JH, Wasko MC, Reinecke J, Wehling P, et al. Clinical trials in the gene therapy of arthritis. Clin Orthop Relat Res. 2000;379 Suppl:S300-7.

309. Evans CH, Ghouze JN, Ghouze E, Robbins PD, Ghiwizzani SC. Osteoarthritis gene therapy. Gene Ther. 2004;11(4):379-89.

310. Evans CH, Ghiwizzani SC, Robbins PD. Gene therapy for arthritis: what next? Arthritis Rheum. 2006;54(6):1714-29.

311. Evans CH, Ghiwizzani SC, Robbins PD. Arthritis gene therapy’s first death. Arthritis Res Ther. 2008;10(3):110.

312. Evans CH, Ghiwizzani SC, Robbins PD. Gene therapy for autoimmune disorders. J Clin Immunol. 2000;20(5):334-46.

313. Evans CH. Gene therapy: what have we accomplished and where do we go from here? J Rheumatol Suppl. 2005;72:17-20.

314. Evans CH, Robbins PD, Ghiwizzani SC, Wasko MC, Tomaino MM, Kang R, et al. Gene transfer to human joints: progress toward a gene therapy of arthritis. Proc Natl Acad Sci U S A. 2005;102(24):8698-703.
316. Sant SM, Suarez TM, Moalli MR, Wu BY, Blaivas M, Laing TJ, et al. Molecular lysis of synovial lining cells by in vivo herpes simplex virus-thymidine kinase gene transfer. Hum Gene Ther. 1998;9(18):2735-43.

317. Mease PJ, Hobbs K, Chalmers A, El-Gabalawy H, Bookman A, Keystone E, et al. Local delivery of a recombinant adeno-associated vector containing a tumour necrosis factor alpha antagonist gene in inflammatory arthritis: a phase 1 dose-escalation safety and tolerability study. Ann Rheum Dis. 2009;68(8):1247-54.

318. Gladman DD, Mease PJ, Ritchlin CT, Choy EH, Sharp JT, Ory PA, et al. Adalimumab for long-term treatment of psoriatic arthritis: forty-eight week data from the adalimumab effectiveness in psoriatic arthritis trial. Arthritis Rheum. 2007;56(2):476-88.

319. Kaiser J. Clinical research: death prompts a review of gene therapy vector. Science. 2007;317(5838):580.

320. Williams DA. RAC reviews serious adverse event associated with AAV therapy trial. Mol Ther. 2007;15(12):2053-4.

321. Williams DA. NIH Recombinant DNA Advisory Committee continues to ponder adverse event associated with AAV gene therapy trial. Mol Ther. 2008;16(3):427-8.

322. Sweeney HL. Gene doping. Sci Am. 2004;291(1):62-9.

323. Adam D. Gene therapy may be up to speed for cheats at 2008 Olympics. Nature. 2001;414(6864):569-70.

324. Friedmann T, Koss JO. Gene transfer and athletics: an impending problem. Mol Ther. 2001;3(6):819-20.

325. Friedmann T, Rabin O, Frankel MS. Ethics: gene doping and sport. Science. 2010;327(5966):647-8.

326. Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. Proc Natl Acad Sci U S A. 1998;95(26):15603-7.

327. Zhou S, Murphy JE, Escobedo JA, Dwarki VJ. Adeno-associated virus-mediated delivery of erythropoietin leads to sustained elevation of hematocrit in nonhuman primates. Gene Ther. 1998;5(5):665-70.

328. Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, et al. Constitutive expression of pHVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation. 1998;97(12):1114-23.

329. Baoutina A, Alexander IE, Rasko JE, Emslie KR. Potential use of gene transfer in athletic performance enhancement. Mol Ther. 2007;15(10):1751-66.

330. de la Chapelle A, Traskelin AL, Juvonen E. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. Proc Natl Acad Sci U S A. 1993;90(10):4495-9.

331. Slot O. Apocalypse now: fears of gene doping are realised. The Times. 2006 Feb 2.

332. Hanson RW, Hakimi P. Born to run: the story of the PEPCK-Cmus mouse. Biochimie. 2008;90(6):838-42.

333. Baoutina A, Alexander IE, Rasko JE, Emslie KR. Developing strategies for detection of gene doping. J Gene Med. 2008;10(1):3-20.