Review article

CURRENT CONCEPTS OF GENE THERAPY AND CARTILAGE REPAIR

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Lesions of the articular cartilage are commonly encountered in orthopaedics and sports medicine.^{1,2} Damage to articular cartilage leads to premature osteoarthritis and may cause a decrease in the quality of life with long-term costs of health care. The management of chondral and osteochondral lesions represents a challenge to clinicians and scientists.^{3,4} Although current concepts of treatment for these injuries are promising, no treatment has yet resulted in complete restoration of the hyaline cartilage and the subchondral bone to a normal state.⁵

The advent of gene therapy provides new possibilities for the treatment of injuries to cartilage.⁶ Extended scientific contributions have been made towards the understanding of the biology of articular cartilage and to new approaches to treatment. The delivery of growth factors, cells, scaffolds, and therapeutic genes into lesions of articular cartilage promises to change the treatment of an aspect of medicine which historically has been limited to frustrating outcomes. Despite significant progress in the treatment of osteochondral lesions, many unsolved problems remain, especially those of the safety of gene transfer.⁷ This review focuses on the basic premises underlying gene therapy and describes the current state of genetic engineering for defects of cartilage.

Biology of cartilage healing

Hyaline cartilage in adults does not have a blood supply, lymphatic drainage or neural elements and the chondrocytes are protected from the nutritional synovial fluid and reparative recognition by their extracellular matrix. The potential for healing is therefore very limited. Only in cases of minor chondral damage with minimal loss of the matrix compo-

nents are chondrocytes able to synthesise new proteoglycans and to restore the cartilage completely.⁸

The underlying vascularised subchondral area is involved in the healing of cartilage defects. A repair process can be initiated from the subchondral marrow by resident progenitor and blood cells which occupy the chondral defect. However, this new reparative tissue, which often contains fibrous elements, undergoes relatively fast degeneration and fails to function as hyaline cartilage.⁹

Current concepts of treatment

Based on knowledge of the pathophysiology of articular cartilage, many clinicians have attempted to improve healing by drilling,¹⁰ abrasion¹¹ or microfracture of the subchondral bone.¹² The aim of these techniques is to recruit bone-marrow stem cells from the subchondral area to stimulate the formation of repair tissue. However, this fibrocartilaginous repair tissue contains a high proportion of fibrous elements and is functionally inferior to normal hyaline cartilage.¹³ With bone-marrow stimulating techniques, symptoms can be improved only temporarily and the long-term function of the joint is reduced.⁵ Therefore this treatment can be recommended only as a timesaving procedure to delay the implantation of a prosthesis.

The transplantation of articular cartilage with autologous and allogenic osteochondral grafts has shown promising results in the treatment of circumscribed cartilage lesions.¹⁴⁻¹⁸ Compared with the outcome of subchondral bone penetration, osteochondral grafts provide repair with a fully-formed articular cartilage matrix and viable chondrocytes potentially capable of maintaining the matrix.^{8,19} Reports of recent clinical studies indicate that these techniques can restore the articular surface of the joint. However, the potential morbidity of the donor sites is the major concern in the clinical outcome of this approach.

Periosteal or perichondrial soft-tissue grafts can also be used to deliver osteochondral progenitor cells to the sites of cartilage defect.²⁰⁻²² These mesenchymal cells are capable of a broad range of chondrogenic expression and of producing new cartilaginous tissue. Studies reporting small groups of patients with osteochondral lesions treated with periosteal and perichondral grafts suggest that an improvement of symptoms can be achieved for a short time. However, the

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Table I. Experimental studies in the literature of the effect of growth factors on cartilage healing in vivo

Author/authors	Application	Cells	Growth factors	Species	Effects
Cuevas et al ²⁹	Intra-articular osmotic pump	—	bFGF	Rabbit	Repair stimulation
Hunziker and Rosenberg ³⁰	Fibrin clot	—	bFGF, TGF- β , IGF-1	Rabbit	Improved
Athanasίου et al ³¹	PLG polymers	—	TGF- β	Goat	Improved
van Beuningen et al ³²	Intra-articular injection	—	TGF- β , BMP-2	Mouse	Biosynthesis +
Fujimoto et al ³³	Intra-articular injection	—	bFGF	Rabbit	Improved
Nixon et al ³⁴	Autogenous fibrin	—	IGF-1	Horse	Biosynthesis +
Sellers et al ³⁵	Collagen sponge	—	BMP-2	Rabbit	Improved
Frenkel et al ²⁶	Type-I collagen	Chondrocytes	BMP-2	Rabbit	Improved
Hunziker ³⁶	Fibrin clot	—	TGF- β	Mini-pig	Improved
Weisser et al ³⁷	Agarose gel	Chondrocytes	bFGF, TGF- β , BMPs	Rabbit	Only bFGF clearly beneficial
Fortier et al ³⁸	Polymerised fibrin	Chondrocytes	IGF-1	Horse	Improved
Mierisch et al ³⁹	Calcium alginate beads	—	TGF- β	Rabbit	Improved
Perka et al ⁴⁰	Fibrin gel	Periosteal cells	TGF- β	Rabbit	Integration +

long-term durability of these graft materials remains uncertain because of poor integration and degeneration over time.^{3,22}

Autologous chondrocyte implantation was introduced during the last decade for the treatment of cartilage lesions.²³ This technique uses standard cell cultures of autologous chondrocytes, which are injected beneath a periosteal flap²³ or seeded on a collagen matrix, sutured over the articular defect.²⁴ Although the clinical results of this treatment are encouraging, there is no histological evidence of the production of hyaline cartilage.^{8,19}

Growth factors

The use of growth factors in combination with tissue engineering seems to be the most promising method in the future for the treatment of lesions of cartilage.²⁵⁻²⁷ Growth factors are cytokines with regulatory functions for healing in tissues of the musculoskeletal system. These small peptides are synthesised by resident cells at the site of the injury such as mesenchymal stem cells and chondrocytes, or by the infiltrating inflammatory cells which stimulate proliferation, migration, matrix synthesis and differentiation of the cells.^{19,25} Many of these growth factors can affect the metabolism of chondrocytes and influence healing of cartilage.^{4,19,25,28}

Among the known growth factors, insulin-like growth factor 1 (IGF-1), transforming growth factor beta (TGF- β), basic fibroblast growth factor (bFGF) and bone morphogenetic protein 2 (BMP-2) have been shown to influence the healing of cartilage in vivo (Table I).^{26,29-40} In animal experiments all of these cytokines were able to improve the histological appearance of cartilage regeneration tissue. Although it is very difficult to determine the biological effect of single cytokines under in vivo conditions, available data show that IGF-1 and TGF- β mostly modulate the synthesis of the cartilage matrix,^{30,32,36,38,41} while bFGF has a powerful mitogenic factor which stimulates the differentiation of chondrocytes.^{29,33,37} BMP-2 seems to be involved in the growth and differentiation of mesenchymal cells to chondroblasts and osteoblasts.^{26,32,35,37} Sellers et al³⁵ used

recombinant human BMP-2 for the treatment of full-thickness defects of articular cartilage in rabbits and found an accelerated formation of new subchondral bone with an improved histological appearance of the overlying articular surface. Others have shown that bone morphogenetic proteins stimulated the ingrowth of subchondral bone reducing the thickness of the repair cartilage and preventing the formation of normal tidemarks.³⁷ Van Beuningen et al³² observed that chondrocytes induced by BMP-2 were found predominantly in the region where the growth plates meet the joint space.

Although the direct application of specific growth factor proteins has a beneficial effect on the healing process of cartilage in vivo, a method for complete restoration of hyaline cartilage is still not available.^{8,19} The clinical use of growth factors is mainly limited by the problem of delivery.⁴² Because of the complexity of cartilage regeneration, a continuous delivery of stimulating substances is required, at least temporarily, to reach the optimum level for the treatment of osteochondral defects. At this point, the short biological half-life of growth factors represents the limiting feature for the success of such treatment. Various strategies, including carrier matrices, pumps and heparin, have been investigated in order to achieve constant levels of growth factors at injured sites.⁴³ Although these approaches have been capable of improving the local concentration of the growth factor proteins, the results of the delivery techniques remain limited and often clinically irrelevant. The application of gene transfer, which is a new technology, represents a unique opportunity for the local administration of growth factors.^{42,44,45}

Gene therapy

Gene therapy is a technique relying on the alteration of cellular genetic information.⁷ It is applicable in the treatment of cartilage lesions by transferring genes, encoded for specific growth factors, into chondrocytes or progenitor cells. With this transfer, a theoretically unique ability is available for the delivery of specific gene products into chondrogenic cells at therapeutic levels for defined periods of time.⁴² For

Table II. Experimental studies in the literature of the effect of gene transfer to chondrogenic cells in vitro

Author/authors	In vitro conditions	Cells	Vectors	Species	Effects
Arai et al ⁴⁹	Monolayer culture	Chondrocyte-like cells	Adenovirus TGF- β	Man	Matrix synthesis +
Doherty et al ⁵⁰	Resurfacing on explants	Chondrocytes	Adenovirus lacZ	Man	Feasibility +
Moller et al ⁵¹	Monolayer culture	Chondrocytes	Retrovirus TGF- β	Rabbit	Matrix synthesis +
Smith et al ⁵²	Monolayer culture	Chondrocyte	Adenovirus IGF-1, TGF- β , BMP-2	Rabbit	Matrix synthesis +
Madry and Trippel ⁵³	Resurfacing on explants	Chondrocytes	FuGENE6 lacZ	Man, cow	Feasibility +
Brower-Toland et al ⁵⁵	Monolayer culture	Chondrocytes	Adenovirus IGF-1	Horse	Biosynthesis +
Madry et al ⁵⁴	Resurfacing on explants	Chondrocytes	DNA IGF-1	Man	Biosynthesis +
Stove et al ⁵⁶	Alginate culture	Chondrocytes	FuGENE6 lacZ	Rabbit	Feasibility +

Table III. Experimental studies in the literature showing the results of genetic engineering of cartilage in vivo

Author/authors	Matrix/scaffold	Cells	Vectors	Species	Effects
Tomita et al ⁵⁷	Direct injection	–	Liposomes SVT gene	Rat	Feasibility +
Ikeda et al ⁶¹	Direct injection	–	Adenovirus TGF beta	Pig	Feasibility +
Mason et al ⁵⁸	PGA scaffold	Periosteal	Retrovirus lacZ	Rabbit	Feasibility +
Mason et al ⁶²	PGA scaffold	Periosteal	Retrovirus BMP-7	Rabbit	Regeneration +
Goomer et al ⁵⁹	PLA scaffold	Perichondrial	DNA lacZ	Rabbit	Feasibility +
Lee et al ⁶³	Direct injection	Fibroblasts	DNA TGF- β	Rabbit	Regeneration +
Adachi et al ⁶⁰	Type-I collagen gel	Muscle-derived stem cells	Retrovirus lacZ	Rabbit	Histology +

gene expression, the transferred DNA material must enter the nucleus where it can be transcribed. After transcription, the generated m-RNA is transported outside the nucleus, and serves as a matrix for the production of proteins (e.g. growth factors) in the ribosome (Fig. 1).

Viral and non-viral vectors can be used for the delivery of genetic material into cells. Non-viral gene transfer systems, such as liposomes, naked DNA or DNA ligands, are usually easier to produce and have a lower toxicity and immunogenicity, but the efficiency of their gene delivery is hindered by a low rate of infection unless the transduced cells are selected.^{46,47} Currently, viral gene vectors, including retrovirus, adenovirus, adeno-associated virus, and herpes virus, present a more efficient method of gene transfer.⁴⁸

Gene delivery into the cartilage and the subchondral bone is potentially possible by an ex vivo or in vivo approach. The vectors can either be directly infected into the host tissue (in vivo) or the cells from the injured tissue are removed, genetically altered in vitro, and reinjected into the site of injury (ex vivo). While the direct method is less technically demanding, indirect gene delivery is safer,

because the gene manipulation takes place under controlled conditions outside the organism. With the ex vivo approach, growth factors can be delivered with endogenous cells capable of responding to the stimuli and participating in the healing process in the injured tissue.

Gene therapy in cartilage repair

In vitro. Various experimental studies have confirmed the capability of chondrocytes to be transduced with transgenes in vitro (Table II).⁴⁹⁻⁵⁶ After establishment of the feasibility of gene transfer for chondrocytes with viral and non-viral vectors using marker genes,^{50,53,56} the biological effect of therapeutic genes was demonstrated.^{49,51,52,54,55} Following gene transfer of IGF-1 and TGF- β , chondrocytes in monolayer cultures^{49,51,52,55} or on the surfaces of cartilage explants⁵⁴ were able to increase matrix biosynthesis and to maintain the chondrocytic phenotype, especially the production of collagen type II.

In vivo. Successful delivery of genes into the articular cartilage was first reported by Tomita et al⁵⁷ using a haemagglutinating virus (HVJ; Sendai virus) liposome suspension

Fig. 1

Diagram of non-viral gene therapy. DNA of a growth factor is packed into a vector (here a liposome) which allows the DNA to enter the target cell and its nucleus. In the nucleus, the DNA is transcribed to RNA and transported to ribosomes where the new protein is produced.

Fig. 2

Diagram of induction of gene expression. Figure 2a – The tet-system uses a chimaeric transactivator (tTA) to activate transcription of the gene of interest from a silent promoter (CMV-promoter). In the absence of tetracycline (doxycycline) the system is inactivated and there is no transcription of the gene of interest (e.g. *lacZ*). Figure 2b – In the presence of doxycycline the system becomes activated. Figure 2c – The transactivator binds to the tet-responsive element in the silent promoter and activates transcription and protein expression in the presence of doxycycline (Dox).

containing the *SV40 large T antigen (SVT)* gene which was injected intra-articularly into the knees of rats. The expression of the *SVT* gene was detected immunohistochemically in chondrocytes in the superficial and middle zones of articular cartilage in the knees for up to three weeks and no pathological changes were observed. The feasibility of gene transfer in articular cartilage and in osteochondral defects using the reporter gene *lacZ* was then confirmed in subsequent studies (Table III).⁵⁸⁻⁶⁰ The first trial of the transfer of a potentially biologically effective gene was presented by Ikeda et al.⁶¹ They administered *TGF- β* gene directly into the joints of Hartley guinea-pigs using an adenoviral vector and showed the survival of this gene transfer for two weeks under in vivo conditions. However, the effect on cartilage turnover was not described in this study.

Mason et al.⁶² were the first to show a biological effect of an effective growth factor in cartilage healing using rabbit

mesenchymal stem cells transduced with retroviral vectors encoded for the gene of bone morphogenetic protein-7 (BMP-7), seeded on polymer scaffold grafts implanted into osteochondral defects in rabbit knees. The grafts containing BMP-7 modified cells consistently showed complete or almost complete regeneration of bone and articular cartilage after 8 and 12 weeks, whereas the grafts from the control groups had only a poor repair as judged by macroscopic, histological and immunohistological criteria. This effect of successful gene transfer was confirmed by the experimental work of Lee et al.⁶³ who injected fibroblasts expressing active TGF- β into the knees of rabbits with artificial defects of cartilage and demonstrated improved regeneration of cartilage after four to six weeks. However, the histological results of all reported experiments have not shown complete restoration of hyaline cartilage.

Controllable gene therapy in cartilage defects

The major concerns in the use of gene therapy are the safety of the gene transfer and the control of gene expression.^{42,46,47} While gene therapy may represent the 'last-chance' option in the treatment of malignancies or fatal muscular dystrophies, the risks of side-effects and potential consequences are unacceptable in elective joint surgery. Vectors integrating into the genome of the cells bear the risk of insertional mutagenesis and the development of a malignancy or an abnormal regulation of cell growth and toxicity because of chronic over-expression of the growth factor.⁷ Moreover, unlike the treatment of chronic disease, the desirable transgene expression is needed for only a few weeks until healing is achieved.

Regulation, i.e. control of the timing and the level of gene expression, is preferable to constitutive gene expression. One of the promising strategies for the regulation of gene therapeutic methods is pharmacologically-controlled gene expression.⁶⁴

Various inducible systems for gene expression have been described which can provide opportunities for expressing toxic genes, growth arrest genes, and therapeutic products in a regulated fashion which was previously not possible.⁶⁴ Many systems suffer from relatively high basal levels of expression of the transferred gene in the un-induced state and from the pleiotropic effects of the inducer on endogenous gene expression.⁶⁵ The tetracycline(tet)-inducible system has the advantage of being dependent on a drug (tetracycline or doxycycline) and to be both highly specific and non-toxic (Fig. 2). In the tet-responsive system, a chimaeric tet-controlled transactivator (tTA) encoded by one plasmid binds to the tet operator (tetO) sequence or tet-responsive element of a second plasmid, inducing expression of the gene of interest under the control of a minimal CMV promoter. Regulation of gene expression by tet is fast, reversible and can be modulated over several orders of magnitude. Tet-controlled expression has been demonstrated in different cells,⁶⁶ in transgenic mice,⁶⁷ in the heart,⁶⁸ the

Fig. 3a

Fig. 3b

Photomicrographs of *tetlacZ*-transfected chondrocytes in cell culture. Figure 3a – A control group without doxycycline. Cells express a slight blue basal β -galactosidase activity (leakiness). Figure 3b – *LacZ* expression in 10% of the chondrocytes after induction of gene expression with doxycycline (1 μ g/ml) (Xgal $\times 80$).

Fig. 4

Photograph of a femoral condyle three weeks after implantation of the tissue-engineered constructs. To exclude unspecific X-gal staining, hybrids were created with non-transfected cells in the upper defect (A) and *tetlacZ*-transfected cells in the lower defect (B). β -galactosidase activity and a X-gal positive staining appeared only in the defect with the transfected cells after treatment of the animals with doxycycline.

brain⁶⁹ and skeletal muscle,⁶⁵ but it has not been previously demonstrated in chondrocytes and osteochondral defects. Tetracycline derivatives like doxycycline are readily absorbed and broadly distributed to the synovial fluid with minimal toxicity at the concentration needed to regulate the activity

Fig. 5

Photomicrograph of inducible *lacZ* expression in chondrocytes after treatment with doxycycline (1 mg/kg) subcutaneously for one week (x, β -galactosidase positive blue cells (= induced gene expression of *lacZ*); X-gal and haematoxylin and eosin, $\times 67$).

of the synthetic promoter. Since doxycycline is well expressed in the synovial fluid, this antibiotic is suitable for the induction of transgene expression into joints.⁶⁵

Recently, the first example of a tetracycline-inducible gene expression in chondrocytes was demonstrated under in vitro and in vivo conditions.⁷⁰ In vitro, chondrocytes non-virally transfected with the marker gene *lacZ*, expressed this reporter gene, produced β -galactosidase and were stained blue after incubation with X-Gal solution only after the addition of doxycycline to the medium (Fig. 3). In vivo, transfected autologous chondrocytes, seeded on type-I collagen sponges and implanted in artificial osteochondral defects in the trochlea femoris of New Zealand white rab-

bits, expressed the marker gene *lacZ* and became β -galactosidase-positive exclusively in animals which obtained doxycycline (Figs 4 and 5). This experiment can be now used for the development of controllable delivery of therapeutic genes such as BMP, TGF- β or IGF-1 in cartilage defects and for learning about the mechanism of the growth-factor cascade in the process of articular healing. At this point however, the availability of vectors carrying effective genes is another obstacle, but great progress has been achieved in many laboratories working on the engineering of these vectors.⁶

Future development

Future research will continue to investigate the optimal combination of scaffolds, matrices, cells, adhesives and growth factors for the repair of defects of articular cartilage. The use of genetic engineering, a combination of gene therapy and tissue engineering, has the potential of becoming a valuable clinical tool for the treatment of chondral lesions and of other orthopaedic disorders.⁴⁵ However, the principal requirement to promote gene therapy in orthopaedics is the establishment of an unrestricted control over the transgene expression, which may be achievable in the near future.

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