Chapter 2
Gene Therapy in Articular Cartilage Repair

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Abstract The restoration of damaged articular cartilage remains one of the biggest challenges in modern clinical orthopaedics. There is no pharmacological treatment that promotes the repair of cartilage, and non-operative treatment inevitably leads to the development of premature osteoarthritis. Current treatment modalities include microfracture, transplantation of osteochondral grafts and autologous chondrocyte implantation (ACI), each having its own benefits and shortcomings. New biological approaches to cartilage repair that are based on the use of cells and molecules that promote chondrogenesis and/or inhibit cartilage breakdown offer a promising alternative to current treatment options. Chondrogenesis is a precisely orchestrated process which involves many growth factors and signaling molecules, and by modifying the local cellular environment, it is possible to enhance formation of more natural cartilage tissue within the defect. These bioactive molecules are difficult to administer effectively. For those that are proteins or RNA molecules, gene transfer has emerged as an attractive option for their sustained synthesis at the site of repair. To accomplish this task,

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two main strategies have been explored. The direct or *in vivo* approach delivers exogenous DNA directly into the joint. In this case synovial lining cells are the main site of gene transfer; depending on the vector, cells around or within the defect may also be genetically modified. During indirect or *ex vivo* delivery, cells are recovered, genetically manipulated outside the body, and then returned to the defect. Delivery of the genetic material to the living cell can be accomplished by use of either viral or non-viral vectors. While viral vectors are much more effective, they raise several safety concerns. Numerous preclinical animal studies have confirmed the effectiveness of these approaches in joints, and several phase I and II clinical gene therapy studies in the local treatment of arthritis provide reason for cautious optimism. This chapter will provide insight into the field of gene therapy in cartilage repair, and its potential for safe and effective clinical translation.

Keywords Cartilage defects • Gene therapy • Vectors • Growth factors

Key Points

- Although the lack of a natural repair process in cartilage is not due to a single, recessive gene, regeneration may be stimulated by gene transfer.
- There is plethora of possible candidate genes for promoting chondrogenesis, cell proliferation, maturation and matrix synthesis along with the inhibition of cartilage degradation.
- Gene therapy requires a dependable and safe delivery system to carry the therapeutic gene(s) into the target cells where they will be expressed.
- Transduction is application of viral vectors while the use of non-viral vectors is called transfection.
- There are two main strategies for gene delivery to joint cells: a direct, or *in vivo*, and an indirect, or *ex vivo*, approach.
- The duration and level of gene expression are important aspects of gene therapy. Cartilage repair would likely require modest levels of transgene expression for limited periods of time, which is more easily achieved than long-term expression.
- Articular chondrocytes and mesenchymal stem cells (MSCs) are currently the two most promising cell types for transplantation approaches.
- When speculating on the possible vector system to be used in clinical translation, recombinant adeno-associated viruses (AAV) seem like the most likely candidate.

2.1 Introduction

Gene therapy is based on the premise that it is possible to compensate for a defective gene in a recessive Mendelian disease by the delivery and expression of a functional one. The first successful gene therapy clinical trial took place in the United States in 1990 involving two patients who suffered from a rare immune disorder called adenosine deaminase severe combined immunodeficiency (ADA-SCID). By using retrovirally-mediated transfer of wild-type adenosine deaminase (ADA) cDNA into the T cells of the patients, it was possible to normalize the number of blood T cells, as well as to improve and normalize many cellular and humoral immune responses [1]. Gene therapy for ADA-SCID and X-linked SCID has now become the standard of care for these diseases. Promising clinical data have recently been published for hemophilia, β-thalessemia, Leber congenital amaurosis, and lipoprotein lipase deficiency [2–6]. These successes validate the concept of using gene therapy for monogenetic, recessive diseases where a single, defined gene is defective. But it is difficult to apply to cartilage repair because its lack of a natural repair process is not due to a single, recessive gene and there is no obvious candidate, single therapeutic gene.

For a very long time articular cartilage was thought of as a quiscent tissue with no possibility of regeneration after injury. The realization that cartilage is a metabolically active tissue, with various matrix components continually being turned over at different rates, has created the paradigm shift of using biological approaches to repair cartilage. Tissue remodelling involves co-ordinated production of matrix metalloproteinases (MMPs) and the ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) family of proteinases, coupled to the synthesis of new proteoglycans and proteins. Molecules involved in cartilage matrix breakdown include MMP-1 (collagenase-1), MMP-3 (stromelysin- 1), MMP-9 (gelatinase 92 kD), and MMP-13 (collagenase- 3). The activity of these proteinases is restrained by the action of tissue inhibitors of metalloproteinases (TIMPs). Many factors are involved in regulation of cartilage turnover (Table 2.1). These include humoral factors such as insulin-like growth factor-1 (IGF-I) and cytokines including interleukin-1 (IL-1), tumor necrosis factor (TNF), and transforming growth factor β (beta) (TGF-β (beta)), which are produced by chondrocytes, synovial cells and other cells found within joints. With age, articular chondrocytes loose their function; their anabolic and mitotic activities decline, expression of senescence-associated enzymes increases and telomere length decreases; aggrecans decrease in size and aggregation, and collagen cross linking increases [7–12]. These are associated with structural changes such as fibrillation and thinning of cartilage and decline of surface repair.

Spontaneous repair of chondral defects is very limited while osteochondral defects involving underlying bone fill with bone marrow that clots, leading to healing with fibrous tissue, or, at best, fibrocartilaginous tissue. Such articular defects predispose to osteoarthritis (OA). There is no pharmacological treatment for cartilage defects, and current surgical modalities include microfracture, transplantation

Table 2.1 Mechanisms of action and candidate genes for cartilage repair

Mechanism of action	Candidate gene	References
Anabolic factors	Chondrogenic transcription factors: SOX5, SOX6, SOX9	[13, 23–31]
	Growth factors:	[10, 32–36]
	IGF-1	[37–40]
	BMP-2, -4, -7	[32, 41–48]
	TGF-β	[49, 50]
	FGF-2	
Anticatabolic factors	Inhibition of proinflammatory cytokines:	[21, 51, 52]
	IL-1Ra	[53–55]
	sIL-1R	
	sTNFR	
Cytoprotection/ Proliferation factors	Inhibition of apoptosis: bcl-2	[67]
	Heat shock proteins:	[24, 26]
	HSP70, GRP78	[69, 70]
	Telomerase: hTERT	[63]
	Cell cycle regulator: p21	

of ostechondral grafts and various cell-based options (ACI being the most common) with or without a scaffold, each having its own benefits and shortcomings [13]. If chosen wisely, each of these techniques may yield good clinical results in terms of pain reduction and improvement of joint function. However, to date none of the proposed techniques results in production of fully matured hyaline cartilage, and there is a continuing need for new and innovative approaches to treat cartilage defects.

Biological approaches that are based on the use of cells and molecules that promote chondrogenesis and/or inhibit cartilage breakdown offer promising, novel treatment options and form a good basis for the application of gene therapy in articular cartilage repair. Stimulation of chondrogenesis, cell proliferation, maturation and synthesis of an authentic extracellular matrix, along with the inhibition of cartilage degradation, are the main strategies employed to accomplish this task. All of these processes are complex, being regulated by a number of different molecules; hence there is a plethora of possible candidate genes for promoting cartilage repair. Selecting the appropriate gene for this purpose is a major challenge to using gene therapy for repairing cartilage.

2.2 General Principles of Gene Therapy

Gene therapy requires a dependable and safe delivery system to carry the therapeutic gene(s) into the target cells where they will be expressed. Commonly used vectors can be viral or non-viral (Table 2.2). When viral vectors are applied, gene delivery is called transduction; the use of non-viral vectors is called transfection. Transfection can occur through natural processes, such as endocytosis, and

Table 2.2 Properties of the main viral vectors used in gene therapy

Virus	Key properties of wild-type virus	Advantages	Disadvantages
Adenovirus	Double stranded genome	Straightforward production at high titers	Inflammatory and antigenic
	~35 kb long	Transducing non-dividing cells	
	Non-enveloped	Wide choice of serotypes	
	Over 50 serotypes		
	~100 nm in size		
	Genome remains episomal		
Herpes simplex virus (HSV)	Double stranded DNA genome	Very efficient transduction of dividing and non-dividing cells	Complex genome – difficult to produce
	~150 kb long	Has natural latency in neurons	Cytotoxic
	Enveloped	Very large carrying capacity	
	~200 nm in size		
	Genome remains episomal		
Adeno-associated virus (AAV)	Single-stranded DNA genome	Perceived to be safe (wild-type virus cause no known disease)	Difficult to produce
	4.8 kb long	Transduces non-dividing cells	Carrying capacity is insufficient for certain applications
	Non-enveloped	Thought to have low immunogenicity, but this is being re-evaluated	
	Growing number of serotypes identified		
	~20 nm in size		
Oncoretrovirus	RNA genome	Straightforward production of vectors at moderate titers	Risk of insertional mutagenesis
	~8–10 kb long	Pseudotyped vectors have wide host range	Require host-cell division
	Enveloped		
	~100 nm in size		
Lentivirus	RNA genome	Straightforward production of vectors at moderate titers	Risk of insertional mutagenesis, but nonintegrating vectors are being developed, and have proved effective in animal models
	~8–10 kb long	Pseudotyped vectors have wide host range and are often very efficient	
	Enveloped	Transduces non-dividing cells	
	~100 nm in size		

efficiency can be enhanced by physical methods, such as electroporation, the use of a gene gun, and liposomes [14–19]. While non-viral vectors are perceived to be safer and easier to manufacture, viral vectors are much more efficient.

Recombinant retroviruses, such as those derived from Moloney murine leukemia virus, were the first to be used in human gene therapy clinical trials. Even though they ensure persistence of the transgene within transduced cells, they infect only dividing cells [20]. Another important property is the random integration of retroviral genetic material into the host genome, an event that might lead to insertional mutagenesis and the activation of tumor genes [21]. Lentivirus, a specific class of retrovirus that includes Human Immunodeficiency Virus, does not require host cell division for efficient transduction. These vectors transduce synovium very effectively after intra-articular injection but, like other retroviruses, pose the risk of insertional mutagenesis [22, 23]. Non-integrating lentiviral vectors have been developed to overcome this concern.

Recombinant adenoviruses have been the vectors most commonly used in clinical trials. They deliver their genomes as episomes, infect both dividing and non-dividing cells, and have large carrying capacity. However, they tend to excite a strong immune response and normally this leads to short-term transgene expression because transduced cells are cleared by the immune system.

Recombinant AAV present several advantages as gene delivery vehicles. Because wild-type AAV produces no known human diseases, they are perceived to be safe, and their DNA is maintained in a stable, episomal form in the nuclei of cells they transduce. Various serotypes of AAV have been shown to transduce chondrocytes, MSCs and synoviocytes. New technologies have enabled easier production of AAV [24–28].

Regardless of the vector used, there are two main strategies for gene delivery to joint cells: a direct, or *in vivo*, and an indirect, or *ex vivo*, approach. *In vivo* delivery is a simpler, less costly, one-step procedure in which vectors are delivered straight into the joint and can modify all available cells. A disadvantage of this strategy is that vectors are introduced into the patient where their subsequent activity cannot be easily controlled. Using *ex vivo* approaches allows for better control of gene transfer. Cells are genetically modified outside the body and then introduced into the joint. Although nominally safer, this approach is more complex and expensive than the *in vivo* approach. The choice of delivery method is based on a number of considerations including the type of the vector to be used, the transgene and the target cells. Delivery of growth factors might be more effective and safer when limited to the defect itself; implantation of cells that have been genetically modified outside of the body might better accomplish such localized delivery.

The duration and level of gene expression are additional, important aspects of gene therapy, which are defined by the therapeutic application. For example, certain monogenic diseases such as lysosomal storage diseases (e.g., Gaucher's disease) or osteogenesis imperfecta may require life-long expression of corrected gene in order to produce sustained clinical improvement [29]. On the other hand, treatment of malignant diseases may require very large amounts of transgene expression for very limited periods of time, in order to eliminate tumor cells without causing significant adverse effects [30]. Along these lines, cartilage repair would likely require modest levels of transgene expression for limited periods of time, which is more easily

achieved than long-term expression [31–33]; indeed, this may already be achievable using current technology.

Depending on the application, regulation of transgene expression may be important. One option is to use exogenous molecules to control transgene expression. Tetracycline-controlled activation of transgene expression is the most commonly used system in eukaryotic cells [34]. For this system, transcription is reversibly turned either on or off (Tet-On or Tet-Off) in the presence of the antibiotic tetracycline or one of its derivatives (e.g. doxycycline). An alternative strategy relies on the natural responsiveness of selected promoters to endogenous stimuli, such as pro-inflammatory cytokines. In theory, such systems could be activated in the presence of certain pathophysiological events e.g. exacerbation of OA or rheumatoid arthritis (RA) [35].

2.3 Candidate Genes for Therapeutic Intervention

A vast number of bioactive cues are known to be involved in the process of chondrogenesis and the maintenance of cartilage homeostasis. Although these signals are pleiotrophic, interactive and redundant, here they will be described according to their principal mechanism of action (Table 2.1).

2.3.1 Anabolic Factors

Chondrogenic transcription factors (*Sex determining region Y- box 5*, *6*, *9* (*SOX5*, *6*, *9*)). A variety of transcription factors have been enlisted in attempts to stimulate anabolic pathways in cartilage. With regard to anabolic transcription factors, most of the focus has been placed on targeting the SOX genes, SOX9 and co-factors SOX5 and SOX6, which are essential for chondrocyte differentiation and cartilage formation. During embryogenesis, SOX9 is expressed in all chondroprogenitor cells and its expression coincides with expression of collagen II [36–38]. Human chondrocytes from OA cartilage have been successfully transduced with retro-, lenti-, adeno- and AAV carrying SOX genes. *In situ* overexpression of SOX9 in normal and OA articular cartilage stimulated proteoglycan and type II collagen synthesis in a dose-dependent manner. These effects were not associated with changes in chondrocyte proliferation. These effects of SOX genes have been shown in MSCs derived from bone marrow and adipose tissue [19, 39–44].

Growth factors (*IGF-I*, *bone morphogenic proteins* (*BMPs*), *TGF-β* (*beta*), *fibroblast growth factor – 2* (*FGF-2*), *growth differentiation factor–5* (*GDF-5*)). Numerous growth factors have been employed in attempts to stimulate anabolic pathways in cartilage. IGF-I is expressed in developing and mature cartilage; it stimulates both cell proliferation and synthesis of aggrecan and collagen type II. Also, IGF-I is a survival factor for chondrocytes. It cannot induce cartilage formation from MSCs, however, so its principal target cells are chondrocytes [45, 46]. Transfection of articular chondrocytes with a plasmid vector containing the cDNA

for human IGF-I and subsequent transplantation of transfected cells onto the surface of articular cartilage explants led to the formation of a new tissue layer on the cartilage explant surface. Subsequent analysis showed thicker cartilage, higher percentage of collagen type II, and increased DNA and glycosaminoglycan (GAG) synthesis in the underlying explants [47]. Allogeneic chondrocytes transfected with plasmid IGF-I and encapsulated in alginate have been transplanted into rabbit osteochondral defects, leading to improved articular cartilage repair and acceleration of the formation of the subchondral bone after 14 weeks [16]. Intra-articular injection of adenoviral vector expressing human IGF-I promoted proteoglycan synthesis without significantly affecting inflammation or cartilage breakdown in rabbits. In addition, no adverse effects were observed 7 days after the treatment [48]. Recombinant AAV-mediated overexpression of IGF-I proved to have long term anabolic effects on chondrocyte cultures from human OA cartilage [49].

Chondrogenic differentiation, maturation and maintenance feature among the wide-ranging biological activities of BMPs. BMP-2, -4 and -7 have been mostly investigated in the context of cartilage regeneration. Both chondrocytes and MSCs of different origins have been successfully transduced with these genes. For example, chondrocytes modified with adenovirus carrying BMP-7 were transplanted onto cartilage explants and maintained *in vitro*. After 3 weeks, thicker neotissue was formed, positive for type II collagen and proteoglycan but negative for type X collagen [50]. When this method was used *in vivo* in an equine model, early post-treatment results were very positive, similar to those found in an *ex vivo* model. However, 8 months later the results were disappointing. Few implanted cells persisted and there was no difference between repair tissue in controls and BMP-7 treated animals [51]. With the use of MSCs retrovirally transduced to express BMP-4 in a rat model, cartilage repair was better than in controls after 6 months [52]. Side effects of BMP gene transfer to joints include osteophyte formation as a result of BMP-transfected cells engaging the synovium, and causing the differentiation of MSCs towards hypertrophic chondrocytes and osteoblasts [53].

All three isoforms of TGF-β (beta) have potent chondrogenic properties. They stimulate matrix synthesis and mitosis of chondrocytes and induce MSC differentiation into chondrocytes [45]. Adenoviruses, retroviruses, AAV and plasmids have been successfully employed in the genetic modification of chondrocytes and MSCs with TGF-β (beta). Chondrocytes modified to overexpress TGF-β(beta)1 increase their hyaline extracellular matrix synthesis in culture [54, 55]. Repair of cartilage was achieved *in vivo* when bone marrow MSCs modified with adenovirus and plasmid to express TGF-β(beta)1 were implanted into chondral and ostechondral defects [56, 57]. Moreover, retrovirally transduced allogenic chondrocytes expressing TGF-β(beta)1 were successully introduced into the joints of patients with OA in a clinical trial [58]. However, TGF-β(beta)1, either applied directly as a protein or via local overexpression, is not suitable for direct intraarticular application as it triggers adverse synovial reactions [59–61].

FGF-2 is a potent chondrocyte mitogen. *In vitro* and *in vivo* studies have shown that FGF-2 gene transfer may be applicable for the treatment of articular cartilage disorders in which cellular repopulation is a therapeutic goal. This beneficial effect

is mediated primarily through fibroblast growth factor receptor 3 (FGFR3), while some anti-anabolic effects observed are mediated primarily through fibroblast growth factor receptor 1 (FGFR1) [62]. Combined transfection of other anabolic factors with FGF-2 improved cartilage healing *in vivo* and less degenerative changes were observed in adjacent cartilage tissue [63].

GDF-5, (BMP-14 or cartilage derived morphogenic protein – 1(CDMP-1)) is known to be an important regulatory factor during the embryologic development of the appendicular skeleton and has been shown to be involved in chondrogenesis [64–66]. It promotes aggregation of mesenchymal cells and enhances chondrocyte differentiation during develeopment and in adult MSCs [67–70]. Bone derived MSCs transfectes with GDF-5 gene enhanced the repair of osteochondral defects [18]. Two different studies successfully injected adenovirus particles carrying the GDF-5 gene in rat tendons and mice degenerated discs respectfully, causing healing in terms of higher collagen II and GAG content [71, 72].

2.3.2 Anticatabolic Factors

Inhibition of proinflammatory cytokines (*interleukin-1 receptor antagonist* (*IL-1Ra*), *soluble interleukin-1 receptor* (*sIL-1R*), *soluble tumor necrosis factor receptor* (*sTNFR*)). IL-1Ra was the first gene used in a clinical trial for gene therapy in joint diseases, paving the path for other genes to follow [73, 74]. Inflammatory cytokines are highly expressed in RA and their role in OA is increasingly appreciated. Their activities have been successfully reduced in animal models of OA and RA, by transfer of genes encoding IL-1Ra, sIL-1R, sTNFR, mostly by delivering them directly into the joint using different viral and non-viral vectors [27, 75–79]. This protected hyaline matrix synthesis, thereby promoting cartilage repair. Since enhanced matrix breakdown may result from both biological and biomechanical signaling, the most effective control of degradation could be achieved by increase of downstream regulators such as TIMPs. Up-regulation of the gene for TIMP is a logical approach to the inhibition of MMP-mediated cartilage degradation. Certain members of the ADAMTS family are also inhibited by TIMPs. Chondrocytes and synovial fibroblasts have been transduced *in vitro* with the TIMP-1 gene and inhibitor IkappaBalpha respectively which resulted in the decreased activity of several MMPs [80, 81].

2.3.3 Cytoprotection/Proliferation Factors

Additional genes of recent interest for improving cartilage repair are those that affect the senescence and life cycle of chondrocytes, protecting these cells from stressful stimuli and apoptosis. Chondrocytes have been successfully modified with B-cell lymphoma 2 (BCL-2) [82], 70 kDa heat shock protein (HSP70) [83], human

telomerase reverse transcriptase (hTERT) and 78 kDa glucose-regulated protein (GRP78) [84, 85] target genes *in vitro*. HSP70 has also been evaluated *in vivo*. This therapy resulted in cytoprotection and better extracellular matrix synthesis. Synoviocytes, adenovirally transduced with cyclin dependent kinase inhibitor 1 (p21), down regulate expression of several inflammatory cytokines including IL-1β (beta), as well as MMP-1 and -3 [86]. There are numerous studies showing that the best anabolic response can be achieved with combinations of genes encoding different factors [63, 87–89].

2.3.4 Post Transcriptional Gene Regulation: MicroRNAs

MicroRNAs are the focus of emerging novel therapeutic strategies, including for cartilage repair. MicroRNAs form a class of non-coding, single strand RNAs that regulate gene expression at the post-transcriptional level by binding to specific sequences within target transcripts. MicroRNAs can act as both positive and negative factors in cartilage homeostasis [90, 91]. Studies on human chondrocytes and in animal models have shown that microRNAs have roles in chondrogenensis and both the anabolic and catabolic events of articular metabolism. During chondrogenesis, microRNA-140 expression in MSC cultures increases in parallel with the expression of SOX9 and collagen type II, alpha 1 (COL2A1). Normal human articular cartilage express microRNA-140, but this expression is significantly reduced in OA tissue. *In vitro* treatment of chondrocytes with IL-1β (beta) suppresses microRNA-140 expression. Conversely, transfection of chondrocytes with microRNA-140 down-regulates IL-1β (beta)-induced ADAMTS5 expression [92–95].

MicroRNA-145 has shown to affect differentiation of MSCs by acting directly on SOX9. Overexpression of microRNA-145 in MSCs decreases the expression of COL2A1, aggrecan (AGC1), cartilage oligomeric matrix protein (COMP), collagen type IX, alpha 2 (COL9A2), and collagen type XI, alpha 1 (COL11A1), and reduces GAG contents synthesis. In contrast, the inhibition of microRNA-145 significantly enhances the mRNA expression of the aforementioned genes and increases GAG production [96, 97]. MicroRNA-145 acts as a direct SOX9 repressor in normal healthy human articular chondrocytes. Experimentally increased microRNA-145 levels cause greatly reduced expression of tissue-specific microRNAs (microRNA-675 and microRNA-140), while increasing levels of the hypertrophic markers Runt related transcription factor 2 (RUNX2) and MMP13, characteristic of the changes occurring in OA [98].

Additional microRNAs have begun to be identified as having a role in cartilage homeostasis. Yamasaki et al. [99] have shown that microRNA-146a is intensely expressed in low grade OA cartilage but less in high grade OA, and its expression decreases in accordance with the level of MMP-13 expression. The expression of microRNA-146 was markedly elevated by IL-1β stimulation in human chondrocytes *in vitro*. Nakasa et al. [100] showed that microRNA-146a inhibits

osteoclastogenesis and has some anticatabolic properties in collagen-induced arthritic joints in mice. The overexpression of microRNA-9, microRNA-98 and microRNA-146 in human chondrocytes can reduce IL-1β (beta) yet increase TNF-α (alpha) mRNA. MicroRNA-9, upregulated in OA tissue, inhibits the secretion of metalloproteinase MMP-13 by isolated human chondrocytes. In addition, the inhibition or overexpression of microRNA-9 can regulate MMP-13 and type II collagen content [101]. MicroRNA-27a reduced MMP-1 and Insulin-like growth factor-binding protein 5 (IGFBP-5) synthesis, controlling arthritis in an indirect way. IL-1β (beta)-induced apoptosis was significantly reduced in rabbit chondrocytes when microRNA-34a was silenced [91, 97, 102].

2.4 Gene Therapy Strategies for Articular Cartilage Repair

2.4.1 Gene Delivery to the Synovium

When delivering genes directly to synovium, possible responding cells are synoviocytes and chondrocytes exposed to transgene products diffusing from synovial cells. Synovium has a large surface area compared to cartilage and is more amenable to gene delivery. Chondrocytes are present at low density and are lodged inside a dense matrix which makes them less accessible to vectors. When attempting to influence cartilage metabolism via gene delivery to synovium, it makes most sense to deliver cDNAs encoding secreted factors, such as IL-1Ra or IGF-1. Direct gene delivery to synovium has been mostly used for treating patients with RA [79, 81, 103]. *Ex vivo* approach using synovial fibroblasts in Phase I clinical trial was successfully initiated in 1996 [73, 74].

2.4.2 Gene Delivery to Chondrocytes

Despite the success of procedures such as ACI, using autologous chondrocytes as target cells for gene delivery requires additional procedures to retrieve the cells from the joint, and cause additional damage to the joint. An alternative strategy could involve taking chondrocytes from different locations in the body, such as the cartilage of nasal septum or ribs [104]. Other issues to be addressed regarding chondrocytes are time span in culture, potential dedifferentiation of cells that can occur with extended culture, and mode of application. One strategy is to load and implant the cells on matrices or use some kind of glue to keep them in place [63, 105]. Recently promising results of a phase I clinical trial have been published in which allogenic chondrocytes retrovirally transduced to express TGF-β(beta)1 were delivered to the knee joints of subjects with advanced OA [58].

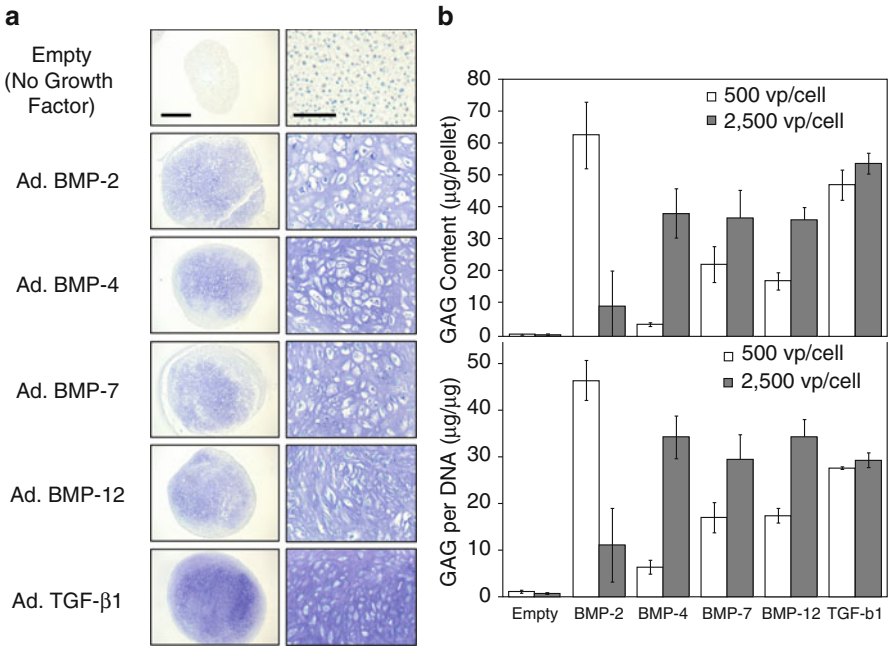

Fig. 2.1 Chondrogenic induction of human bone marrow-derived MSCs by growth factor overexpression: comparison of different BMPs with TGF-β (beta) 1. Human MSCs were transduced with adenoviral vectors encoding either no transgene (empty) or one of several known chondrogenic growth factors, then were cultured as cell aggregates for 4 weeks under standard chondrogenic conditions. (**a**) The resulting cell pellets were sectioned and stained with Toluidine Blue, which binds with sulfated glycosaminoglycan (GAG) chains. The two columns show representative pellets for the most chondrogenic dose of each virus at different magnifications (*left* scale bar = 500 μm; *right* scale bar = 100 μm). While all the presented BMPs induced chondrogenesis and proteoglycan deposition by human MSCs, they generally led to a more hypertrophic phenotype than did TGF-β (beta) 1 overexpression. (**b**) GAG levels within digested pellets were measured quantitatively by dimethylmethylene blue binding. Total GAG content per pellet (*upper panel*) and deposition relative to DNA content (*lower panel*) are shown for the same growth factors in (**a**) delivered using either 500 or 2,500 viral particles (vp)/cell. The GAG deposition response to BMP overexpression was highly dependent on the amount of viral vector and the corresponding level of BMP secretion. Cells were less sensitive to TGF-β (beta) 1 secretion levels. TGF-β (beta) 1 overexpression promoted higher cell density within pellets after 4 weeks, so that maximum GAG/DNA levels were relatively lower than for BMP-overexpressing groups

2.4.3 Gene Delivery to Mesenchymal Progenitors

MSCs offer an attractive alternative to chondrocytes as vehicles for *ex vivo* gene delivery to sites of cartilage damage. These cells can easily be retrieved from bone marrow, periosteum, synovium, adipose tissue, or skeletal muscle and differentiated into chondrocytes in tissue culture [106, 107] (Fig. 2.1). MSCs in combination with

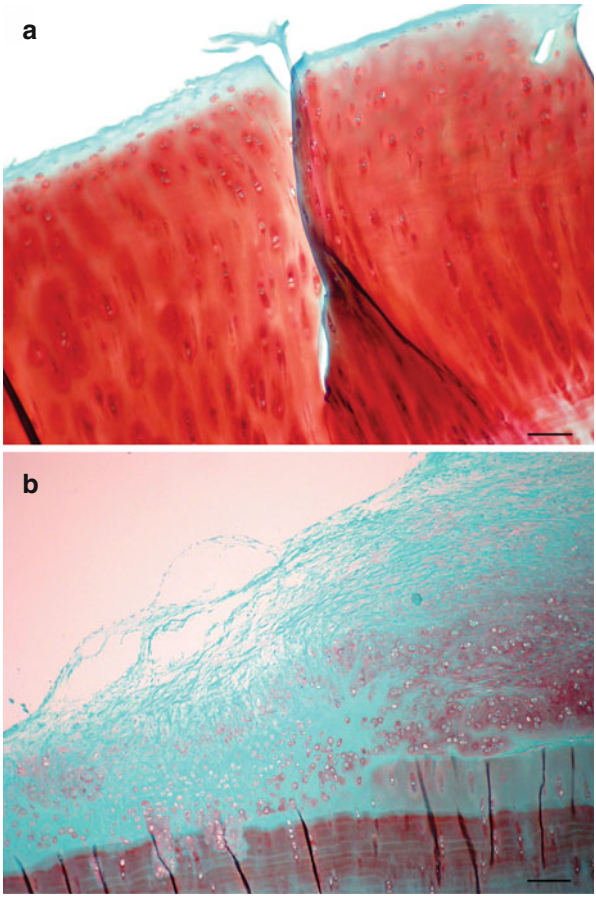

Fig. 2.2 Hyaline nature of cartilage healing after treatment with bone marrow clot adenovirally transduced with TGF**β**(beta)1 compared to healing after no treatment. Critical-size full-thickness cartilage defects in sheep were treated either with bone marrow clot genetically modified to secrete TGF**β**(beta)1 (**a**) or left untreated (**b**). After 6 months histology revealed formation of hyaline cartilage (*stained red*) in genetically treated defects and mixture of fibrous tissue (*stained green*) and fibrocartilage in untreated defects. Staining: Safranin O; magnification: 100×; scale bar = 100 **μ**m

different scaffolds have been successfully employed to treat chondral and osteochondral defects in animal models *in vivo* [18, 56, 57, 108, 109] (Fig. 2.2). One potential challenge for using MSCs concerns the prevention of hypertrophic maturation that is typically associated with their chondrogenic differentiation [110]. According to the literature, MSCs from synovium form chondrocytes without undergoing hypertrophic differentiation. One way of delivering MSCs form bone marrow to the cartilage defects is clot technology designed by Pascher et al. [109]. After aspiration, bone marrow is transduced with vector carrying certain gene and left briefly at room temperature to clot. Clot is then placed into the defect without any fixation.

As an alternative to MSCs, recent progress has been made regarding the use of induced pluripotent stem cells (iPSCs) for treatment of cartilage defects. Wei et al. formed iPSCs from OA cartilage and then differentiated them into chondrocytes using lentiviral transduction of TGF-β (beta)1 in an alginate matrix [111].

2.5 Challenges for the Clinical Application of Gene Therapy to Promote Articular Cartilage Repair

Even though gene transfer to joints is local, there is still concern about systemic effects and safety issues are important impediments to successful clinical translation. The whole field of gene therapy carries the perception of being risky, unsafe and difficult to deliver. However, thorough review of available data suggests that this perception is partially exaggerated. There have been over 1 700 clinical trials worldwide with more than 10,000 patients being treated, and only few fatalities have been unequivocally connected with gene therapy itself [112]. However, each of these events has been seized by media, creating a negative perception of the whole field. This was highlighted by the 2007 death of a subject in an arthritis gene therapy trial [113]. Although subsequent investigation exonerated locally administered gene therapy from being responsible for this death, it was a huge step back in efforts to translate gene therapy into clinical practice for treating joint conditions. It also emphasizes the importance of selecting not only an appropriate gene vector system, target gene and delivery method, but also suitable subjects for these trials. Thus, the *ex vivo* approach, although has some drawbacks compared to *in vivo*, might represent a safer option for cartilage repair. Additionally, some genes when applied *in vivo* had significant local side effects compared to *ex vivo* approach. Adenovirally mediated delivery of TGF- β(beta)1 or BMP-2 to the synovial lining, for instance, was found to generate joint fibrosis, extreme swelling, osteophytes and cartilage degeneration [59, 108, 114, 115]. Regarding the choice of a proper target gene, it may be more suitable to target anti-inflammatory genes into the synovium where they can have more general intra-articular effect, while use of growth factors should be localized to chondrocytes.

Several critical questions must be answered in order to select an effective gene therapeutic strategy. What are the optimal treatment modalities for different types of cartilage damage? For example, how are large defects treated relative to small defects? Which cells should be used or targeted? Which vectors best target these cells? What is the transgene of choice? Moreover, should gene therapy strategies be modified to reflect cartilage anisotropy? Articular cartilage is organized in 4 layers (superficial, intermediate, deep and mineralized cartilage); the most significant structural and molecular difference is between deep layer and mineralized cartilage. Type II collagen is present in the deep zone, while type type X collagen predominates in mineralized cartilage; additionally hypertrophic chondrocytes express alkaline phosphatase. Would cartilage implants constructed so that lower layer cells are modified or stimulated to preferentially express type X collagen and alkaline phosphatase, while upper layer cells express collagen type II, constitute the most appropriate therapy? This would definitely be a more scientifically and technologically challenging, laborious and costly approach.

Articular chondrocytes and MSCs are currently the two most promising cell types for transplantation approaches. Since it has been shown that MSCs exhibit

immunosuppressive properties, they may survive when transplanted into allogeneic hosts, although this is controversial. Allografting would make clinical translation much easier, since this approach avoids damaging already impaired joints to obtain autologous cells. It is not difficult to envision commercially available, genetically modified MSCs ready to be transplanted into localized cartilage defects with one-step, minimally invasive surgical procedures.

When speculating on the possible vector system to be used in clinical translation, AAV seems like the most likely candidate [25]. AAV causes no known human disease, has appropriate packaging capabilities, and transduces non-dividing cells. It has been thought to have low immunogenicity, but this is being re-evaluated. Another drawback is its complicated and costly production (see Table 2.1).

Use of scaffolds in cartilage repair is one the most exciting areas in orthopaedic research, both for scientists and clinicians [116]. Most current research is focused on resorbable scaffolds whose main function is to provide temporary, three-dimensional templates on which cells can adhere and synthesize extracellular matrix (ECM). As the scaffold resorbs, it is progressively replaced by newly formed, functional tissue. This approach, termed matrix-assisted chondrocyte transplantation is currently used in clinical practice to treat localized cartilage defects [117]. Autologous chondrocytes are attached to different types of matrices (e.g. collagen, hyaluronic acid etc.) and transplanted into the defect. Combining genetically modified cells with tissue engineered matrix might be a more effective strategy than cell delivery alone [88]. This would allow complete filling of the defect and three-dimensional orientation of genetically modified cells, thus ensuring more natural environment for production of ECM. Furthermore, optimal mechanical properties of the scaffold would make handling easier and surgical procedures more convenient to perform.

2.6 Conclusion

Translating gene therapy for treating cartilage lesions into clinical practice is not easy. Cartilage defects are not life threatening diseases, and treatment modalities developed so far serve their main purpose – minimizing the pain and improving the quality of life. Most of these biological approaches provide either cells alone, or construct made of cells and temporary scaffolds. Gene therapy has emerged as a feasible option to add as the final ingredient in this system, providing sustainable local expression of bioactive cue(s). A key challenge for translating this into clinical practice will depend on the development of safe and effective gene delivery systems with long-lasting expression of therapeutic transgenes, the identification of effective yet safe combinations of therapeutic genes, identification of the ideal target cell(s) (chondrocytes, MSCs or synoviocytes) and identifying the most appropriate carriers which better support the chondrogenic process within the defect. The regulatory issues, timelines and costs should not be underestimated as barriers to translation.

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