Gene Transfer Strategies for Articular Cartilage Repair

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Abstract

Gene therapy protocols are well suited to deliver genes coding for therapeutic factors over time in a spatially defined manner within sites of cartilage injury resulting from acute trauma or during osteoarthritis. The focus of this chapter is to examine the benefits of gene therapy to improve cartilage repair in such lesions, based on promising experimental and clinical evidence in relevant models in vivo using growth, transcription, and signalling factors capable of stimulating the chondrogenic and chondro-reparative processes locally. A continuous, combined effort between scientists and orthopaedic surgeons may allow to bring gene therapy from encouraging data at the bench to a successful, safe translation in the broadly affected human population.

9.1 Introduction

Articular cartilage defects like after trauma or during osteoarthritis (OA) have a limited capacity for self-repair. The idea of applying gene transfer strategies to enhance cartilage repair by local application of therapeutic (growth, transcription, signalling) factors originates from the possibility to extend therapeutic transgene expression and subsequent effects over time compared with the injection of recombinant agents showing relatively short half-lives. Increasing, promising experimental data have shown the benefits of providing such therapeutic sequences using various gene transfer systems in relevant in vitro, in situ, and in vivo models of focal

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and OA cartilage lesions via direct (cell-free) and indirect (cell-associated) procedures, allowing to rejuvenate the affected cells and to improve the repair of the damaged tissues. The availability of clinically suited vectors and of workable strategies may allow in a close future to provide adapted, effective novel options to treat cartilage injuries in patients.

9.2 Articular Cartilage Repair

Adult hyaline cartilage, the tissue that affords a smooth gliding of articulating surfaces and protects the subchondral bone against mechanical stress in the joint, has a limited intrinsic ability to heal in the absence of vascularization that may provide chondro-regenerative cells such as progenitor cell populations in response to injury (Heijink et al. 2012; Pape et al. 2010). The articular cartilage is formed by chondrocytes embedded in a self-produced, complex extracellular matrix in different layers, with a calcified basal layer towards the underlying subchondral bone. The cartilage matrix contains proteoglycans bound to 70–80% water, collagen fibrils (mostly type II but also type VI, IX, XI, and XIV collagen), and other molecules such as the cartilage oligomeric matrix protein (COMP), link protein, decorin, fibromodulin, fibronectin, and tenascin. Cartilage lesions can be circumscribed (focal defects) or generalized (osteoarthritis, OA, a chronic, inflammatory/catabolic whole degenerative joint disorder with a gradual degradation of the cartilage, subchondral bone, synovium, ligaments, tendons, and muscles) (Madry et al. 2012; Heijink et al. 2012; Loeser et al. 2012).

The repair of cartilage is considered as the production of a tissue that shares structural similarities with the hyaline articular cartilage. Regeneration, however, is a complete reproduction of the hyaline cartilage. Natural repair of chondral defects occurs by migration of cells from the synovial membrane but leading to a tissue that does not integrate with the surrounding cartilage and to larger defects. Osteochondral defects are filled with a clot from the bone marrow containing chondrogenically and osteogenically competent bone marrow-derived mesenchymal stem cells (BM-MSCs), but again a fibrocartilaginous repair tissue is produced with early signs of OA. OA cartilage has also restricted repair capabilities leading to an irreversible degradation of the cartilage and a remodelling of the osteochondral unit.

Current interventions for chondral defects include marrow stimulation (subchondral drilling, microfracture, and abrasion arthroplasty), and the transplantation of autologous chondrocytes with or without supportive matrix (autologous chondrocyte implantation, i.e. ACI) and those for osteochondral defects is based on the implantation of uninjured osteochondral cylinders and of subchondral bone grafts combined with ACI (Madry et al. 2011a) (for details, see Chap. 5). OA treatments are either conservative (non-pharmacological and pharmacological options using non-steroidal anti-inflammatory drugs, opioid analgesics, or intraarticular corticosteroid or hyaluronic acid (HA) injections) or surgical like by osteotomy (Madry et al. 2011a).

As none of these procedures are capable of fully managing any of these lesions, novel, effective options are needed to improve cartilage repair, like those afforded by gene therapy that may need to take into account the differences between the nature, size, number, and location of the injury and the stage of the disease (Madry and Cucchiarini 2013, 2015; Frisch et al. 2015b; Cucchiarini et al. 2014; Madry et al. 2011b; Cucchiarini and Madry 2005).

9.3 Gene Therapy: Principles and Experimental Approaches for Cartilage Repair

Gene therapy aims at treating human disorders by applying gene transfer techniques in patients in vivo. Foreign genes may penetrate the target cell to reach the nucleus where they stay extrachromosomal (more or less stable episomal forms) or become integrated within the host genome in specific or unspecific locations (stable forms) as features inherent to the class and biology of the vector employed. Targeting by gene transfer usually affects a high number of cells that are sufficient for the production of the transgene product and for consequent therapeutic applications. The best characterized vectors available for gene therapy protocols mostly include nonviral constructs and approaches and viral vectors (Table 9.1) (Cucchiarini and Madry 2005; Madry and Cucchiarini 2013, 2015; Frisch et al. 2015b; Madry et al. 2011b).

Table 9.1 Common vectors available for gene therapy

Types		Advantages	Limitations	Integration
Nonviral vectors		• Not infectious • Not toxic • Easy to produce • Large capacity	• Low efficiency • Short-term expression	–
Viral vectors	Adenovirus	• High efficiency	• Replication competence • Toxic • Immunogenic • Short-term expression	–
	Retro-/*Lentivirus*	• High efficiency • Long-term expression	• Replication competence • Insertional mutagenesis	Yes
	HSV	• High efficiency • Large capacity	• Cytotoxic • Short-term expression	–
	rAAV	• High efficiency • Long-term expression • Low immunogenic	• Difficult to produce • Size limitation	Mostly episomal

Abbreviations: *HSV Herpes simplex virus*, *rAAV* recombinant adeno-associated virus

9.3.1 Gene Transfer Vectors

9.3.1.1 Nonviral Systems

Transfection is the transfer of foreign genes via nonviral vectors (cationic lipids and liposomes, polymers, polyamines, polyethylenimines, nanoparticles). These systems are large, easy to generate, and safe (lack of acquiring replication competence like for viral vectors, no immunogenicity), allowing for repeated administration. Yet, they display a relatively low efficacy compared with viral vectors. Also, as mostly episomal forms, they commonly promote short-term transgene expression. These vectors are thus rather employed in indirect gene transfer protocols by implantation of ex vivo modified cells in the recipient.

9.3.1.2 Viral Vectors

Transduction is the term characterizing viral-based gene transfer. Viral vectors employ their natural entry pathways in the cells. Adenoviral vectors have a high gene transfer efficacy, enabling direct approaches in vivo, but they are highly immunogenic and remain episomal, restricting transgene expression to only about 1–2 weeks. Retroviruses instead can integrate into the host genome, allowing for the maintenance of the transgene over time. Still, integration is associated with a risk of insertional mutagenesis and of tumour gene activation. In addition, these vectors transduce only dividing cells (progenitor cells but not differentiated chondrocytes and bone cells) at a restricted host range, making them more adapted for indirect, ex vivo approaches, further allowing to increasing their otherwise low gene transfer efficacy. Lentiviral vectors that derive from the *Human immunodeficiency virus* (HIV) instead can integrate in nondividing cells at higher efficiency, but concerns remain due to the potential for insertional mutagenesis and to the problem of introducing material carrying HIV sequences. Large *Herpes simplex virus* (HSV) vectors can target nondividing cells, but still induce cytotoxic responses and allow only for very transient transgene expression. Small recombinant adeno-associated viral (rAAV) vectors have various advantages as they are safe and low immunogenic in the complete absence of viral gene sequences. This is particularly important as cartilage lesions are not life-threatening problems. They can transduce both dividing and nondividing cells and are mostly kept as very stable episomes, allowing for long-term transgene expression.

9.4 Strategies to Improve Cartilage Repair

Target cells relevant for cartilage repair permissive to gene transfer include articular chondrocytes to repopulate the injured cartilage, bone cells (osteoblasts/osteocytes to reconstruct the subchondral bone), synoviocytes (as a source of therapeutic factors), other affected cells (meniscal fibrochondrocytes, tendon/ligament cells, muscle cells), and progenitor cells (BM-MSCs that may commit towards mesodermal cell lineages

as isolated/expanded suspensions or directly in marrow aspirates/concentrates) (Arai et al. 2000; Baragi et al. 1995; Cucchiarini et al. 2014; Doherty et al. 1998; Gouze et al. 2002; Hildebrand et al. 1999; Madry et al. 2004a; Madry and Trippel 2000; Mason et al. 2000; Nita et al. 1996; Orth et al. 2014; Rey-Rico et al. 2015a; Stender et al. 2007). These cells may be directly genetically modified within the lesions by direct gene transfer vector administration or first isolated and/or expanded for indirect gene transfer ex vivo prior to reimplantation. This may offer to employ other cell types such as MSCs from other tissues like the adipose tissue, synovium, periosteum, and perichondrium (Cucchiarini and Madry 2005; Madry and Cucchiarini 2013, 2015; Cucchiarini et al. 2014; Frisch et al. 2015b; Madry et al. 2011b). Various approaches have been developed experimentally to deliver therapeutic gene sequences in sites of articular cartilage injury (Fig. 9.1), among which administration of a therapeutic

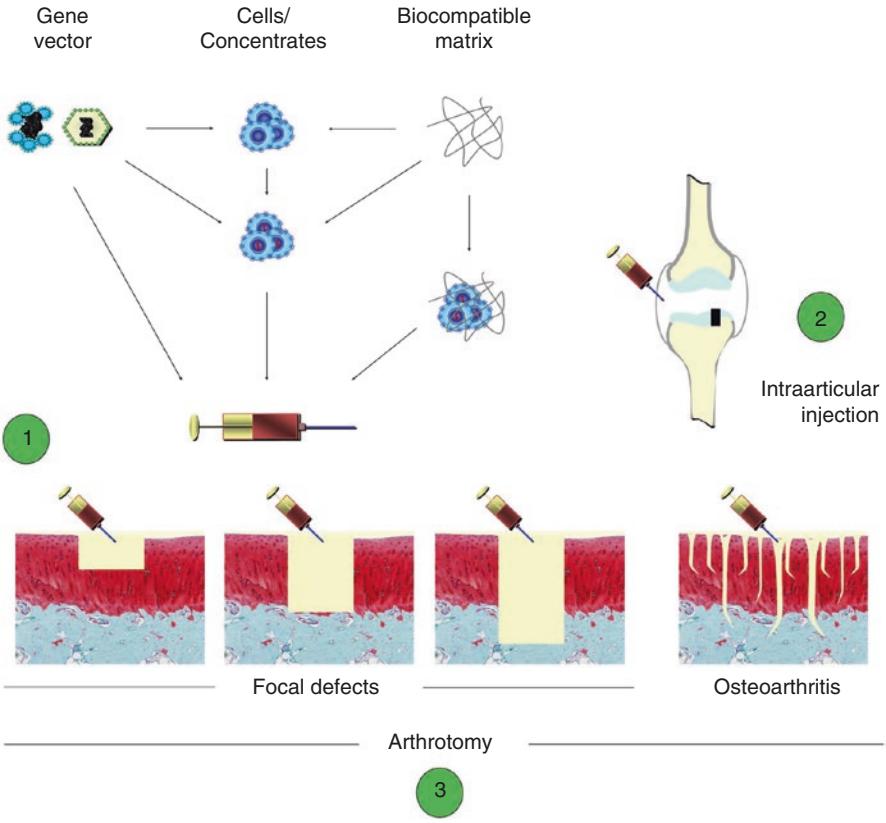

Fig. 9.1 Experimental approaches for the delivery of therapeutic gene sequences in sites of articular cartilage injury. (*1*) Preparation of a therapeutic composition with gene vectors, cells (suspension or as concentrates), and a biocompatible matrix. The composition may be provided (*2*) intraarticularly or (*3*) via arthrotomy in either focal defects or in OA cartilage

compound intraarticularly or via arthrotomy using either the gene vector itself or genetically modified cells as a suspension or using a biocompatible matrix (Cucchiarini and Madry 2005, 2014b; Johnstone et al. 2013; Madry and Cucchiarini 2013, 2015; Cucchiarini et al. 2014; Frisch et al. 2015b; Madry et al. 2011b).

9.4.1 Gene Therapy for Cartilage Repair: Evidence In Vitro

Pathways that might be targeted to improve cartilage repair by gene therapy include the activation of cell proliferation and survival, the stimulation of anabolic responses, and the prevention of inflammation and tissue degradation via single or combined approaches.

9.4.1.1 Activation of Cell Proliferation and Survival

These processes may be stimulated by gene transfer of mitogenic growth factors (insulin-like growth factor I (IGF-I), basic fibroblast growth factor (FGF-2), bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-β)) (Madry et al. 2001, 2004b; Cucchiarini et al. 2009, 2011; Weimer et al. 2012; Shi et al. 2013; Neumann et al. 2013; Venkatesan et al. 2013; Frisch et al. 2014a, b, 2015a), telomerase (hTERT) (Piera-Velazquez et al. 2002), inhibitors of apoptosis (bcl-2) (Surendran et al. 2006), or of the heat shock protein 70 (HSP70) (Grossin et al. 2006). Most remarkably, delivery and overexpression of the key IGF-I and TGF-β factors via potent rAAV-mediated gene transfer allowed for a stable reproduction of the cell proliferative indices in human OA articular cartilage explant cultures in situ, reaching levels and patterns typical of normal cartilage over an extended period of 90 days when the cells are embedded in their natural matrix (Weimer et al. 2012; Venkatesan et al. 2013).

9.4.1.2 Stimulation of Anabolic Responses

Production of matrix components may be enhanced by application of sequences for the matrix molecules themselves or of the enzymes that synthesize them (Venkatesan et al. 2004), of pro-anabolic growth and signalling factors (IGF-I, FGF-2, BMPs, TGF-β, parathyroid hormone-related peptide (PTHrP), Indian hedgehog (Ihh)) (Smith et al. 2000; Nixon et al. 2000; Shuler et al. 2000; Mi et al. 2000; Madry et al. 2001; Brower-Toland et al. 2001; Palmer et al. 2005; Cucchiarini et al. 2005; Ulrich-Vinther et al. 2005; Wang et al. 2011; Weimer et al. 2012; Steinert et al. 2012; Neumann et al. 2013; Venkatesan et al. 2013; Frisch et al. 2014a, b, 2015a), or of tissue-specific transcription factors (sex-determining region Y-type high mobility group box—SOX family, zinc-finger protein 145 (ZNF145)) (Li et al. 2004; Cucchiarini et al. 2007; Liu et al. 2011; Rey-Rico et al. 2015a; Shi et al. 2015).

Among these many factors, here again administration of IGF-I and TGF-β sequences via rAAV vectors may be best suited in light of reports showing close-to-normal, long-term reconstruction of the extracellular matrix in human OA articular cartilage for at least 90 days in situ (Weimer et al. 2012; Venkatesan et al. 2013), together with the use of the cartilage-specific SOX9 transcription factor that also exhibits highly effective chondro-regenerative activities that are essential for cartilage repair (Cucchiarini et al. 2007; Rey-Rico et al. 2015a).

9.4.1.3 Prevention of Inflammation and Tissue Degradation

Reduction of catabolic processes may be achieved with inhibitors of matrix-degrading enzymes (Kafienah et al. 2003) and of pro-inflammatory cytokines (interleukin-1 receptor antagonist—IL-1Ra) (Baragi et al. 1995; Roessler et al. 1995; Glass et al. 2014). Glass et al. recently provided interesting evidence that combining gene therapy with functional tissue engineering provided powerful tools to produce cartilage with immunomodulatory properties via scaffold-mediated IL-1Ra gene transfer in MSCs, permitting chondrogenesis upon pathologic activation by IL-1 (Glass et al. 2014). Prevention of osteophyte formation in OA may be also considered like by application of antagonists of the TGF-β/BMP pathway (latency-associated peptide—mLAP-1, Smads) (Scharstuhl et al. 2003) or of an IL-1Ra formulation (Fernandes et al. 1999).

9.4.1.4 Combined Approaches

Multifactorial approaches have been attempted by cotransfer of activators of proliferative/anabolic processes (IGF-I/FGF-2/BMPs/SOX) (Ikeda et al. 2004; Cucchiarini et al. 2009; Orth et al. 2011; Shi et al. 2012, 2013) or of activators of anabolic/proliferative pathways with inhibitors of catabolism (IGF-I/FGF-2/IL-1Ra) (Nixon et al. 2005; Haupt et al. 2005; Chen et al. 2010). Approaches that aim both at counteracting deleterious inflammation while restoring the altered metabolic balance might be desirable to address the spectrum of pathomechanisms triggered in sites of cartilage injury. Specifically, several groups showed that cells cotransduced with IGF-I/IL-1Ra via adenoviral vectors were more potent to reverse IL-1-mediated proteoglycan depletion in cartilage compared with single gene treatments (Nixon et al. 2005; Haupt et al. 2005; Chen et al. 2010).

9.4.2 Gene Therapy for Cartilage Repair: Evidence In Vivo

Both direct and indirect gene transfer strategies have been tested in animal models of cartilage injury to provide therapeutic genes for enhanced cartilage repair. Direct strategies are less invasive, but necessitate that the vectors are adapted to effectively reach the target cells within their dense matrix. In this case, the small rAAV vectors are probably the most adequate gene vehicles to achieve this goal. Indirect strategies might be more desirable to repopulate tissue lesions, having the additional advantages of introducing modified cells rather than free vector particles while permitting extensive control of the cells prior to reimplantation and allowing the use of biocompatible scaffolds.

9.4.2.1 Direct Gene Transfer Strategies

Such approaches have been tested to enhance cartilage repair in experimental models of focal defects (Cucchiarini et al. 2005, 2013; Morisset et al. 2007; Cucchiarini and Madry 2014a; Griffin et al. 2015) and of OA (Fernandes et al. 1999; Frisbie et al. 2002; Grossin et al. 2006; Chen et al. 2008, 2010; Hsieh et al. 2009, 2010; Shen et al. 2011; Oh et al. 2012; Santangelo et al. 2012; Zhang et al. 2015) using

sequences for growth factors (IGF-I, FGF-2, TGF-β) (Cucchiarini et al. 2005; Chen et al. 2010; Cucchiarini and Madry 2014a; Griffin et al. 2015; Zhang et al. 2015), IL-1Ra (Fernandes et al. 1999; Frisbie et al. 2002; Chen et al. 2010; Santangelo et al. 2012; Zhang et al. 2015), SOX9 (Cucchiarini et al. 2013), HSP70 (Grossin et al. 2006), silencers of NF-kappaBp65 (Chen et al. 2008), inhibitors of inflammatory pain processes (pro-opiomelanocortin, POMC) (Shen et al. 2011), antagonists of the canonical Wnt pathway (Dickkopf1, Dkk-1) (Oh et al. 2012), kallistatin or angiogenic inhibitors (thrombospondin-1, TSP-1) (Hsieh et al. 2009, 2010), or combined approaches (IGF-I/IL-1Ra, TGF-β/IL-1Ra) (Morisset et al. 2007; Zhang et al. 2015), allowing for improved cartilage repair in these different systems. Most significantly, treatment of experimental focal (osteochondral) defects by direct application of an rAAV vector carrying the potent SOX9 transcription factor improved the processes of cartilage repair in rabbit knee joints for a stable period of 16 weeks without detrimental effects (Cucchiarini et al. 2013). Regarding OA, therapeutic success has been reported by various groups based on the application of IL-1Ra-coding nonviral and adenoviral vectors both in OA guinea pigs, rabbits, and horses, promoting a net reduction in the disease severity together with improvements in cartilage preservation (Fernandes et al. 1999; Frisbie et al. 2002; Santangelo et al. 2012; Zhang et al. 2015).

9.4.2.2 Indirect Gene Transfer Strategies

Administration of genetically modified cells has been also reported to treat experimental models of focal defects (Mason et al. 2000; Lee et al. 2001; Hidaka et al. 2003; Georgi et al. 1992; Madry et al. 2005, 2013; Guo et al. 2006; Kaul et al. 2006; Kuroda et al. 2006; Goodrich et al. 2007; Evans et al. 2009; Vogt et al. 2009; Che et al. 2010; Ivkovic et al. 2010; Noh et al. 2010; Liu et al. 2011; Orth et al. 2011; Ortved et al. 2015; Sieker et al. 2015) and of OA (Bandara et al. 1993; Zhang et al. 2004; Matsumoto et al. 2009) using genetic modification of chondrocytes (Hidaka et al. 2003; Madry et al. 2005, 2013; Kaul et al. 2006; Goodrich et al. 2007; Che et al. 2010; Noh et al. 2010; Orth et al. 2011; Ortved et al. 2015), synoviocytes (Bandara et al. 1993; Zhang et al. 2004), various progenitor cells as suspensions or as marrow aspirates/concentrates (Mason et al. 2000; Katayama et al. 2004; Guo et al. 2006; Kuroda et al. 2006; Vogt et al. 2009; Matsumoto et al. 2009; Ivkovic et al. 2010; Liu et al. 2011; Sieker et al. 2015), and tissue grafts (Evans et al. 2009) to overexpress growth factors (IGF-I, FGF-2, BMPs, TGF-β) (Mason et al. 2000; Lee et al. 2001; Hidaka et al. 2003; Madry et al. 2005, 2013; Guo et al. 2006; Kaul et al. 2006; Kuroda et al. 2006; Goodrich et al. 2007; Evans et al. 2009; Vogt et al. 2009; Che et al. 2010; Ivkovic et al. 2010; Noh et al. 2010; Ortved et al. 2015), IL-1Ra (Bandara et al. 1993; Zhang et al. 2004), SOX9 (Liu et al. 2011), Ihh (Sieker et al. 2015), the cartilage-derived morphogenetic protein 1 (CDMP-1) (Katayama et al. 2004), IGF-I/FGF-2 (Orth et al. 2011), IL-1Ra/IL-10 (Zhang et al. 2004), or BMP-4/sFlt-1 (an antagonist of the vascular endothelial growth factor, VEGF) (Matsumoto et al. 2009), allowing for improved cartilage repair in these various models. Significant advances have been made in experimental cartilage repair when implanting IGF-I-expressing vectors in articular chondrocytes or BMP-2-modified

tissues (fat, muscle, fibrin clots) in focal defects in rabbits and horses using nonviral, adenoviral, retroviral, and rAAV vectors (Goodrich et al. 2007; Evans et al. 2009; Vogt et al. 2009; Madry et al. 2013; Ortved et al. 2015), leading to improved tissue healing. In OA models, mostly synovial cells overexpressing IL-1Ra via retroviral vectors have been provided to prevent cartilage breakdown in OA rabbits (Bandara et al. 1993; Zhang et al. 2004), while injection of muscle-derived MSCs producing BMP-4/sFlt1 in OA rats led to durable cartilage repair (Matsumoto et al. 2009).

9.5 Gene Therapy: Current Advances in Clinical Trials for Cartilage Repair—Perspectives

Compared with the large body of available experimental data, relatively few trials have been initiated to treat patients with focal cartilage lesions and OA, reflecting the difficulty to translate research ideas and visions into clinical and commercial reality (Evans et al. 2012, 2013; Cucchiarini et al. 2014; Bara et al. 2015). While no trials are ongoing for focal defects, phase I and II clinical trials have been recently published to treat OA patients by injecting retrovirally modified chondrocytes to produce TGF-β1 (Ha et al. 2012, 2015; Lee et al. 2015; Cherian et al. 2015), showing a trend towards efficacy with improvements in pain, function, and physical ability. Yet, in light of multiple reports showing adverse effects of TGF-β in experimental models in vivo (synovial inflammation and fibrosis, osteophyte formation) (van Beuningen et al. 1998; Bakker et al. 2001; Mi et al. 2003; Blaney Davidson et al. 2007; Remst et al. 2013), other approaches might be necessary to improve the safety of the outcomes and prevent such detrimental, undesirable effects in patients.

Remarkably, rAAV vectors have emerged as well-suited systems for clinical applications as these vectors are highly effective in direct, less invasive protocols compared with retroviral vectors and considered as safe delivery compounds, receiving market authorization by the European Union Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) for the treatment of lipoprotein lipase (LPL) deficiency (Glybera®) (Yla-Herttuala 2012; Buning 2013). Interestingly, a phase I study by direct administration of an rAAV IL-1Ra construct has been initiated to treat OA patients (Evans et al. 2013). It remains to be seen whether such a trial will allow for successful outcomes as a major concern to the clinical use of this vector class is the significance of pre-existing and induced immune responses against the viral capsid proteins of AAV, with a clear impact on clinical efficacy and safety (Ferreira et al. 2014a, b). While an rAAV-based gene therapy might be provided in conditions of immunosuppression to improve efficacy (Ferreira et al. 2014b), the safety of the immunosuppressed patients remains a concern as OA is a nonlethal disease. A newly developed, highly potent approach to circumvent these issues is to provide rAAV using effective controlled release strategies by delivery of the vectors from adapted biomaterials as a means to mask the immunogenic capsid epitopes in the host without impairing the efficacy of gene transfer and target cell modification (Rey-Rico et al. 2015b, c; Rey-Rico and

Cucchiarini 2015; Diaz-Rodriguez et al. 2015). Tissue-engineering strategies may also prove beneficial to improve the repair of cartilage lesions and thus address the challenge of providing functional, adapted cartilage replacement therapies as largely tested in preclinical settings in relevant models (Johnstone et al. 2013; Cucchiarini and Madry 2014b; Madry and Cucchiarini 2014). Eventually, as complete regeneration of an original hyaline cartilage has not been reported thus far in preclinical models, further research is clearly needed to identify the most effective genes or combinations of genes for therapy, suggesting that a better understanding of the joint pathologies is essential by continuous efforts between orthopaedic surgeons, scientists, and regulatory organizations to advance the current approaches and trials in patients (Cucchiarini et al. 2014).

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